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2022 KALAS

International Symposium

July 20(Wed) ~ 23(Sat)

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NSF 49 · EN 12469 · E 323747 · KS J 0012

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Safety Evaluation

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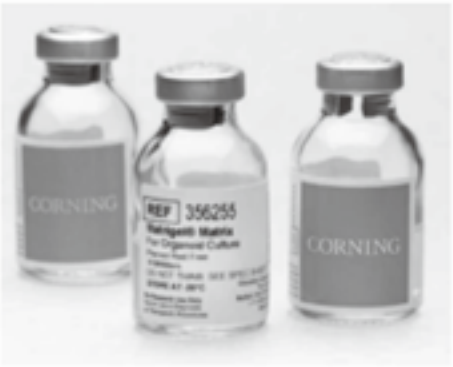
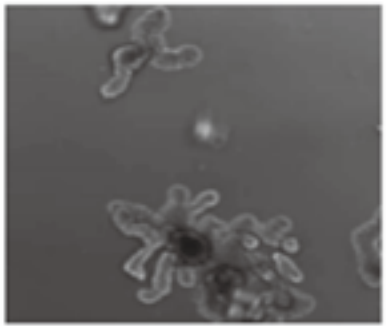
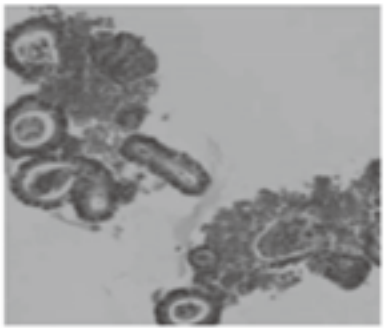
Corning® Matrigel® Matrix For Organoid Culture

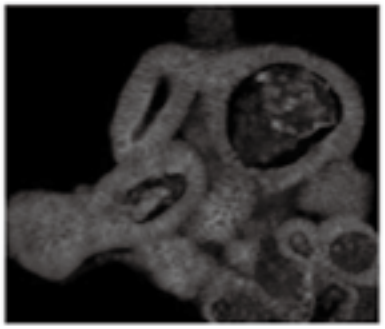
CORNING



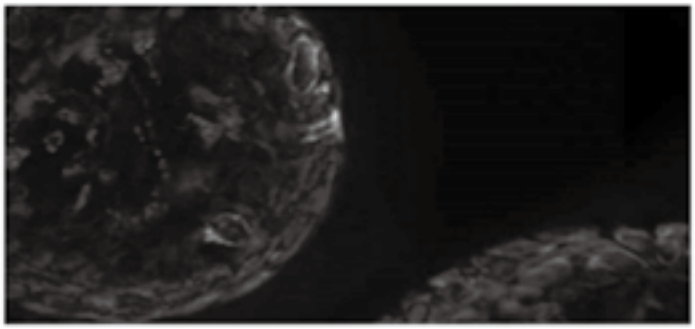
- 일반적으로 사용되는 오가노이드 배양 프로토콜로 '3D Dome' 형성 및 유지
- Maxtrix 강도의 지표인 elastic modulus 정보 제공
- 형성된 Matrix는 37°C 14일 동안 안정
- Negative for bacteria, fungi, mycoplasma
- Endotoxin Unit 테스트 완료 (Limulus Amoebocyte Lysate assay)







Intestinal organoids grown in Corning Matrigel matrix for organoid culture show typical budding morphology and marker expression (Vimentin, Mucin-2, Villin, Chromogranin, and Lysozyme)[3].



Airway organoids grown in Corning Matrigel matrix for organoid culture shown to express typical differentiation markers of basal (green), ciliated (red) and goblet (orange) cells[4].

Ordering Information

Cat. No.	Description	Qty/Pk	Qty/Cs
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NISO Rack (자동급수 장치형)

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Decon Chamber


Double Bio Safety Cabinet


Rotary Cage Washer


Cabinet Cage Washer


Tunnel Washer


Rack,Cage Washer


Bedding Dispenser

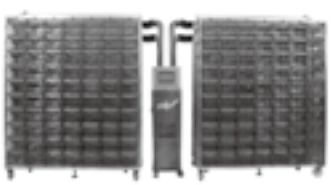
2. IVC Rack (Individual Ventilation Cage System)


ECO-friendly animal Lab


NISO Standard Rack


NISO Auto Watering Rack


MVCS Top type


MVCS Separated type


MVCS Cabinet type


Disposable Rack(ABSL-3,4, Standard type)

3. Breeding Equipment


Guinearpig Rack


Rabbit Auto Washing Rack


Chicken Rack


Dog Cage


Pig Incubator & Cage


Monkey Cage

4. ISOLATOR (ABSL-3,4)


Rodent ISO Rack


Rabbit ISO Rack


Chicken ISO Rack


Operating Isolator


Dog Isolator


Pig & Mini Pig Isolator


Monkey Isolator

5. Managing Equipment


Work Station


Disposal Cleaner


BSC


Sink


Cart

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Operating Table


Back Draft Chamber


CO_2 gas Cabinet


Operating Tool

7. Testing Equipment


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Smoking Tester

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Inhalation Chamber

Inhalation Toxicity Testing System

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Clean Bench

Motic
MORE THAN MICROSCOPY

AUTOMATIC TISSUE SLIDE SCANNER

미래를 여는 생명과학 기업
(주) 킴앤프렌즈
The Company of Life Science
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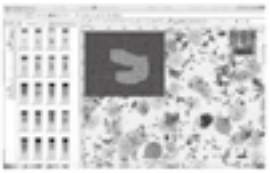
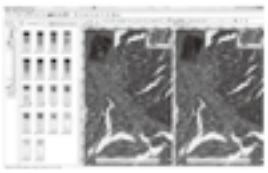



STEREOTAXIC INSTRUMENTS

Stoelting SINCE 1886

#51500D
Digital New Standard Stereotaxic, Rat and Mouse

#51730
Just for Mouse Stereotaxic instrument

#53850D
Stoelting Rodent Warner Control Box X2 w/cable

#53311
Quintessential Stereotaxic Injector (QSI)

#51500
New Standard Stereotaxic, Rat and Mouse

#51700
Motorized Lab Standard Stereotaxic., Rat,

#51625
Mouse and Neonatal Rat Adaptor

#51725
Just for Mice Stereotaxic Instrument

F · S · T
Fine Science Tools

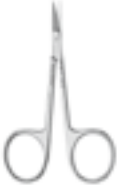
MOST POPULAR ITEMS

Vannas Spring Scissors

Cutting Edge: 2.5mm
Tip Diameter: 0.05mm
Length: 8cm

No. 15000-08

Extra Fine Bonn Scissors



Straight
Sharp/Sharp: 8.5 cm
Curved
Sharp/Sharp: 8.5 cm

No. 14084-08 / 14085-08

Fine Iris Scissors

Straight
Sharp/Sharp: 10.5 cm
Cutting Edge: 23mm

No. 14088-10

Tungsten Carbide Fine Scissors

Straight
Sharp/Sharp: 9 cm

No. 14568-09

Straight
Sharp/Sharp: 11 cm

No. 14568-12

Friedman-Pearson Rongeur

Straight: 14 cm
Curved: 14 cm

No. 16220-14

Friedman Rongeur

Curved
2.5 mm Cup
13 cm

No. 16000-14

Dumont #5 Fine Forceps

Biology tip
0.05 x 0.01 mm
Inox
11 cm

No. 11254-20

Suture Tying Forceps

Angeld 45°
Platform
Tip width: 0.4 mm
9 cm

No. 11063-07

대구경북과학기술원 실험동물센터

DGIST LABORATORY ANIMAL RESOURCE CENTER

DGIST LARC

ONE STOP SOLUTION

1 동물반입을 위한 청정화 및 첨단사육시스템

첨단 동물 사육 시스템

규모 : 5,440 ㎡, IVC Cage

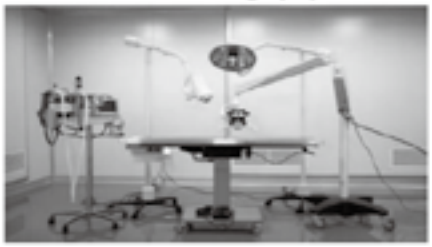
수용 능력 : 35,000 Mice, 3,500 Rats, 10 Guinea pigs, 24 Rabbits, 10 Mini pigs

실험동물 청정화 및 고속증식 서비스

2 소-중동물 모델별 전용 수술/처치 장비 및 시설

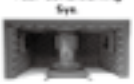
Stereotaxic Surgery Station

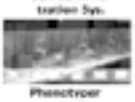
Middle-Animal Surgery System



3 다면적 표현형 분석 장비 및 활용지원 (행동/대사/생체 이미징)

소동물행동분석



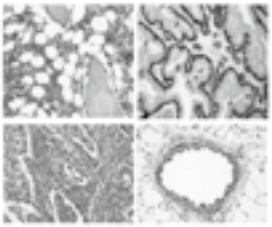


In vivo 생체 이미징

대사사육통합분석

4 생체 유래 조직분석 장비 및 기술지원 (조직배양/부검/조직병리)

조직병리분석



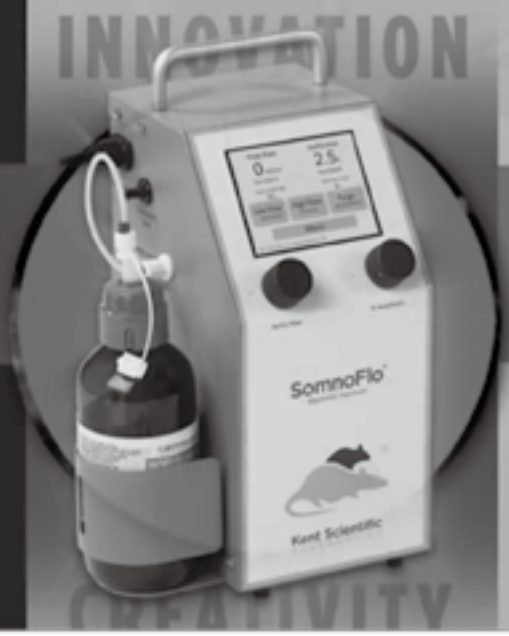
기타 자세한 사항은 larc.dgist.ac.kr 홈페이지에서 확인 바랍니다.

문의 : 053-785-0520, dgist_larc@dgist.ac.kr

Introducing the SomnoFlo®

Low-Flow Electronic Vaporizer

Compact • Precise
Accurate • Maintenance free



INNOVATION TECHNOLOGY CREATIVITY

- Low flow rates
  1 mL/hr 미만의 isoflurane 사용으로 비용 절감
- Built-in air compressor
  Room air or compressed gas 사용 가능
- Automated anesthetic delivery system
  0.1% 단위로 정밀한 마취제 전달
- Induction chamber purge
  WAG 노출 최소화

"실내 공기를 이용, 산소탱크가 필요 없음.
2-3 button 만 이용하면 됨.
매우 쉽고 편리한 디지털 마취기
소형으로 이동이 자유롭다"

- Filling and Delivery
  - SomnoFlo : Direct from bottle (100ml & 250ml)
  - SomnoSuite : Galss Syringe (5ml & 10ml)
- Warming System
  - SomnoFlo : Not included
  - SomnoSuite : RightTemp included (Warming pad & Sensors)

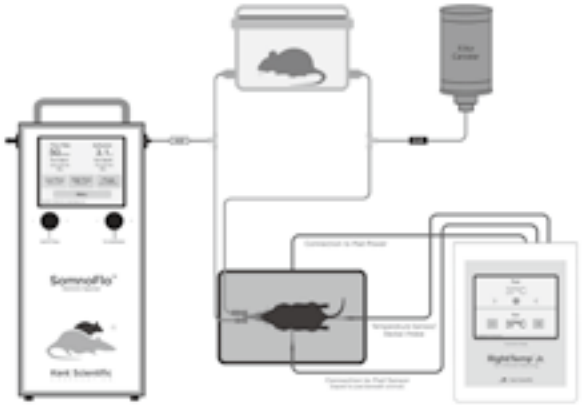
SomnoFlo

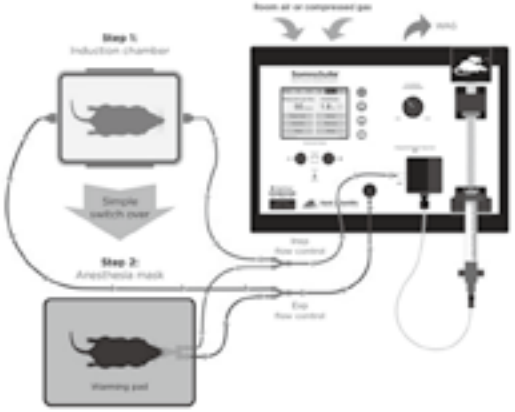
SomnoFlo SF-01 Starter Kit SF-MSEKIT / SF-RATKIT

SomnoSuite

SomnoSuite SS-01 Starter Kit SOMNO-MSEKIT / RATKIT

Easy Setup





(주)이츠바이오 www.itsbio.co.kr (T) 02-3462-8658 (F) 02-3462-8659 info@itsbio.co.kr

www.kalas.or.kr

2022 KALAS International Symposium

July 20(Wed) ~ 23(Sat)

ICC JEJU · Jeju Island · Korea

UNBIAS Unveiling Bio-network using Animal System

Organized by

KOREAN ASSOCIATION FOR LABORATORY ANIMAL SCIENCE

Sponsored by

KOFST Korean Federation of Science & Technology Societies

National Institute of Food and Drug Safety Evaluation

KMPC 국가마우스표현형분석사업단 KOREA MOUSE PHENOTYPING CENTER

WE GREEN JEJU Sustainable MICE CITY

KIT 안전성평가연구소 Korea Institute of Toxicology

INVITATION

It is our great pleasure to warmly invite you to the 2022 KALAS International Symposium, entitled with '**UNBIAS (Unveiling Bio-network using Animal System)**' at Jeju International Convention Center (Jeju ICC) in Jeju island from July 20 (Wed) to 23 (Sat) in 2022.

The International Symposium is composed with 2 plenary lectures: 'Microbiome and nutrition in animal health and diseases' (Prof. Won-Jae Lee, Seoul Natl. Univ.) and 'Defining and engineering the gut stem cell microenvironment' (Prof. Tae-Hee Kim, The Hospital for Sick Children & University of Toronto), and 16 program sessions including Cancer model, Zebra-fish, Marmoset, Gut-brain axis, Mitochondrial dynamics and therapy, IBD preclinical models, Liver diseases, IACUC, Research ethics, KLAT educational sessions, etc.

The symposium will be supported by several institutions, agencies and organizations including KOFST, NIFDS, JCVB, KIT, KMPC, etc. and I would like to thank all those concerned in this symposium.

We hope that all participants will enjoy this KALAS international symposium that give an opportunity to broaden your academic knowledge, and carry back with you fond memories from Jeju island. Once again, I sincerely appreciate for those who support and contribute to this international symposium.

KilSoo Kim
President, KALAS

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	위원	강경수	신구대학교
	위원	김재길	한신메디칼㈜
	위원	김찬수	베트컴코리아㈜
	위원	박천귀	㈜쓰리샤인
	위원	유병천	㈜아이티스텐다드
	위원	이 오	㈜오리엔트바이오
	위원	이현주	㈜샘타코바이오코리아
	위원	한남옥	㈜코아텍
법제위원회	위원장	**김배환**	계명대학교
	위원(간사)	김기석	안전성평가연구소
	위원(간사)	서민수	대구경북첨단의료산업진흥재단
	위원(간사)	정세훈	대구보건대학교
연구윤리위원회	위원장	**이원우**	서울대학교
	위원(간사)	박준원	강원대학교
	위원	천명선	서울대학교
	위원	전주홍	서울대학교
사업위원회	위원장	**박종환**	전남대학교
	위원(간사)	김동재	대구경북과학기술원
동물복지위원회	위원장	**제정환**	서울대학교병원
	위원(간사)	김시윤	건국대학교
	위원	천명선	서울대학교
	위원	엄민영	한국식품연구원
포상위원회	위원장	**이태훈**	전남대학교
	위원(간사)	김지수	한국생명공학연구원
	위원	복진웅	연세대학교
	위원	성제경	서울대학교
연구정책위원회	위원장	**최양규**	건국대학교
	부위원장	석승혁	서울대학교
	위원(간사)	나이랑	서울대학교병원
	위원(간사)	이경선	오송첨단의료산업진흥재단
	위원(간사)	정의숙	대구경북첨단의료산업진흥재단
교육위원회	위원장	**이병한**	오송첨단의료산업진흥재단
	위원(간사)	조윤주	서정대학교
	위원	박세일	연세대학교
	위원	이재일	서울대학교
	위원	이영아	신구대학교
	위원	이재원	한국건설생활환경시험연구원
회원위원회	위원장	**최우성**	Shine TnC
	위원	주영신	가톨릭대학교
공보위원회	위원장	**고필옥**	경상대학교
지회	서울/경기지회장	**송문용**	한국건설생활환경시험연구원
	충청지회장	**정재황**	충북도립대학
	강원지회장	**양세란**	강원대학교
	전라지회장	**김종춘**	전남대학교
	경상지회장	**권동락**	대구가톨릭대학교
	제주지회장	**지영흔**	제주대학교

KALAS COUNCIL MEMBERS

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성명	소속
강경선	서울대학교
강병철	서울대학교
강부현	㈜켐온
강종구	㈜바이오톡스텍
강진석	남서울대학교
고필옥	경상대학교
고혁완	연세대학교
구재형	대구경북과학기술원
권동락	대구가톨릭대학교병원
권명상	강원대학교
권중기	전북대학교
김곤섭	경상대학교
김근형	충북대학교
김길수	대구경북첨단의료산업진흥재단
김대중	충북대학교
김동환	건양대학교
김명옥	경북대학교
김무강	충남대학교
김배환	계명대학교
김옥진	원광대학교
김용범	안전성평가연구소
김윤배	충북대학교
김정훈	서울대학교
김종성	한국실험동물전임수의사협의회
김종춘	전남대학교
김진만	(주)코아텍
김천호	강원대학교
김철규	제주대학교
김충용	㈜제니아
김태완	경북대학교
김태환	경북대학교
김형진	한국생명공학연구원
김환묵	가천대학교
남기택	연세대학교
남기환	한국생명공학연구원
남상윤	충북대학교
남정석	광주과학기술원(GIST)
류재웅	경북대학교
박대훈	동신대학교
박재학	서울대학교
박정규	서울대학교
박종일	한국한의약진흥원 한약비임상시험센터
박종환	전남대학교

성명	소속
박준석	대구경북첨단의료산업진흥재단
박천귀	㈜쓰리샤인
배재성	경북대학교
배춘식	전남대학교
서준교	한림대학교
석승혁	서울대학교
성제경	서울대학교
성하정	지지에스㈜
송문용	한국건설생활환경시험연구원
송승우	㈜지나패스
송시환	㈜켐온
송창선	건국대학교
송창우	안전성평가연구소
신영수	신구대학교
신재호	을지대학교
양만표	충북대학교
양세란	강원대학교
염수청	서울대학교
오구택	이화여자대학교
오승현	가천대학교
원청길	경상대학교
위명복	강원대학교
유영춘	건양대학교
윤문석	농림축산검역본부
윤여성	서울대학교
윤원기	한국생명공학연구원
윤준원	서울대학교
이경선	오송첨단의료산업진흥재단
이국현	서울대학교
이근욱	한림대학교
이동섭	서울대학교
이만휘	경북대학교
이민재	강원대학교
이범준	충북대학교
이병한	오송첨단의료산업진흥재단
이병희	환경부 국립생물자원관
이상래	아주대학교
이순신	순천향대학교
이영순	한국실험동물협회
이영재	가천대학교
이원우	서울대학교
이정규	중앙실험동물㈜
이종권	식품의약품안전평가원

성명	소속
이철호	한국생명공학연구원
이태훈	전남대학교
이학모	㈜디자인원헬스케어
이한웅	연세대학교
이현웅	㈜대종기기산업
이현주	㈜샘타코 바이오코리아
이　호	국립암센터
장인석	경상국립대학교
장자준	서울대학교
장재진	㈜오리엔트 바이오
전경희	연세대학교
전현정	한국식품연구원
정기원	㈜엠제이엘티디
정용현	한국한의약진흥원
정은주	안전성평가연구소
정재황	충북도립대학
정지윤	공주대학교
정태천	영남대학교
제정환	서울대학교병원
조규혁	안전성평가연구소
조성대	서울대학교
조재진	서울대학교
조정식	(전) 식약처 실험동물자원실
진희경	경북대학교
차신우	안전성평가연구소
차지영	가천대학교
천병년	㈜우정바이오
최경철	충북대학교
최병인	가톨릭대학교 성의교정
최양규	건국대학교
최연식	한국폴리텍대학 바이오캠퍼스
최우성	Shine TnC
최재훈	한양대학교
한남옥	㈜ 코아텍
한범석	호서대학교
한상섭	전북대학교
한진수	건국대학교
허승호	서울아산병원
허　용	대구가톨릭대학교
현병화	KAIST
황대연	부산대학교
황종익	고려대학교

발전기금 납입 회원

2022. 7. 1. 기준

성명	소속	금액(단위:원)
Alan Lee Chedester	NIH	300,000
Toru Takeo	Kumamoto University	USD 1,000
강병철	서울대학교	2,311,200
강종구	㈜바이오톡스텍	1,000,000
강진석	남서울대학교	200,000
권구범	NTCV Vaccine Co.	100,000
권중기	전북대학교	200,000
김길수	경북대학교	200,000
김대용	서울대학교	350,000
김대중	충북대학교	517,000
김덕원	삼양약화학	50,000
김배환	계명대학교	802,280
김윤배	충북대학교	267,680
김종성	삼성생명과학연구원	267,680
김종춘	전남대학교	300,000
김형식	부산대학교	300,000
남정석	광주과학기술원	869,950
박재학	서울대학교	5,955,525
박충권	녹십자 EM	300,000
서경덕	천안연암대학	100,000
서준교	한림대학교	1,769,950
석승혁	서울대학교	2,100,000
성제경	서울대학교	300,000
성하정	크로엔리서치	200,000
손우찬	울산대학교	100,000
송시환	㈜켐온	100,000
송창우	안전성평가연구소	3,650,000
신영수	신구대학교	467,680
신재호	을지대학교	200,000
안병우	충북대학교	200,000
염수청	서울대학교	200,000
오승현	가천대학교	200,000
오양석	한림대학교	1,791,299
원무호	강원대학교	3,000,000

성명	소속	금액(단위:원)
위명복	강원대학교	391,200
유영춘	건양대학교	200,000
이민재	강원대학교	500,000
이범준	충북대학교	200,000
이병한	오송첨단의료산업진흥재단	367,680
이상구	㈜바이오톡스텍	200,000
이상필	㈜한주씨엠아이	4,000,000
이수해	식품의약품안전처	100,000
이정규	㈜중앙실험동물	1,000,000
이철호	한국생명공학연구원	200,000
이한웅	연세대학교	5,000,000
인증위원회	인증위원회	3,000,000
인증위원회	동물실험길잡이 인세	36,587,000
장동덕	국군의학연구소	200,000
장자준	서울대학교	882,400
정기원	㈜엠제이엘티디	1,000,000
정재황	충북도립대학	100,000
정지윤	공주대학교	200,000
제정환	서울대학교병원	837,630
조기행	서울대학교	300,000
조윤주	서정대학교	200,000
차신우	안전성평가연구소	200,000
천병년	㈜우정비에스씨	2,000,000
최경철	충북대학교	1,000,000
최양규	건국대학교	769,950
최연식	한국폴리텍바이오대학교	200,000
최우성	대구경북첨단 의료산업진흥재단	200,000
한남옥	코아텍	30,000,000
한범석	건양대학교	200,000
허　용	대구가톨릭대학교	200,000
현병화	오송첨단의료산업진흥재단	30,300,000
황대연	부산대학교	200,000
황인구	서울대학교	1,900,000
(재)한국건설생활환경시험연구원		2,000,000

학술상, 편집위, 실험동물연구장학생 후원금 납입 내역

2022. 7. 1. 기준

소속	금액(단위:원)	년도
㈜쓰리싸인	25,000,000	2005-2009
㈜코아텍	10,000,000	2016
중앙실험동물㈜	197,000,000	2007-2021

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2022. 07. 01 기준

성명	소속
강경수	신구대학교
강병철	서울대학교
강종구	충북대학교
강진주	경북대학교
고은아	제주대학교
곽동미	경북대학교
구재형	대구경북과학기술원 (DGIST)
권구범	우정BSC
권동락	대구가톨릭대학교병원
권명상	강원대학교
권은아	서울대학교병원
권재성	연세대학교
권태준	대구경북첨단의료산업진흥재단
김건아	을지대학교
김경원	서울아산병원
김근형	충북대학교
김길수	대구경북첨단의료산업진흥재단
김대원	강릉원주대학교
김대중	충북대학교
김명옥	경북대학교
김무강	(재)충남동물자원센터
김배환	계명대학교
김보라	국립암센터
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<tr><td>13:00-14:00</td><td colspan="3">Registration</td><td></td></tr>
<tr><td>14:00-15:40</td><td>(ENG)
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Current status of pig to NHP heart and kidney xenotransplantation</td><td>(ENG)
Symposium 2
[IACUC]
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Shell Scheme
(Space+Frame)
:16:00~</td></tr>
<tr><td>15:40-16:00</td><td colspan="2">Refreshment</td></tr>
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Studies on brain and vascular functions using novel mouse models</td><td>(KOR)
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준비위원회
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TIME TABLE

July 21 (Thu)

HALL	Halla Hall A	Halla Hall B	Samda Hall	Meeting Room (3F)	Meeting Room (3F)	Lobby
08:30-09:00						
09:00-09:40	**Registration**					**Poster 부착시간** (09:00-11:00)
09:40-10:00	Opening					
10:00-11:00	**(KOR) Plenary Lecture 1**					
11:00-11:50	**(ENG) Symposium 5** Mitochondrial dynamics and therapy	**(KOR) Symposium6 [KMPC1]** Non mammalian -zebrafish	**(KOR) Symposium 7 [NIFDS1]** Preclinical non-human primate research for pharmaceutics development			**Poster & Booth**
11:50-12:40					**인증위원회 소회의** (12:30~13:30 / 303호)	
12:40-13:00	Refreshment & Exhibition visit					
13:00-13:20	**Luncheon Seminar 1** (K-MEDI hub)	**Luncheon Seminar 2** (KRICP)	**Luncheon Seminar 3** (KOSABIO & FOLAS)			
13:20-14:00	Lunch Break					**Poster Presentation1** (13:20-14:20)
14:00-14:40	**Academy Award Presentation**			**식품의약품 안전평가원** Satellites meeting (14:00~17:00 / 301호)	**동물복지 위원회 소회의** (14:00~15:00 / 303호)	
14:40-14:50	Refreshment					
14:50-16:30	**(ENG) Symposium 8** Current topics of the microbiota-gut brain axis in health and disease	**(KOR) Symposium 9 [KMPC2]** *In vitro* and *in vivo* models for cancer research	**(KOR) Symposium 10 [NIFDS2]** Development of medicines to respond to global public health crises			**Poster & Booth**
16:30-17:00	Refreshment					
17:00-19:00	**General Meeting & Welcome Reception (Tamna Hall 5F)**					

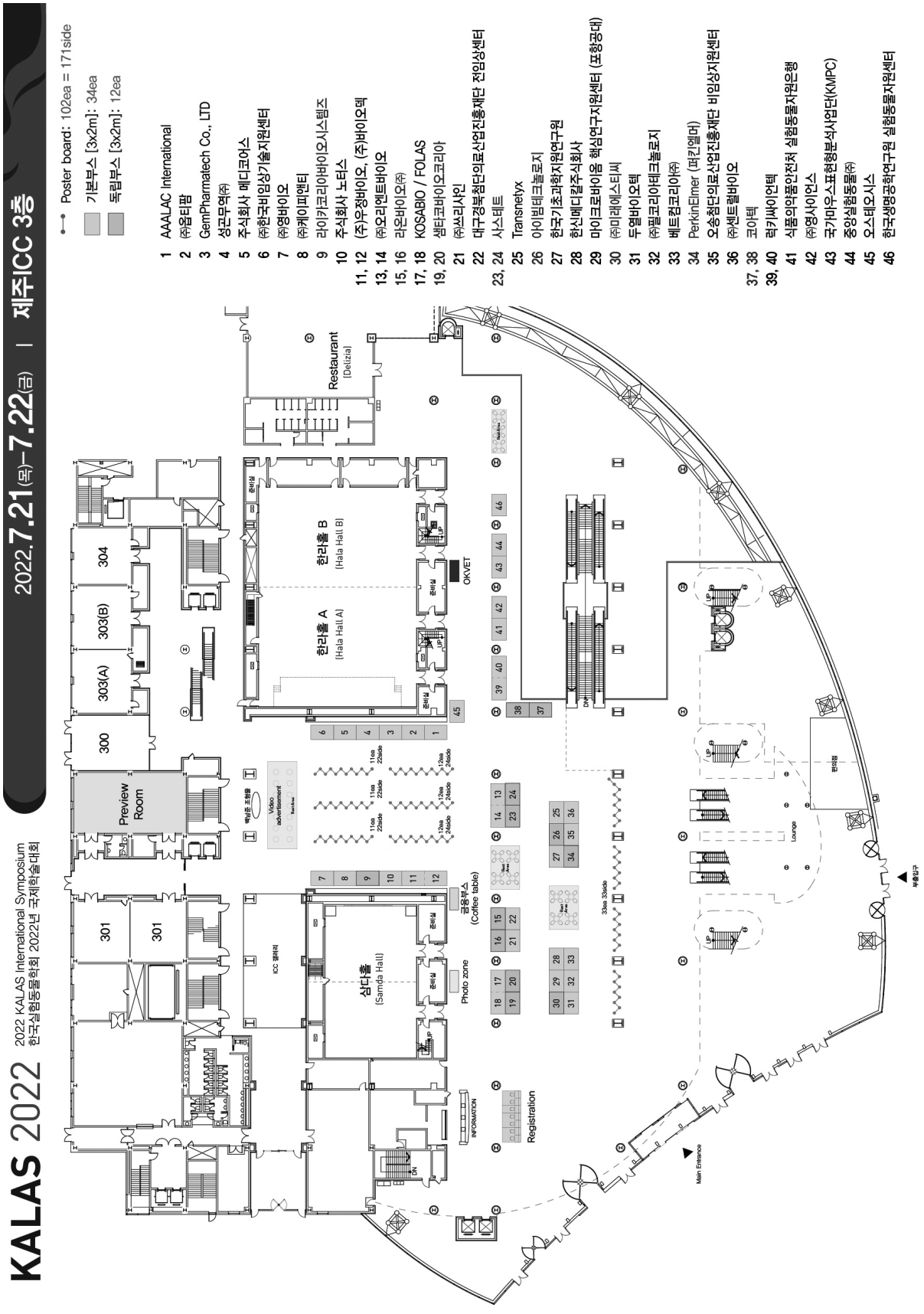
July 22 (Fri)

HALL	Halla Hall A	Halla Hall B	Samda Hall	Meeting Room (3F)	Lobby
08:30-09:00	Registration				
09:00-10:40	**(ENG) Symposium 11** IBD preclinical models and new treatment modalities	**(KOR) Symposium 12** Current translational research for human diseases using marmoset	**(KOR) Symposium 13 [Special Subcommittee]** Animal facility design and operation strategy for user safety	**산학협력 간담회** (10:00~10:30 / 301호)	**Poster 부착시간** (09:00-11:00)
10:40-11:00	Refreshment & Exhibition visit				
11:00-12:00	**(ENG) Plenary Lecture 2**				**Poster & Booth**
12:00-12:10	Refreshment				
12:10-12:30	**Luncheon Seminar 4** (IT standard)	**Luncheon Seminar 5** (Ajou Univ. RSCP)	**Luncheon Seminar 6** (THREE SHINE INC)		
12:30-14:00	Lunch Break				**Poster Presentation2** (13:00-14:00)
14:00-15:30	**(ENG) Symposium 14** The precision medicine initiative for liver disease through metabolic analysis	**(KOR) Symposium 15 [KLAT Education2]** Laboratory animal facility management	**(KOR) Symposium 16** Research ethics and responsible research		**Poster & Booth**
15:30-15:50		**기술원 QUIZ EVENT**			
15:50-16:20	**Giveaway & Closing Ceremony**				

July 23 (Sat)

HALL	Halla Hall A
9:30-11:00	Workshop

HALL INFORMATION



PROGRAM

July 20 (Wed)

• Symposium 1

14:00-15:40 | Halla Hall A ENG

Current status of pig to NHP heart and kidney xenotransplantation

· Organizer : Ik-Jin Yun (Konkuk University Hospital)
· Chair : Ik-Jin Yun (Konkuk University Hospital) / Hyunil Kim (Optipharm Co., Ltd)

Xenotransplantation steps from research to clinical application	Wayne Hawthorne	University of Sydney
Current status and results of genetic engineered pig to monkey preclinical kidney xeno-transplantation in Korea	Ik-Jin Yun	Konkuk University Hospital
Progress and breakthrough in preclinical and clinical xenotransplantation of pig hearts with multiple genetical modifications	Paolo Brenner	Clinic of Grosshadern, University of Munich (LMU)
Results of pig to monkey heterotopic cardiac xenotransplantation in Korea	Hyun Keun Chee	Konkuk University Medical Center

• Symposium 2 [IACUC]

14:00-15:40 | Halla Hall B ENG

COVID-19's impacts on IACUC communication

· Organizer / Chair : Seung Hyeok Seok (Seoul Natl. Univ.)

A COVID-19 pandemic: the survey for animal use and care staff in Korea	Na Ahn	Jangan Univ.
Conduct of IACUC meetings and semiannual facility inspections under the pandemic situation	Gwi Hyang Lee	The Catholic Univ.
Utilizing various modes of communication at SNU IACUC office during the COVID-19 pandemic	Ji Min Lee	Seoul Natl. Univ.
Guidance for IACUC regarding animal care and use during COVID-19 Pandemic – AAALAC International expectation	Montip Gettayacamin	AAALAC International

• Symposium 3

16:00-17:40 | Halla Hall A KOR

Studies on brain and vascular functions using novel mouse models

· Organizer / Chair : Young Jae Lee (Gachon Univ.) / Young Hoon Sung (University of Ulsan & Asan Medical Center)

Animal models for studying the onset of arteriovenous malformations	Yong Hwan Kim	Barrow Neurological Institute
Harmful effects of polystyrene nanoplastics on developing brain	Da Yong Lee	KRIBB
Mouse models of 22q11.2 deletion syndrome for studying neuroanatomical abnormalities	Tae-Yeon Eom	St Jude Children's Research Hospital
Reward learning improves social information processing in the medial prefrontal cortex	Doyun Lee	IBS

• Symposium 4 [KLAT Education1 With KIT]

16:00-17:40 | Halla Hall B KOR

Advanced experimental techniques and practical considerations in preclinical animal study

· Organizer / Chair : Kang-Hyun Han (KIT)

Efficacy and toxicity evaluation of anticancer drugs in tumor-bearing mice	Soon-Oh Hong	Kolon Life Science
Delivery of antisense oligonucleotides to the mouse brain and neurobehavioral evaluations	Jeong-Wook Ghim	SoVarGen
Intrathecal injection and neurologic examination in beagle dogs	Won-Tae Kim	KIT

PROGRAM

July 21 (Thu)

• Opening — 9:40-10:00 | Halla Hall A

• Plenary Lecture 1 — 10:00-11:00 | Halla Hall A — KOR

· Organizer : Jinwoong Bok (Yonsei Univ.) | · Chair : Je Kyung Seong (Seoul Natl. Univ.)

Microbiome and nutrition in animal health and diseases	Won-Jae Lee	Seoul Natl. Univ.

• Symposium 5 — 11:00-12:40 | Halla Hall A — ENG

Mitochondrial dynamics and therapy

· Organizer : Kyoung-Jin Oh (KRIBB) | · Chair : Jae Bum Kim (Seoul Natl. Univ.)

In adipocytes, DNMT1 is an epigenetic safeguard of mitochondrial dynamics	Jae Bum Kim	Seoul Natl. Univ.
Mitochondria-targeted drug delivery systems for anti-tumor therapy	Han Chang Kang	The Catholic Univ.
Regulation of metabolic switch in aged skeletal muscle by RNA binding protein	Jiyun Ahn	KFRI
Fluorescence imaging of mitochondrial DNA base excision repair (BER) process	Yong Woong Jun	Stanford Univ.

• Symposium 6 [KMPC1] — 11:00-12:40 | Halla Hall B — KOR

Non mammalian -zebrafish

· Organizer : Hyunju Ro (Chungnam Natl. Univ.) | · Chair : Min Jung Kim (Sookmyung Women's Univ.)

Disease modeling of rare neurological disorders in zebrafish	Cheol-Hee Kim	Chungnam Natl. Univ.
Zebrafish as a model for neurodegenerative diseases and neurotoxicity	Hae Chul Park	Korea Univ.
A novel gene switch optimized for zebrafish transgenesis	Hyunju Ro	Chungnam Natl. Univ.

• Symposium 7 [NIFDS1] — 11:00-12:40 | Samda Hall — KOR

Preclinical non-human primate research for pharmaceutics development

· Organizer : Byeong-Cheol Kang (Seoul Natl. Univ.) | · Chair : Jong Kwon Lee (NIFDS)

Validation of scrub typhus vaccine in nonhuman primates infection model	Nam-Hyuk Cho	Seoul Natl. Univ.
Safety evaluation of vaccines for nonhuman primates	Doo-Wan Cho	KIT
Preclinical research with marmoset in seoul national university hospital marmoset model network center (SNUH MMNC)	Byeong-Cheol Kang	Seoul Natl. Univ.
Studying multiple sclerosis (MS) with advanced MRI and pathology using marmoset model	Seung-Kwon Ha	University of Pittsburgh

• Luncheon Seminar 1 — 13:00-13:20 | Halla Hall A

· Organizer : Preclinical Research Center, K-MEDI hub

I want to know there, Preclinical Research Center, K-MEDI hub	Joon-Suk Park	Preclinical Research Center, K-MEDI hub

• **Luncheon Seminar 2**	13:00-13:20 | Halla Hall B	
· Organizer : KRICP, Korea Institute of Radiological & Medical Sciences		
Toward national upgrade of *in vivo* animal experiments in drug development and evaluation	Kyeong Min Kim	Korea Radioisotope Center for Pharmaceuticals

• **Luncheon Seminar 3**	13:00-13:20 | Samda Hall	
· Organizer : KOSABIO & FOLAS | · Chair : Sang Rae Lee (AJOU Univ.)		
Cage Washers 101 – Comprehensive review of cage washing solutions	Mike Douglas	Allentown, LLC

• **Poster Presentation 1**	13:20-14:20 | Lobby	
· Chair : Jae-Hoon Choi (Hanyang Univ.)		

• **Academy Award Presentation**	14:00-14:40 | Halla Hall A	
· Chair : KilSoo Kim (K-MEDI hub)		
Inflammatory response in the mid colon of ICR mice treated with polystyrene microplastics for two weeks	Yun Ju Choi	Pusan Natl. Univ.
Establishment of particulate matter-induced lung injury model in mouse	Se Yong Park	Seoul Natl. Univ.
Surgical removal of a telemetry system in a cynomolgus monkey (*Macaca fascicularis*): a 12-month observation study	Doo-Wan Cho	KIT

• **Symposium 8** 14:50-16:30 | Halla Hall A ENG Current topics of the microbiota-gut brain axis in health and disease		
· Organizer / Chair : Jeong-Soo Lee (KRIBB)		
Gut feelings about the brain: Rodent models of the microbiome-gut-brain axis	John Cryan	University College Cork
Beneficial effects of human gut microbiota *Akkermansia muciniphila* on cognitive function in neurodegenerative mouse models	Chul-Ho Lee	KRIBB
Microbiome interactions with the nervous system in health and disease	Elaine Hsiao	UCLA
Transfer of a healthy microbiota reduces amyloid and tau pathology in an Alzheimer's disease animal model	Jin-Woo Bae	Kyung Hee Univ.

• **Symposium 9 [KMPC2]** 14:50-16:30 | Halla Hall B KOR *In vitro* and *in vivo* models for cancer research		
· Organizer : Seung Hyun Oh (Gachon Univ.) · Chair : Je Kyung Seong (Seoul Natl. Univ.) / Seung Hyun Oh (Gachon Univ.)		
Patient-derived cancer organoid hub platform for refractory or rare cancer	Yun-Hee Kim	National Cancer Center Korea
Preclinical murine models for cancer research : CDX vs Syngeneic model	Seung Hyun Oh	Gachon Univ.
Patient-derived xenograft model for cancer research	Sung Yup Cho	Seoul Natl. Univ.
Companion animals as alternative models for translational cancer research	Kyong-Ah Yoon	Konkuk Univ.

PROGRAM

• **Symposium 10 [NIFDS2]**		14:50-16:30 | Samda Hall KOR
Development of medicines to respond to global public health crises		
· Organizer : Jun Won Yun (Seoul Natl. Univ.) | · Chair : Jun-Young Seo (Yonsei Univ.)		
Nanobiotechnology for diagnosis and vaccine against Viral Disease X in animal and human	Daesub Song	Seoul Natl. Univ.
Current status of mRNA vaccine development for the preparedness of emerging infectious diseases in Korea	Kee-Jong Hong	Gachon Univ.
Development of next generation COVID-19 vaccine using adenovirus vector platform	Kwang-Soo Shin	CELLID

• **General Meeting & Welcome Reception**	17:00-19:00 | Tamna Hall 5F

July 22 (Fri)

• **Symposium 11**		09:00-10:40 | Halla Hall A ENG
IBD preclinical models and new treatment modalities		
· Organizer : Yi Rang Na (Seoul Natl. Univ. Hospital) | · Chair : Seung Hyeok Seok (Seoul Natl. Univ.)		
The role of macrophages in gut inflammation	Seung Hyeok Seok	Seoul Natl. Univ.
Understanding neuro-immune crosstalk in the gut using animal models to develop novel therapeutic strategies for intestinal inflammation	Gianluca Mateoli	KU Leuven
Gene therapy of genetic diseases via CRISPR systems	Daesik Kim	Sungkyunkwan Univ.
Application of ex-vivo generated macrophages as potential cell therapy	Yi Rang Na	Seoul Natl. Univ. Hospital

• **Symposium 12**		09:00-10:40 | Halla Hall B KOR
Current translational research for human diseases using marmoset		
· Organizer : Jeong Hun Kim (Seoul Natl. Univ.) | · Chair : Byeong-Cheol Kang (Seoul Natl. Univ.)		
Translational research for retinopathy using marmoset	Jeong Hun Kim	Seoul Natl. Univ.
Characterization and age-related changes of primary marmoset retinal pigment epithelial cells	Ha Young Jang	Seoul Natl. Univ. Hospital
Generation of immortalized marmoset cell lines by a CRISPR-Cas9-mediated gene targeting	Young Hoon Sung	University of Ulsan & Asan Medical Center
Development of genetically engineered marmoset models of early onset Alzheimer's disease: Initial experiences	Jung Eun Park	University of Pittsburgh

● **Symposium 13 [Special Subcommittee]** Animal facility design and operation strategy for user safety	09:00-10:40 | Samda Hall	KOR
· Organizer : Yirang Na (Seoul Natl. Univ. Hospital) | · Chair : Yang-Kyu Choi (Konkuk Univ.)		
Efficacy evaluation and inhalation toxicity study for new inhaled drug development of respiratory diseases	Sung-Hwan Kim	KIT
Establishing and maintaining a gnotobiotic mouse facility	Hyunjhung Jhun	KFRI
Animal imaging facility	Jae Jun Lee	Osong Medical Innovation Foundation
Facility for non human primates	Ji-su Kim	KRIBB, PRC

● **Plenary Lecture 2**	11:00-12:00 | Halla Hall A	ENG
· Organizer / Chair : Huyk-Wan Ko (Yonsei Univ.)		
Defining and engineering the gut stem cell microenvironment	Tae-Hee Kim	The Hospital for Sick Children & University of Toronto

● **Luncheon Seminar 4**	12:10-12:30 | Halla Hall A	
· Organizer : ITstandard | · Chair : Jun-Gyo Suh (Hallym Univ.)		
Development of LMO management program for experiment and research	Byung chun Yoo	ITstandard. Co.,Ltd.

● **Luncheon Seminar 5**	12:10-12:30 | Halla Hall B	
· Organizer : Ajou Univ. Regulatory Strategy Center for Combination Product (RSCP)		
History of xeno-islets and recent new researches from transgenic pigs' islets	Hyunil Kim	Optipharm Inc.

● **Luncheon Seminar 6**	12:10-12:30 | Samda Hall	
· Organizer : THREE SHINE INC | · Chair : Dae Youn Hwang (Pusan Natl. Univ.)		
미래 실험동물실 경쟁력	Chun Gui Park	THREE SHINE INC

● **Poster Presentation 2**	13:00-14:00 | Lobby
· Chair : Jae-Hoon Choi (Hanyang Univ.)	

PROGRAM

• Symposium 14

14:00-15:50 | Halla Hall A ENG

The precision medicine initiative for liver disease through metabolic analysis

· Organizer / Chair : Won-Il Jeong (KAIST) / Hyon-Seung Yi (Chungnam Natl. Univ.)

Metabolic crosstalks in regulation of hepatic lipid homeostasis	Nika Danial	Dana-Farber Cancer institute (Harvard Medical School)
Altered fuel metabolism in the pathogenesis of NAFLD	Dong Wook Choi	Chungnam Natl. Univ.
Role of mitochondrial stress response in hepatic steatosis and liver cancer progression	Hyon-Seung Yi	Chungnam Natl. Univ.
Hepatic glutamate and mGluR5 in liver fibrosis	Won-Il Jeong	KAIST

• Symposium 15 [KLAT Education2]

14:00-15:50 | Halla Hall B KOR

Laboratory animal facility management

· Organizer : Byeong-Cheol Kang (Seoul Natl. Univ.) | · Chair : Hyung-Sik Kim (Pusan Natl. Univ.)

Facility management and environmental enrichment for nonhuman primate	Young-Su Yang	KIT
Laboratory animal facility management of environmental monitoring and COVID-19 pandemic	Kyoungmin Roh	Seoul Natl. Univ. Hospital
Disinfection and sterilization in laboratory animal facilities	Hyunjhung Jhun	KFRI
Quiz Event		Planning Committee

• Symposium 16

14:00-15:50 | Samda Hall KOR

Research ethics and responsible research

· Organizer : Ju-Hong Jeon (Seoul Natl. Univ.)
· Chair : Ju-Hong Jeon (Seoul Natl. Univ.) / Won-Woo Lee (Seoul Natl. Univ.)

Research misconduct under the academic promotion act and the national R&D innovation act	Ju-Hong Jeon	Seoul Natl. Univ.
How does research ethics committee operate?	Chin Ho Cho	Seoul Natl. Univ.
How to identify and deal with conflicts of interest in research	Hyobin Lee	Chungnam Natl. Univ.

• Giveaway & Closing Ceremony

15:50-16:20 | Halla Hall A

July 23 (Sat)

• Workshop

09:30-11:00 | Halla Hall A

CONFERENCE AND EVENT INFORMATION

1. 개회식 (Opening Ceremony)

July 21 (Thu) 9:40~10:00 / Halla Hall A

- 개회사: 복진웅 학술위원장
- 인사말: 김길수 이사장

2. 총회 및 환영만찬 (General Meeting & Welcome Reception)

July 21 (Thu) 17:00~19:00 / Tamna Hall (5F)

- 개회사: 남기택 총무위원장

1) 총회 안건

2) 학술상 시상식

① 공로상

시상 : 김길수 이사장

성 명	소 속
이국현	서울대학교

② 젊은과학자상

시상 : 김길수 이사장

성 명	소 속
최윤주 박세용 조두완	부산대학교 서울대학교 KIT

③ LAR 다수논문게재상

시상 : 김길수 이사장

성 명	소 속
황대연	부산대학교

④ LAR 논문다수인용상

시상 : 김길수 이사장

성 명	소 속
고필옥 김종춘 최영현	경상대학교 전남대학교 동의대학교

⑤ 실험동물연구장학생

시상 : 복진웅 학술위원장

구분	성 명	소 속
그룹 I	곽진아 손현경 진유정 황세미	서울대학교 경상대학교 부산대학교 건국대학교

⑥ 한국실험동물기술원상

시상 : 강병철 인증위원장

성 명	소 속
정민재 장윤성	한림대학교 전북대학교병원

⑦ 한국실험동물기술원 우수합격자상

시상 : 강병철 인증위원장

구분	성 명	소 속
1급	허인재 김예현	디티앤씨알오 ㈜ 켐온
2급	김유정 견다연	서정대학교 서울호서직업전문학교

3) 환영만찬

- 축사 : (사)대한수의학회 김곤섭 이사장
- 사회: 기획위원회

3. 폐회식 (Closing Ceremony)

July 22 (Fri) 15:50~16:20 / Halla Hall A

1) 우수포스터상 시상
 - 시상: 복진웅 학술위원장
2) 경품추첨
3) 폐회사
 - 인사말: 김길수 이사장
 - 폐회사: 복진웅 학술위원장
4) 기념촬영

4. 관련위원회 회의 및 모임 (Commitee & Meeting)

· KALAS 평의원회 (Council Meeting)

July 20 (Wed) 11:30~13:00 / 5F 오션뷰 (Ocean view)

참석대상: 한국실험동물학회 평의원

· KALAS-AFLAS 2023 준비위원회

July 20 (Wed) 16:00~17:00 / Room 303

참석대상: 한국실험동물학회 AFLAS 준비위원회 위원

· KALAS 이사회 (Board Meeting)

July 20 (Wed) 18:00~19:00 / 부영호텔

참석대상: 한국실험동물학회 이사 9명 및 감사 2명

· KALAS 인증위원회

July 21 (Thu) 12:30~13:30 / Room 303

참석대상: 한국실험동물학회 인증위원회 간사

· KALAS 동물복지위원회

July 21 (Thu) 14:00~15:00 / Room 303

참석대상: 한국실험동물학회 동물복지위원회 위원

· 식품의약품안전평가원 Satellites meeting

July 21 (Thu) 14:00~17:00 / Room 301

참석대상: 식품의약품안전평가원 관계자

공동주관: 식품의약품안전평가원 약리 연구과,
충북대학교 약학대학, 성균관대학교 약학대학,
대구한의대학교, k-bio

주제: 신종마약류 의존성평가 모델개발과 응용

· KALAS 우수포스터상 선정 회의

July 22 (Fri) 14:00~14:30 / Room 300 (사무국)

참석대상: 학술위원회 및 포스터 심사위원

5. 점심식사 안내 (Luncheon Seminar)

런천세미나		기업명	장소	티켓 수령처
July 21 (Thu) 13:00~13:20	LS 1	K-MEDI hub	Halla Hall A	09:30부터 기업부스 (선착순 100명)
	LS 2	KRICP	Halla Hall B	09:00부터 등록데스크 (선착순 100명)
	LS 3	KOSABIO & FOLAS	Samda Hall	09:30부터 기업부스 (선착순 100명)
July 22 (Fri) 12:10~12:30	LS 4	ITStandard	Halla Hall A	09:00부터 등록데스크 (선착순 100명)
	LS 5	Ajou Univ. RSCP	Halla Hall B	09:00부터 등록데스크 (선착순 100명)
	LS 6	THREE SHINE INC	Samda Hall	09:30부터 기업부스 (선착순 100명)

※ 런천 티켓은 한정 수량으로 조기 품절될 수 있습니다.
※ 런천 세미나 강연을 청취하신 후 퇴실 시 도장 수령하고, 식당에서 티켓 확인 후 식사가 제공 됩니다.
도장이 없는 경우 식사가 불가능한 점 참고 바랍니다.
※ 식사장소 : 5F 오션뷰 (Ocean view)

LS 1> K-MEDI hub
주제 : I want to know there, Preclinical Research Center, K-MEDI hub
연자 : Joon-Suk Park (Preclinical Research Center, K-MEDI hub)

LS 2> KRICP
주제 : Toward national upgrade of *in vivo* animal experiments in drug development and evaluation
연자 : Kyeong Min Kim (Korea Radioisotope Center for Pharmaceuticals)

LS 3> KOSABIO & FOLAS
주제 : Cage Washers 101 – Comprehensive review of cage washing solutions
연자 : Mike Douglas (Allentown, LLC)

LS 4> ITStandard
주제 : Development of LMO management program for experiment and research
연자 : Byung chun Yoo (ITstandard. Co.,Ltd)

LS 5> Ajou Univ. RSCP
주제 : History of xeno-islets and recent new researches from transgenic pigs' islets
연자 : Hyunil Kim (Optipharm Inc.)

LS 6 > THREE SHINE INC
주제 : 미래 실험동물실 경쟁력
연자 : Chun Gui Park (THREE SHINE INC)

6. 영상광고

- 운영방식: 강연장과 전시장 휴게공간에서 전시 기간 동안 기업 광고를 상영
- 신청기업: 대구경북첨단의료산업진흥재단 전임상센터, ㈜코사바이오 (KOSABIO/FOLAS)

7. 기념품 협찬

- 협찬기관 : 농림축산검역본부
- 후원물품: 스트레스볼 2,000개
- 배포방식: 등록데스크에서 수령

8. 실험동물연구장학생 포스터 발표

발표 시간	7월 21일(목) 13:20-14:20
발표 장소	제주컨벤션센터 (ICC JEJU) 3F Lobby
포스터 번호	PS-R-001 (유전자질환모델) PS-R-002 (해부생리) PS-R-003 (독성병리) PS-R-004 (해부생리)
	총 4개
부착 시간	7월 21일(목) 9:00-11:00
철거 시간	7월 21일(목) 17:40-18:00

※ 철거 시간 이후의 포스터 분실은 책임지지 않습니다.

· 포스터 발표는 좌장의 진행에 따라 포스터당 7분 (5분 발표, 2분 질의응답)으로 진행되며,
발표시간에 포스터 앞에 대기하여 주시기 바랍니다.
· **실험동물연구장학생 시상**: July 21 (Thu) 17:00~19:00 / Tamna Hall (5F)

9. 포스터 발표 (Poster Session)

발표 시간	포스터 발표1	포스터 발표2
	7월 21일(목) 13:20-14:20	7월 22일(금) 13:00-14:00
발표 장소	제주컨벤션센터 (ICC JEJU) 3F Lobby	
포스터 번호	PS-A-001~049 (해부생리) PS-B-001~053 (독성병리) PS-C-001~008 (미생물) PS-D-001~031 (유전자질환모델) PS-E-001~026 (시설운영 및 기타)	PS-A-050~098 (해부생리) PS-B-054~106 (독성병리) PS-C-009~019 (미생물) PS-D-032~062 (유전자질환모델) PS-E-027~053 (시설운영 및 기타)
	총 167개	총 171개
부착 시간	7월 21일(목) 09:00-11:00	7월 22일(금) 09:00-11:00
철거 시간	7월 21일(목) 16:30-17:00	7월 22일(금) 15:50-16:20

※ 철거 시간 이후의 포스터 분실은 책임지지 않습니다.

· **포스터 심사**: 포스터 발표는 좌장의 진행에 따라 포스터당 4분(3분 발표, 1분 질의응답)으로 진행되며,
내용의 과학성, 연구 성과, 발표자의 발표력 등을 기준으로 심사위원이 평가하여 우수포스터를 선정합니다.
발표 시간에 자리에 없는 경우, 미부착으로 간주합니다.

· **미부착 포스터**: 포스터 보드에 2회 이상 (개수로 적용) 미부착 시, 교신저자(연구책임자)에게 향후 1년간 포스터 제출 불가의 제재가 주어집니다.

· **우수포스터상 시상**: July 22 (Fri) 15:50~16:20 / Halla Hall A
우수포스터의 경우 폐회식에서 선정자를 호명합니다. 호명 시 자리에 없으면 다음 우수자에게 상이 수여되오니, 학술대회 종료일까지 학술대회에 꼭 참석해 주시기 바랍니다.
(상장과 상금 15만원 수여, 대리수상불가)

10. 이벤트 및 경품추첨 안내 (Event & Giveaway)

한국실험동물학회에서는 재미있고 즐거운 학술대회를 위해 풍성한 이벤트와 경품을 마련하였사오니, 회원님들의 많은 참여를 부탁드립니다.

(1) 환영만찬 노래자랑 이벤트

· **진행일시**: July 21 (Thu) 17:00 총회 후 환영만찬

(2) 실험동물기술원 교육강연 (Symposium 15) 퀴즈이벤트

· **진행일시**: July 22 (Fri) 14:00~ Halla Hall B 교육강연 2 후 진행
· **참여방법**: 스마트폰 앱/Play스토어에서 퀴즈를 위한 카훗(Kahoot) 앱 다운로드 후 참여
· **경품**: 에어팟2세대, 백화점상품권 10/5/3/1만원권, OKVET 협찬도서 등

(3) 폐회식 경품추첨

· **진행일시**: July 22 (Fri) 15:50 폐회식에서 추첨
· **참여방법**: 초록집 내 삽입 되어있는 설문지와 명찰 뒷면 부스 Stamp Card에 30개 이상의 도장 채우기
→ 설문지 작성 및 Stamp Card 도장 두 가지 모두 확인 후 명찰 뒷면 응모권을 등록 데스크에 비치된 경품함 넣기
· **경품**: 갤럭시탭 S8, 백화점상품권 30/10/5/1만원권, OKVET 협찬도서 등

※ KALAS Exhibition Hall (전시장) 운영 규칙

전시장에 입장시에는 항상 명찰을 잘 보이도록 착용하여야 한다. 전시장에 참여한 단체의 활동은 배정된 구역 내에서 진행되어야 한다. 해당 소속 전시 부스 이외의 타사 부스에 허락 없이 들어갈 수 없다. 사진촬영은 금지한다. 해당 부스의 전시책임자의 허가 없이 타 참여사의 제품 혹은 장비를 사진, 동영상, 혹은 기타 방식으로 기록 보관할 수 없다. 전시장을 포함하여 컨벤션홀 내부 전체는 모두 금연이다.

Safety & Security:
모든 참여사의 인원들은 전시장 내부에서 항상 명찰을 잘 보이도록 착용하여야 한다.
발급받은 명찰은 어떠한 방식으로든 변경하거나 삭제할 수 없다.
참여 단체의 전시에 참여하는 인원은 해당 전시사의 허가 없이 타사 부스에 들어갈 수 없다.

KALAS 규정:
"전시장에 참여하는 참여사 인원과 학회 참가자는 모두 KALAS의 규정에 따라야 한다. 규정에 어긋나거나 학술대회 및 전시장에 심각한 문제를 일으키는 경우, KALAS의 권한으로 전시사 및 참가자는 페널티를 부여 받을 수 있다. 페널티는 전시사나 참가자를 전시홀에서 퇴장시키거나 사전 공지없이 전시 권한을 즉각 종료 시키고 전시를 마감할 수 있으며, 위의 사항들은 중복되어 부여될 수도 있다. 모든 규정의 실행은 최종적으로 학회 섭외위원회의 결정에 의거한다."

TRAFFIC INFORMATION

[제주 국제컨벤션센터(ICC)]

- 주소 : 제주특별자치도 서귀포시 중문관광로 224 (중문동)
- 전화 : 064-735-1000
- 홈페이지 : iccjeju.co.kr

[교통안내]

1. 리무진 버스 (600번 제주공항 ↔ 중문관광단지)

- 탑승장: 공항정문 1층 5번 게이트 왼쪽 리무진 버스 승차장
- 운행표

공항→제주 썬호텔(구 더호텔)→롯데시티호텔(구 그레이스호텔)→한라병원→정존마을→동광환승정류소→
창천리→예래입구→중문관광단지입구→여미지식물원입구→그랜드 조선 제주 입구→더 쇼어호텔→
신라호텔→스위트호텔→하나호텔 한국관광공사→롯데호텔→한국콘도입구(남)→플레이케이팝박물관(서)→
씨 에스호텔→ **제주국제컨벤션센터 중문대포해안주상절리대**→대포항→배튼개입구(히든호텔)→
약천사→월평마을→강정농협→왕대왓→켄싱턴리조트(약근천)→서건도→월드컵경기장→
샛기정공원(구뉴경남호텔)→서귀포부두(화인호텔)→서복전시관→파라다이스호텔입구→서귀포칼호텔

※ 항공기 이착륙 시간 변경. 기상이변, 행사관계 등으로 수송수요에 변동이 생길 경우에는 증회 또는 운행시간이 조정될 수 있음.
※ 공항 출발 22:20, 22:50분 차량은 테디벨리골프장 정류소 정차.

- 운행시간 (매 18-30분 간격으로 운행)
  (1) 제주국제공항출발 (06:00-22:50)
  (2) ICC JEJU 출발 (06:35-22:16)
  ※ 전체 운영시간표 사이트 참고 → http://bus.jeju.go.kr/schedule/viewNew/600

- 이용요금: 편도(성인) 4,500원~5,500원 (컨벤션센터까지 약 50분-1시간 소요)
  ※ 탑승 시, 기사님께 하차지를 말씀하시기 바랍니다.

- 이용문의: 삼영교통 (064) 746-9369

2. 택시 (제주공항 ↔ 중문)

- 택시승차장 장거리, 단거리 확인: 제주공항 택시승차장에서 이용시
  장거리 승차장에서 출발하여 오십시오. 요금은 미리 정해져 있으므로 승차전에 확인하세요.
- 이용안내: 약 40-45분 (거리 40㎞)

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SYMPOSIUM 1

| July 20 (Wed) 14:00-15:40 | Halla Hall A | ENG |

Current status of pig to NHP heart and kidney xenotransplantation

· Organizer : Ik-Jin Yun (Konkuk University Hospital)
· Chair : Ik-Jin Yun (Konkuk University Hospital) / Hyunil Kim (Optipharm Co., Ltd)

1	Xenotransplantation steps from research to clinical application	Wayne Hawthorne University of Sydney
2	Current status and results of genetic engineered pig to monkey preclinical kidney xeno-transplantation in Korea	Ik-Jin Yun Konkuk University Hospital
3	Progress and breakthrough in preclinical and clinical xenotransplantation of pig hearts with multiple genetical modifications	Paolo Brenner Clinic of Grosshadern, University of Munich (LMU)
4	Results of pig to monkey heterotopic cardiac xenotransplantation in Korea	Hyun Keun Chee Konkuk University Medical Center

S1-1

Xenotransplantation steps from research to clinical application

Wayne John Hawthorne

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Recently we have seen a resurgence of clinical xenotransplantation with several clinical trials in the USA. However, for xenotransplantation to be safely introduced for widespread clinical application we require continued guidance and close interaction between the international guiding bodies, the International Xenotransplantation Association (IXA), The Transplantation Society (TTS) and the World Health Organization (WHO). This is essential to ensure oversight of what is required; to ensure appropriate safety measures are taken and safety monitoring are undertaken. This is particularly relevant with the current pandemic where the introduction of potential unknown viruses could play a role in immunosuppressed patients. We require long-term monitoring of xenotransplantation trials, with established outcomes and a registry which is updated regularly, in line with the current IXA recommendations. Additionally, continuous updates to world guidelines and regulatory guidance documents are indicated, in line with the ongoing technological advancements and findings from current pre-clinical and clinical studies, to ensure the most up-to-date implementation of guidance at the global level. This is clearly crucial, as evidenced from the outcomes of the Changsha Global Consultation with Continued involvement of representatives from all disciplines are essential to discuss progress and innovations in the field, to mirror this progress with respect to regulatory oversight, and foster this at the global level under the umbrella of the three international guiding organizations of the IXA, TTS and WHO. This lecture will provide an update on how these processes have occurred and provide data from preclinical cases in Islet Xenotransplantation with a focus on the use of Neonatal Islet Cell Clusters (NICC) and transgenic pig technology transplanted into diabetic Non-Human Primates.

Key words : Diabetes, Neonatal Islet Cell Clusters, Islet Cell Transplantation, Xenotransplantation

S1-2

Current status and results of genetic engineered pig to monkey preclinical kidney xeno-transplantation in Korea

Ik Jin Yun

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Background: For establishing the clinical xeno kidney transplantation, pig to NHP(Nonhuman Primate) preclinical experiments are very important last step to verify and prove the value of xenotransplantation. However, results of NHP preclinical experiments of solid organ xenotransplantation are not such satisfactory as to initiate the clinical trials yet. In Korea, although GalTKO pigs are developed early in the middle of 2000th, NHP trials of solid organs have been done only since 2011.

Materials & Methods: Since 2011, 24 kidney pig to NHP xenotransplantation experiments have been done in Xenotransplantation research team of Konkuk University Hospital. Donor transgenic pigs are delivered from three institutes (8 cases from National Institute of Animal Science, 15 cases from Optipharm® and one case from Cronex®) and all the transgenics are commonly GalT Knockout (GTKO) based. The kind of knock in genes are CD39, CD46, CD55, CD73 and thrombomodulin and two to four transgenic modifications with GTKO. We have used the cynomolgus monkey as recipient animal. After the general anesthesia, dissection and cold perfusion for the left kidney of transgenic pig is done. We extract the right kidney of host monkey before intraabdominal anastomosis of pig kidney to aorta and IVC. After 2 weeks observation, second look operation is done, and the remained left kidney of monkey is removed. We have used the immunosuppressants of CD154 ab, rituximab, ATG, Tacrolimus, MMF and steroid.

Results: Average survival durations are 22 days. For the exception of early failure cases less than 2 days survival due to the technical failure, 19 cases are survived more than seven days and the average survival durations for them are 34 days. The longest survival animal had lived during 86 days after transplantation and next is 84 days. And among kidney recipients, nearly 40% survive more than 3 weeks and more recently experimented cases have showed longer survivals. For the transplanted kidney survivals after the second look operation of all the removal of host kidney, functioning grafts are confirmed. There are no signs of hyper-acute rejection for the transplanted graft.

Conclusions: This survival results are relatively poor yet, but longest cases of survival are recent cases and so results are improving. Although the beginning pig to NHP xeno kidney transplantation experiment in Korea is very late comparing with the western and until 2020, our team is only group for the experiments with insufficient resources and results, with the supports of governmental fund and volunteering activities of clinical experts of clinical kidney transplantation, we expect the continuing experiments and studies to make comparative good results and contribute the beginning of clinical trials of kidney xenotransplantation.

Key words : Transgenic pig, NHP, Preclinical study, Kidney, Xenotransplantation

S1-3

Progress and breakthrough in preclinical and clinical xenotransplantation of pig hearts with multiple genetical modifications

Paolo Brenner

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Introduction: A 90-days-survival of 60% in a life-supporting orthotopic pig-to-baboon model (oXHTx) is necessary before starting clinical cardiac xenotransplantation. We achieved this with a new costimulation blockade immunosuppression (CD40mAb). Typical problems like perioperative xenograft dysfunction (PCXD) and cardiac xenograft (over)growth (CXO) were solved with a cold perfusion preservation and anti-proliferative therapy.
Methods: We transplanted 8 baboons with GalKO/hCD46/hTM-transgenic pig hearts. Immunosuppression consisted of ATG, rituximab, mycophenolate-mofetil (MMF), cortisone and CD40mAb(2C10). To prevent PCXD we used a non-ischemic 8oC cold perfusion solution with oxygenated blood. To inhibit CXO/hypertrophy, antihypertensive drugs and temsirolimus were given.
Results: Using cold perfusion no PCXD occurred. After growth control 6 recipient baboons reached end of study (90 days), 2 were prolonged up to d182/d195. Two baboons died on d14/d26 of sepsis (pCMV-positive donor hearts). No hyperacute or delayed rejection occurred. All baboons were in excellent general conditions (Nature 564;430-3). With the same immunosuppression and cold perfusion in 2021 Mohiuddin in Baltimore/USA achieved with 7-10-fold genetically modified donor pigs in oXHTx a maximal survival of 264 days.
The second, clinical breakthrough was a worldwide first-in-man cardiac xenotransplantation (January 7th, 2022) in Baltimore: Mr. Bennett (57 years old, non-transplantable on ECMO support) received a pig heart with 10 gene modifications as “compassionate use”. During implantation a typ-A-aortic-dissection occurred as complication. During recovery next weeks xenograft showed normal function. Bowl problems led to laparatomy and mobilisation was difficult. After day 50 situation deteriorated and after change to a palliative concept patient died (pod60, histologically no major signs of xenograft rejection). This 2-months-survival should be classified as success despite of the marginal patient (immobilized, ECMO, redo-operation, non-compliance) and the complicated operation.
Conclusion: After 25 years of experimental research we achieved in Munich in the last 5 years a major progress and breakthrough with a constant reproduceable long-term-survival of 3–6 months. This was the essential milestone on the way to clinical cardiac xenotransplantation, which was started with the patient in Baltimore and push this new key technology also for other organs (kindney, lung, islet cells). In Munich a first clinical pilot trial is planned in 2024 and still in 2022 a compassionate use.

Key words : Cardiac xenotransplantation, Orthotopic pig-to-baboon model, Xenograft rejection, Xenograft dysfunction, Xenograft overgrowth

S1-4

Results of pig to monkey heterotopic cardiac xenotransplantation in Korea

Hyun Keun Chee

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Background : The absolute shortage of donors compared with patients requiring transplantation is currently an unsolved problem, and the only possible solution may be xenotransplantation. To establish a successful clinical trial, a preclinical study using nonhuman primates is essential. Starting in November 2011, our team initiated heterotopic abdominal heart xenotransplantation, the first in the Republic of Korea.

Methods : A total of 22 xenotransplantation procedures have been performed since 2011. Single transgenic pig (alpha-galactosidase transferase knockout [GalT KO], n = 16), double transgenic pig (GalT KO + CD46, n = 3, and GalT KO + CD39, n = 2), and triple transgenic pig (GalT KO + CD46 + CD70, n = 1) models were used. Our baseline regimen of immunosuppressants comprised CD154 ab, rituximab, anti-thymocyte globulin, tacrolimus, mycophenolate mofetil, and steroids.

Results : The mean graft survival was 16 ± 16.27 days, and the mean graft survival was significantly longer in cases performed since 2014 (7.5 ± 8.03 days vs 24.67 ± 17.50; P = .01). Although the donor heart ischemic time was decreased per annum, no correlations could be found between ischemic time and survival days of the graft. Double or triple genetic manipulated hearts exhibited significantly better survival (11.63 ± 11.29 days vs 30.83 ± 20.34 days; P = .03). When the ratio of heart weight (grams) to nonhuman primate weight (kilograms) was lower, the results tended to be better (P < .05). The rate of immediate postoperative bleeding (9%, n = 2) causing death was relatively high in the earlier period, but there have been no serious surgical complications affecting graft survival since 2013.

Conclusions : Investigation of effective and optimal target genes for each organ to further progression toward better results is important. In addition, the immunosuppressive regimen needs to be further studied and constantly refined.

Key words : Xeno-heart transplantation, Xenotransplantation, Transgenic pig, Non human primate

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SYMPOSIUM 2 [IACUC]

| July 20 (Wed) 14:00-15:40 | Halla Hall B | ENG |

COVID-19's impacts on IACUC communication

· Organizer / Chair : Seung Hyeok Seok (Seoul Natl. Univ.)

1	A COVID-19 pandemic: the survey for animal use and care staff in Korea	Na Ahn Jangan Univ.
2	Conduct of IACUC meetings and semiannual facility inspections under the pandemic situation	Gwi Hyang Lee The Catholic Univ.
3	Utilizing various modes of communication at SNU IACUC office during the COVID-19 pandemic	Ji Min Lee Seoul Natl. Univ.
4	Guidance for IACUC regarding animal care and use during COVID-19 Pandemic – AAALAC International expectation	Montip Gettayacamin AAALAC International

S2-1

A COVID-19 pandemic: the survey for animal users and care staff in Korea

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As part of a study to find out how the ongoing COVID-19 pandemic has affected animal experiments, a survey was conducted among the Institutional Animal Care and Use Committee (IACUC) members, researchers including the principal investigator, and animal facility managers and workers. First, it was investigated how the COVID-19 pandemic has brought about changes to the IACUC and the management and operation of animal facilities. Next, through an in-depth interview with the selected interviewees, it was investigated what kind of preparation is needed when a similar situation occurs in the future.
The results of this study can be used to propose and prepare necessary countermeasures in the event of similar repetitive situations in the future as well as the understanding of current effect of COVID-19 pandemic on the circumstances of the management and operation of the animal facility and the IACUC.

Key words : Animal experiment, Animal facility, COVID-19, Institutional Animal Care and Use Committee, Laboratory animals

S2-2

Conduct of IACUC meetings and semiannual facility inspections under the pandemic situation

Gwi Hyang Lee

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The COVID-19 pandemic has impacted the global animal research community in the aspect of ethical review and animal care. Most IACUCs have been challenged by social distancing followed by the government restrictions on sanitary rules. The IACUC has responsibility for the welfare of animals at its facility within the institution and it has been a legal requirement in Korea since 2008 (Animal Protection Act, Article 25). As of 2020, there were 449 IACUCs registered to the Animal Protection & Welfare Division of the Animal and Plant Quarantine Agency. The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) issued the final rule amending the Animal Welfare Act (AWA) regulations to implement a requirement for contingency plans for the handling of animals and train their employees on implementing those plans during emergencies. There are limited information and resources available on how institutions and IACUCs can prepare their crisis management plan considering animal welfare issues for this long-lasting pandemic situation in Korea. The authors have investigated government guidance and resources for the IACUC functions relating to convened meetings and responsibilities of assessing laboratory animal facilities in responding to COVID-19 Pandemic Contingency Planning. The Korea Information Center for the 3Rs and the BIC Study Foundation have provided the Korean version of the 3Rs resources since 2012. The institutional policies were also searched through the official websites. Our findings are summarized with comparison tables and may develop the Korean version of checklists based on Korean research culture. It may be a helpful resource for the Korean IACUCs on how to prepare regulatory requirements considering ethical viewpoints to ensure the continuation of animal care and the maintenance of animal welfare under the pandemic situation for achieving both good animal welfare and for good quality science.

Key words : COVID-19 pandemic, IACUC function, IACUC meetings, Korea Information Center for the 3Rs, Semiannual facility inspections

S2-3

Utilizing various modes of communication at SNU IACUC office during the COVID-19 pandemic

Ji Min Lee

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As the unprecedentedly strong contagious coronavirus is spread, the Korean government enforced physical distance between individuals, that is, social distancing, for a long period of time from March, 2020 to April 2022. This policy had an immediate impact on human-to-human communication as a primary response to prevent the infection and spread of the COVID-19 virus. The communication method between individuals can be classified into a face-to-face method in which verbal/non-verbal signals are exchanged through direct contact with the other person and a remote method in which an electronic product such as a computer or mobile device is used to communicate indirectly without direct contact with the other person. Seoul National University Institutional Animal Care Use Committee (SNU IACUC) has converted various committee activities such as meetings and educations to a remote format while observing social distancing according to the government guidelines. Since the SNU IACUC was first established in 2008, the deliberation has maintained the online submission and review method, allowing the same deliberation activities to continue despite COVID-19, but some activities, such as inspection, had many restrictions due to social distancing. SNU IACUC members and administrators of Seoul National University conducted a self-evaluation to see if these committee activities were appropriate in terms of communication. Based on that results, we would like to review institutional and practical supplementary points and suggest a method to efficiently respond to a similar pandemic situation and operation direction of the post-COVID-19 social environment.

Key words : COVID-19, Pandemic, IACUC, Communication model, Remote meeting

S2-4

Guidance for IACUC regarding animal care and use during COVID-19 Pandemic – AAALAC International expectation

Montip Gettayacamin

AAALAC International

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AAALAC International is a private, non-profit organization that promotes the humane and ethical treatment of animals in science through voluntary program assessment, accreditation and education. AAALAC International has been recognized around the world as a symbol of high-quality animal care and use for research, teaching and testing, as well as promoting animal welfare and maintaining safety. Twenty-five accredited institutions are located in Republic of Korea. There are more than 230 accredited programs in Asia and Australia, and over 1,050 institutions in 51 countries.

This presentation outlines the expectation and function of Institutional Animal Care and Use Committee (IACUC) during COVID-19 pandemic. AAALAC International accredited institutions must have functional IACUC that comply with all relevant local regulations as well as the recommendations of the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011). Responsibility of the IACUC is to oversee and routinely evaluate the animal care and use program as described in the *Guide*. During the pandemic, IACUC activities could be conducted at less than normal situation. At many institutions, several research protocols were postponed. IACUC should consider modified measures for the purpose of social and physical distancing. Virtual communication technology has been used at several institutions when they cannot be face-to-face. All accredited institutions must have plan to define actions as necessary to prevent animal pain, distress, and death. Several institutions added pandemic management in the emergency preparedness plan, and modified the written procedures several times due to personnel shortage.

When a significant, unexpected, adverse event occurs resulting in negative consequences to animal welfare or human health, the institution should activate an established adverse event assessment and reporting plan that provides guidance on what should be investigated, the timing of reporting and who should receive reports, as well as steps to prevent or mitigate recurrence.

Key words : IACUC, Animal care and use, COVID-19 Pandemic management, AAALAC International, Expectation

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SYMPOSIUM 3

| July 20 (Wed) 16:00-17:40 | Halla Hall A | KOR |

Studies on brain and vascular functions using novel mouse models

· Organizer / Chair : Young Jae Lee (Gachon Univ.)
Young Hoon Sung (University of Ulsan & Asan Medical Center)

1	Animal models for studying the onset of arteriovenous malformations	Yong Hwan Kim Barrow Neurological Institute
2	Harmful effects of polystyrene nanoplastics on developing brain	Da Yong Lee KRIBB
3	Mouse models of 22q11.2 deletion syndrome for studying neuroanatomical abnormalities	Tae-Yeon Eom St Jude Children's Research Hospital
4	Reward learning improves social information processing in the medial prefrontal cortex	Doyun Lee IBS

S3-1

Animal models for studying the onset of arteriovenous malformations

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Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant vascular disorder caused by mutations in *ENG, ACVRL1*, or *SMAD4* gene that encodes proteins involved in TGF-β/BMP signaling. HHT patients develop arteriovenous malformations (AVMs), shunts between arteries and veins without intervening capillaries, in visceral organs and skin. The direct flow of high pressure arterial blood into the veins results in gushing bleeding when AVMs rupture. Development of skin AVMs in adult mice requires secondary insults such as wounding or angiogenic stimulation. It has been presumed that AVMs result from abnormal remodeling of pre-existing capillaries between arterial and venous branches. Previously, we suggested using a dorsal skin window chamber system that AVMs form from abnormal connections between nascent arterial and venous branches rather than from remodeling of existing connecting capillaries in *Alk1*-deficient mice. However, controversies still exist about the initiation of AVM formation because of the complexity of vascular structure and the limited imaging readouts. Here, we revisited this issue with more advanced approaches. We cut the artery and vein and removed surrounding tissues including capillaries. Vascular remodeling and AVMs were visualized with brightfield intravital imaging, hemoglobin saturation mapping and fluorescence-labelled red blood cell (RBC) tracing. Interestingly, the ends of the severed artery and vein were elongated and directly connected each other, then the AV-shunts became dilated and remodeled into fully developed AVMs. Consistent with the aberrant vascular morphology changes, the hemoglobin saturation map and RBC-tracing data confirmed the rapid, highly oxygenated arterial blood flow into the connected veins through the AV-shunt. Taken together, these results further support our previous findings and infers that abnormal connections between nascent arterial and venous vessels in pro-angiogenic milieu is essential for the onset of AVM formation.

Key words : Hereditary hemorrhagic telangiectasia, Arteriovenous malformation, Activin receptor-like kinase 1, Endoglin, Smad4

S3-2

Harmful effects of polystyrene nanoplastics on developing brain

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As global plastic production continues to grow, microplastics released from a massive quantity of plastic wastes have become a critical environmental concern. These microplastic particles are found in a wide range of living organisms in a diverse array of ecosystems. In this study, we investigated the biological effects of polystyrene nanoplastic (PSNP) on development of the central nervous system using cultured neural stem cells (NSCs) and mice exposed to PSNP during developmental stages. Our study demonstrates that maternal administration of PSNP during gestation and lactating periods altered the functioning of NSCs, neural cell compositions, and brain histology in progeny. Similarly, PSNP-induced molecular and functional defects were also observed in cultured NSCs *in vitro*. Finally, we show that the abnormal brain development caused by exposure to high concentrations of PSNP results in neurophysiological and cognitive deficits in a gender-specific manner. Our data demonstrate the possibility that exposure to high amounts of PSNP may increase the risk of neurodevelopmental defects.

Key words : Polystyrene nanoplastic, Brain development, Neural stem cell, Cognitive deficit

S3-3

Mouse models of 22q11.2 deletion syndrome for studying neuroanatomical abnormalities

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The 22q11.2 deletion syndrome (22q11DS), also known as DiGeorge or velocardiofacial syndrome, is the most common microdeletion in humans. The syndrome is associated with multiple physical and neuropsychiatric morbidities. This led us to investigate the pathological and structural connectivity of 22q11DS brain abnormalities. Although meta-analyses show brain alterations in patients with 22q11DS, the mechanisms underlying these structural and functional associations in 22q11DS are largely unknown. Herein, we used murine models of 22a11DS to elucidate the mechanisms responsible for neuroanatomical changes in 22q11DS. We found progressive enlargement of the ventricles in *Df(16)1/+* and *Dgcr8*$^{+/-}$ mice. We identified that haploinsufficiency of the microRNA-processing gene Dgcr8 results in Drd1 elevation, which is brought about by a reduction in Drd1-targeting microRNAs miR-382-3p and miR-674-3p. Replenishing either microRNA in 22q11DS mice normalizes ciliary beating and ventricular size. Knocking down the microRNAs or deleting their seed sites on Drd1 mimicked cilia-beating and ventricular deficits. These results suggest that the Dgcr8–miR-382-3p/miR-674-3p–Drd1 mechanism contributes to deceleration of ciliary motility and age-dependent ventricular enlargement in 22q11DS. Using 22q11DS mouse models, we also found that volume of paraflocculus/flocculus (PF/F) in the cerebellum was considerably decreased, which was also seen in human subjects with 22q11DS. This anatomical change was associated with motor learning deficits measured by vestibulo-occular reflex. We characterized the genes involved in PF/F malformation and found that *Tbx1* haploinsufficiency recapitulated both PF/F and behavioral phenotypes in mice. Histological and immunohistochemistry analyses showed no abnormalities in the cerebellar neurogenesis in the PF/F. However, we found that the bone structure surrounding the PF/F was abnormally developed in 22q11DS mice. Together, our data provide the mechanisms underlying neuroanatomical alterations in 22q11DS. Our study also supports that 22q11DS mice are valid models of the disease that can reproduce abnormal brain morphology, which help us understand the neural pathogenesis of 22q11DS.

Key words : 22q11.2 deletion syndrome, Neuroanatomical changes, Dgcr8, Paraflocculus/flocculus, Tbx1

S3-4

Reward learning improves social information processing in the medial prefrontal cortex

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Social interactions are often intertwined with rewarding sentiments, giving rise to social motivation. Lack of social motivation is the defining trait of autism spectrum disorder (ASD) and one of its diagnostic criteria. The social motivation hypothesis proposes that ASD manifests from the deficit of representing reward value from social stimuli. Treatments for ASD include behavioral interventions, of which the main strategy is positive reinforcement. The medial prefrontal cortex (mPFC) is a central neuroanatomical hub for social and reward circuits, and it integrates the information to modulate complex social behaviors. We developed a novel social cue-reward association paradigm that allows us to investigate social and reward information processing simultaneously. WT or SHANK2 KO mice, a model of ASD, learned to discriminate a social cue and a non-social odor cue associated with reward, from another set of social and non-social odor cues associated with the lack of reward. Using *in vivo* calcium imaging in head-fixed subject mice, we monitored the activity of mPFC neurons during the task. Before training, WT mice were able to form social representation at the cellular level, and the social cues could be decoded at the population level, whereas SHANK2 KO mice could not at both levels. After behavioral training, WT mice formed distinct representations of social and reward information, respectively. SHANK2 KO mice, to our surprise, also developed distinct neural representations for both information. Our results suggest that social cue-reward association training partially rescues social information processing in ASD model mice and that the interlink between the social and reward representations in the mPFC is critical for the neural mechanism of social motivation.

Key words : Social motivation, Autism spectrum disorder, mPFC, Reward, Calcium imaging

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SYMPOSIUM 4
[KLAT EDUCATION1 WITH KIT]

| July 20 (Wed) 16:00-17:40 | Halla Hall B | KOR |

Advanced experimental techniques and practical considerations in preclinical animal study

· Organizer / Chair : Kang-Hyun Han (KIT)

1	Efficacy and toxicity evaluation of anticancer drugs in tumor-bearing mice	Soon-Oh Hong Kolon Life Science
2	Delivery of antisense oligonucleotides to the mouse brain and neurobehavioral evaluations	Jeong-Wook Ghim SoVarGen
3	Intrathecal injection and neurologic examination in beagle dogs	Won-Tae Kim KIT

S4-1

Efficacy and toxicity evaluation of anticancer drugs in tumor-bearing mice

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Continued evaluation of the reliability of preclinical studies in mice for predicting clinical efficacy and toxicity of antitumor drugs has brought about encouragement of greater dependence on efficacy and toxicity studies in mice. Advantages commonly ascribed to the mouse as a model are the ease and affordability of studying large numbers of individuals, and minimization of drug requirements. In general, efficacy evaluation of anticancer drugs has been mainly conducted in mouse tumor models, whereas most toxicity evaluations have been investigated in healthy mice. Some anticancer drugs, however, have the characteristics that react and activate specifically in tumor cells, such as oncolytic viruses that preferentially replicate in cancer cells, but not in normal cells. In such a case, toxicology studies should be performed in a mouse tumor model to provide important information on the safety of anticancer drugs for use in humans. For a toxicology study in tumor-bearing mice, there are several considerations in each step from designing experiment protocols to interpreting the results as reflecting the attributes of mouse tumor models compared to ones with healthy mice. In the presentation, we will discuss the efficacy and toxicity evaluation of anticancer drugs in mouse tumor models.

Key words : Tumor-bearing mice, Efficacy, Toxicity, Anticancer drugs

S4-2

Delivery of antisense oligonucleotides to the mouse brain and neurobehavioral evaluations

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Antisense oligonucleotides (ASOs) are short synthetic single-stranded nucleic acids that can knockdown sequence-specific target RNAs, resulting in modulation of protein expression. ASO-based approaches are ideal options for the treatment of genetic neurological disorders. The blood-brain barrier limits the entrance of most therapeutic agents into the brain. It can be overcome by injecting those agents directly into the cerebrospinal fluid (CSF). In pre-clinical studies, intracerebroventricular (ICV) injection of ASOs is a useful tool for therapy of neurological disorders including neurodegenerative and neuropsychiatric diseases and brain tumors for animal models. Here, we describe ICV injection of ASOs to the mouse brain and verification of injection via immunohistochemistry. Intractable epilepsy model mice, which showed spontaneous seizures and anxiety-like behavior, were prepared by in-utero electroporation. Test ASOs were injected to intractable epilepsy model mice via ICV injection. Seizures and anxiety-like behavior of the animals were rescued by ASO treatment. These results suggest that direct ICV injection can be used for successful delivery of ASOs to the lateral ventricle to assess candidate ASO drugs in the mouse brain.

Key words : Antisense oligonucleotide, Intracerebroventricular injection, Epilepsy

S4-3

Intrathecal injection and neurologic examination in beagle dogs

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Also known as the subarachnoid space, the intrathecal space is the fluid-filled area located between the innermost layer of covering (the pia mater) of the spinal cord and the middle layer of covering (the arachnoid mater). The blood-brain barrier is the major obstacle for drug delivery to the brain and spinal cord. Intrathecal injection technique is delivering drugs directly to spinal cord bypassing blood-brain barrier. Intrathecal injection is useful in spinal anesthesia, chemotherapy, or pain management in clinical field.

For spinal cord related study, intrathecal injection site in beagle dog is mainly L5 ~ L6. Spinal needle is placed between 5 and 6 lumbar vertebrae and confirm cerebrospinal fluid is flowed out. After intrathecal injection, neurologic examination should perform to check any side effects with injection. Neurological examination can be divided by evaluation of mentation, posture and gait, cranial nerves, postural reactions, spinal reflexes, pain on spinal palpation and pain perfection.

Key words : Intrathecal injection, Neurologic examination, Beagle dog

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PLENARY LECTURE 1

| July 21 (Thu) 10:00-11:00 | Halla Hall A | KOR |

· Organizer : Jinwoong Bok (Yonsei Univ.) | · Chair : Je Kyung Seong (Seoul Natl. Univ.)

Microbiome and nutrition in animal health and diseases	Won-Jae Lee Seoul Natl. Univ.

KOREAN ASSOCIATION
FOR LABORATORY ANIMAL SCIENCE

Plenary Lecture 1

Microbiome and nutrition in animal health and diseases

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All metazoan guts have evolved to form a strategic alliance with indigenous microbiota. This evolutionarily-conserved mutualistic phenomenon has long-believed to be achieved by fine-tuned molecular interactions between the host and its microbiota. A corollary to the necessity of a commensal microbiota for host physiological homeostasis is that failure to achieve balanced host-microbe interactions may result in pathophysiological consequences for the host. In fact, it has been observed that altered community structure of gut microbiota is likely to be associated with metabolic and/or inflammatory diseases such as obesity, diabetes, and inflammatory bowel diseases. However, the lack of understanding of critical genes in the microbiome and host genomes makes it difficult to explain the exact mechanism by which the gut microbiota impacts host health. The research on this issue has been hampered mainly by technical difficulties associated with in-depth integrated genetic analysis of both the microbes and host. To overcome these limitations, we have developed the combination of *Drosophila* and its commensal microbiota as a genetic model of host-microbe interaction which enabled us to perform a simultaneous genetic analysis of both host and microbe in an *in vivo* interacting condition. Using this Drosophila-microbiota *in vivo* interacting model system, we could identify commensal microbiomes involved in host development as well as bacterial microbiome involved in gut physiology. The information obtained from *Drosophila*-microbiota model system provided us a novel insight into the underlying mechanistic events of malnutrition-associated diseases in vertebrates including mice and humans. The unexpected roles of the microbiome in malnutrtion in diverse animals from flies to humans will be presented and discussed in the lecture.

Key words : Microbiome, Malnutrition-associated diseases, Dysbiosis

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SYMPOSIUM 5

| July 21 (Thu) 11:00-12:40 | Halla Hall A | ENG |

Mitochondrial dynamics and therapy

· Organizer : Kyoung-Jin Oh (KRIBB) | · Chair : Jae Bum Kim (Seoul Natl. Univ.)

1	In adipocytes, DNMT1 is an epigenetic safeguard of mitochondrial dynamics	Jae Bum Kim Seoul Natl. Univ.
2	Mitochondria-targeted drug delivery systems for anti-tumor therapy	Han Chang Kang The Catholic Univ.
3	Regulation of metabolic switch in aged skeletal muscle by RNA binding protein	Jiyun Ahn KFRI
4	Fluorescence imaging of mitochondrial DNA base excision repair (BER) process	Yong Woong Jun Stanford Univ.

S5-1

In adipocytes, DNMT1 is an epigenetic safeguard of mitochondrial dynamics

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Adipose tissue plays a central role in maintaining systemic energy metabolism. Impaired adipose tissue plasticity, characterized by adipocyte hypertrophy, promotes metabolically defective and, thereby, functionally exhausted adipose tissue remodeling in obesity and its related metabolic diseases. However, the signaling mechanisms that maintain the adipose tissue plasticity required for metabolic fitness are poorly defined. In this work, we identified the DNA methyltransferase 1 (Dnmt1) as a critical regulator of adipose tissue plasticity and epigenetic mechanism for antiobesity. Integrating multi-layer genomic data including WGBS, PCHi-C data and hisotone ChIP-seq data, we comprehensively showed that adipocyte-specific DNA methylation, manifested in distal enhancers and CTCF sites, synergistically cooperates with a cis-regulatory network to establish adipocyte-specific transcriptome required to maintain metabolic function of adipocytes. In particular, Dnmt1 deficiency in adipocytes promoted defects in adipose tissue plasticity characterized by significant increase in adipocyte hypertrophy and these deleterious effects of adipocyte Dnmt1 deficiency provoked systemic hyperlipidemia, insulin resistance, and decreased systemic energy expenditure. Mechanistically, Dnmt1 deficiency impaired mitochondrial bioenergetics by reducing mitochondrial fission and leads to defective lipid metabolism, rendering adipocyte hypertrophy and dysfunction. As we also found the clinical relevance of adipose DNMT1 with adipose tissue plasticity and systemic energy metabolism, including BMI, type 2 diabetes-related traits and markers of adipocyte progenitors in human data sets, Dnmt1 may represent a potential target for epigenetic therapy to combat obesity and related metabolic disorders.

Key words : Mitochondria, Fission, Drp1, Dnmt, TAD

S5-2

Mitochondria-targeted drug delivery systems for anti-tumor therapy

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With expanding necessity for maximizing therapeutic effects and minimizing side effects of the delivered therapeutics, interest in subcellular organelle-targeting drug delivery systems is dramatically growing. Among subcellular organelles, mitochondria are focused because they rule significant controlling functions (e.g., controlling the homeostasis of vital physiological functions, synthesizing bioenergy molecules, determining cell death/viability, etc.) in cells and their destruction and damage in cancer cells are a hot issue in organelle-targeted drug delivery systems. Currently, to target mitochondria, many research groups have been used lipophilic cations such as triphenylphosphonium (TPP). However, TPP-decorated nanoparticles could be limited in their clinical application due to their positive surface charges and remarkable cytotoxicities. The issues have triggered to investigate new mitochondria-targeting moieties such as neutral, anionic, or hydrophilic molecules. Thus, in this presentation, current strategies and issues in mitochondria-targeted drug delivery systems will be introduced by using various nanoscale systems developed in my research group and some examples in their anticancer therapies will be discussed.

Key words : Drug delivery systems, Lipophilic cation, Mitochondria targeting, Nanoparticle, Organelle targeting

S5-3

Regulation of metabolic switch in aged skeletal muscle by RNA binding protein

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Mitochondria regulate a wide range of metabolic functions, such as energy production, redox homeostasis and cell survival. Mitochondrial dysfunction is an important contributor to the development of muscle aging. Hypoxia is associated with the functional decline during aging process. Mitochondria play a role as the site of metabolic switch in cancers. However, the change of mitochondrial metabolism and its underlying mechanisms during muscle aging have not been reported.
We measured mitochondrial metabolism and observed aged skeletal muscle showed metabolic switch from OXPHOS to glycolysis in *C. elegans*, mouse and human. RNA-seq analysis and protein array identified several RNA binding protein (RBP) which were changed during aging process. Among them, we found DEAD-Box Helicase 54 (DDX54) is associated with metabolic switch of aged muscle in *C. elegans*, mouse and human. Silencing of DDX54 increased mitochondrial function and decreased muscle aging. It also reversed metabolic switch to glycolysis in aged muscle. Conversely, overexpression of DDX54 decreased mitochondrial function and increased muscle aging. It profound metabolic switch of aged muscle. We also tested the therapeutic effect of rosmarinic acid with anti-Warburg effect against muscle aging.
This study provides new insights into the mechanism of metabolic switch of aged muscle and possibility of dietary intervention to ameliorate sarcopenia via targeting metabolic impairments.

Key words : Mitochondria, Metabolic switch, Aging, Skeletal muscle, Sarcopenia

S5-4

Fluorescence imaging of mitochondrial DNA base excision repair (BER) process

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Mitochondrial function in cells declines with aging and with neurodegeneration, due in large part to accumulated mutations in mitochondrial DNA (mtDNA) which arise from deficient DNA repair. However, measuring this repair activity is challenging. Here, we describe a strategy for visualizing mitochondrial base excision repair (BER) activity in living cells by a small-molecule light-up fluorescent probe (UBER) that undergoes highly selective covalent bond formation with AP sites in DNA. We find that the probe signals localize to mitochondria in cell culture. Fluorescence enhancement of signals was observed when cells were exposed to oxidative stress, where higher DNA repair levels are expected. Studies with enzyme inhibitors that suppress oxidative DNA damage or glycosylase activity showed modulation of UBER signals as expected. The probe was further employed to explore the effect of defensive enzymes against oxidative damage in DNA (MTH1 and OGG1), revealing altered signals consistent with the enzymes' roles in suppressing damage. Significantly, UBER was used to image brain responses to oxidative stress, revealing distinct repair responses in different brain regions. The results suggest the general use of the probe in measuring mitochondrial DNA repair responses in living systems, with direct relevance to the study of aging and neurodegenerative disease.

Key words : Mitochondrial DNA, DNA repair, Base excision repair, Fluorescent probe

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SYMPOSIUM 6 [KMPC1]

| July 21 (Thu) 11:00-12:40 | Halla Hall B | KOR |

Non mammalian -zebrafish

· Organizer : Hyunju Ro (Chungnam Natl. Univ.)
· Chair : Min Jung Kim (Sookmyung Women's Univ.)

1	Disease modeling of rare neurological disorders in zebrafish	Cheol-Hee Kim Chungnam Natl. Univ.
2	Zebrafish as a model for neurodegenerative diseases and neurotoxicity	Hae Chul Park Korea Univ.
3	A novel gene switch optimized for zebrafish transgenesis	Hyunju Ro Chungnam Natl. Univ.

S6-1

Disease modeling of rare neurological disorders in zebrafish

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Rare diseases are those which affect a small number of people compared to the general population. However, many patients with a rare disease remain undiagnosed, and a large majority of rare diseases still have no form of viable treatment. Approximately 40% of rare diseases include neurologic and neurodevelopmental disorders. In order to understand the characteristics of rare neurological disorders and identify causative genes, various model organisms have been utilized extensively. Recently, genome-wide association studies (GWAS) have provided candidate genes for a variety of rare neurological diseases, several of which are known to be comorbid with abnormal social behavior. In order to validate GWAS candidate genes, we have taken a systematic, targeted knockout (KO) approach to establish various disease models in zebrafish. To date, we have validated the function of human candidate genes involved in Kallmann syndrome, Potocki-Shaffer-syndrome, Miles-Carpenter syndrome, Down syndrome/autism, and Armfield syndrome. Recently, we have also established targeted KO zebrafish for clinically important genes involved in autism spectrum disorders, X-linked intellectual disability, childhood ataxia with CNS hypomyelination, congenital hypothyroidism, attention deficit hyperactivity disorder, schizophrenia, and bipolar disorders. In addition, our zebrafish center also maintains zebrafish KO lines involved in emotional control, e.g. anxiety or aggression, and movement disorders, such as infantile spasm/epilepsy and amyotrophic lateral sclerosis. For phenotypic analysis, we have established and utilize a battery of social interaction tests including shoaling, group behavior, mirror biting, alarm substance, body size preference, color preference, and a three-chamber social choice task. From bench to bedside, our efforts have made contributions to the recently established international patient foundations, ZC4H2 Deficiency Research Foundation and DeSanto-Shinawi Syndrome Foundation. In closing, the Zebrafish Center for Disease Modeling (ZCDM) is trying to contribute to the zebrafish research community for the advancement of technical methods and application of zebrafish KO lines related to human disease.

Key words : Zebrafish, Disease modeling, Rare diseases, Mental disorders, Autism

S6-2

Zebrafish as a model for neurodegenerative diseases and neurotoxicity

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Since the introduction of the zebrafish as a model for the study of vertebrate development and human diseases, genetic manipulation of the embryo to generate stable transgenic and knock-out/knock-in zebrafish model to study their function has been developed. Recently it has become apparent that these powerful methodologies can be used to investigate the pathogenesis of human neurodegenerative diseases and to identify candidate therapeutic approaches. In addition to its application to human disease, zebrafish has been widely used as a tool to detect environmental toxins and to investigate the mechanisms of action of toxins. The benefits of zebrafish for studying human disease are equally useful for studying environmental toxicity. Here, we show how zebrafish are being used to generate neurodegenerative disease models and analyze pathogenesis, as well as to detect the presence of some neurotoxins and identify how environmental exposures affect human health and disease. We will present our data modeling various neurodegenerative diseases including amyotrophic lateral sclerosis, diabetic neuropathy, and demyelination disease in zebrafish.

Key words : Zebrafish, Neurodegenerative disease, Neurotoxicity, Demyelination disease, Amyotrophic lateral sclerosis

S6-3

A novel gene switch optimized for zebrafish transgenesis

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Though various transgene expression switches have been adopted in a wide variety of organisms for basic and biomedical research, intrinsic obstacles of those existing systems, including toxicity and gene silencing, have been limiting their use in vertebrate transgenesis. Here we demonstrate a novel QF-based binary transgene switch (IQ-Switch) that is relatively free of driver toxicity and transgene silencing, and exhibits potent and highly tunable transgene activation by the chemical inducer tebufenozide, a non-toxic lipophilic molecule to developing zebrafish with negligible background. The interchangeable IQ-Switch makes it possible to elicit ubiquitous and tissue specific transgene expression in a spatiotemporal manner. We generated a RASopathy disease model using IQ-Switch and demonstrated that the RASopathy symptoms were ameliorated by the specific $BRAF^{(V600E)}$ inhibitor vemurafenib, validating the therapeutic use of the gene switch. The orthogonal IQ-Switch provides a state-of-the-art platform for flexible regulation of transgene expression in zebrafish, potentially applicable in cell-based systems and other model organisms. By connecting IQ-Switch with other available gene switches, it would be possible to regulate several transgenes with different modality in the organismal level.

Key words : Zebrafish, Transgene, IQ-Switch, Tebufenozide, RASopathy

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SYMPOSIUM 7 [NIFDS1]

| July 21 (Thu) 11:00-12:40 | Samda Hall | KOR |

Preclinical non-human primate research for pharmaceutics development

· Organizer : Byeong-Cheol Kang (Seoul Natl. Univ.) | · Chair : Jong Kwon Lee (NIFDS)

1	Validation of scrub typhus vaccine in nonhuman primates infection model	Nam-Hyuk Cho Seoul Natl. Univ.
2	Safety evaluation of vaccines for nonhuman primates	Doo-Wan Cho KIT
3	Preclinical research with marmoset in seoul national university hospital marmoset model network center (SNUH MMNC)	Byeong-Cheol Kang Seoul Natl. Univ.
4	Studying multiple sclerosis (MS) with advanced MRI and pathology using marmoset model	Seung-Kwon Ha University of Pittsburgh

S7-1

Validation of scrub typhus vaccine in non-human primate infection model

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Scrub typhus is a mite-borne febrile disease caused by O. tsutsugamushi infection. Recently, emergence of scrub typhus has attracted considerable attention in several endemic countries in Asia and the western Pacific. In addition, the antigenic diversity of the intracellular pathogen has been a serious obstacle for developing effective diagnostics and vaccine. Here, we developed a novel recombinant antigen derived from conserved regions of 56 kDa type-specific antigen (TSA56), a major outer membrane protein responsible for genetic heterogeneity and antigenicity, and evaluated it as a protective vaccine antigen. Our findings demonstrate that immunization with conserved blocks of TSA56 (cTSA56) not only provides protective immunity against lethal challenges with the homologous genotype, but also confers significantly better protection against heterologous genotypes than TSA56. Adoptive transfer of CD4+ or CD8+ T cells from immunized mice provided significantly enhanced protection against lethal challenge, whereas immune B cells failed to do so, indicating that cellular immunity against the conserved epitopes plays a protective role. Moreover, immunization with a 10-mer peptide mixture, screened from CD8+ T cell epitopes within the conserved region of TSA56, provided enhanced protection against lethal challenge with O. tsutsugamushi. By using non-human primate (Rhesus macaque) infection model, presenting similar clinical phenotypes and immunological features as human scrub typhus, we also assessed the vaccine efficacy *in vivo*. I will present and discuss the results obtained from the preclinical study.

Key words : Scrub typhus, Vaccine, Non-human primate

S7-2

Safety evaluation of vaccines for nonhuman primates

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In December 2019, a new strain of betacoronavirus, severe acute respiratory syndrome coronavirus 2, which causes coronavirus disease 2019 (COVID-19), emerged in Wuhan, China. Subsequently, the virus quickly spread worldwide and the World Health Organization declared COVID-19 a global pandemic on March 11, 2020. In a public health crisis, vaccine manufacturers are entering clinical trials at an unprecedented speed based on platform technology and accumulated data that have proven safety, such as mRNA vaccines and virus vector vaccines. And regulatory agencies in each country including the Ministry of Food and Drug Safety is shortening the time to enter clinical trials for COVID-19 vaccine candidates through expedited review and prior consultation.

Korea Institute of Toxicology (KIT), the only government-funded research institute in the field of toxicology in Korea, conducted a project, "Supportive project on toxicity assessment of COVID-19 vaccines and therapeutics candidates", under the auspices of Ministry of Science and ICT (the compilation of 3rd extra budget for pandemic stimulus). Through this project, which was conducted with a budget of 4 billion won for a total of one year (24Jul20~23Jul21), the candidates to be supported were selected in consideration of the excellence of the candidates and applicant's development competency ('20.8), the GLP toxicity study was conducted free of charge. Even after the completion of the project, rapid toxicity study for COVID-19 vaccines and therapeutics candidates is provided through Fast Track System.

Although primates should be considered only in the absence of other suitable species according to the guideline on nonclinical evaluation of biopharmaceuticals, WHO designated NHP study as a mandatory item in nonclinical evaluation for COVID-19 vaccines and therapeutics development (WHO R&D Blueprint, '20.1). And more and more requests for toxicity studies on NHP are being made to utilize the characteristics of human-like NHP.

In this session, we would like to elucidate the considerations for the toxicity study for COVID-19 vaccines in NHP and introduce a case of repeated dose toxicity study for COVID-19 vaccine candidate in NHP which was conducted as the project.

Key words : NHP, Vaccine candidate, Toxicity study, Biopharmaceuticals

S7-3

Preclinical research with marmoset in seoul national university hospital marmoset model network center (SNUH MMNC)

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The demand for non-human primate (NHP) in biomedical research have been increased for several decades. Although macaque monkeys such as Cynomolgus and Rhesus have been widely used in NHP research, they have disadvantages as laboratory animals such as securing husbandry space, low reproductive efficiency, difficulties in handling and risk of zoonotic infections. Recently, the common marmoset (*Callithrix jacchus*), has emerged as an attractive NHP to overcome the limits of macaque monkeys. Marmoset have several advantages of simple breeding, easy handling, fewer diseases and small body size compared to macaque monkeys. For this reason, the United States, China, Japan, and Europe countries regard marmoset as a crucial strategic bio-resource and are striving to secure marmoset and establish infrastructure. However, in Korea, domestic colony and breeding system for marmoset, and related infrastructure are not well established. In 2021, National Bio-Resource Project was launched as multi-ministerial funding and SNUH MMNC project was selected to strategize the marmoset as a national bio-resource. First of all, SNUH MMNC project is carried out for three years, and the ultimate goals of the project are: 1. Securing marmoset resources, 2. Standardizing marmoset breeding and quality management systems. 3. Development of marmoset disease model. To achieve these goals, SNUH MMNC team is composed of laboratory animal medicine, microbiology, clinical disease, obstetric and genetically engineering experts. Each expert will meet and communicate regularly and hold SNUH MMNC symposium twice a year. Also SNUH MMNC will organize a public relations team to promote the necessity and role of MMNC to the veterinary and biology major students, and researchers through on-offline. After the end of the funding, the marmoset colony will be maintained through the finance derived from its own production and sales revenue, and the marmoset resource bank will be operated to promote and encourage the researchers utilize the marmoset for research. We expect that these efforts will develop the quality of biomedical science of Korea, and contribute the improvement of public health.

Key words : Preclinical research, Marmoset, SNUH-MMNC

S7-4

Studying multiple sclerosis (MS) with advanced MRI and pathology using marmoset model

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. Although it has been extensively studied, the proximate trigger of the immune response remains uncertain. Experimental autoimmune encephalomyelitis in the common marmoset recapitulates many radiological and pathological features of focal multiple sclerosis lesions in the cerebral white matter, unlike traditional experimental autoimmune encephalomyelitis in rodents. This provides an opportunity to investigate how lesions form as well as the relative timing of factors involved in lesion pathogenesis, especially during early stages of the disease. We used Magnetic resonance imaging (MRI) to track experimental autoimmune encephalomyelitis lesions *in vivo* to determine their age, stage of development, and location, and we assessed the corresponding histopathology post-mortem. Also, we will present novel 3D-printer methodology for accurately sectioning marmoset brain tissues and thus allowing precise matching between histology and MRI.

Key words : Multiple Sclerosis, EAE, MRI, 3D-Printer, Marmoset

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LUNCHEON SEMINAR 1~3

| July 21 (Thu) 13:00-13:20 |

Luncheon Seminar 1 — Halla Hall A

· Organizer : Preclinical Research Center, K-MEDI hub

I want to know there, Preclinical Research Center, K-MEDI hub	Joon-Suk Park Preclinical Research Center, K-MEDI hub

Luncheon Seminar 2 — Halla Hall B

· Organizer : KRICP, Korea Institute of Radiological & Medical Sciences

Toward national upgrade of *in vivo* animal experiments in drug development and evaluation	Kyeong Min Kim Korea Radioisotope Center for Pharmaceuticals

Luncheon Seminar 3 — Samda Hall

· Organizer : KOSABIO & FOLAS | · Chair : Sang Rae Lee (AJOU Univ.)

Cage Washers 101 – Comprehensive review of cage washing solutions	Mike Douglas Allentown, LLC

KOREAN ASSOCIATION
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LS1

I want to know there, Preclinical Research Center, K-MEDI hub

Joon-Suk Park

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A state-of-the-art research and development center in Daegu, Korea – K-MEDI hub – is creating a medical industry ecosystem for the research and development of new drugs and medical devices. At its Preclinical Research Centre (PRC), one strong research focus is evaluating the impact of stress on the welfare of laboratory animals in the context of preclinical research. The aim is to improve international standards and guidelines for experimental conditions in the future, improving both the welfare of laboratory animals and the quality of research results. PRC is certified to provide high-quality veterinary care in a wide variety of laboratory animal species including rodents, rabbits, dogs, pigs, and non-human primates. One of the main goals of the PRC is to establish a cutting-edge animal experimentation system that will support K-MEDI hub's overall objective of developing new drugs and medical devices. In addition, the center designs and implements various strategies to provide customized support for animal studies. With the work carried out at the PRC, new methods can be devised to improve the welfare of laboratory animals, the end goal being for these methods to be adopted globally. The K-MEDI hub PRC was certified by the Ministry of Food and Drug Safety in 2016 and awarded full accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC-i) in 2020. As a result, it is recognized as a nonclinical study institution that encourages the humane treatment of animals in science and maintains a high level for the care and use of laboratory animals. The PRC constitutes animal testing systems for the development of chemically synthesized pharmaceutical products and medical devices.

Key words : Preclinical research, Laboratory animal

LS2

Toward national upgrade of *in vivo* animal experiments in drug development and evaluation

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Korea Radioisotope Center for Pharmaceuticals (KRICP) is a government-affiliated institution that is specialized in the field of radiolabeled ADME, bioimaging and radiopharmaceuticals GLP & GMP. KRICP is the only nonclinical & clinical testing facility able to handle radioisotopes in Korea and houses the state-of-the-art equipment run by experts in the field. We have conducted nonclinical pharmacokinetic and bioimaging-based efficacy evaluations using experimental animals. We have various animal disease models, evaluation methodologies, and radioisotope labeling methods to evaluate drug candidates. In our center, various *in vivo* bioimaging equipment (PET/CT, SPECT/CT, PET/MRI, High Tesla MRI, Dedicated CT, Optical Imager, PET/CT for large animal) been installed and used in the animal experiment for drug evaluation and disease model evaluation. In the evaluation of the *in vivo* pharmacokinetics of drug candidates, the tissue distribution and excretion level of the drug candidates can be quantitatively confirmed. Drug distribution in each tissue over time and its movement can be also visually confirmed through bioimaging using drug candidates labeled with radioisotopes. In conclusion, at KRICP, we are dedicated to help pharmaceutical companies and researchers utilizing radioisotopes in drug R&D and can provide solutions of radiolabeled ADME & efficacy test with animal and clinical samples.

Key words : Radiolabeled ADME, *In vivo* bioimaging, Drug evaluation, Animal experiment

LS3

Cage Washers 101 – Comprehensive review of cage washing solutions

Mike Douglas

Allentown, LLC

Cage washing is a key process of the health management program and bioexclusion practices within an animal facility. Mike Douglas will review washer technologies on today's market and highlight the differences in cage washer functionality that will provide the fundamental knowledge to select a suitable cage washer for your specific needs.

The presentation will review topics such as:

- What do you need to clean – common items that require washing in an animal facility
- The wash process – key elements to achieve effective cleaning
- Good working practices and the limitations when working with cage washers – allowing your cage washer to perform optimally and reliably
- Detergents – the importance of protecting your wash items
- How do you know the washer is working – testing and validation techniques
- Technologies to further improve the efficiencies of a cage wash area

By understanding the above topics, effective washing of animal caging and associated components can be achieved, ensuring you providing the maximum protection for the health status and welfare of your animals, staff and facility, while also improving environmental and financial waste.

Key words : Cage washer

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ACADEMY AWARD PRESENTATION

| July 21 (Thu) 14:00-14:40 | Halla Hall A |

· Chair : KilSoo Kim (K-MEDI hub)		
1	Inflammatory response in the mid colon of ICR mice treated with polystyrene microplastics for two weeks	Yun Ju Choi Pusan Natl. Univ.
2	Establishment of particulate matter-induced lung injury model in mouse	Se Yong Park Seoul Natl. Univ.
3	Surgical removal of a telemetry system in a cynomolgus monkey (*Macaca fascicularis*): a 12-month observation study	Doo-Wan Cho KIT

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A1-1

Inflammatory response in the mid colon of ICR mice treated with polystyrene microplastics for two weeks

Yun Ju Choi

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Background: The oral administration of polystyrene-microplastics (PS-MPs) causes chronic constipation of ICR mice, but there are no reports on their effects on the inflammatory response in the colon. To determine if the oral admin- istration of MPs causes inflammation in the colon, the changes in the apoptosis-associated speck like protein con- taining a caspase recruitment domain (ASC)-inflammasome pathway, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, and inflammatory cytokine expression were evaluated in the mid colon of ICR mice treated with 0.5 μm size PS-MPs for two weeks.

Results: The thicknesses of the mucosa, muscle, flat luminal surface, and crypt layer were decreased significantly ($p < 0.01$) in the mid colon of the MPs treated group compared to the Vehicle treated group. On the other hand, a remarkable increase in the expression levels of NOD-like receptor pyrin domain-containing protein (NLRP) 3, ASC, and Cleaved Caspase (Cas)-1 protein was observed in the MPs treated group. In addition, similar increasing pattern in the levels of p-NF-κB and phospho-inhibitory subunit of NF-κB (p-IkB) α protein was detected. Four inflammatory cytokines, including NF-κB, interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL-1β, showed an increased expression level after the MPs treatment.

Conclusions: Therefore, the present study suggests that PS-MPs can be a novel cause of an inflammatory response in the mid colon of ICR mice.

Key words : Microplastics, Inflammation, Colon, NF-κB, Cytokines

A1-2

Establishment of particulate matter-induced lung injury model in mouse

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Particulate matter (PM) is one of the principal causes of human respiratory disabilities resulting from air pollution. Animal models have been applied to discover preventive and therapeutic drugs for lung diseases caused by PM. However, the induced severity of lung injury in animal models using PM varies from study to study due to disparities in the preparation of PM, and the route and number of PM administrations. In this study, we established an *in vivo* model to evaluate PM-induced lung injury in mice. PM dispersion was prepared using SRM2975. Reactive oxygen species were increased in MLE 12 cells exposed to this PM dispersion. *In vivo* studies were conducted in the PM single challenge model, PM multiple challenge model, and PM challenge with ovalbumin-induced asthma using the PM dispersion. No histopathological changes were observed in lung tissues after a single injection of PM, whereas mild to moderate lung inflammation was obtained in the lungs of mice exposed to PM three times. However, fibrotic changes were barely seen, even though transmission electron microscopy (TEM) studies revealed the presence of PM particles in the alveolar macrophages and alveolar capillaries. In the ovalbumin (OVA)-PM model, peribronchial inflammation and mucous hypersecretion were more severe in the OVA+PM group than the OVA group. Serum IgE levels tended to increase in OVA+PM group than in OVA group. In this study, we established a PM-induced lung injury model to examine the lung damage induced by PM. Based on our results, repeated exposures of PM are necessary to induce lung inflammation by PM alone. PM challenge, in the presence of underlying diseases such as asthma, can also be an appropriate model for studying the health effect of PM.

Key words : Air pollution, Particulate matter, Animal model, Lung injury, Asthma

A1-3

Surgical removal of a telemetry system in a cynomolgus monkey (*Macaca fascicularis*): a 12-month observation study

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Background: Telemetry is a wireless implanted device that measures biological signals in conscious animals and usually requires surgery for its removal when the study is finished. After removing the device, the animals are either used for other studies or euthanatized.
Case presentation: Herein, we report the case of a living cynomolgus monkey (Macaca fascicularis) that was used for the entire experimental period, instead of euthanasia, after surgical removal of an implanted telemetry system. Radiography was used to determine the status of the implanted telemetry, following which, a repair surgery was performed for removing the system; clinical signs were used to preserve the life of the cynomolgus monkey. Postoperative clinical signs, food consumption, hematology, and serum biochemistry were examined during the 12-month observational period. No abnormal readings or conditions were observed in the subject after implant removal.
Conclusions: This study may be a useful case report for living cynomolgus monkeys in telemetry implantations used throughout the study period. We suggest minimizing the suffering and improving the welfare of these animals.

Key words : Cynomolgus monkey, Surgery, Telemetry, Implantation, Welfare

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SYMPOSIUM 8

| July 21 (Thu) 14:50-16:30 | Halla Hall A | ENG |

Current topics of the microbiota-gut brain axis in health and disease

· Organizer / Chair : Jeong-Soo Lee (KRIBB)

1	Gut feelings about the brain: Rodent models of the microbiome-gut-brain axis	John Cryan University College Cork
2	Beneficial effects of human gut microbiota *Akkermansia muciniphila* on cognitive function in neurodegenerative mouse models	Chul-Ho Lee KRIBB
3	Microbiome interactions with the nervous system in health and disease	Elaine Hsiao UCLA
4	Transfer of a healthy microbiota reduces amyloid and tau pathology in an Alzheimer's disease animal model	Jin-Woo Bae Kyung Hee Univ.

S8-1

Gut feelings about the brain: Rodent models of the microbiome-gut-brain axis

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The microbiota-gut-brain axis is emerging as a research area of increasing interest for those investigating the biological and physiological basis of neurodevelopmental, age-related and neurodegenerative disorders. The routes of communication between the gut and brain include the vagus nerve, the immune system, tryptophan metabolism, via the enteric nervous system or via microbial metabolites such as short chain fatty acids. These mechanisms also impinge on neuroendocrine function at multiple levels. Studies in animal models have been key in delineating that neurodevelopment and the programming of an appropriate stress response is dependent on the microbiota. Developmentally, a variety of factors can impact the microbiota in early life including mode of birth delivery, antibiotic exposure, mode of nutritional provision, infection, stress as well as host genetics. Stress can significantly impact the microbiota-gut-brain axis at all stages across the lifespan. Moreover, animal models have been key in linking the regulation of fundamental brain processes ranging from adult hippocampal neurogenesis to myelination to microglia activation by the microbiome. Finally, studies examining the translation of these effects from animals to humans are currently ongoing. Further studies will focus on understanding the mechanisms underlying such brain effects and developing nutritional and microbial-based intervention strategies and how these interact with various systems in the body including the cannabinoid system.

S8-2

Beneficial effects of human gut microbiota *Akkermansia muciniphila* on cognitive function in neurodegenerative mouse models

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Gut microbiome has been studied extensively for the various disease prevention and treatment. One of the most abundant microorganism of the intestinal microbiota, *Akkermansia muciniphila* (*A. muciniphilla*), is considered as a next generation probiotic microbiota, due to its improving effects on metabolic diseases such as obesity, diabetes and dyslipidemia. Recently *A. muciniphilla* is also reported to be related with depression and anxiety-like behavior following social defeat, and its oral administration for 4 weeks sufficiently prevented memory decay in high fat diet-fed animal model. These studies strongly indicate that *A. muciniphilla* have an important role in neuropsychiatric disorders via the gut-brain-axis. Currently the action mechanism of *A. muciniphilla* is presumed to be associated with protection of intestinal mucosal barrier, modulation of the immune system and metabolites including short-chain fatty acids and amino acids. To investigate whether *A. muciniphilla* can improve actually the brain function, we orally administered *A. muciniphilla* in LPS-induced cognitive deficient model and neurodegenerative cognition-impaired aged mice. Indeed, behavioral performance like novel object recognition and Y-maze alteration tests was significantly improved by *A. muciniphilla* administration, as well as the amelioration of neuroinflammation and GFAP gliosis in the brain of LPS-induced cognitive deficient model. The improvement of behavioral performance was also found in aged mice. From these experimental results, it was suggested that *A. muciniphilla* is beneficial for the improvement of cognitive decline induced by inflammation and neurodegenerative changes, and have an underlying crosstalk system with a host through unidentified factors yet. In regard to gut-brain axis, we are now focused on the isolation of *A. muciniphilla*-derived functional molecules responsible for cognitive function improvement.

Key words : Microbiota, Cognitive function, *Akkermansia muciniphilla*, Gut-brain axis

S8-3

Microbiome interactions with the nervous system in health and disease

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The gut microbiota is emerging as an important modulator of brain function and behavior, as several recent discoveries reveal substantial effects of the microbiome on neurophysiology, neuroimmunity and animal behavior. Despite these findings supporting a “microbiome-gut-brain axis”, the molecular and cellular mechanisms that underlie interactions between the gut microbiota and brain remain poorly understood. To uncover these, the Hsiao laboratory is mining the human microbiota for microbial modulators of host neuroactive molecules, investigating the impact of microbiota-immune system interactions on neurodevelopment and examining the microbiome as an interface between gene-environment interactions in neurological diseases. Overall, we aim to dissect biological pathways for communication between the gut microbiota and nervous system, toward understanding fundamental interactions between physiological systems that impact brain and behavior.

Key words : Microbiome, Microbiota, Gut-brain, Neuroimmune, Physiology

S8-4

Transfer of a healthy microbiota reduces amyloid and tau pathology in an Alzheimer's disease animal model

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Cerebral amyloidosis and severe tauopathy in the brain are key pathological features of Alzheimer's disease (AD). Despite a strong influence of the intestinal microbiota on AD, the causal relationship between the gut microbiota and AD pathophysiology is still elusive. Using a recently developed AD-like pathology with amyloid and neurofibrillary tangles (ADLP^{APT}) transgenic mouse model of AD, which shows amyloid plaques, neurofibrillary tangles and reactive gliosis in their brains along with memory deficits, we examined the impact of the gut microbiota on AD pathogenesis. Composition of the gut microbiota in ADLP^{APT} mice differed from that of healthy wild-type (WT) mice. Besides, ADLP^{APT} mice showed a loss of epithelial barrier integrity and chronic intestinal and systemic inflammation. Both frequent transfer and transplantation of the faecal microbiota from WT mice into ADLP^{APT} mice ameliorated the formation of amyloid β plaques and neurofibrillary tangles, glial reactivity and cognitive impairment. Additionally, the faecal microbiota transfer reversed abnormalities in the colonic expression of genes related to intestinal macrophage activity and the circulating blood inflammatory monocytes in the ADLP^{APT} recipient mice. These results indicate that microbiota-mediated intestinal and systemic immune aberrations contribute to the pathogenesis of AD in ADLP^{APT} mice, providing new insights into the relationship between the gut (colonic gene expression, gut permeability), blood (blood immune cell population) and brain (pathology) axis and AD (memory deficits). Thus, restoring gut microbial homeostasis may have beneficial effects on AD treatment.

Key words : Alzheimer's disease, Transgenic mouse model, Gut microbiota, Faecal microbiota transfer

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SYMPOSIUM 9 [KMPC2]

| July 21 (Thu) 14:50-16:30 | Halla Hall B | KOR |

In vitro and *in vivo* models for cancer research

· Organizer : Seung Hyun Oh (Gachon Univ.)
· Chair : Je Kyung Seong (Seoul Natl. Univ.) / Seung Hyun Oh (Gachon Univ.)

1	Patient-derived cancer organoid hub platform for refractory or rare cancer	Yun-Hee Kim National Cancer Center Korea
2	Preclinical murine models for cancer research : CDX vs Syngeneic model	Seung Hyun Oh Gachon Univ.
3	Patient-derived xenograft model for cancer research	Sung Yup Cho Seoul Natl. Univ.
4	Companion animals as alternative models for translational cancer research	Kyong-Ah Yoon Konkuk Univ.

S9-1

Patient-derived cancer organoid hub platform for refractory or rare cancer

Yun-Hee Kim

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Patient-derived preclinical models such as patient-derived cancer cells in 2D culture (PDC), patient-derived xenograft (PDX), and patient-derived organoid (PDO) have been developed to better understand cancer evolution and predict clinical outcomes. PDO, in particular, has emerged as a new model system in precision medicine because it can permanently preserve tumor heterogeneity *in vitro* while still preserving the original tumor's heterogeneity. Drug sensitivity testing, clinical drug high-throughput screening, epigenetic research, and genetic engineering can all benefit from these patient-derived cancer organoids. Despite the benefits of the PDO system, there are still limitations and shortcomings, such as the fact that its establishment necessitates a high level of technical expertise and that obtaining cancer samples is challenging due to the restricted number of specimens available. Furthermore, individual researchers' access to clinical material is quite limited. In the case of refractory or rare cancer, these issues are more difficult to solve. The goal of our research is to establish a promising cancer organoid platform that can overcome these challenges by optimizing organoid culture and preservation techniques and providing a representative collection of well-characterized models for diverse cancers. More than 100 instances organoids with extended passage times for pancreatobiliary tract cancer, advanced breast and ovarian cancer, mouth cancer, and other cancers are available on our organoid hub platform. This hub platform has the potential to bridge the gap between clinical demand and basic research by providing an optimization model for various tumors, allowing precision medicine, and facilitating research collaboration among various research groups and institutes.

Funding Source: This research was supported by grants from National cancer Centre (NCC-2210980), and National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2020M3A9A5036362).

Key words : Patient-derived organoid platform, Preclinical model, Refractory cancer, Rare cancer

S9-2

Preclinical murine models for cancer research : CDX vs Syngeneic model

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Cancer is the most frequently diagnosed form of disease and the leading cause of death in the world, so studies to identify candidate genes and to develop novel therapeutic agents are actively underway. Numerous mouse models have been developed because models can closely mimic many types of human tumors, greatly expand their *in vivo* research potential, and play an important role in molecular mechanism research, diagnostic marker discovery, and treatment development. More and more experimental mouse models, including spontaneous and chemically induced carcinogenesis, tumor transplantation, and genetically engineered mice, are being used for testing candidate compounds and proof of concept of appropriate diagnostic and treatment biomarkers. Here, I would like to introduce and explain a simple mouse xenograft model and a syngeneic model that can be used in experiments involving metastasis and immunotherapy to inform researchers interested in cancer study.

Key words : Xenograft, Mouse, Cancer, Syngeneic, Model

S9-3

Patient-derived xenograft model for cancer research

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Cancer precision medicine is a diagnostic and therapeutic approach based on the stratification of patients using genomic and molecular profiling of tumors. To develop the diagnostic and therapeutic tools for the application of cancer precision medicine, proper preclinical mouse models that reflect the tumor heterogeneity are required. Patient-derived xenograft (PDX) models are generated by the engraftment of patient tumors into immune-deficient mice, which retain several aspects of patient tumors' characteristics including inter-tumoral and intra-tumoral heterogeneity. We established PDXs from gastric cancer (GC) patients and developed prediction model to determine the responsiveness to 5-FU and oxaliplatin-based chemotherapy based on profiling of PDXs. When the PDXs are defined as either responders or non-responders according to tumor volume change after treatment, the responsiveness of PDXs is significantly consistent with the respective clinical outcomes of the patients. An integrative genomic and transcriptomic analysis of PDXs reveals that pathways associated with cell-to-cell and cell-to-extracellular matrix interactions enriched among the non-responders in both cancer cells and the tumor microenvironment. We develop a 30-gene prediction model to determine the responsiveness to 5-FU and oxaliplatin-based chemotherapy and confirm the significant poor survival outcomes among cases classified as non-responder-like in three independent GC cohorts. Our study may inform clinical decision-making when designing treatment strategies.

Key words : Patient-derived xenografts, Precision oncology, Cancer drug development, Cancer genomics

S9-4

Companion animals as alternative models for translational cancer research

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Companion animals that share human lifestyles and environments can develop spontaneous cancers. Cancer has become one of the leading causes of morbidity and mortality in companion animals with extended life expectancy. Numerous studies have shown that naturally occurring canine cancers share multifactorial traits with human cancers: histopathologic characteristics, genetic complexity, and even therapeutic targets. Therefore, the comparative oncology has the advantages not only for humans providing spontaneous cancer models, but also for companion animals providing new treatment options.

Preclinical and clinical research with companion animals can serve as a complement to the traditional mouse cancer models that lack key characteristics such as long latency, heterogeneity, and immune features. Recent studies also suggest the dog as an attractive model for cancer immunotherapy research.

We established the effective drug screening system using canine patient-derived cells (PDC) to predict the most effective anticancer treatment for each patient in the KU animal cancer center. PDCs were generated from various resected canine malignancies including hepatocellular carcinoma, lung adenocarcinoma, and transitional cell carcinoma (TCC). Molecular markers and drug responses of PDCs have contributed to select drugs suitable for clinical application. Established canine cancer cell lines from PDCs can be useful for drug development and target discovery. Furthermore, we try to contribute for the intermediate stage between traditional preclinical studies and human clinical trials by organizing clinical trial center for companion animals. Comparative oncology based translational research will simultaneously drive the implementation of precision medicine in veterinary oncology and advances in human cancer research.

Key words : Canine cancer, Spontaneous cancer model, Translational cancer research, Patient-derived cells

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SYMPOSIUM 10 [NIFDS2]

| July 21 (Thu) 14:50-16:30 | Samda Hall | KOR |

Development of medicines to respond to global public health crises

· Organizer : Jun Won Yun (Seoul Natl. Univ.) | · Chair : Jun-Young Seo (Yonsei Univ.)

1	Nanobiotechnology for diagnosis and vaccine against Viral Disease X in animal and human	Daesub Song Seoul Natl. Univ.
2	Current status of mRNA vaccine development for the preparedness of emerging infectious diseases in Korea	Kee-Jong Hong Gachon Univ.
3	Development of next generation COVID-19 vaccine using adenovirus vector platform	Kwang-Soo Shin CELLID

S10-1

Nanobiotechnology for diagnosis and vaccine against Viral Disease X in animal and human

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Unlike in the past, emergences of viral infections have been and are occurring at very frequent intervals, causing enormous deaths and disability worldwide. Against a constant background of established infections, periodical emerging the epidemics of highly pathogenic influenza viruses (HPAI) greatly expand the global burden of infections. Accurate and rapid diagnosis of viral infections can result in effective and appropriate prevention and quarantine measures. This study compares and discusses about rapid diagnostics for the differential patho-typing between HPAI and LPAI using nanobiotechnology. The field of nanotechnology encompasses those technologies to fabricate materials, including sphere, cubic and nanoscale particles. Therefore, nanobiotechnology has the potential to offer not merely advances in diagnostics and vaccination to control infectious diseases but also in delivering various capabilities as below; Highly pathogenic avian influenza virus (HPAIV) infections have occurred continuously and crossed the species barrier to humans, leading to fatalities. A PCR-based molecular test is currently the most sensitive diagnostic tool for HPAIV; however, the results must be analyzed in centralized diagnosis systems by a trained individual. This requirement leads to delays in quarantine and isolation. To control the spread of HPAIV, rapid and accurate diagnostics suitable for field testing are needed, and the tests must facilitate a differential diagnosis between HPAIV and low pathogenic avian influenza virus (LPAIV), which undergo cleavage specifically by trypsin- or furin-like proteases, respectively. In this study, we have developed a differential avian influenza virus (AIV) rapid test kit and evaluated it *in vitro* and using clinical specimens from HPAIV H5N1-infected dogs. We demonstrated that this rapid test kit provides highly sensitive and specific detection of HPAIV and LPAIV and is thus a useful field diagnostic tool for H5N1 HPAIV outbreaks and for rapid quarantine control of the disease. In addition to diagnostics, development of better adjuvant accompanied with vaccine for enhancing immunogenicity has been greatly required for the control of influenza infection. Herein, we also address nano-complex of amphiphilic grafted poly (amino acid) and hydrophobic squalene (PA/S-NC) as a potent adjuvant that can act as a robust strategy for induce humoral (Th2) and cellular (Th1) immune responses as well as a delivery agent of antigens. CASq performed great biocompatibility, particle stability, and produced a high degree of antigen-specific antibodies and T cell immune responses in mice when CASq was co-administered with inactivated whole influenza virus antigen (CA04), in which CASq exhibited complete protection against lethal infection.

Key words : HPAIV, Nanobiotechnology, Diagnostics, Adjuvant, Vaccination

S10-2

Current status of mRNA vaccines for the rapid preparedness of emerging infectious diseases in Korea

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As one of the ways to break the exceptionally long Corona-19 situation, the mRNA vaccine was strongly highlighted which supplied the vaccine much faster than expected before. Although the supply of mRNA vaccines only from Moderna and Pfizer was not enough for global demand, this new vaccine was recognized as a very important technology as a new vaccine development method to prepare for another pandemic of future infectious diseases.
Compared to recombinant synthetic vaccines and whole vaccines including live-attenuated and killed vaccines, the development and production capacity of mRNA vaccines in Korea is still far from a global level, also there are high entry barriers in related patents. In this domestic situation, the Ministry of Health and Welfare and the Korea Disease Control and prevention Agency have cooperated and stared "Korea mRNA Vacine Initiative (KmVAC)" as a new project team to internalize mRNA vaccine technology, related preclinical/clinical testing system, and rapid production platform as soon as possible. As a First step, KmVAC will rapidly develop the domestic mRNA vaccine development technology and build the related fast testing system for the rapid approval through the next 4-year performance of project.
KmVAC supports seven preclinical stage research teams on new mRNA antigen development and related technologies that are expected to prepare the future pandemic, also two clinical stage research teams developing Mock-up or multivalent mRNA vaccines for variants of influenza and coronaviruses. This project is promoting establish the platforms and technology to develop and produce domestic mRNA vaccines reaching clinical phase 2 within two years.

Key words : mRNA vaccine, Emerging infectious diseases, Rapid preparedness, Disease X

S10-3

Development of next generation COVID-19 vaccine using adenovirus vector platform

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Several COVID-19 platforms have been licensed across the world thus far, but vaccine platform research that can lead to effective antigen delivery is still ongoing. In addition, the emergence of different SARS-CoV-2 variants requires the development of vaccine platforms that can rapidly respond to variants. Here, we constructed adenovirus vector-based COVID-19 vaccine that could modulate humoral immunity by harboring SARS-CoV-2 antigens onto a chimeric adenovirus 5/35 platform that was effective in cellular immunity. By replacing the S1/S2 furin cleavage sequence of the SARS-CoV-2 Spike (S) protein mounted on vaccine with the linker sequence, high antigen expression was confirmed in various cell lines. The high levels of antigen expression contributed to antigen-specific antibody activity in mice and non-human primates (NHPs) with a single vaccination of vaccine. Furthermore, the adenovirus-induced Th1 immune response was specifically raised for the S protein, and these immune responses protected the NHP against live viruses. The established adenovirus vector platform could be extended to develop different variant-specific COVID-19 vaccine. For instance, AdCLD-CoV19-1 OMI, the Omicron-variant specific vaccine, has been developed by using Ad5/35 platform and was proved for its ability to induce neutralizing activity to SARS-CoV-2 Omicron variant in preclinical studies. The safety and immunogenicity of AdCLD-CoV19-1 in human will be tested in clinical studies.

Key words : COVID-19, Vaccine, Adenovirus vector

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SYMPOSIUM 11

| July 22 (Fri) 09:00-10:40 | Halla Hall A | ENG |

IBD preclinical models and new treatment modalities

· Organizer : Yi Rang Na (Seoul Natl. Univ. Hospital)
· Chair : Seung Hyeok Seok (Seoul Natl. Univ.)

1	The role of macrophages in gut inflammation	Seung Hyeok Seok Seoul Natl. Univ.
2	Understanding neuro-immune crosstalk in the gut using animal models to develop novel therapeutic strategies for intestinal inflammation	Gianluca Mateoli KU Leuven
3	Gene therapy of genetic diseases via CRISPR systems	Daesik Kim Sungkyunkwan Univ.
4	Application of ex-vivo generated macrophages as potential cell therapy	Yi Rang Na Seoul Natl. Univ. Hospital

S11-1

The role of macrophages in gut inflammation

Seung Hyeok Seok

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Intestinal macrophages have roles in maintaining tissue homeostasis, inflammation, and especially inducing resolution after inflammation. Resolution of inflammation in general is an active process controlled by local recruitment of monocytes and accumulation of alternatively activated macrophages with pro-resolving capacity. However, in individuals with a genetic and environmental predisposition, regulation of intestinal immunity is impaired, leading to chronic relapsing immune activation and pathologies of the gastrointestinal tract, such as inflammatory bowel disease (IBD). Dysfunctional resolution of intestinal inflammation and altered mucosal healing are essential features in the pathogenesis of IBD. As evidence suggests a causal link between defects in the resolution of intestinal inflammation and altered monocyte–macrophage differentiation in patients with IBD, macrophages have been considered as a novel potential target to develop new treatment approaches.
In this talk, I discuss the molecular and cellular mechanisms involved in the differentiation and function of intestinal macrophages in homeostasis and inflammation, and their role in resolving the inflammatory process.

Key words : Intestinal macrophages, Inflammation, Homeostasis, Inflammatory bowel disease

S11-2

Understanding neuro-immune crosstalk in the gut using animal models to develop novel therapeutic strategies for intestinal inflammation

Gianluca Matteoli

Department of Chronic Diseases, Metabolism and Ageing; Translational Research Center for Gastrointestinal Disorders (TARGID); KU Leuven, Belgium

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One of the main tasks of the immune system is to discriminate and appropriately react to "danger" or "non-danger" signals. This is crucial in the gastrointestinal tract, where the immune system is confronted with a myriad of food antigens and symbiotic microflora that are in constant contact with the mucosa, in addition to any potential pathogens. This large number of antigens and commensal microflora, which are essential for providing vital nutrients, must be tolerated by the intestinal immune system to prevent aberrant inflammation. Hence, the balance between immune activation versus tolerance should be tightly regulated to maintain intestinal homeostasis and to prevent immune activation indiscriminately against all luminal antigens. Loss of this delicate equilibrium can lead to chronic activation of the intestinal immune response resulting in intestinal disorders, such as inflammatory bowel diseases (IBD). In order to maintain homeostasis, the immune system has evolved diverse regulatory strategies including additional non-immunological actors able to control the immune response. Accumulating evidence strongly indicates a bidirectional link between the two systems in which the brain modulates the immune response via the detection of circulating cytokines and via direct afferent input from sensory fibers and from enteric neurons. In the current presentation, I will highlight the most recent findings regarding the cross-talk between the nervous system and the mucosal immune system and will discuss the potential use of these neuronal circuits and neuromediators as novel therapeutic tools to reestablish immune tolerance and treat intestinal chronic inflammation.

Key words : Inflammatory bowel disease, Intestinal immune system, Neuropeptide, Oral tolerance, Parasympathetic system, Peptidergic pathway, Sympathetic system

S11-3

Gene therapy of genetic diseases via CRISPR systems

Daesik Kim

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There are more than 7,000 genetic disorders, including hemophilia and sickle cell disease, and many of the genetic disorders are fatal and not curable. Therefore, it's important to develop gene targeting methods that could cure genetic diseases.
The type II CRISPR-Cas9 system, a form of adaptive immunity in eubacteria and archaea against foreign DNA elements, has been successfully repurposed for genome editing in human cell lines, animals, and plants. Here I will present CRISPR systems mediated gene therapy methods, which could cure genetic diseases.

Key words : CRISPR, Cas9, Gene therapy

S11-4

Application of ex-vivo generated macrophages as potential cell therapy

Yi Rang Na

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Reparative macrophages play an important role in regulating tissue repair of inflammatory diseases. In this talk, we discuss the feasibility of macrophage cell therapy to aid tissue regeneration in a disease of gastrointestinal track. To provide pre-clinical evidence of the efficacy and safety of ex-vivo generated macrophages, mouse bone marrow derived macrophages were prepared and adoptively transferred to colitic mice. Before injection, macrophages were treated with IL-4 for 24 hours (M2) or not (M0) to compare their anti-inflammatory and pro-regenerative capacity. Both types of transferred macrophages efficiently migrated to the inflamed area and persisted up to 40 days. M2 macrophages, not M0, reduced disease severity by lowering reactive oxygen species of abdomen, IL-6 production and infiltration of neutrophils. Pro-regenerative capacity of transferred M2 macrophages were also confirmed by determining recovery of shortened intestine. In addition, background strain-unmatched macrophages did not induce acute rejection reaction or any other side effects, revealing their low immunogeneicity. Finally, we will briefly introduce several interesting approaches to create efficient pro-regenerative macrophages other than classical IL-4 treatment. In conclusion, this translational study establishes an importance proof-of-concept in an application of ex-vivo generated macrophages for inflammatory diseases.

Key words : Cell therapy, Macrophage, Inflammation, Regeneration

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SYMPOSIUM 12

| July 22 (Fri) 09:00-10:40 | Halla Hall B | KOR |

Current translational research for human diseases using marmoset

· Organizer : Jeong Hun Kim (Seoul Natl. Univ.) | · Chair : Byeong-Cheol Kang (Seoul Natl. Univ.)

1	Translational research for retinopathy using marmoset	Jeong Hun Kim Seoul Natl. Univ.
2	Characterization and age-related changes of primary marmoset retinal pigment epithelial cells	Ha Young Jang Seoul Natl. Univ. Hospital
3	Generation of immortalized marmoset cell lines by a CRISPR-Cas9-mediated gene targeting	Young Hoon Sung University of Ulsan & Asan Medical Center
4	Development of genetically engineered marmoset models of early onset Alzheimer's disease: Initial experiences	Jung Eun Park University of Pittsburgh

S12-1

Translational research for retinopathy using marmoset

Jeong Hun Kim

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Department of Ophthalmology, College of Medicine, Seoul National University
Fight against Angiogenesis-Related Blindness (FARB) Laboratory, Clinical Research Institute, Seoul National University Hospital

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From the FDA approval of anti-VEGF aptamer to wet-type age-related macular degeneration (AMD) of choroidal neovascularization, anti-VEGF aptamer and antibody have been widely used against all kinds of vaso-proliferative retinopathy. Actually, current therapies directed at controlling vascular abnormalities in vaso-proliferative retinopathy target VEGF and can slow the progression of these diseases. While the general role of VEGF in development has been well described, the specific function of locally synthesized VEGF in the eye is incompletely understood. Recently, RNA-guided genome surgery using CRISPR-Cas9 nucleases has shown promise for the treatment of diverse genetic diseases. Yet, the potential of such nucleases for therapeutic applications in non-genetic diseases including AMD, diabetic retinopathy (DR) as well as retinopathy of prematurity (ROP) is largely unexplored. Those vision-threatening retinopathies such as AMD, DR, and ROP are leading causes of blindness in adults and children, which is associated with retinal over-expression of, rather than mutations in, the VEGFA gene. Currently, novel trials for therapeutics on retinopathy should be needed using human diseases mimicking animal models beyond rodents

Herein I would like to provide some my recent experimental results of translational research using marmoset on retinopathy. In addition, new project to establish genetically modified marmoset mimicking retinopathy would be introduced.

Key words : Translational research, Retinopathy, Marmoset

S12-2

Characterization and age-related changes of primary marmoset retinal pigment epithelium cells

Ha Young Jang

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We isolated and cultured primary retinal pigment epithelium (RPE) cells from marmoset monkeys as *ex vivo* steps prior to animal experiments to assess the mechanisms leading to RPE dysfunction in aging and age-related macular degeneration (AMD) and to develop advanced therapies, including RPE transplantation. The RPE plays a critical role in maintaining retinal function as a metabolic gatekeeper between photoreceptors and choriocapillaris. The RPE and Bruch's membrane (BM) suffer cumulative damage throughout their lifetime, which is known to induce AMD. In contrast to palliative pharmacological treatments, transplantation of the RPE has curative potential for AMD. However, autologous RPE transplants may have the disadvantage of carrying the same genetic information that may have led to AMD manifestation. The successful therapeutic modalities of cell application in AMD may be approached through intermediate stages in *ex vivo* research involving genetic engineering, *in vitro* therapy for RPE rejuvenation, and prosthesis of BM to improve the RPE transplants. Because marmosets have a human-like visual system and the potential to create transgenic primate models, they can be an ideal model for genetic engineering research for cell therapy that crosses *in vivo* and *in vitro* studies. Experiments using primary RPE cells have been recommended because it has recently become apparent that there are profound limitations and caveats to the relevance and reproducibility of experiments using the ARPE19 cell line, a spontaneously arising human RPE-derived cell line. We isolated primary marmoset RPE from the eyes of 7-year-old marmosets and 6-day-old marmosets. Prior to genetic manipulation, we confirmed culture conditions for the formation of a suitable RPE monolayer with a hexagonal shape similar to *in vivo*. In addition, we assessed the characteristic changes in primary marmoset RPE cells according to the age of the marmosets.

Key words : Marmoset, Retinal pigment epithelium, Age-related macular degeneration, Characterization, Age-related changes

S12-3

Generation of immortalized marmoset cell lines by a CRISPR-Cas9-mediated gene targeting

Young Hoon Sung

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Common marmoset (Callithrix jacchus) is a non-human primate that has the diverse advantage in modeling human diseases. Although diverse *in vitro* experiments should be conducted using immortalized cell lines prior to *in-vivo* animal testing, there are only a few immortalized marmoset cell lines available at present. In this study, we established efficient and convenient procedures for immortalizing primary marmoset dermal fibroblasts by targeted inactivation of p53 gene and INK4A/ARF locus using CRISPR-Cas9. Furthermore, genotoxic stresses stabilized p53 proteins in INK4A/ARF-deficient marmoset cells and normally activated the expression of p53 target genes. Therefore, our results indicate that Cas9-mediated inactivation of p53 gene or INK4A/ARF locus is a robust tool for establishing immortalized marmoset cell lines with defined genetic alterations.

Key words : Marmoset, Immortalization, CRISPR, Cas9, p53, INK4a, Genotoxic stress

S12-4

Development of genetically engineered marmoset models of early onset Alzheimer's disease: initial experiences

Jung Eun Park

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Alzheimer's disease (AD), the most common form of dementia in older adults, affects over 50 million people worldwide. The primary symptoms of AD are memory loss, cognitive impairment, mood/behavioral changes, and death. Despite intensive research, AD pathology is still poorly understood, and, to date, there is no cure. There's a strong need to identify better animal models to bridge the translational gap and identify potential treatments. We hypothesize that the common marmoset (Callithrix jacchus) is the ideal non-human primate model for AD. We used CRISPR/cas9 gene-editing techniques to engineer marmosets harboring mutations in the presenilin-1 gene that causes familial AD in humans. Compared to age/sex-matched wild-type non-carrier marmosets, these animals express elevated plasma levels of beta-amyloid (Ab). These alterations were observed as early as 8 weeks postnatal (the earliest time point tested) and confirmed in culture media from skin-derived fibroblasts from the same individuals. These data demonstrate that our genetically engineered marmosets, born with KI point mutations in PSEN1 that cause EOAD, display the early emergence of AD biomarkers seen in human carriers. These observations raise the possibility that these unique animals will be a platform for revealing the earliest molecular and cellular events that are the root causes of AD onset and progression. This work will allow the testing of disease mechanisms and extend the usefulness of marmosets as translational AD models.

Key words : Marmoset, Alzheimer's disease, Genetic engineering

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SYMPOSIUM 13
[SPECIAL SUBCOMMITTEE]

| July 22 (Fri) 09:00-10:40 | Samda Hall | KOR |

Animal facility design and operation strategy for user safety

· Organizer : Yirang Na (Seoul Natl. Univ. Hospital) | · Chair : Yang-Kyu Choi (Konkuk Univ.)

1	Efficacy evaluation and inhalation toxicity study for new inhaled drug development of respiratory diseases	Sung-Hwan Kim KIT
2	Establishing and maintaining a gnotobiotic mouse facility	Hyunjhung Jhun KFRI
3	Animal imaging facility	Jae Jun Lee Osong Medical Innovation Foundation
4	Facility for non human primates	Ji-su Kim KRIBB, PRC

S13-1

Efficacy evaluation and inhalation toxicity study for new inhaled drug development of respiratory diseases

Sung-Hwan Kim

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Lower respiratory tract infections and chronic obstructive pulmonary disease are leading causes of death worldwide, and their numbers are rising. Recently, the importance of respiratory diseases has been highlighted as a result of COVID-19 and ultrafine particles. New drug development of respiratory diseases is becoming very important, and there has been unprecedented growth in the market for respiratory diseases therapy. For treatment of respiratory disease, the preferred route of drug administration is by inhalation. Unlike systemic treatments, inhaled medicines are rapidly directed to the airways, allowing for rapid onset. Targeting a drug directly to the lungs allows for lower doses to be administered, limiting potential side effects. This strategy also avoids the complicating factors of plasma binding and first-pass metabolism, which can restrict the efficacy of oral or parenteral preparations of a drug. Nevertheless, there are three major reasons why the development of new inhaled drug in respiratory disease could be difficult. 1) Animal models of respiratory disease for early drug testing are not very satisfactory. An appropriate animal model is required for the development of new inhaled drug. 2) The inhalation toxicity study requires a costly and highly specialized infrastructure. 3) The selection and evaluation of the most appropriate respiratory drug delivery devices is critical to successful inhaled drug treatment. Therefore, I hope that this presentation will help you better understand the development process of respiratory disease treatment.

Key words : Efficacy evaluation, Inhalation toxicity study, New inhaled drug development, Respiratory diseases

S13-2

Establishing and maintaining a gnotobiotic mouse facility

Hyunjhung Jhun

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The gut microbiota is a complex and dynamic community composed of bacteria whose activity profoundly influences human health and diseases. Germ-free mouse models are generally considered the gold standard for microbiota studies. Germ-free animals originate from gnotobiotic animals obtained by colonization with pure culture or cocktails of bacterial strains. Gnotobiotic animals provide a valuable research tool to help elucidate the host-microbiota relationship. However, maintaining and monitoring the gnotobiotic mice facility is complex and labor-intensive.
This presentation will provide a general guide to establishing and maintaining a gnotobiotic mouse facility with critical requirements regarding costs, space, equipment, sterilization, personnel, and operational procedures.

Key words : Gnotobiotic facility, Germ free, Gut microbiota, Equipment, Sterilization

S13-3

Animal imaging facility

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Bio-imaging is used in various fields. In the development of new drugs, bio-imaging is widely used because it can obtain information that is difficult to obtain by other methods, such as drug-target interaction, drug delivery to target, and pharmacokinetic information. In addition, it is widely used in basic research, new drug development, and treatment fields by providing a non-invasive method for clinical application of research results in the laboratory.

For preclinical imaging, high statistical power can be obtained with a small number of animals, which reduces costs and enables ethical experiments.

Based on these advantages, there is no doubt about the potentially beneficial role of small animal imaging in preclinical research. However, imaging equipment installation is not straightforward. Some imaging equipment such as microscopes can be used simply after installing the equipment on a workbench. However, most of the imaging equipment requires a space for data processing and simple animal procedures.

The first consideration when designing an imaging center or imaging facility is deciding what type of research it will support. Next, it is necessary to decide which imaging method to use and whether a specific imaging system is required. Imaging systems are often quite expensive with a variety of modalities and vendors, so careful selection is required to ensure the optimal combination of systems. Additionally, when designing imaging centers, the process can be divided into several categories: vendor-specific site requirements (cooling, power, space), computer infrastructure requirements for storage and database information, animals used, radiation and biosafety requirements. In this lecture, I will talk about the points to consider when installing imaging facilities and evaluating imaging.

Key words : Bio-imaging, Imaging facility, Designing, Safety

S13-4

Facility for non human primates

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Bio-industry is emerging as an alternative to solve modern problems regarding population, aging, food, environment, and energy. Bio-industry, which is the core of the fourth industrial revolution, is a field that will lead to advancements in human health and economic prosperity. Primates that are essential for preclinical studies in the bio-health industry are strategic national resources. Biotechnology-leading countries (US, Germany, Japan, etc.) have already established and studied facilities for primate research since the early 1960s. In 2005, the Korean government established the Korea National Primate Research Center (NPRC) at the Korea Research Institute of Bioscience and Biotechnology (KRIBB). The government began construction on the Primate Resources Center (PRC) in 2015 in response to globalization trends, such as the weaponization of primate resources and the restriction of imports. The facility was completed in 2018. The PRC has the largest non-human primate infrastructure in Korea. Non-human primates in the PRC are managed by extensive microbiological monitoring (e.g., infectious viruses and bacteria) to ensure specific pathogen-free (SPF) non-human primate resources. In addition, the PRC has made efforts to construct collaborative networks and support industries, academia, and institutes focusing on non-human primate research, including neurodegenerative disease modeling, regenerative medicine, and pharmaceutical research related to incurable diseases. All these elements together (housing, food, enrichment, training) provide the suitable conditions for healthy mental balance of our animals, and a healthy social balance within the animal house, conditions that are essential for successful research. My hopes are that I have also enriched your world on the subject of environmental enrichment for monkeys.

Key words : SPF monkey, Cynomolgus monkey, Rhesus monkey, Non human primates infrastructure

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PLENARY LECTURE 2

| July 22 (Fri) 10:00-11:00 | Halla Hall A | ENG |

· Organizer / Chair : Huyk-Wan Ko (Yonsei Univ.)

Defining and engineering the gut stem cell microenvironment	Tae-Hee Kim The Hospital for Sick Children & University of Toronto

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Plenary Lecture 2

Defining and engineering the gut stem cell microenvironment

Tae-Hee Kim

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The gut stem cell microenvironment secretes key signals for maintaining proper stem cell self-renewal and differentiation. Dysregulation of these niche signals can lead to serious diseases such as inflammatory bowel disease and cancer. We and others have recently demonstrated that specific mesenchymal cells in proximity to stem cells constitute a gut stem cell niche, but how it is influenced by environmental factors such as gut microbiota is still unclear. By utilizing mouse genetic and organoid co-culture models, and performing single cell analysis, we demonstrate that gut microbiota promotes stem cell differentiation through the regulation of macrophages and specific mesenchymal niche cells. If this stem cell niche is impaired, newborns could be exposed to necrotizing enterocolitis, a deadly inflammatory disease. While our microbiome analysis reveals that Lactobacillus abundance is dramatically reduced in the NEC mouse model, the treatment of mice with Lactobacillus rescues NEC-like pathology through the activation of macrophage and mesenchymal stem cell niches. In order to enhance this gut microbiota-mediated regeneration, we have successfully engineered gut microbiota to secrete stem cell niche factors and validated their utility *in vitro* and *in vivo*. Taken together, our work has not only defined the gut microbiota-mediated regulation of stem cell niches, but also developed a novel probiotic approach for the promotion of stem cell-based regeneration.

Key words : Gut stem cell niches, Wnt signaling, Inflammation, Gut microbiota

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LUNCHEON SEMINAR 4~6

| July 22 (Fri) 12:10-12:30 |

Luncheon Seminar 4 — Halla Hall A

· Organizer : Itstandard | · Chair : Jun-Gyo Suh (Hallym Univ.)

Development of LMO management program for experiment and research	Byung chun Yoo ITstandard. Co.,Ltd.

Luncheon Seminar 5 — Halla Hall B

· Organizer : Ajou Univ. Regulatory Strategy Center for Combination Product (RSCP)

History of xeno-islets and recent new researches from transgenic pigs' islets	Hyunil Kim Optipharm Inc.

Luncheon Seminar 6 — Samda Hall

· Organizer : THREE SHINE INC | · Chair : Dae Youn Hwang (Pusan Natl. Univ.)

미래 실험동물실 경쟁력	Chun Gui Park THREE SHINE INC

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LS4

Development of LMO management program for experiment and research

Byung chun Yoo

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Universities and businesses can have multiple animal experiments facilities. There is a need for an integrated management system to manage efficient animal experiments facilities. An Internet-based management system is effective for managing animal laboratory facilities with different physical locations. Seoul National University has about 13 animal experiments facilities. Each management system is constructed and utilized in four animal experiments facilities. In animal laboratory facilities that do not utilize the system, they are documented. As the law is strengthened and the number of tasks to be recorded increases, the task force of animal laboratory facilities is getting more and more popular. The introduction of an integrated system can reduce workloads related to animal experiments and enable transparent animal use management. Animal experimentation involves many tasks such as education, IACUC, animal purchase, animal carry onto experiments facilities, animal take-off, carcass treatment. The college needs to know and report on the status of animal experiments conducted at each facility. The Law on <experiments on animals, and nationality of genetically modified organisms> also increased the number of LMO animal services. All production, storage, transport and disposal of LMO animals must be managed. Using the integrated management system, it is possible to conveniently manage LMO animals by using the records of animal purchase, import, export, and breeding necessary for animal experiment. It is expected that both researchers and business people will need to use an integrated system of animal laboratory facilities for safe and rational animal experiments.

Key words : LMO, Integrated management system, IACUC

LS5

History of xeno-islets and recent new researches from transgenic pigs' islets

Hyunil Kim

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Porcine islet xenotransplantation is a promising treatment for type 1 diabetes as an alternative to human pancreatic islet transplantation and long-term insulin therapy. Several research groups have explored porcine islets as an alternative to the inconsistent and chronic shortage of pancreases from human organ donors. But the number of donors and supply is insufficient compared with that of recipients and their needs. Because of these situations, research for xenotransplantation is being conducted.

Recently, xenotransplantation research has attracted attention due to the human clinical trials. In case of xeno-islets study, pigs have very similar insulin sequences compared to human. Additionally, pigs have very similar size of blood vessels and anatomical structure.

We believe xeno-islets can be very strong candidate for the type 1 diabetes patients. With this session, we will share the current research situation and brief history of xeno-islets' research trials.

Key words : Transgenic Pig, islets, Xenotransplantation, Type1 DM

LS6

미래 실험동물실 경쟁력

CHUN-GUI PARK

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Key words : Lab animal equipment, IVC Rack, Avtar 3D, Lab animal breeding room

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SYMPOSIUM 14

| July 22 (Fri) 14:00-15:50 | Halla Hall A | ENG |

The precision medicine initiative for liver disease through metabolic analysis

· Organizer / Chair : Won-Il Jeong (KAIST) / Hyon-Seung Yi (Chungnam Natl. Univ.)

1	Metabolic crosstalks in regulation of hepatic lipid homeostasis	Nika Danial Dana-Farber Cancer institute (Harvard Medical School)
2	Altered fuel metabolism in the pathogenesis of NAFLD	Dong Wook Choi Chungnam Natl. Univ.
3	Role of mitochondrial stress response in hepatic steatosis and liver cancer progression	Hyon-Seung Yi Chungnam Natl. Univ.
4	Hepatic glutamate and mGluR5 in liver fibrosis	Won-Il Jeong KAIST

S14-1

Metabolic crosstalks in regulation of hepatic lipid homeostasis

Nika N. Danial

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Hepatic lipid metabolism is regulated by hormonal and nutrient signals that converge on transcriptional and post-transcriptional control of lipid acquisition, storage, catabolism and export pathways. These processes are tightly controlled at transcriptional and post-transcriptional levels by hormonal and nutrient cues to prevent excess hepatic lipid accumulation (steatosis). However, the spectrum of nutrient control of these pathways beyond FA fluxes is not fully understood. Within this context, increasing evidence indicates a role for nutrient signals in transcriptional and epigenetic regulation of hepatic lipid metabolism. We identified the transcriptional co-regulator Host cell factor 1 (HCF-1) as a component of a nutrient sensitive promoter complex at lipogenic genes, which recruits OGT to ChREBP, regulating ChREBP O-GlcNAcylation and transcriptional activity in response to glucose/carbohydrates in hepatocytes/liver. HCF-1 is also required for recruitment of epigenetic modifiers to this complex. Recent ChIP-seq and RNA-seq integrative analyses also suggest that, beyond *de novo* lipogenesis, additional gene programs related to hepatic lipid metabolism are also stimulated by glucose in an HCF-1 dependent manner. I will discuss our ongoing studies on the mechanisms and consequences of HCF-1 directed transcriptional regulation of hepatic lipid metabolism.

Key words : HCF-1, ChREBP, O-GlcNAcylation, Lipogenesis, Lipoproteins

S14-2

Altered fuel metabolism in the pathogenesis of NAFLD

Dong Wook Choi

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Alteration of hepatic fuel metabolisms has long been associated with non-alcoholic fatty liver disease (NAFLD) progression, although the precise causalities and the underlying mechanisms are still enigmatic. In this presentation, I will briefly talk about our recent approaches on taking metabolic snapshots allowing for grasping the disease relevant changes in hepatic fuel metabolism using metabolomics coupled to a primary hepatocytes model where an excessive lipid build-up metabolically recapitulates dysregulated lipid metabolism in NAFLD. Among them, intracellular branched chain amino acids (BCAAs) are significantly altered, which may attribute to activation of a transcriptional program that provokes BCAAs metabolism. Notably, a genetic manipulation that reverses the change of BCAA metabolism in the model ameliorates dysregulated fatty acid oxidation (FAO), suggesting that the BCAA metabolism may play significant roles in unleashing the metabolic phenotype during NAFLD progression. Such observation is also associated with the liver from mouse model and human patients with NAFLD, highlighting the altered hepatic BCAAs metabolism as a potent therapeutic target of NAFLD.

Key words : Non-alcoholic fatty liver diseases (NAFLD), Fatty acid oxidation (FAO), Branched chain amino acids (BCAAs)

S14-3

Role of mitochondrial stress response in hepatic steatosis and liver cancer progression

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The mammalian mitochondrial ribosome (also known as the mitoribosome) is specialized in the translation of the 13 mitochondria-encoded membrane proteins, which are essential for the regulation of cellular respiration. Mitoribosomes are macrostructures of dual genetic origin, formed by three mitoribosomal RNA components encoded in the mtDNA and 89 specific mitoribosomal proteins encoded in the nuclear DNA. Although mitochondrial respiratory defects are frequently observed in human cancers, mechanisms underlying the involvement of mitoribosomal dysfunction in hepatocellular carcinoma (HCC) remain poorly understood.

In the present lecture, I will show the tumor immune microenvironment in the presence of liver-specific mitoribosomal defects. The rationale for this lecture will be based on the following evidence: 1) the expression of genes encoding for mitoribosomal proteins, mitoribosome assembly factors, and mitochondrial translation factors is modified in numerous cancers; and 2) mitochondrial dysfunction is associated with the pathogenesis and progression of a wide spectrum of liver diseases.

I believe that this lecture will provide an important conceptual advance in metabolism and mitochondrial biology, and would be of great interest to the audiences, particularly given the translational relevance of immunometabolism.

Key words : Mitochondria, T cell, Immunometabolism, Liver cancer

S14-4

Hepatic glutamate and mGluR5 in liver fibrosis

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The important roles of metabotropic glutamate receptor 5 (mGluR5) and its ligand glutamate in HSCs have recently been reported in several liver diseases. However, the mechanism linking the glutamine/glutamate metabolism and mGluR5 in liver fibrosis remains unclear. Here, we discovered that mGluR5 activation in natural killer (NK) cells ameliorates liver fibrosis through increased cytotoxicity and interferon-γ (IFN-γ) production in both mice and humans. In carbon tetrachloride (CCl_4) treatment or 5-week methionine-deficient and choline-deficient diet, liver fibrosis was more aggravated in mGluR5 knockout (KO) mice with significantly decreased frequency of NK cells compared with wild-type mice. Consistently, NK cell-specific mGluR5 KO mice had aggravated CCl_4 -induced liver fibrosis with decreased production of IFN-γ. Conversely, *in vitro* activation of mGluR5 in NK cells significantly increased the expression of anti-fibrosis-related genes including Ifng, Prf1, and Klrk1 and the production of IFN-γ through the mitogen-activated extracellular signal-regulated kinase/extracellular signal-related kinase pathway, contributing to the increased cytotoxicity against activated HSCs. However, we found that the uptake of glutamate was increased in activated HSCs, resulting in shortage of extracellular glutamate and reduced stimulation of mGluR5 in NK cells. Consequently, this could enable HSCs to evade NK cell cytotoxicity in advanced liver fibrosis. *In vivo*, pharmacologic activation of mGluR5 accelerated CCl_4 -induced liver fibrosis regression by restoring NK cell cytotoxicity. In humans, mGluR5 activation enhanced the cytotoxicity of NK cells isolated from healthy donors, but not from patients with cirrhosis with significantly reduced mGluR5 expression in NK cells. In conclusion, mGluR5 plays important roles in attenuating liver fibrosis by augmenting NK cell cytotoxicity, which could be used as a potential therapeutic target for liver fibrosis.

Key words : Glutamate, mGluR5, NK cell, Liver, Fibrosis

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SYMPOSIUM 15
[KLAT EDUCATION2]

| July 22 (Fri) 14:00-15:50 | Halla Hall B | KOR |

Laboratory animal facility management

· Organizer : Byeong-Cheol Kang (Seoul Natl. Univ.) | · Chair : Hyung-Sik Kim (Pusan Natl. Univ.)

1	Facility management and environmental enrichment for nonhuman primate	Young-Su Yang KIT
2	Laboratory animal facility management of environmental monitoring and COVID-19 pandemic	Kyoungmin Roh Seoul Natl. Univ. Hospital
3	Disinfection and sterilization in laboratory animal facilities	Hyunjhung Jhun KFRI

S15-1

Facility Management and Environmental Enrichment for Nonhuman Primate

Young-Su Yang

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Nonhuman primates (NHPs) are the most similar experimental animals to humans and are helping in various scientific research and medical developments. Particularly, in relation to COVID-19, there has been a critical demand for NHPs as experimental animals, resulting in a corresponding scarcity internationally. In order to use these primates for experiments, selection of healthy individuals and facility management are important. NHP health can be classified into physical health and mental health. For physical health, professional facility management based on primate characteristics such as cage designs according to the behaviors, supply of balanced feed, proper environmental conditions such as temperature and humidity, prevention of diseases and quarantine of new animals are important. Since NHPs are animals with high perceptual ability, relatively more attention needs to be given to their mental health as compared to other animals. To encourage a species-specific behavior of NHPs, a collective effort known as enrichment is recommended. Enrichments can be divided into environmental enrichment, instrumental enrichment, nutritional enrichment, social enrichment, and training enrichment. In addition, safety management and regulatory medical check-up for animal facility workers performing actual animal treatments are also important parts of NHPs study. In the present seminar, facility management and enrichment programs for NHPs are discussed in detail.

Key words : NHPs, Health, Management, Enrichment

S15-2

Laboratory animal facility management of environmental monitoring and COVID-19 pandemic

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Monitoring of environmental conditions in animal holding spaces and other environmentally sensitive areas in the facility should be considered. Automated monitoring systems, which notify personnel of excursions in environmental conditions, including temperature, relative humidity and photoperiods, are advisable to prevent animal loss or physiologic changes as a result of system malfunction. The functions and accuracy of such systems should be regularly verified. All factors (relative humidity, temperature, housing, air quality; ammonia concentration, ventilation; 10-15 per hour, cage population density, vibrations, noise, water, diet, enrichment, light/dark cycle and so on) influencing research results should be monitored on a regular basis.

The COVID-19 pandemic could constitute such a disaster if it creates severe shortages in staffing and in supply chains. Each facility builds up substantial reserves of crucial animal-care and laboratory supplies. These include personal protective equipment as well as food, water and bedding for the animals. Back-up for services such as animal care and health checks will be necessary. And if there are no longer enough staff members to provide basic animal care, depopulation might be the only option. Laboratory animal facilities had to find plausible mitigating measures to safeguard the welfare of animals in their care, to prevent animal suffering if staff could not reach the animal, albeit with limited time.

Key words : Housing and husbandry, Zoonotic disease, Nonhuman primates

S15-3

Disinfection and sterilization in laboratory animal facilities

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Laboratory animal facilities for the care and use of all animals in research, teaching, and testing must be conducive to the well-being and safety of the animals, provide an appropriately-appointed and safe workplace for people, and establish a stable research environment. In order to accommodate these needs, it is essential to make procedures for the effective disinfection and sterilization of animal facilities and equipment used for research to prevent microbial agents that may cause sub-clinical and clinical diseases that could jeopardize the health of the animals and personnel.
This presentation will provide a difference between disinfection and sterilization, types of disinfectants, and how to select the appropriate sterilization methods and indicators to assure sterility in laboratory animal facilities.

Key words : Disinfection, Sterilization, Indicators, Laboratory animal facilities, Microbial agents

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SYMPOSIUM 16

| July 22 (Fri) 14:00-15:50 | Samda Hall | KOR |

Research ethics and responsible research

· Organizer : Ju-Hong Jeon (Seoul Natl. Univ.)
· Chair : Ju-Hong Jeon (Seoul Natl. Univ.) / Won-Woo Lee (Seoul Natl. Univ.)

1	Research misconduct under the academic promotion act and the national R&D innovation act	Ju-Hong Jeon Seoul Natl. Univ.
2	How does research ethics committee operate?	Chin Ho Cho Seoul Natl. Univ.
3	How to identify and deal with conflicts of interest in research	Hyobin Lee Chungnam Natl. Univ.

S16-1

Research misconduct under the academic promotion act and the national R&D innovation act

Ju-Hong Jeon

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Compliance with research ethics is becoming increasingly essential for responsible research in the implementation of national R&D projects. In particular, researchers must not commit research misconduct, which is a serious violation that breaches the credibility of research and public trust. In Korea, research ethics is regulated by two Korean laws, the Academic Promotion Act and the National R&D Innovation Act. The aim of this presentation is to explain how research misconduct is defined in the two legal systems. Through this presentation, I would like to help understand the concept of research misconduct and practice research ethics.

Key words : Research misconduct, Research ethics, R&D, Korean law

S16-2

How does research ethics committee operate?

Chin Ho Cho

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Since Hwang's Stem Cell gate in 2005, various systems for research ethics have been established in Korea and one of the well-known systems is the Committee on Research Integrity (CRI). Based on Government's Instruction, 'Guidelines for securing Research Ethics', it was required to establish the CRI in research institutions, especially Universities. According to the 2020 survey of the National Research Fund, the CRI was established in about 95% of universities. Although the CRI is established in most universities, members of each do not clearly know how the CRI operates. Therefore, the purpose of this lecture is to ensure that researchers do not violate research ethics by understanding the CRI operating system.

Key words : Research ethics system, Committee on Research Integrity(CRI)

S16-3

이해충돌 방지법 시행에 따른 이해충돌 관리

Hyobin Lee
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2022년 5월 19일 이해충돌 방지법 시행에 따라 대학과 연구기관에서는 이해충돌 예방을 위한 관련 규정을 마련하고 있다. 학회에서도 이에 따라 관련한 규정을 마련하고 서약서 등을 만들어 준비할 필요가 있다. 일반적으로 연구와 관련되어서는 4가지 이해충돌이 있다. 재정적 이해충돌은 논문과 관련하여 재정적 이해관계가 있는 경우로 연구자가 논문을 발표하면서 생길 수 있는 재정적 이해관계를 모두 밝혀야 한다. 인적 이해충돌은 편집자, 심사자, 저자 간에 발생할 수 있는 이해관계를 이미 파악하고 심사자와 저자 간에 사적 이해충돌이 발생하는 경우 심사를 못하게 하는 등의 조치가 필요하다. 편집자가 자신이 편집자로 있는 논문에 출판하는 경우 심사자를 배정하지 않아야 하며, 미성년 저자가 있는 경우 이를 파악하여 논문에 밝혀야 한다. 논문과 관련되어 저자의 소속기관이 다양한 경우 직무상 이해충돌이 발생할 수 있으므로 이를 논문에 밝힐 필요가 있다. 지적 이해충돌은 연구자의 이념, 신념, 지적 성향이 달라 편향적 심사를 할 수 있는 경우 지적 이해충돌이 발생할 수 있으므로 심사에서 제외 하거나 이를 논문에 밝혀야 한다.

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실험동물연구장학생
POSTER PRESENTATION

| July 21 (Thu) 13:20-14:20 | ICC JEJU 3F Lobby |

· Chair : Jae-Hoon Choi (Hanyang Univ.)		
1	PS-R-001 (유전자질환모델)	Jina Kwak Seoul Natl. Univ.
2	PS-R-002 (해부생리)	Hyunkyoung Son Gyeongsang Natl. Univ.
3	PS-R-003 (독성병리)	You Jeong Jin Pusan Natl. Univ.
4	PS-R-004 (해부생리)	Semi Hwang Konkuk Univ.

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실험동물연구장학생 포스터 발표 안내

1. 포스터 발표 안내

발표시간	7월 21일(목) 13:20-14:20
발표 장소	제주컨벤션센터 (ICC JEJU) 3F Lobby
포스터 번호	PS-R-001 (유전자질환모델) PS-R-002 (해부생리) PS-R-003 (독성병리) PS-R-004 (해부생리)
	총 4개
부착 시간	7월 21일(목) 9:00-11:00
철거 시간	7월 21일(목) 17:40-18:00

※ 철거 시간 이후의 포스터 분실은 책임지지 않습니다.

2. 포스터 발표 및 시상

· 포스터 발표는 좌장의 진행에 따라 포스터당 7분 (5분 발표, 2분 질의응답)으로 진행되며, 발표시간에 포스터 앞에 대기하여 주시기 바랍니다.
· 장학생은 총회에서 진행하는 시상식에 반드시 참석해 주시기 바랍니다. (대리수상불가)
· 포스터보드에 2회 이상 (개수로 적용) 미부착 시, 교신저자(연구책임자)에게 향후 1년간 포스터 제출 불가의 제재가 주어집니다.

3. 포스터 작성안내

· Poster board의 크기는 **95 cm (가로) X 210 cm (세로)**이며, 특히 제목이 가로 넓이를 초과하지 않도록 준비하여야 합니다.
· 모든 포스터는 지정된 날짜, 기간 동안에 지정된 board에 부착하여야 합니다.
· 포스터 내용은 abstract, purpose, results(figures and tables), conclusions, references의 순서로 작성합니다.
· 전방 2 m 위치에서 쉽게 읽을 수 있도록 굵고 명확한 글씨체를 이용하여 제작합니다.
· 모든 포스터의 부착 및 철거는 당일 지정된 시간에 발표자가 수행하여 주기 바랍니다.

| 실험동물연구장학생 Poster

Poster no.	Title	Speaker
PS-R-001	Moderate improvement of idiopathic bond lytic lesion by alendronate in common marmoset (*Callithrix jacchus*)	Jina Kwak
PS-R-002	Chlorogenic acid attenuates neuroinflammation by preventing nuclear factor kappa B activation in stroke animal model	Hyunkyoung Son
PS-R-003	Effects of inhalated Microplastics on the inflammatory response and toxicity of ICR mice	You Jeong Jin
PS-R-004	The regulation of TAZ expression by estrogen in mouse uterus	Semi Hwang

PS-R-001

Moderate improvement of idiopathic bond lytic lesion by alendronate in common marmoset (*Callithrix jacchus*)

Jina Kwak[1,2], Jong-Min Kim[2,3], Joo-Il Kim[1,2], Hyun-Jin Lim[1,2], Byeong-Cheol Kang[1,2,4,5*]

[1]*Graduate School of Translational Medicine, Seoul National University College of Medicine, Seoul, Korea*
[2]*Department of Experimental Animal Research, Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea*
[3]*Xenotransplantation Research Center, Seoul National University College of Medicine, Seoul, Korea*
[4]*Seoul National University Hospital Marmoset Model Network Center, Seoul, Korea*
[5]*Designed Animal Resource Center, Institute of GreenBio Science Technology, Seoul National University, Pyeongchang-gun, Gangwon-do, Korea*

Common marmoset (*Callithrix jacchus*) is an useful non-human primate model for studying neuroscience, reproduction, infectious disease and toxicological science. Veterinary management of marmoset is essential for producing reliable and reproducible experimental results. Sometimes, metabolic bone disease(BMD) such as vitamin D-dependent rickets type II(VDDR2) or fibrous osteodystrophy(FOD) could naturally occurred in captive marmoset due to their unique metabolic characteristics. Here,e we found early-age onset idiopathic bone resorption in adult male and female sibling marmosets, never been used in other experiments. Both marmosets showed loss of body weights, sudden lameness and immobile in their arm or leg. In compute tomography(CT) result, well-demarcated lesion was observed in entire body and serum ALP level was increased. Alendronate, an inhibitor of osteoclast-mediate bone resorption and acts as a bisphosphonate was orally administrated (1.2mg/kg) twice a week to treat this unknown skeletal disease. One month after administration, both marmoset stopped lameness and showed normal behavior. CT result revealed their lesions were recovered and ALP level was recovered to a normal level. The characteristics of idiopathic bone resorption, description of veterinary care and special nutrients would helpful for management of marmosets.

Corresponding author : Byeong-Cheol Kang

Keywords : Common marmoset, Idiopathic bone resorption, Alendronate, Veterinary management

PS-R-002

Chlorogenic acid attenuates neuroinflammation by preventing nuclear factor kappa B activation in stroke animal model

Hyunkyoung Son, Ju-Bin Kang, Murad-Ali Shah, Dong-Ju Park, Phil-Ok Koh*

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Ischemic stroke is the most common type of stroke and is caused by vascular closure. Chlorogenic acid is a polyphenolic compound that is present in various plants. It is used as a traditional oriental medicine because of its anti-oxidant and anti-inflammatory properties. We investigated whether chlorogenic acid mediates neuroprotective effects by regulating pro-inflammatory proteins. Focal cerebral ischemia was induced through middle cerebral artery occlusion (MCAO) surgery in adult rats. Chlorogenic acid (30 mg/kg) or vehicle was injected into the abdominal cavity 2 h after MCAO. Rats were sacrificed 24 h after MCAO surgery and brain tissues were isolated immediately. MCAO caused histopathological changes in the ischemic cerebral cortex, and chlorogenic acid attenuated these changes. Chlorogenic acid reduced MCAO-induced reactive oxygen species generation and oxidative stress increase in the cerebral cortex. Furthermore, cerebral ischemia increased the expression of ionized calcium-binding adapter molecule-1 (Iba-1) and glial fibrillary acidic protein (GFAP), which are microglia and astrocyte activation markers, respectively. However, chlorogenic acid prevented MCAO-induced these increases. MCAO damage also increased the expression of nuclear factor-κB (NF-κB), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α). Chlorogenic acid treatment attenuated these increases caused by MCAO. These proteins are representative pro-inflammatory markers. This study confirmed that chlorogenic acid exerts an anti-oxidative effect and elucidated anti-inflammatory effect through regulating NF-κB, IL-1β, and TNF-α on cerebral ischemia. Thus, we can suggest that chlorogenic acid has neuroprotective effects by reducing oxidative stress and controlling pro-inflammatory proteins against cerebral ischemic damage. This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (NRF-2021R1F1A105878711)

Corresponding author : Phil-Ok Koh

Keywords : Cerebral ischemia, Chlorogenic acid, Neuroinflammation, Nuclear factor-κB

PS-R-003

Effects of inhalated Microplastics on the inflammatory response and toxicity of ICR mice

Dae youn Hwang[1*], You Jeong Jin[1], Ji Eun Kim[1], Yu Jeong Roh[1], Hee Jin Song[1], Ayun Seol[1], Jumin Park[2], Yong Lim[3]

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[3]*Department of Clinical Laboratory Science, College of Nursing and Healthcare Science, Dong-Eui University, Busan, Korea.*

Microplastics (MPs) are known to cause inflammatory response and toxicity, but the effects of inhalation treatment have been rarely studied. To investigate whether inhalation of polystyrene (PS)-MPs can induce an inflammation and toxicity, alterations on several key parameters for toxic and inflammatory response were analyzed in ICR mice inhaled with 0.5 mm size of PS-MPs for 2 weeks (4, 8 and 16 mg/mL). MPs were accumulated in the liver (1.15±3.4 mg/g), kidney (16.96±4.0), intestine (43.54±34.26) and lung (12.78±1.0 mg/g) of inhalated ICR mice without any changes in body or organ weight. The number of total cells and macrophage were significantly increased in BALF of MP treated group compared to Vehicle treated group. Also, a necrotic pyknosis and hyaline droplets on histopathological analyses was observed in the H&E stained liver tissue and kidney of MP inhalated group, while slight increase on the thickness of the respiratory epithelium and infiltration of the inflammatory cells were detected in lung tissue of the same group. The level of inflammatory markers such as MUC-2 and Klf4 were higher in MP treated group than Vehicle treated group. Furthermore, the level of several inflammatory cytokines including IL-4, IL-5, IL-10, IL-18, TNF-α, and IL-1β were remarkably increased in the lung tissue of MP treated group compared to Vehicle treated group. Therefore, these results suggest that inhalation of PS-MPs may induce significant inflammatory response in ICR mice without any significant toxicity.

Corresponding author : Dae youn Hwang

Keywords : Lung, Toxicity, Inflammation, Inhalation, Polystyrene microplastics

PS-R-004

The regulation of TAZ expression by estrogen in mouse uterus

Semi Hwang, Byeongseok Kim, Siyoung Lee, Giwan Lee, Hyeukjung Kim, Youngsok Choi*

Department of Stem Cell and Regenerative Biotechnology, Konkuk University, Seoul 05029, Republic of Korea

The mouse uterus undergoes many morphological and functional changes. The dynamic modulation is precisely regulated by two steroid hormones, estrogen and progesterone, during the estrous cycle. TAZ known as a transcriptional coactivator, which shuttles between the cytoplasm and the nucleus under the Hippo signaling. Nuclear TAZ is involved in regulation of cell proliferation, organ overgrowth, survival to stress and dedifferentiation. However, the phosphorylation of TAZ at Ser89 leads destabilization of TAZ protein by sequestering into the cytoplasm resulting in proteasomal degradation. Several recent studies indicated that estrogen is associated with regulation of TAZ activation via GPER (G protein-coupled estrogen receptor 1) by inhibiting TAZ phosphorylation. But the regulation of TAZ in mouse uterus remains unknown. In this study, we investigated TAZ expression and its regulation in mouse uterus. TAZ mRNA and protein did not show significant change during the estrous cycle. However, immunofluorescence analysis revealed that TAZ nuclear localization is significantly increase at the estrus stage. Interestingly, the phosphorylation of TAZ was changed according to the estrous stage, and it was lowest at the proestrus stage. And the expression level of TAZ mRNA and protein increased time-dependent by estrogen treatment. The expression level of TAZ mRNA was highest at 4 hours (4h) and protein is significantly accumulated in 6h and 12h after estrogen treatment. Also, immunofluorescence staining showed that nuclear TAZ was significantly increased in 6h and 12h after estrogen treatment compared to control(oil). In conclusion, TAZ expression is regulated and activated by estrogen in mouse uterus, but further analysis is required to determine whether the TAZ expression is regulated by nuclear estrogen receptors ERα and ERβ, which are sequence-specific DNA-binding transcription factors or GPER, which is seven-transmembrane-domain receptor that mediates non-genomic estrogen related signaling. This research was supported by a grant from National Research Foundation of The Ministry of Science, ICT & Future Planning (2021R1A2C1011916).

Corresponding author : Youngsok Choi

Keywords : TAZ, Estrogen, Mouse uterus

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포스터 초록
POSTER PRESENTATION

Poster Presentation 1	
July 21 (Thu) 13:20-14:20 ICC JEJU 3F Lobby	
· Chair : Jae-Hoon Choi (Hanyang Univ.)	
1	PS-A-001~049 (해부생리)
2	PS-B-001~053 (독성병리)
3	PS-C-001~008 (미생물)
4	PS-D-001~031 (유전자질환모델)
5	PS-E-001~026 (시설운영 및 기타)

Poster Presentation 2	
July 22 (Fri) 13:00-14:00 ICC JEJU 3F Lobby	
· Chair : Jae-Hoon Choi (Hanyang Univ.)	
1	PS-A-050~098 (해부생리)
2	PS-B-054~106 (독성병리)
3	PS-C-009~019 (미생물)
4	PS-D-032~062 (유전자질환모델)
5	PS-E-027~053 (시설운영 및 기타)

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포스터 초록 (Poster Presentaion) 안내

1. 포스터 발표 안내

발표시간	포스터 발표1	포스터 발표2
	7월 21일(목) 13:20-14:20	7월 22일(금) 13:00-14:00
발표 장소	제주컨벤션센터 (ICC JEJU) 3F Lobby	
포스터 번호	PS-A-001~049 (해부생리) PS-B-001~053 (독성병리) PS-C-001~008 (미생물) PS-D-001~031 (유전자질환모델) PS-E-001~026 (시설운영 및 기타)	PS-A-050~098 (해부생리) PS-B-054~106 (독성병리) PS-C-009~019 (미생물) PS-D-032~062 (유전자질환모델) PS-E-027~053 (시설운영 및 기타)
	총 167개	총 171개
부착 시간	7월 21일(목) 09:00-11:00	7월 22일(금) 09:00-11:00
철거 시간	7월 21일(목) 16:30-17:00	7월 22일(금) 15:50-16:20

※ 철거 시간 이후의 포스터 분실은 책임지지 않습니다.

2. 포스터 심사 및 시상

· **포스터 심사**: 포스터 발표는 좌장의 진행에 따라 포스터당 4분(3분 발표, 1분 질의응답)으로 진행되며, 내용의 과학성, 연구 성과, 발표자의 발표력 등을 기준으로 심사위원이 평가하여 우수포스터를 선정합니다. 발표 시간에 자리에 없는 경우, 미부착으로 간주합니다.
· **미부착 포스터**: 포스터 보드에 2회 이상 (개수로 적용) 미부착 시, 교신저자(연구책임자)에게 향후 1년간 포스터 제출 불가의 제재가 주어집니다.
· **우수포스터상 시상**: July 22 (Fri) 15:50~16:20 / Halla Hall A
· 우수포스터의 경우 폐회식에서 선정자를 호명합니다. 호명 시 자리에 없으면 다음 우수자에게 상이 수여되오니, 학술대회 종료일까지 학술대회에 꼭 참석해 주시기 바랍니다. (상장과 상금 15만원 수여, 대리수상불가)

3. 포스터 작성안내

· Poster board의 크기는 **95 cm (가로) X 210 cm (세로)**이며, 특히 제목이 가로 넓이를 초과하지 않도록 준비하여야 합니다.
· 모든 포스터는 지정된 날짜, 기간 동안에 지정된 board에 부착하여야 합니다.
· 포스터 내용은 abstract, purpose, results(figures and tables), conclusions, references의 순서로 작성합니다.
· 전방 2 m 위치에서 쉽게 읽을 수 있도록 굵고 명확한 글씨체를 이용하여 제작합니다.
· 모든 포스터의 부착 및 철거는 당일 지정된 시간에 발표자가 수행하여 주기 바랍니다.

| Anatomy / Physiology

Poster no.	Title	Speaker
PS-A-001	Ultrasound mediated miR146a-5p nanoparticles protect photothrombotic cerebral infarction	Dong Woon Kim
PS-A-002	Residues F130A and N138A of 5-HT3A interact ergot alkaloid chanoclavine	Jiwon Lee
PS-A-003	Naringin as a novel analgesic candidate through antioxidative and analgesic effects	Youngseo Park
PS-A-004	Nicotinamide riboside improves fetal growth under hypoglycemic condition	Eui-Ju Hong
PS-A-005	Sex hormone-binding globulin inhibits the entry of zoonotic coronavirus via AXL	Eui-Ju Hong
PS-A-006	CHIP ameliorates neuronal damage in H_2O_2-induced oxidative stress in HT22 cells and gerbil ischemia	Kyu Ri Hahn
PS-A-007	Differential effects of Cu,Zn superoxide dismutase on cuprizone-induced demyelination and reduction of adult neurogenesis in mice	Kyu Ri Hahn
PS-A-008	Phlorotannin can improve the intestinal epithelial barrier during laxative effects in loperamide-induced constipation of SD rats	Ji Eun Kim
PS-A-009	An optimized herbal medicine containing *Scutellaria baicalensis, Alisma canaliculatum*, and *Atractylodes macrocephala* Koidz has a potent antiplatelet and antithrombotic activity	Yeon-Ji Kim
PS-A-010	An optimized herbal medicine HTB attenuates hyperlipidemia by activating the AMPK/SREBP2/PCSK9 signaling pathway	Yeon-Ji Kim
PS-A-011	AMP-activated protein kinase activation in skeletal muscle modulates exercise-induced uncoupled protein 1 expression in brown adipocyte in mouse model	Youn Ju Kim
PS-A-012	Microbiota mediates aerobic exercise capacity via modulation of skeletal muscle glucose metabolism in mice	Youn Ju Kim
PS-A-013	Effects of parabens on inflammasome	Gilyoung Lee
PS-A-014	The Neuroprotective effects of exosomes derived from TSG101-overexpressing human neural stem cells in a stroke model	Dongsun Park
PS-A-015	Laxative effects of the methanol extract of green pine cones in loperamide-induced constipation of SD rats	Jumin Park
PS-A-016	*Atractylodes macrocephala Koidz* induces apoptosis in human gastric cancer cells through activation of the ROS and MAPK signaling pathway	Na Ri Choi
PS-A-017	Improvement of acute kidney injury by mesenchymal stem cell treated with dopamine D1 receptor agonist	Tae Min Kim
PS-A-018	Mixture of Corni Fructus and Schisandrae Fructus ameliorates testosterone-induced benign prostatic hyperplasia through regulating 5α-reductase 2 and androgen receptor	Hyun Hwangbo
PS-A-019	Improvement of particulate matter 2.5-induced keratoconjunctivitis sicca accompanying retinal and lipid metabolism disorders by Schisandrae Fructus	Da Hye Kim
PS-A-020	Inhibition of monosodium urate-induced NLRP3 inflammasome activation through NOX3/4-dependent mitochondrial oxidative stress in RAW 264.7 and bone marrow-derived macrophages by diallyl trisulfide	Min Yeong Kim
PS-A-021	Construction and performance evaluation of 3D bio-printed small-caliber artificial blood vessel graft	Kyeongwoong Yang
PS-A-022	Retinoic acid alleviates the reduction of the ubiquitin-proteasome system due to ischemic brain injury	Ju-Bin Kang
PS-A-023	Quercetin prevents glutamate toxicity-induced neuronal cell damage by regulating parvalbumin expression	Ju-Bin Kang
PS-A-024	The roles of enteric nervous system in radiation-induced enteropathy	Hyosun Jang
PS-A-025	The effect of functional foods containing collagen on osteoarthritis rat models	Sun Ju So
PS-A-026	CD47;Rag2;IL2rg triple KO mice preconditioning with busulfan injection could be a noble platform for generating hematopoietic stem cells engrafted humanized mice	Kang-hyun Kim, Seung-Ho Heo
PS-A-027	Embryotoxic and teratogenic effects of Scolopendra water extract in mice	Jeong Min Lee
PS-A-028	Olaparib, a selective PARP-1 inhibitor, aggravates radiation induced intestinal injury	Bohye Kim
PS-A-029	Expression of ATG9A and ATG9B in mouse oocytes and reproductive tissues	Sujin Son
PS-A-030	Effect of Lactobacillus fermentum BCC-LF-01 on alveolar bone loss in ligature-induced periodontitis mice	Bo Hyun Jung

Poster no.	Title	Speaker
PS-A-031	Indoxyl sulfate, a uremic toxin, induces trained immunity of monocytes through AhR-dependent arachidonic acid pathway	Hee Young Kim
PS-A-032	Hepatoprotective effects of catechin in carbon tetrachloride-induced hepatic damage in rats	Eunjun Jang
PS-A-033	Resveratrol alleviates the symptoms of experimental autoimmune neuritis	Seukchan Kim
PS-A-034	The role of leucine influx and its catabolism for regulating Th17 responses of human $CD4^+$ T cells	Yeon Jun Kang
PS-A-035	Different anti-tumor immune responses of fractionated high-dose radiation applied with intervals of 1- or 5-day in FSaII-bearing C3H mice	Hyun Kyung Kim
PS-A-036	The differentiation of epidermis of minipig skin during pregnancy	Jinhyung Rho
PS-A-037	An octopus-derived peptide with antidiuretic activity in rats	Seonmi Jo
PS-A-038	Evaluation of antitumor activity of N3095 as a novel orally active pyruvate dehydrogenase kinase inhibitor in mice of colorectal cancer	Kwang Hee Son
PS-A-039	Peripheral nerve-derived stem cell spheroids induce functional recovery and repair after spinal cord injury in rodent	Seong Taek Kim
PS-A-040	Developing an ADHD animal model by inducing neuroendocrine dysregulation during the prenatal period	Hye-Ji KIM
PS-A-041	Anti-inflammatory and anti-obesity effects of Pachydictyon coriaceum extract	So Yeon Kim
PS-A-042	Inhibitory effects of Endarachne binghamiae extract on inflammation and obesity	Sang Seop Lee
PS-A-043	Effects of Sargassum horneri extract on LPS-induced inflammation in RAW 264.7 cells and high fat diet-induced obesity in mice	So Yeon Lee
PS-A-044	Tumor-derived noncanonical Notch ligand DLK1 attenuates tumor growth by regulating macrophages	Misu Kim
PS-A-045	Dohongsamul-tang ameliorated cardiac function through calcineurin/NFATc4 signaling pathway in TAC-induced left ventricular hypertrophy rat	Mi Hyeon Hong
PS-A-046	Adipose stem cell-derived exosomes in combination with Hyaluronic acid for soft tissue augmentation: *in-vivo* study	Seung Hwan Kim
PS-A-047	Encapsulation of metformin within alginate shell-microcapsule with a thin oil layer ameliorates inflammatory bowel disease and improves gut microbiome	Eun-Ju Kim
PS-A-048	Oral delivery of pentoxifylline loaded microcapsules prevents inflammatory bowel disease and improves gut microbiome	Seong-Ryeong Lim
PS-A-049	Association of haptoglobin phenotype with neurological and cognitive outcome in patients with subarachnoid hemorrhage	Sung Woo Han
PS-A-050	The combination of prebiotics with probiotic complex affected differently intestinal hydrolase activity, microbial population and immunological biomarkers in SD rats fed an AIN-diet	InSurk Jang
PS-A-051	Mild traumatic brain injury and subsequent acute pulmonary inflammatory response	Seung Hyuk Lim
PS-A-052	Involvement of lipocalin-2 in experimental autoimmune uveitis	Sungmoo Hong
PS-A-053	Regulation of γc expression via IL-4 signaling in regulatory T cells	Jeong Ha Ryu
PS-A-054	IL-4-induced Forkhead box protein O regulates γc expression in T cells.	So Min Lee
PS-A-055	$NKP46^+NK1.1^+$ cells derived from definitive hemogenic endothelium of mouse aorta gonad mesonephros were markedly increased by 3-Deazaneplanocin A hydrochloride	Soo-Been Jeon
PS-A-056	Protective effects of an aqueous extract of *Protaetia brevitarsis seulensis* larvae against radiation-induced testicular injury in mice	Sohi Kang
PS-A-057	Effects of Sparganii Rhizoma on osteoclast formation and osteoblast differentiation and on an OVX-induced bone loss model	Eom Ji Kim
PS-A-058	Design and evaluation of an AI model for automated tail vein administrator in rodent models	Jonguk Kim
PS-A-059	Metabolites analysis and pharmacokinetic studies of [^{18}F]FP-CIT in preclinical models	Jae Hun Ahn
PS-A-060	Albiflorin promotes osteoblast differentiation and healing of rat femoral fractures through enhancing BMP-2/Smad and Wnt/β-Catenin signaling	Minsun Kim
PS-A-061	Effects of chloroform fraction of *Fritillariae Thunbergii* Bulbus on atopic symptoms in a DNCB-induced atopic dermatitis-like skin lesion model and in vitro models	Sooyeon Hong

| Anatomy / Physiology

Poster no.	Title	Speaker
PS-A-062	Association between sex-biased Cux2 expression and pancreatic cell damage in type 2 diabetic mice	Boyoung Kim
PS-A-063	Phenotyping of fecal microbiota of Korean wild mouse (KWM/Hym)	Eun Sun Park
PS-A-064	Neuroprotective effects of Populus tomentiglandulosa on neurotoxicity in an amyloid beta-induced Alzheimer's disease mouse	Ji Hyun Kim
PS-A-065	D-Allulose ameliorates hyperglycemia through IRE1α sulfonation-RIDD- sirt1 decay axis in the skeletal muscle	Geum-Hwa Lee, Hwa-Young Lee
PS-A-066	The desalted Salicornia herbacea water extract prevent osteoporosis by reduced osteoclast differentiation and ROS	Yun-Ji Lee
PS-A-067	Visualization of interscapular brown adipose tissue (iBAT) with TSPO targeting ligand by Cerenkov luminescence imaging (CLI) in the UCP1 Thermomouse	Seok-Yong Lee
PS-A-068	Codonopsis laceolata water extract ameliorates asthma severity by Inducing Th2 Cells' and pulmonary epithelial Cells' apoptosis via NF-κB/COX-2 pathway	So-Hyeon Bok
PS-A-069	Usage of natural volatile organic compounds as biological modulators of disease	Soon-Young Lee
PS-A-070	Transcranial alternating current stimulation exerts symptom relieving effects in the brain of a rat transient MCAo model	Yoon Beom Lee
PS-A-071	Characterization of rabbit diabetic mellitus model using alloxan	Seonghwa Lee, Gwang-Hoon Lee
PS-A-072	HPVSC-derived cyclophilin-A can recover ovarian function in mice with cyclophosphamide-induced premature ovarian failure	Ji Yun Park
PS-A-073	LncRNA *PTPRE-AS1* expression is significantly increased in IL-4 induced M2 macrophages	Hye Jin Choi
PS-A-074	Inhibitory effects of endarachne binghamiae extract on inflammation and obesity	Sang-seop Lee
PS-A-075	Melatonin significantly down-regulates long non-coding RNA *Cox2* in M1 macrophages stimulated with LPS	Dohyeong Kim
PS-A-076	Cardiovascular hemodynamic evaluation using PV loop analysis in rats	Woori Jo
PS-A-077	Anti-obesity effects of *Lactobacillus paracasei* subsp. *paracasei*, L. casei 431 on high fat diet-induced obese rats	Yun Jeong Shin
PS-A-078	Effect of glycemic variability on infarct volume in ischemic stroke model	Ye Jin Kim
PS-A-079	6-Shogaol suppresses osteoclastogenesis and alveolar bone resorption in mice with ligature-induced periodontitis	Zhi Bin Liu
PS-A-080	Anti-inflammatory effect of Stachys affinis extract on DSS-induced colitis in mice	Yun-Seong Lee
PS-A-081	Effect of grain mixture powder with sword bean on loperamide-induced constipation in C57BL/6 mice	Yun-Seong Lee
PS-A-082	Costunolide suppresses colorectal cancer cells growth and induces apoptosis *in vitro* and *in vivo*	Lei Ma
PS-A-083	*In vitro* and *In vivo* costunolide inhibits the growth of colorectal cancer cells and induces apoptosis	Domg Wook Kim
PS-A-084	Targeting protein Kinase B pathway with costunolide induces apoptosis Of oral cancer cells *in vitro* and *in vivo*	Ke Huang
PS-A-085	Effect of oocyte maturation, *in vitro* embryo development by ovum pick-up of follicle-stimulating hormone-treated Hanwoo (Bos taurus coreanae)	ChaeYeon Kim
PS-A-086	Agrimonia eupatoria alleviates hepatic fat accumulation and inflammation in the CDAHFD-diet-induced murine NASH model	Min-Jeong Jo
PS-A-087	The anti-obesity effect of Lactobacillus plantarum strain BK21 postbiotics isolated from Kimchi on high-carbohydrate-diet induced obesity in mice	Min-Jeong Jo
PS-A-088	Characterization of regucalcin as a novel tubulin deacetylase	Min-Jeong Jo
PS-A-089	Conjugation of Pheophorbide A and SN38 with hyaluronan nanoparticles for photodynamic- and cascadic chemotherapy in cancer stem-like ovarian cancer	Hosun Jung
PS-A-090	Visualization of a novel human monoclonal antibody against Claudin-3 using human ovarian cancer-bearing mice	Sera Oh
PS-A-091	Metabolic index using Glucose-Thymidine Ratio (GTR) as a potential imaging biomarker to assess response to immune checkpoint inhibitor therapy in mouse melanoma model	Sera Oh

Poster no.	Title	Speaker
PS-A-092	Immunomodulatory role of Fermented Garlic Extract on macrophage cell proliferation, cytokine secretion and Natural Killer cell cytotoxicity	Pallavi Gurung, Junmo Lim
PS-A-093	Comparison of the characteristcs between porcine bone marrow-derived Mesenchymal stem cells and peripheral blood mononuclear cells for transplantation therapies	Young Kyu Kim
PS-A-094	Hydrogen peroxide regulates gonadotropin-releasing hormone neurons excitability in postnatal and concentration-dependent manner in mice	Santosh Rijal
PS-A-095	GABA- and glycine-mimetic responses of honokiol on the substantia gelatinosa neurons of the trigeminal subnucleus caudalis in mice	Nhung Ha Thuy Le
PS-A-096	Visual phenotyping analysis of Korean wild mouse KWM/Hym	Munkhdelger Jamiyansharav
PS-A-097	Coenzyme Q10-Micelles complex ameliorates osteoarthritis by targeting for RIP3 and p-MLKL regulated necroptosis	Hyun Sik Na
PS-A-098	Age- and sex-relative effect of naringenin on substantia gelatinosa neurons of trigeminal subnucleus caudalis in immature mice	Seon-Ah Park

| Toxicology / Pathology

Poster no.	Title	Speaker
PS-B-001	Schisandrin C interact with V225A and V288Y on serotonin receptor	Sanung Eom
PS-B-002	Binding sites D177 and F199 are the major binding sites of kaempferol on human 5-HT3A receptors	Junho Lee
PS-B-003	Study of parasite pheromone receptor and gating mechanism to receptors of neurotransmitters	Junho Lee
PS-B-004	Transferability, and within/between-laboratory validation studies of improved in chemico alternative assay, spectro-DPRA, for skin sensitization test	Yu-Jin Hong, Hye-Jeong Sin
PS-B-005	Samhwangsasim-tang improves cognitive function through BDNF-mediated pathway in scopolamine-induced mouse model	Malk Eun Pak
PS-B-006	No apparent cellular immunotoxicity in mice subchronically exposed to polyethylene or polytetrafluorethylene microplastics through gastric intubation	Da Eun Lee
PS-B-007	Monitoring of maternal antibody for 8th industrialization line Korean Native Chicken in Poultry Research Institute during 4 weeks	Are-Sun You
PS-B-008	Novel role of *Dipterocarpus tuberculatus* as a stimulator of focal cell adhesion through the regulation of MLC2/FAK/Akt signaling pathway	Ayun Seol
PS-B-009	Analysis of methenamine residues in horse muscle by targeted sampling plan in 2021	Sunjin Park
PS-B-010	Optimized characterization of NK cell-derived nano-vesicles as a new mRNA delivery system	Min-Kyung Park
PS-B-011	Targeting tumor-intrinsic PD-L1 suppresses the progression and aggressiveness of head and neck cancer cells by inhibiting the GSK3β-dependent Snail degradation	Min-Hye Ahn
PS-B-012	The antitumor effect of Genipin in human oral squamous cell carcinoma	Dong-Guk Park
PS-B-013	Artificial intelligence-based assessment of dependence with cocaine self-administered marmosets data	Juhui Gim
PS-B-014	The effects of Angelica Gigas Nakai extract on the myelosuppressive mice	Seojin Park
PS-B-015	Administraion of Gastrodia elata blume extract attenuated the acute kidney injury induced by vancomycin in rats	Yeon Su Lee
PS-B-016	Administraion of Gastrodia elata blume extract attenuated the ovalbumin-induced allergic asthma in rats	Da Eun Jung
PS-B-017	Effect of manganese and lead exposure on the expression of serotonin receptors in the stariatum of C57BL/6 mice	Kisok Kim
PS-B-018	Maternal DMEP exposure alters neural proliferation and synaptic function in the mice	MoonYi Ko

| Toxicology / Pathology

Poster no.	Title	Speaker
PS-B-019	Toxicity evaluation of cigarette butts using chironomus riparius	Dong Hyun Kim, Da seul Kim, Hyung Hoon Jin
PS-B-020	Prediction of skin sensitization potential of silicon dioxide and titanium dioxide nanoparticles through the local lymph node assay: 5-bromo-2-deoxyuridine flow cytometry method	Manisha Adhikari
PS-B-021	Electro-acupuncture stimulation of HT7 alleviate sleep deprivation against acute caffeine exposure by regulating BDNF-mediated ER stress in the rat medial septum	Yeonhee Ryu
PS-B-022	A comparative study on clinical pathology under two different diets in cynomolgus monkeys (*Macaca fascicularis*)	Da-Hee Kim
PS-B-023	Mechanisms of cutaneous neurogenic inflammatory spots expression on referred visceral pain: primary and secondary uterine pain in the adult virgin rat	Kwang-Ho Choi
PS-B-024	Aromadendrin reduces airway inflammation in an experimental mouse of allergic asthma	Jin-Mi Park, Juhyun Lee
PS-B-025	Aromadendrin has anti-inflammatory effect on airway inflammation in an experimental animal models of COPD	Jinseon Choi, Juhyun Lee
PS-B-026	Inhibition of liver X receptor attenuates nonalcoholic steatohepatitis by regulating differentiation of monocyte-derived macrophages	Kyurae Kim
PS-B-027	Actue toxictiy study of Asparagus officinalis L. root extracts in Sprague Dawley rats	Jae Hee Lee
PS-B-028	The NLRP3 inflammasome is inhibited in natural products, protecting against acute gout	Hyeonjin Kim
PS-B-029	Natural products protect against acute gout by inhibiting the activation of the NLRP3 inflammasome	Soo Hyun Jeong
PS-B-030	Ginger and its two active components as novel autophagic and apoptotic mediators in oral squamous cell carcinoma	Hyun-Ji Kim
PS-B-031	Development of pcl nasolacrimal stent for the treatment of epiphora	Seungkuk Bae
PS-B-032	Symptom of collagen induced arthritis in DBA-1J mouse was alleviated by bee venom	Sokho Kim
PS-B-033	Repeated dose toxicity study in beagle dogs by intrathecal lumbar puncture: a potential non-rodent model for intrathecal drug delivery	Min-Kyung Cho
PS-B-034	Antiepileptic and anxiolytic effects of hinokinin	Abdulaziz Jabborov
PS-B-035	Evaluation of dependence potential induced by abused drugs with intracranial selfstimulation	Yong-Qing Zhang
PS-B-036	Anti-cancer effects of a novel multi-targets agent, KMU-191 and its electrophysiological safety	Shin Kim
PS-B-037	Liver toxicity from PFOS exposure in animal studies: A systematic review and meta-analysis	Se-A Lee
PS-B-038	Amelioration of tunicamycin-induced liver injury by taurine supplementation in mice	So min Lee
PS-B-039	Dietary restriction alleviates acetaminophen-induced hepatotoxicity	Ji Eun Bae
PS-B-040	Synthetic cannabinoids-induced reward behavior is associated with cannabinoid receptor 1 receptor and dopamine transporter function	Aeseul Kim
PS-B-041	Evaluation of the regenerative potential of cell therapeutics using repair-associated cell-containing intestinal organoid	Ji-Su Ahn
PS-B-042	A novel strategy to potentiate the therapeutic efficacy of secretome from canine stem cells against atopic dermatitis utilizing microencapsulation technologies	Su-Jeong Oh
PS-B-043	Atopy-induced stress increases hippocampal neuroinflammatory damage in an atopic dermatitis mouse model	Hye-Sun Lim, Gunhyuk Park
PS-B-044	Pathogenic effect of crystallin alpha B in fibrosis and angiogenesis in mice age related macular degeneration model	Eunhye Yu
PS-B-045	Effect of inhaled 1,2-Dichlorobenzene on cytochrome P450s and lipid [eroxidation in B6C3F1 mice	Eun-Sang Cho
PS-B-046	Improvements to improve the survival rate of rats in the carcinogenicity test (inhalation toxicity)	Sung-Bae Lee
PS-B-047	28-Days inhalation toxicity of 2-Methoxyethanol in B6C3F1 mice	Daesik Rha

Poster no.	Title	Speaker
PS-B-048	Subacute(28-days) inhalation toxicity of 1-Ethoxy-2-propanol in wistar rats	Daesik Rha
PS-B-049	Cigarette smoke-mediated alteration of reactive oxygen species (ROS) regulates immunomodulatory and hematopoietic stem cell supporting properties in human mesenchymal stem cells	Hyun Sung Park
PS-B-050	Gunryeong-tang suppresses cardio-renal syndrome in rats with pulmonary arterial hypertension	Se Won Na
PS-B-051	The factors determining the biological fate of nanodiamond: sp3/sp2 carbon ratio vs hydrodynamic size	Jiyoung Jeong
PS-B-052	The toxicity evaluation of cement and nano-cement after intratracheal instillation to rat	Eun Sol Bae
PS-B-053	The toxic effects of fragmented microplastics under ultraviolet oxidation	Yeonjeong Ha
PS-B-054	Acute lung injury induced by 2D materials(single and multi-layered Ti3C2 MXene	Song-yeon Kim
PS-B-055	Effects of 2-week repeated intratracheal instillation of vehicles on mice lung	Hyeon-Young Kim
PS-B-056	Effect of neonatal exposure of di-(2-methoxyethyl)phthalate on susceptibility to nicotine-induced locomotor sensitization and nicotine self-administration in rats	Tae Wan Kim
PS-B-057	A novel synthetic cathinone, α-pyrrolidinobutiothiophenone, produces psychomotor, rewarding, and reinforcing properties in rodents and increases dopamine level in the striatum of mice	Oc-Hee Kim
PS-B-058	The cytotoxic effects of nanoplastics in mouse preimplantation embryos	Hyeong-ju You
PS-B-059	The cytotoxic effects of particulate matter 10 on rhesus monkey in skin fibroblast	Jiin Lee
PS-B-060	Dysfunction of female reproductive system is caused by exposure to airborne nanoplatics in mice	Changsic Youn
PS-B-061	Damage to olfactory organs of adult zebrafish induced by diesel particulate matter	Hyejin Lee
PS-B-062	Cimicifugae Rhizoma extract attenuates oxidative stress and airway inflammation via the upregulation of Nrf2/HO-1/NQO1 and downregulation of NF-B phosphorylation in ovalbumin-induced asthma	So-Won Pak
PS-B-063	Titanium dioxide nanoparticles exacerbate allergic airway inflammation via TXNIP upregulation in a mouse model of asthma	Woong-Il Kim
PS-B-064	Effect of 28-day repeated oral dose toxicity of aluminum chloride(AlCl3) in rat	Yea-Gin Yang
PS-B-065	Efficacy evalution of candidate A in DSS-induced inflammatory bowel disease mouse model	Gukdo Kim
PS-B-066	Comparison of biochemical analysis in rodents using diluted serum	Kil-Woong Ha
PS-B-067	Human induced-pluripotent stem cells (iPSc) derived-hepatocyte as an *in vitro* model for evaluation of cytochrome P450 induction by hepatotoxicant	Nam-Ju Kim
PS-B-068	Withdrawal from treatment with 3-Fluoroethamphetamine induces hyperactivity and depression-like behavior in mice	Hyejin Joo
PS-B-069	Hepatoprotective effect of germinated soybean embryo extracts in HFD-fed obese mice	Hyunwoo Cho
PS-B-070	A comparative study of artificial intelligence analysis for diagnosis of liver fibrosis in rats	Tae-Yang Jung
PS-B-071	Hepatic steatosis screening analysis study applying artificial intelligence to rats	Tae-Yang Jung
PS-B-072	A study for selecting optimal classification AI model for 1-naphthyl isothiocyanate-induced liver necrosis in the mouse	Tae-Yang Jung
PS-B-073	Using drug discrimination techniques to evaluate the abuse related effects of 3-Fluoroethamphetamine in rats	Wonjong Lee, Hyun Kyu Min
PS-B-074	The protective effect of *Achyranthes japonica* aqueous extract (USL) in the dry eye model	Bongkyun Park
PS-B-075	Toxicological evaluation of extracellular vesicles derived from canine adipose tissue-derived mesenchymal stem cells (ASC-EVs)	Sung Bae Kim
PS-B-076	Neurotoxic effects of 3-Fluoroethamphetamine in mice: behavioral pharmacological approach	Cheolmin Jo
PS-B-077	Dependence potential of 4-Fluoroethylphenidate (4F-EPH); Behavior approaches in mice	Jin-Mook Kim

| Toxicology / Pathology

Poster no.	Title	Speaker
PS-B-078	Particulate matters-mediated oxidative stress induces airway inflammation and pulmonary dysfunction through TXNIP/NF-κB and SIRT1/p53/caspase-3 pathways in mice	Ji-Hye Ha
PS-B-079	*Spiraea prunifolia var. simpliciflora* downregulates inflammatory responses and oxidative stress in a mouse model of PPE/LPS-induces chronic obstructive pulmonary disease	Ba-Wool Lee
PS-B-080	Effect of mexedrone, a mephedrone analog, on abuse-related behaviors in rodents	Kyung Oh Jeon
PS-B-081	Anti-atopic effect of Persicaria longiseta (Bruijn) Kitag in atopic dermatitis murine model induced by 2,4-dinitrochlorobenzene	Min Hee Hwang
PS-B-082	Establishment of transfection condition for study to investigate effect of miR-143 on canine mammary gland malignant tumor	Gi Taek Oh
PS-B-083	Inhibitory effect of gastrodin and 4-hydroxybenzyl alcohol on tumor cell proliferation	Gi Yeon Kwon
PS-B-084	Setting conditions for performing miRNA-210 transfection experiment	Min Young Heo
PS-B-085	Image-based evaluation of adjuvant efficacy in a vaccine candidate against norovirus infection	In Woo Kim
PS-B-086	Dopamine responsiveness to 3-FEA in rodents: Investigations in the nucleus accumbens	Dong-Hyun Youn
PS-B-087	Evaluation of the potential immunotoxicity in mice with single exposure to poplypropylene microplastics through intragastric intubation	Sarina Kusma
PS-B-088	Cyclophilin A-induced M2 macrophage polarization protects inflammation-induced preterm birth in mice	So Hee Park
PS-B-089	The deficiency of ABCG1 and ABCG4 transporters by rare earth oxide nanoparticles induces the pulmonary alveolar proteinosis	Soyeon Jeon
PS-B-090	Comparison of disease severity by induction period in the Bleomycin-induced mouse model of idiopathic pulmonary fibrosis	HARAM KIM
PS-B-091	*Aloe vera* prevents lung fibrosis by suppressing TGF-β/Smad pathway	Soo-Jung Yang
PS-B-092	External electrical stimulation suppresses hepatic lipid accumulation by modulating lipid metabolism in non-alcoholic fatty liver animal models	Hana Lee
PS-B-093	The development of alcohol-associated hepatocellular carcinoma mouse model	Wonhyo Seo
PS-B-094	Assessment of the lung toxic effect of whole cigarette smoke extract on repeated intratracheal instillation	Jinhee Kim
PS-B-095	Processed Aloe vera gel accelerates reversal of liver fibrosis *in vitro* and *in vivo* through regulation of TGFβ1 pathway	Hoang Lan Vo
PS-B-096	Methoxychlor and Bisphenol A have more negative effects on male reproductive system when used together	Ju Yeon Hong
PS-B-097	Understanding the etiology and establishing the therapeutic strategies for menopausal xerostomia using ovariectomized mice	Yoojin Seo
PS-B-098	Adverse affects of chlopyrifos on reproductive and immune system	Min Woo OH
PS-B-099	Compound K-enriched Korean red ginseng regulates induces apoptosis of lung cancer cells through STAT3 down-regulation	Jung Ho Hwang
PS-B-100	The impact of vanadium oxide exposure on sperm motility and function in mice	Eungyung Kim
PS-B-101	Chlorpyrifos affects reproductive system in female mice	So yeon Ryu
PS-B-102	The effects of micro-current stimulation on alleviating non-alcoholic steatoheptitis in CDAHFD-induced mice model	Doyong Kim
PS-B-103	A comprehensive mechanism in inhibiting gap junction intercellular communication by DMBA	Dong-Hoon Won
PS-B-104	Identification of potential mechanism through miRNA-target gene profile of cisplatin-induced hepatotoxicity and nephrotoxicity and discorvery of circulating miRNA diagnostic biomarker	Da-Bin Hwang
PS-B-105	Brain structural alteration by cocaine administration is regulated by dihydropyrimidinase-related protein 2 and crystallin alpha B in the marmoset	Sun Mi Gu
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| Microbiology

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PS-C-002	Anti-malarial effect of PJ extract by inhibiting platelet activation in rodent model	Youn-Kyoung Goo
PS-C-003	Regulation of the SARS-CoV-2 pseudovirus infection by using engineered exosomes	Kyoung-myeon Kim
PS-C-004	The pneumococcal pep27 mutant infection enhances intracellular uptake and immune mediation, and provides apoptosis resistance via Mcl-1 upregulation in Raw264.7 cells	Ki El Nam
PS-C-005	Comparisons of diagnostic methods for identifying *Pasteurella pneumotropica* in experimental mice in Korea	Se hee PARK
PS-C-006	Galectin-4 enhances the immunostimulatory function of M2 macrophages to upregulate the antiviral $CD4^+$ T cell response and antibody production	In-Gu Lee
PS-C-007	Identification of fecal microbiomes characteristics and transcriptome in blood of growing Jindo dogs	Soyoung Choi
PS-C-008	Identification of age-related compositional changes of intestinal microbiota in piglets	Jin A Lim
PS-C-009	Development of mouse lethal models for Japanese encephalitis virus and Dabie bandavirus	Jung-Eun Kim
PS-C-010	Effect of genetic background differences between FVB and C57BL/6 mice in SARS-CoV-2 infection	Ah-Reum Kang
PS-C-011	Comparative analysis of complex IBD model in Il2rg-deficient mouse and C1qa/Rag2 double knockout mouse	Sun-Min Seo
PS-C-012	Establishment of a Hataan Orthohantavirus infection model in mice	Young Jo Song
PS-C-013	Health monitoring system of "K-MEDI hub Preclinical Research Center" for high quality improvement of laboratory animals	Hyejin Kim
PS-C-014	Protective effect of *Lactobacillus kunkeei* on Dextran Sodium Sulfate-induced colitis mouse model	Tae-Sung Lee
PS-C-015	Hydroxy fatty acid produced by gut bacteria protects diet-induced obesity through improving energy expenditure	Yeonmi Lee
PS-C-016	Gut bacteria-derived leucic acid attenuates diet-induced obesity and improves insulin sensitivity	Hye Rim Jang
PS-C-017	A bacteria-mediated tryptophan derivative, tryptamine, reduces fat accumulation in diet-induced obese mice	Jongjun Lee
PS-C-018	Use of nanobody as a treatment for influenza disease	Jae Hyun Hwang
PS-C-019	*Lactobacillus* spp. isolated from Honey bee can ameliorate high fat diet-induced obesity	Do-Hyeon Jung

| Genetic disease model

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PS-D-002	Similarities and differences in immunity between Rag2 knock out mice derived from two different sources	Yu Jeong Roh
PS-D-003	Loperamide-induced constipation activates inflammatory signaling pathways in transverse colon of SD rats via Complement C3 and its receptors	Hee Jin Song
PS-D-004	Mig-6 in BAT controls brown adipogenesis and thermogenesis in mice.	Sorim Choung
PS-D-005	Ablation of *CrebH* accelerates the progression of inflammatory bowel disease–associated liver injury	Sanghee Lee
PS-D-006	Novel biomarkers for pre-diabetes and diabetes mouse model	Jae-Ho Lee
PS-D-007	Protective role of *DAX-1* deficiency against acetaminophen-induced liver injury in animal model	Young Joo Suh
PS-D-008	Anti-inflammatory effect of *DAX1* against ConA-induced acute liver injury in mice	Hyo Jeong Yun

| Genetic disease model

Poster no.	Title	Speaker
PS-D-009	LRH-1 regulates hepatic triglyceride metabolism via modulation of the expression of Perilipin 5	Eun-Ho Lee
PS-D-010	Insulin resistance improving effects of *Circium japonicum* extract from type 2 diabetic mice model	Ji-Hye Choi
PS-D-011	Thymosin beta 4 regulates NLRP 3 inflammasome through multiple singaling pathways	Ji-Hye Choi
PS-D-012	KLF10 as a tumor suppressor gene and its TGF-B signaling	Woon Kyu Lee
PS-D-013	Establishment of canine cancer organoids using patient-derived cells from hepatocellular carcinomas and mammary gland tumors	Kieun Bae
PS-D-014	Antioxidation and anti-inflammatory effects of gamma-irradiated silk sericin and fibroin in H2O2-induced HaCaT Cell	Ji-Hye Choi
PS-D-015	Effect of protecting phosphatidylcholine against liver and kidney cell damage by advanced glycation end products (AGEs)	Ji-Hye Choi
PS-D-016	Effects of particulate matter (PM) on the Tau-BiFC transgenic mouse	Kyu Hyeon Kim
PS-D-017	Comparison of phenotype expression between Leprdb/Korl and Leprdb/J mice	Jae-Hong Min
PS-D-018	Establishment and characterization of six canine hepatocellular carcinoma cell lines	Ja Young Lee
PS-D-019	Cytosolic $Ca2^{+}$ levels signal by Per2 down regulation is related to amphetamine-like drugs induced reward behavior	Eunchong Hong
PS-D-020	Establishment and evaluation of a novel mouse model of Fabry disease	Dong-Won Seol
PS-D-021	Breeding and characterization of Spinocerebellar Ataxia type 1 model mice	Min Hyeak Lee
PS-D-022	Auditory or audiovisual stimulation ameliorates cognitive impairment and neuropathology at different stages of ApoE4 knock-in mice	Yeonkyeong Lee
PS-D-023	Anti-adipogenic effects of citrus flavonoid, Nobiletin in 3T3-L1 cells	Se-A Lee
PS-D-024	Production of transgenic piglets for BRAF mutation-based melanoma model using Jeju native pig fetal fibroblast	Dongjin Oh
PS-D-025	Assessment of OKT-3 induced cytokine release syndrome in hPBMC- humanized mouse model	Seo Yule Jeong, Myeongjin Choi
PS-D-026	Adipocyte PHLPP2 inhibition prevents obesity-induced fatty liver	KyeongJin Kim
PS-D-027	A mouse model with genetic defect of mitochondrial complex I to study neurodegeneration.	Won-Seok Choi
PS-D-028	Effect of gunryeong-tang on heart and kidney damage in diabetic mice model	AiLin Tai
PS-D-029	Modulation of PI3K/PTEN-mTOR signaling pathway in the antibody class switching in activated B cells	Dong-Gyu Kim
PS-D-030	Augmented antitumor effect of unripe *Rubus coreanus* Miquel combined with oxaliplatin in a humanized PD-1/PD-L1 knockin colorectal cancer mouse model	Eun-Ji Lee
PS-D-031	The anti-tumor effects of sotorasib in a patient-derived organoid and xenograft mouse model of KRASG12C pancreatic cancer	Mi Rim Lee
PS-D-032	The regulatory role of nuclear factor erythroid-2-related factor in autoimmune disease animal model	Seon Hyeok Kim
PS-D-033	Preclinical study of NGUL, a novel matched-pair theranostic agent labeled with Cu-64 and Cu-67 targeting prostate cancer	Hye Yeon Seo
PS-D-034	Study on intact retinal vessels and vasculopathies in adult zebrafish eye	Seung-Hyun Jung
PS-D-035	Effects of Glycine max germinated extract and Angelica gigas extract mixture on osteoblast and osteoclast	Sangmin Lee
PS-D-036	Therapeutic effects of LED fusion of two wavelength bands on Atopic dermatitis of NC/Nga mice	Sangmin Lee
PS-D-037	Deciphering transcriptome profiles of whole blood in administration to heroin of cynomolgus monkey	Se-Hee Choe
PS-D-038	CXCR4 regulates the maintenance of stemness and radio-resistance in chordoma cells	Chan Woong Jung
PS-D-039	Endothelial PTP mitigates vascular inflammation via USF1/A20 axis-mediated NF-κB inactivation	Min Ji Cho

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PS-D-040	20 (S)-ginsenoside Rh2 exerts anti-cancer activity through the Axl signaling pathway in colorectal cancer cells	Na-eun Cho
PS-D-041	Monitoring induction of 'cold' to 'hot' tumors by irradiation	Ho Rim Oh
PS-D-042	Hepatic PTPA ameliorates high-fat diet-induced hepatosteatosis and disruption of glucose homeostasis by the activation of the FGF21	Byungtae Hwang
PS-D-043	Ovarian cycle control, collection, *in vitro* maturation and *in vitro* fertilization for oocytes of common marmoset	Heejong Eom, Dohyun Lee
PS-D-044	MTX loaded nanoparticles (MTX-NPs) ameliorate rheumatoid arthritis by simultaneously upregulation of Treg and Breg	SeungCheon Yang
PS-D-045	Modification of atopic dermatitis animal model using MC903: focusing on shortening of induction period and reduction of adverse effects	Soyeon Kim
PS-D-046	MTOR/STAT3 targeting suppresses scleroderma via reciprocal regualtion of TH17 and fibroblast with biguanide compounds	Jeong Won Choi
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PS-D-048	Plasmid DNA nanoparticles for nonviral oral gene therapy	Young Jae Park
PS-D-049	IL-17A promotes *Helicobacter pylori*-induced gastric carcinogenesis via interactions with IL-17RC	Hyo-Jung Kwon
PS-D-050	Loss of Rab25 reduces tumorigenesis in MMTV-PyMT breast cancer model.	Chanyang Uhm
PS-D-051	Discovery of early diagnostic markers of hepatocellular carcinoma based on serum exosomal microRNAs	Jin-Seong Hwang
PS-D-052	The inhibited invasion of human glioblastoma cells by copper sulfate with chelators in zebrafish model	Heabin Kim, Jei Ha Lee
PS-D-053	Overexpression of cathepsin S is associated with toll-like receptor 7 in systemin lupus erythematosus	Hyeonjin Kim
PS-D-054	Generation of FIX-/- rat as a novel pre-clinical model for severe hemophilia B with anti-FIX inhibitors	Hee Sook Bae
PS-D-055	*In vivo* genome editing at the APOC3 locus for long-term correction of hemophilia B	Kyu Jun Lee
PS-D-056	DDX53 confers the self-renewal and drug-resistance of ovarian cancer stem-like cells	Youngmi Kim
PS-D-057	A report on non-clinical study of kidney xenotransplantation from genetically-engineered pigs to monkeys	Ju young Lee
PS-D-058	Prolyl hydroxylation primes CMGC kinases for activation through Tyrosine autophosphorylation	Sang Bae Lee
PS-D-059	Effect of obesity on anaplastic thyroid cancer progression in immunocompetent orthotopic model	Seong Hyeon Kim
PS-D-060	Creation of mouse models and analysis methods for the investigation of brain disorders	Kyungrim Yi
PS-D-061	Behavioral and neuropathological analysis for Alzheimer's disease preclinical research by the 5xFAD mouse model	Chang-Hoon Shin
PS-D-062	Metformin ameliorates monosodium-iodoacetate-induced Osteoarthritis via regulation of mitochondria and the autophagy-inflammation- cell death axis	Keun Hyung Cho

| Facility / Management / Others

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PS-E-002	Novel stent-based electrode for radiofrequency ablation in the rat esophagus: a preliminary study	Dong-Sung Won
PS-E-003	Current operational issues of the Institutional Animal Care and Use Committee in Korea and suggestions for improvement	Sangho Roh
PS-E-004	Transcriptomic insight into the ion channel genes across tissue types and developmental stages	Eun A Ko
PS-E-005	Vitamin D suppresses pain and cartilage destruction in OA animals model via regulation of autophagic flux and cell death	JooYeon Jhun
PS-E-006	Extracts of *Ficus erecta* Thunb. leaves ameliorate cognitive impairment and neuronal loss in an amyloid-β-Induced Alzheimer's disease-like animal model	Eunjin Sohn
PS-E-007	The protective effect of *Tilia amurensis* honey protective effect on infection of influenza A virus infection through activation of interferon signaling	Eun-Bin Kwon
PS-E-008	National primate infrastructure for biomedical and basic science	Sang-Je Park
PS-E-009	Support center for vaccine commercialization and development	Min-Kyung Park
PS-E-010	Assessment tool based non-invasive molecular imaging for pharmacokinetic evaluation of oligonucleotides	Sun Mi Park
PS-E-011	Comparative study of domestic and foreign national residue programs	Gye-Hyeong Woo
PS-E-012	Domestic and abroad investigations on bioresidue testing before shipment	Gye-Hyeong Woo
PS-E-013	Research trends of animal-assisted interventions in agro-healing	Hyun A Lee
PS-E-014	68Ga-Lactosylated albumin agent for the evaluation of liver function using quantitative imaging	Jin Sil Kim
PS-E-015	Comparison of biodistribution according to liposome size for the development of nano-based therapeutics	Ji Yoon Kim
PS-E-016	The application of alternatives to animal testing for skin sensitization evaluation on pesticides	So Hye Hong
PS-E-017	Study on a new toxicological assessment of endocrine disruptors (EDs) pesticides by *in vitro* OECD guideline	Ji Yeon Yang
PS-E-018	Effect of lactic acid bacteria on the pharmacokinetics and metabolism of ginsenosides in mice	Jin-hyang Park
PS-E-019	Pharmacokinetics of polynemoraline C in mice	Ji-Hyeon Jeon
PS-E-020	Development of diabetes pig model; pathological study on alloxan or streptozotocin-induced diabetic pig	Na-Rae Shin
PS-E-021	Analysis of CCR2 splice variant expression patterns and functional properties	Hee-Kyung Park
PS-E-022	Polymeric micelles including osthol-based drug combination for lung cancer therapy	Jae Min Lee
PS-E-023	Increased bioavailability of chrysin by preparing spray dried solid dispersion of chrysin	Jong-Geon LEE
PS-E-024	Cynomolgus macaque model for COVID-19 Delta variant	Seung Ho Baek
PS-E-025	Role of the modified Saengmaeksan(mSMS) in L-NAME-induced hypertension model	Jin Ki Jung
PS-E-026	Analysis of the animal experiment protocols review by IACUC in 2021 in Korea	Soo hee Leem
PS-E-027	Establishment of a NanoBiT-Based Cytosolic Ca^{2+} sensor by optimizing Calmodulin-binding motif and protein expression levels	NGUYEN PHUONG LAN
PS-E-028	Nicotinamide Mononucleotide (NMN) enhances cloned mice embryo quality and improves the number of inner cell mass (ICM) cells	Yoo Bin Choi
PS-E-029	Functional characterization of CXCR3 splicing variants and their ligands	THI HUONG NGUYEN
PS-E-030	Establishment of the Mouse Pathogen Standard Cooperation Center (MPSC) in Korea	Sun-Min Seo
PS-E-031	New types of guided bone regeneration membrane in alveolar bone defect of beagle dog	Yong Sub Byun
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Poster no.	Title	Speaker
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PS-E-034	Luteolin supplementation during oocyte maturation improves developmental competence of cloned embryos by reducing oxidative stress in pigs	Pil-Soo Jeong
PS-E-035	Bisphenol A –induced developmental impairment can be alleviated with IGF-1 treatment in porcine embryos	Min Ju Kim
PS-E-036	Cadmium exposure causes defect in porcine oocyte maturation and subsequent embryonic development by inducing ER stress	Min-Ah Gwon
PS-E-037	Proposal of in *vitro* and *in vivo* studies design to investigate the effect of FLASH radiotherapy	Yuri Lee
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PS-E-039	Suggestions for efficient life-cycle management of GLP standard operating procedures	Hee-Jeong Ahn
PS-E-040	Predicting the abuse liability of new psychoactive compounds	Jeongha Kim
PS-E-041	Highly efficient human hematopoietic stem and progenitor cell generation from induced pluripotent stem cells using a simple scalable monolayer culture system	Myoung Hee Han
PS-E-042	Therapeutic efficacy of graphene quantum dots for alleviation of acute graft-versus-host disease in a xenogeneic mouse model	Aaron Yu
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PS-E-046	Performance study of anticoagulant (ASTM F1830) for establishment of *in vitro* blood circulation loop system	Jeong Hwa Kim
PS-E-047	Non-human primate diagnostic testing services of K-MEDI hub preclinical research center	Minji Seong
PS-E-048	Characterization of a rat plantar heel pain model using fascial distortion and efficacy test	Na-Hye Park
PS-E-049	Clinical evaluation of rapid diagnostic test kit for canine parvovirus and coronavirus	Chaeyeong Min
PS-E-050	Evaluation of biocompatibility for electrospinning PLLA-based absorbable dural substitute in rat brain dura mater defect model	Minjoo Cho
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PS-E-052	HepaRG cell line as an *in vitro* model for measurement of CYP450 enzyme activity by metabolic induction	Byung-suk Jeon
PS-E-053	Combined regenerative effect of human umbilical cord blood-derived mesenchymal stem cells, polydeoxyribonucleotides, and microcurrent therapy on chronic rotator cuff tear in a rabbit model	Dong Rak Kwon

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PS-A-001

Ultrasound mediated miR146a-5p nanoparticles protect photothrombotic cerebral infarction

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Stroke is one of the most common causes of death and the treatment of ischemic stroke is difficult due to the blood-brain barrier (BBB). Recently, focused ultrasound (FUS) and microbubble (MB) are non-invasive method and have been used to improve BBB opening and help drug localization in brain. As microRNA 146a-5p is a master post-transcriptional regulator of immune and inflammatory response involved genes, in this study, I try to reduce infarct damage through FUS and MB combined delivery of microRNA 146a-5p PLGA nanoparticles (miR146a-5p NPs) in a photothrombotic ischemic stroke (PTS) mice model. First, it was confirmed whether the BBB transmittance and the expression of nanoparticles were increased using Evans blue (EBs) and GFP vector-encapsulated PLGA nanoparticles (GFP NPs) after FUS and MB. It was successfully stained with EBs in the cerebral cortex. GFP expression showed that Cisterna magna (intrathecal.IT) NPs injection induced mainly microglia compared to astrocytes and neurons, and intravenous (IV) injection after sonication increased the expression of NPs than IV injection. Second, I synthesized miR146a-5p NPs and characterized with size, zeta potential and SEM images. Third, I revealed miR146a-5p NPs significantly decreased infarct volume of cerebral cortex in PTS mice. Moreover, application of miR146a-5p NPs prevent the activation of NF-κB/p38 MAPK pathways and pro-inflammatory cytokines. In the evaluation of motor dysfunction, the IT injection group and the FUS group were significantly relieved than the IV injection group. The infarct volume was significantly decreased in the IT injection group and the FUS group compared to the IV injection group, and the FUS group decreased more than the IT injection group. Taken together, FUS-mediated BBB opening resulted in effective drug localization of miR146a-5p NPs, suggesting that microglia-targeted miR146-5p NPs have neuroprotective effects and therapeutic value in the treatment of ischemic stroke.

Corresponding author : Dong Woon Kim

Keywords : Ultrasound, Nanoparticle, Photothrombosis

PS-A-002

Residues F130A and N138A of 5-HT3A interact ergot alkaloid chanoclavine

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Irritable bowel syndrome (IBS) is a chronic disease that causes abdominal pain and an imbalance of defecation patterns due to gastrointestinal dysfunction. The cause of IBS remains unclear, but intestinal-brain axis problems and neurotransmitters have been suggested as factors. In this study, chanoclavine, which has a ring structure similar to 5-hydroxytryptamine (5-HT), showed an interaction with the 5-HT3A receptor to regulate IBS. Although its derivatives are known to be involved in neurotransmitter receptors, the molecular physiological mechanism of the interac-tion between chanoclavine and the 5-HT3A receptor is unknown. Electrophysiological experi-ments were conducted using two-electrode voltage-clamp analysis to observe the inhibitory ef-fects of chanoclavine on Xenopus oocytes in which the h5-HT3A receptor was expressed. The co-application of chanoclavine and 5-HT resulted in concentration-dependent, reversible, volt-age-independent, and competitive inhibition. The 5-HT3A response induced by 5-HT was blocked by chanoclavine with IC50 values of 107.2 μM. Docking studies suggested that chanoclavine was positioned close F130 and N138 in 5-HT3A receptor binding site. The double mutation of F130A and N138A significantly attenuated interaction of chanoclavine compared to a single mutation or the wild-type. These data suggest that F130 and N138 are important sites for ligand binding and activity. Chanoclavine and ergonovine have different effects. Asparagine, the 130th amino acid sequence of the 5-HT3A receptor, and phenylalanine, the 138th, is an important role in the binding of chanoclavine but ergonovine has no interaction with any amino acid sequence of the 5-HT3A receptor. The results of electrophysiological studies and of in silico simulation showed that chanoclavine has the potential to inhibit the hypergastric stimulation of the gut by inhibit-ing the stimulation of signal transduction through 5-HT3A receptor stimulation. These findings suggest chanoclavine as a potential anti-emetic agent for excessive gut stimulation and offer in-sight into the mechanisms of 5-HT3A receptor inhibition.

Corresponding author : Junho Lee

Keywords : Ergot alkaloids, Serotonin receptor, Irritable bowel syndrome

PS-A-003

Naringin as a novel analgesic candidate through antioxidative and analgesic effects

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Transient receptor potential vanilloid member 1 (TRPV1) is activated in response to capsaicin, protons, temperature, and free reactive oxygen species (ROS) released from inflammatory mol-ecules after exposure to harmful stimuli. The expression level of TRPV1 is elevated in the dorsal root ganglion, and its activation through capsaicin and ROS mediates neuropathic pain in mice. Its expression is high in peripheral and central nervous systems. Although pain is a response evolved for survival, many studies have been conducted to develop analgesics, but no clear re-sults have been reported. Here, we found that naringin selectively inhibited capsai-cin-stimulated inward currents in Xenopus oocytes using two-electrode voltage-clam. The results of this study showed that naringin has IC50 value of 33.3 μM on TRPV1. The amino acid residues D471 and N628 of TRPV1 were involved in its binding to naringin. Our study bridged the gap between the pain suppression effect of TRPV1 and the preventive effect of naringin on neuro-pathic pain and oxidation. Naringin had the same characteristics as a model selective antagonist, which is claimed to be ideal for development of analgesics targeting TRPV1. The expression level of TRPV1 is elevated in the dorsal root ganglion, and its activation through capsaicin and ROS mediates neuropathic pain in mice. Thus, this study suggests the applicability of naringin as a novel analgesic candidate through antioxidative and analgesic effects of naringin.

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Keywords : Antioxidant, Transient receptor potential vanilloid member 1

PS-A-004

Nicotinamide riboside improves fetal growth under hypoglycemic condition

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Nicotinamide riboside (NR) is considered a super-supplement that prevents obesity and diabetes. While NR has been investigated for various effects depending on nutritional conditions, metabolic research on women and pregnant women has rarely been discussed. In this study, we focused on the glycemic control of NR in females and found the protective role of NR in pregnant animals under hypoglycemic conditions. Metabolic-tolerance tests were performed in vivo under progesterone (P4) exposure after ovariectomy (OVX). NR enhanced resistance to energy deprivation, such as starvation, and showed a slight increase in gluconeogenesis in naïve control mice. However, NR reduced hyperglycemia and significantly induced gluconeogenesis in OVX mice. While NR reduced hyperglycemia in the P4-treated OVX mice, it reduced insulin response and substantially increased gluconeogenesis. Similar to animal experiments, NR increased gluconeogenesis and mitochondrial respiration in Hep3B cells. The gluconeogenic function of NR is mediated by tricarboxylic acid cycle (TCA) cycle enrichment, as residual pyruvate could induce gluconeogenesis. NR recovered fetal growth by increasing blood glucose levels when artificial hypoglycemia was induced during pregnancy. Our study revealed the glucose-metabolic function of NR in hypoglycemic pregnant animals, suggesting NR as a dietary supplement to improve fetal growth. Because diabetic women suffer from hypoglycemia due to insulin therapy, NR has therapeutic potential for use as a glycemic control pill.

Corresponding author : Eui-Ju Hong

Keywords : Nicotinamide riboside, Hypoglycemia, Energy metabolism

PS-A-005

Sex hormone-binding globulin inhibits the entry of zoonotic coronavirus via AXL

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Although vaccines and drugs have been developed to prevent and treat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, the host factors involved in the zoonotic transmission of unknown coronaviruses require further attention. To enter host cells, SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) and tyrosine-protein kinase receptor UFO (AXL), one of the tumor-associated macrophage (TAM) receptors. As sex hormone-binding globulin (SHBG) inhibited TAM receptors in a recent study, we investigated whether SHBG restricts coronavirus entry through AXL inhibition. In transgenic (*SHBG*-Tg) mice, SHBG protein was present in the lavage fluid of the respiratory system and reduced pulmonary AXL level. SHBG nebulization could decrease pulmonary AXL levels. *In vitro*, SHBG incubation in DBT cells decreased AXL protein levels and restricted mouse hepatitis virus (MHV) propagation and entry. The virus' suppressive effect of SHBG depended on AXL as it was not observed in AXL inhibitor (bemcentinib)-treated cells. Intranasal exposure of SHBG also reduced pulmonary AXL protein and interfered with pulmonary MHV entry after *ex vivo* viral inoculation. SHBG significantly reduced pseudotyped SARS-CoV-2 entry in (AXL dominant) A549, but not in Vero cells. In conclusion, our study reports SHBG as a novel host anti-viral factor that reduces coronavirus entry through AXL inhibition, suggesting SHBG as a human therapeutic protein against the diverse family of coronaviruses.

Corresponding author : Eui-Ju Hong

Keywords : SHBG, Coronavirus, AXL

PS-A-006

CHIP ameliorates neuronal damage in H_2O_2-induced oxidative stress in HT22 cells and gerbil ischemia

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Carboxyl terminus of Hsc70-interacting protein (CHIP) is a highly conserved protein, and it is linked to the connection between molecular chaperones and proteasomes to degrade the chaperone-bound proteins with its structure. In the present study, we synthesized the transactivator of transcription (Tat)-CHIP fusion protein to be effectively delivered into the brain and examined the effects of CHIP protein against oxidative stress in HT22 cells induced by hydrogen peroxide (H_2O_2) treatment and against ischemic damage in gerbils by 5 min of occlusion of both common carotid arteries to elucidate the possibility of using Tat-CHIP as a therapeutic agent against ischemic damage. Tat-CHIP was effectively delivered to HT22 hippocampal cells in a concentration- and time-dependent manner, and protein degradation was confirmed in HT22 cells. In addition, Tat-CHIP significantly ameliorated the oxidative damage induced by 200 μM H_2O_2 and decreased DNA fragmentation and reactive oxygen species formation. In addition, Tat-CHIP showed neuroprotective effects against ischemic damage in a dose-dependent manner and significant ameliorative effects against ischemia-induced glial activation, oxidative stress (hydroperoxide and malondialdehyde), and pro-inflammatory cytokine (interleukin-1β, interleukin-6, and tumor necrosis factor-α) release in the hippocampus. These results suggest that Tat-CHIP could be a therapeutic agent that can protect neurons from ischemic damage.

Corresponding author : Dae Won Kim, In Koo Hwang

Keywords : Carboxyl-terminus of Hsc70-interacting protein, Oxidative stress, Ischemic insult, HT22 cells, Gerbils

PS-A-007

Differential effects of Cu,Zn superoxide dismutase on cuprizone-induced demyelination and reduction of adult neurogenesis in mice

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Cuprizone causes consistent demyelination and oligodendrocyte damage in the mouse brain. Cu,Zn-superoxide dismutase 1 (SOD1) has neuroprotective potential against various neurological disorders, such as transient cerebral ischemia and traumatic brain injury. In this study, we investigated whether SOD1 has neuroprotective effects against cuprizone-induced demyelination and adult hippocampal neurogenesis in C57BL/6 mice, using the PEP-1-SOD1 fusion protein to facilitate the delivery of SOD1 protein into hippocampal neurons. Eight weeks feeding of cuprizonesupplemented (0.2%) diets caused a significant decrease in myelin basic protein (MBP) expression in the stratum lacunosum-moleculare of the CA1 region, the polymorphic layer of the dentate gyrus, and the corpus callosum, while ionized calcium-binding adapter molecule 1 (Iba-1)-immunoreactive microglia showed activated and phagocytic phenotypes. In addition, cuprizone treatment reduced proliferating cells and neuroblasts as shown using Ki67 and doublecortin immunostaining. Treatment with PEP-1-SOD1 to normal mice did not show any significant changes in MBP expression and Iba-1-immunoreactive microglia. However, Ki67-positive proliferating cells and doublecortinimmunoreactive neuroblasts were significantly decreased. Simultaneous treatment with PEP-1-SOD1 and cuprizonesupplemented diets did not ameliorate the MBP reduction in these regions, but mitigated the increase of Iba-1 immunoreactivity in the corpus callosum and alleviated the reduction of MBP in corpus callosum and proliferating cells, not neuroblasts, in the dentate gyrus. In conclusion, PEP-1-SOD1 treatment only has partial effects to reduce cuprizone-induced demyelination and microglial activation in the hippocampus and corpus callosum and has minimal effects on proliferating cells in the dentate gyrus.

Corresponding author : Yeo Sung Yoon

Keywords : Cuprizone, SOD1, C57BL/6 mouse, Demyelination, Neurogenesis

PS-A-008

Phlorotannin can improve the intestinal epithelial barrier during laxative effects in loperamide-induced constipation of SD rats

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To involve the improvement of IEB during the laxative effects of phlorotannin (Pt) derived from Ecklonia cava in the chronic constipation, alterations on the expression of regulatory proteins for tight junction (TJ) and adherens junction (AJ), and inflammatory cytokines were measured in the loperamide (Lop)-induced constipation SD rats after Pt treatment. Pt treatment induce laxative effects including recovery of stool parameters and gastrointestinal transit and histological structure of colon in Lop-induced constipation rats. Also, a significant recovery was detected on the histological structure of IEB including mucus layer, epithelial cells and lamina propria in the mid colon of Lop+Pt treated rats. Also, the expression levels of E-cadherin, p120-catenin and b-catenin for AJ as well as occuludin, claudin and ZO proteins for TJ in epithelial cells were remarkably improved after Pt treatment although the rate of increase is different. Furthermore, Pt treatment induce the increase on the expression level of several inflammatory cytokines such as TNFa, IL-4, IL-13, IFN-r, IL-1b, IL-19 ad IL-16 in the mid colon of Lop+Pt treated rats. Therefore, these results provide the first evidence that the improvement of IEB can involve in the laxative effects of Pt in Lop-induced constipation SD rats.

Corresponding author : Dae Youn Hwang

Keywords : Intestinal epithelial barrier, Constipation, Phlorotannins, Tight junction, Adherens junction

PS-A-009

An optimized herbal medicine containing *Scutellaria baicalensis, Alisma canaliculatum,* and *Atractylodes macrocephala* Koidz has a potent antiplatelet and antithrombotic activity

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Platelet-derived thrombosis plays an important role in the pathogenesis of cardiovascular diseases. HTB is an optimized herbal medicine containing *Scutellaria baicalensis, Alisma canaliculatum, and Atractylodes macrocephala* Koidz. It is widely used in traditional medicine due to its anti-inflammatory and antioxidant effects. However, its antiplatelet and antithrombotic activities have not been completely validated. The current study aimed to examine the inhibitory effect of the novel herb formula HTB against platelet activation and thrombus formation. The antiplatelet activities of HTB via platelet aggregation, granule secretion, reactive oxygen species generation, and intracellular calcium mobilization were evaluated. Moreover, the antithrombotic effect of HTB via $FeCl_3$-induced arterial thrombus formation *in vivo* in mice was assessed. The inhibitory effect of HTB against primary hemostasis was investigated based on transection tail bleeding time. Results showed that HTB treatment significantly inhibited glycoprotein VI-mediated platelet aggregation, granule secretion, reactive oxygen species generation, and intracellular calcium mobilization. Biochemical studies revealed that HTB inhibited glycoprotein VI-mediated platelet signal transduction during cell activation. Further, its antioxidant effect might be derived by reducing the phosphorylation of the $p47^{phox}$/Hic5 axis signalosome. Oral HTB treatment was effective in decreasing $FeCl_3$-induced arterial thrombus formation without prolonging the tail bleeding time. Therefore, HTB can be an effective therapeutic agent against thrombotic diseases.

Corresponding author : Kyungho Kim

Keywords : Platelet, Thrombosis, *Scutellaria baicalensis*, *Alisma canaliculatum*, *Atractylodes macrocephala* Koidz

PS-A-010

An optimized herbal medicine HTB attenuates hyperlipidemia by activating the AMPK/SREBP2/PCSK9 signaling pathway

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The prevalence of hyperlipidemia is rising globally, and traditional herbal medicine is gaining the spotlight as a treatment for dyslipidemia. An optimized herbal medicine HTB containing *Scutellaria baicalensis, Alisma canaliculatum,* and *Atractylodes macrocephala* Koidz has been frequently utilized in traditional medicine due to its anti-inflammatory and antioxidant properties. However, its antihyperlipidemic activity has not been completely validated. The current study aimed to examine the inhibitory effect of the novel herb formula HTB against hyperlipidemia. Mice were given either poloxamer 407 (P407) intraperitoneally or a high-fat diet to induce acute or chronic hyperlipidemia. Compared to the P407-control group, HTB treatment considerably reduced high plasma and hepatic triglyceride (TC) and cholesterol (CHOL) levels. Studies using a high-fat diet showed that HTB treatment significantly reduced weight gain, plasma TC and LDL-CHOL, hepatic lipid contents, tissue damage marker, and inflammatory cytokines, but improved glucose tolerance, muscle weight, and physical activity. Biochemical studies revealed that HTB down-regulated the expression of fatty acid synthase, SREBP2, and HMGCR while simultaneously up-regulated the expression of PGC1α and ABCA1. Additionally, we found that HTB treatment promoted hepatic AMPK activity, then blocked PCSK9 activity, which regulates LDL receptor degradation. According to proteomic analysis, HTB treatment enhanced protein profiles involved in mitochondria L-carnitine and fatty acid oxidation. Taken together, we demonstrated that HTB ameliorates hyperlipidemia in P407- or/and high fat-induced obese mice via AMPK/SREBP2/PCSK9 signaling pathway. Hence, HTB could be used as a new lipid-lowering drug for the prevention and treatment of hyperlipidemia-associated disorders.

Corresponding author : Kyungho Kim

Keywords : Hyperlipidemia, Herbal medicine, AMPK, Cholesterol, Triglyceride

PS-A-011

AMP-activated protein kinase activation in skeletal muscle modulates exercise-induced uncoupled protein 1 expression in brown adipocyte in mouse model

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Aerobic exercise is an effective intervention in preventing obesity and is also an important factor associated with thermogenesis. There is an increasing interest in the factors and mechanisms induced by aerobic exercise that can influence the metabolism and thermogenic activity in an individual. Recent studies suggest that exercise induced circulating factors (known as 'exerkines'), which are able to modulate activation of brown adipose tissue (BAT) and browning of white adipose tissue. However, the underlying molecular mechanisms associated with the effect of exercise-induced peripheral factors on BAT activation remain poorly understood. Furthermore, the role of exercise training in BAT activation is still debatable. Hence, the purpose of our study is to assess whether exercise training affects the expression of uncoupled protein 1 (UCP1) in brown adipocytes via release of different blood factors. Four weeks of exercise training significantly decreased the body weight gain and fat mass gain. Furthermore, trained mice exhibit higher levels of energy expenditure and UCP1 expression than untrained mice. Surprisingly, treatment with serum from exercise-trained mice increased the expression of UCP1 in differentiated brown adipocytes. To gain a better understanding of these mechanisms, we analysed the conditioned media obtained after treating the C2C12 myotubes with an AMP-activated protein kinase (AMPK) activator (AICAR; 5-aminoimidazole-4-carboxamide ribonucleotide), which leads to an increased expression of UCP1 when added to brown adipocytes. Our observations suggest the possibility of aerobic exercise-induced BAT activation via activation of AMPK in skeletal muscles.

Corresponding author : Je Kyung Seong

Keywords : Exercise, UCP1, AMPK, BAT activation, Exerkine

PS-A-012

Microbiota mediates aerobic exercise capacity via modulation of skeletal muscle glucose metabolism in mice

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Microbiota is an important enhancer of exercise performance and is a regulator of host physiology and energy metabolism through beneficial metabolite production by bacterial fermentation. In this study, we discovered that germ-free (GF) mice had a reduced capability for aerobic exercise as well as low oxygen consumption rates and glucose availability. Surprisingly, GF mice showed lower body weight gain and lower fat mass than specific pathogen-free (SPF) mice. Therefore, we hypothesized that these paradoxical phenotypes could be mediated by a compensatory increase in lipolysis in adipose tissues owing to impaired glucose utilization in the skeletal muscle. Our data revealed that gut microbiota depletion impairs host aerobic exercise capacity via the deterioration of glucose storage and utilization. These findings indicated that GF mice used fat as an alternative means for energy production. Additionally, this adaptation of alternative fuel utilization controls obesity in GF mice and results in impaired aerobic exercise capacity.

Corresponding author : Je Kyung Seong

Keywords : Germ-free, Microbiota, Exercise, Glucose metabolism, Skeletal muscle

PS-A-013

Effects of parabens on inflammasome

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Parabens are synthetic chemicals widely used as preservatives in cosmetics, pharmaceuticals, and foods. Although parabens, i.e., ethyl- and methyl-parabens, are considered relatively safe, study of possible health hazards has been undertaken due to the frequent exposure to parabens and their accumulation in the body. In this study, we elucidated the effect of parabens on inflammasome induction of inflammatory responses in innate immunity, such as interleukin (IL)-1β maturation and gasdermin D (GSDMD)-mediating pyroptosis. Parabens attenuated the inflammatory responses to intracellular lipopolysaccharide (LPS) triggering of non-canonical (NC) inflammasome activation, but did not alter canonical inflammasome (i.e., NLRP3, NLRC4 and AIM2) responses. The NC inflammasome is assembled by the interaction of murine caspase (Casp)-11 (Casp4/5 in human) with cytosolic LPS, inducing endotoxin sepsis. Parabens selectively inhibited NC inflammasome activation in both human and murine macrophages and diminished the peritoneal IL-1β production in LPS-injected mice. Parabens blocked the cleavage of GSDMD, Casp1, and Casp4, but did not change the expression of Casp11 or the activity of Casp1. Taken together, the results indicate that parabens could disrupt Gram-negative pathogen infection through the inhibition of NC inflammasome activation.

Corresponding author : Geun-Shik Lee

Keywords : Parabens, Inflammasome, Caspase-11, Interleukin-1beta, Macrophages

PS-A-014

The Neuroprotective effects of exosomes derived from TSG101-overexpressing human neural stem cells in a stroke model

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Although tissue-type plasminogen activator was approved by the FDA for early reperfusion of occluded vessels, there is a need for an effective neuroprotective drug for stroke patients. In this study, we established tumor susceptibility gene (TSG)101-overexpressing human neural stem cells (F3.TSG) and investigated whether they show enhanced secretion of exosomes and whether treatment with exosomes during reperfusion alleviates ischemia-reperfusion-mediated brain damage. F3.TSG cells secreted higher amounts of exosomes than the parental F3 cells. In N2A cells subjected to oxygen-glucose deprivation (OGD), treatment with exosomes or co-culture with F3.TSG cells significantly attenuated lactate dehydrogenase release, the mRNA expression of pro-inflammatory factors, and the protein expression of DNA damage-related proteins. In a middle cerebral artery occlusion (MCAO) rat model, treatment with exosomes, F3 cells, or F3.TSG cells after 2 h of occlusion followed by reperfusion reduced the infarction volume and suppressed inflammatory cytokines, DNA damage-related proteins, and glial fibrillary acidic protein, and upregulated several neurotrophic factors. Thus, TSG101-overexpressing neural stem cells showed enhanced exosome secretion; exosome treatment protected against MCAO-induced brain damage via anti-inflammatory activities, DNA damage pathway inhibition, and growth/trophic factor induction. Therefore, exosomes and F3.TSG cells can affect neuroprotection and functional recovery in acute stroke patients.

Corresponding author : Dongsun Park

Keywords : TSG101, Neural stem cells, MCAO, Neuroprotection, Exosomes

PS-A-015

Laxative effects of the methanol extract of green pine cones in loperamide-induced constipation of SD rats

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Pinus densiflora is widely consumed as a health supplement or food for health promotion and has recently been used as a cosmetic fuel because it contains numerous proanthocyanidins, which are a major class of polyphenols with potent antioxidant activity. To investigate the role of the methanol extracts of green pine cone (MPC, unripe fruits of *Pinus densiflora*) on the regulation of laxative activity in chronic constipation, we investigated alteration on the excretion parameters, gastrointestinal transit ratio, histological structure and mucin secretion were examined in the loperamide (Lop)-induced constipation of SD rats after treatment of MPC's three different concentrations (125, 250, and 500 mg/kg body weight). This extract contained several bioactive compounds including diterpenoid compounds such as dehydroabietic acid, taxodone and ferruginol. These bioactive compounds exhibited high inhibitory activity against DPPH and ABTS radicals. MPC treatment induced significant improvements in stools number and weight when compared to Lop+Vehicle treated group. Also, the gastrointestinal transit and intestine length were remarkably recovered in Lop+MPC treated group, while a similar recovery pattern was observed in the histopathological structure of mid colon of same group. Furthermore, the mucin secretion level and the expression level of Muc and aquaporin (AQP) mRNA were increased after MPC treatment. Taken together, these results provide the first evidence that MPC may important role on its laxative effects in chronic constipation models.

Corresponding author : Dae Youn Hwang

Keywords : Constipation, Pine cone, *Pinus densiflora*, Laxative effects, Loperamide

PS-A-016

Atractylodes macrocephala Koidz induces apoptosis in human gastric cancer cells through activation of the ROS and MAPK signaling pathway

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Atractylodes macrocephala Koidz (AMK) is a traditional medicine used to treat various diseases, including gastrointestinal diseases, cancer, osteoporosis, Alzheimer's disease, and others. However, the anti-gastric cancer and apoptotic effects of the ethanol extract of the AMK remain unknown. DNA content analysis indicated that AMK increased the sub G1 population of AGS cells. In addition, levels of proapoptotic cascade components, including caspase-3, caspase-9 and poly ADP ribose polymerase, were augmented by AMK treatment. Mitochondrial membrane potential was reduced, and the ratio of Bcl-2 associated X protein (Bax)/ B cell lymphoma- 2 (Bcl-2) were increased, also intracellular reactive oxygen species (ROS) production was also increased by AMK treatment. The AMK induced cytotoxic effects and ROS production could be attenuated by N-acetyl-cysteine (NAC), an ROS scavenger. Taken together, these results indicate that AMK is a potent apoptotic herbal medicine, which exerts its effects via the ROS mediated mitochondrial pathway. These findings suggest that AMK induced apoptosis in AGS cells and thus might serve as a novel anticancer agent to promote apoptosis of gastric cancer cells.

Corresponding author : Byung Joo Kim

Keywords : *Atractylodes macrocephala Koidz*, Gastric cancer, Apoptosis, Proliferation, AGS

PS-A-017

Improvement of acute kidney injury by mesenchymal stem cell treated with dopamine D1 receptor agonist

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Cell-based therapeutics are an unique alternative for renal diseases including acute kidney injury (AKI). Among various sources, mesenchymal stem cells (MSCs) are accepted as safe and ideal option. However, its use in vivo is limited due to low survival. We investigated whether fenoldopam mesylate (FD), a selective dopamine D1 receptor agonist FD, can stimulate the therapeutic efficacy of MSCs in a mice model of cisplatin-induced AKI. We found that both renal function markers and acute tubular necrosis was improved by FD-treated MSCs, in comparison with non-treated MSCs. In addition, other parameters including tubular apoptosis/injury, macrophage infiltration, and serum concentration of tumor necrosis factor-a were markedly diminished than those administrated with non-treated MSCs. In conclusion, we suggest that the function of MSCs can be enhanced by FD and this strategy can be used in preparing robust therapeutic MSCs. This work was also supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2021R1A2C2093867).

Corresponding author : Tae Min Kim

Keywords : Mesenchymal stem cells, Dopamine D1 receptor, Acute kidney injury

PS-A-018

Mixture of Corni Fructus and Schisandrae Fructus ameliorates testosterone-induced benign prostatic hyperplasia through regulating 5α-reductase 2 and androgen receptor

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Benign prostatic hyperplasia (BPH) characterized by an enlarged prostate gland is common in elderly men. Corni Fructus (CF) and Schisandrae Fructus are known to have various pharmacological effects, including antioxidant and anti-inflammatory activities. In this study, we evaluated the inhibitory efficacy of CF, SF, and their mixture (MIX) on the development of BPH using an *in vivo* model of testosterone-induced BPH. Six-week-old male Sprague-Dawley rats were randomly divided into seven groups. To induce BPH, testosterone propionate (TP) was injected to rats except for those in the control group. Finasteride (Fina), saw palmetto (SP), CF, SF, and MIX were orally administered along with TP injection. At the end of treatment, histological changes in the prostate and the level of various biomarkers related to BPH were evaluated. Our results showed that BPH induced by TP led to prostate weight and histological changes. Treatment with MIX effectively improved TP-induced BPH by reducing prostate index, lumen area, epithelial thickness, and expression of BPH biomarkers such as 5α-reductase type 2, prostate-specific antigen, androgen receptor, and proliferating cell nuclear antigen compared to treatment with CF or SF alone. Moreover, MIX further reduced levels of elevated serum testosterone, dihydrotestosterone, and prostate-specific antigen in BPH compared to the SP, a positive control. BPH was also improved more by MIX than by CF or SF alone. Based on the results, MIX is a potential natural therapeutic candidate for BPH by regulating 5α-reductase and AR signaling pathway.

Corresponding author : Yung Hyun Choi

Keywords : 5α-reductase 2, Androgen receptor, Benign prostatic hyperplasia, Corni Fructus, Schisandrae Fructus

PS-A-019

Improvement of particulate matter 2.5-induced keratoconjunctivitis sicca accompanying retinal and lipid metabolism disorders by Schisandrae Fructus

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Particulate matter 2.5 ($PM_{2.5}$) is the biological toxic substances in the air pollutants, and causes to keratoconjunctivitis sicca (KCS). To date, studies on the development of treatment for $PM_{2.5}$-mediated KCS are extremely limited. Schisandrae Fructus (SF) has been used as traditionally herbal medicine in northeastern Asia. SF has various pharmacological effects, but its effect on the ocular system have not been well defined. Therefore, in this study, we evaluated the effect of oral administration of SF ethanol extract (SFE) in the eye, hematology and biochemistry in KCS rats induced by topical exposure to $PM_{2.5}$. During the same period, Sprague-Dawley rats were administered topically in both eyes with $PM2.5_{2.5}$ four times daily for 14 days. During the same period, SFE and lutein were orally administered once a day. On the 0, 7, and 14th day, tear production was measured. On the 14th day, hematological, biochemical and histological changes were analyzed. In addition, the histological change in the eye including cornea, conjunctiva, lacrimal gland, and retina was analyzed. SFE ameliorated the $PM_{2.5}$ exposure-induced reduction of tear secretion and corneal epithelial damage, goblet cell loss in conjunctiva and overexpression of inflammatory factors in lacrimal gland. In addition, SFE markedly improved $PM_{2.5}$-mediated ganglion cell loss and recovered the thickness of inner plexiform layer. Furthermore, the levels of serum total cholesterol and low-density lipoprotein cholesterol were markedly up-regulated by $PM_{2.5}$, but this change was down-regulated by SFE administration. In summary, oral administration may have protective effects against $PM_{2.5}$-induced KCS via stabilization of the tear film and suppression of inflammation, and may in part contribute to improving retinal disorder and dyslipidemia.

Corresponding author : Yung Hyun Choi

Keywords : Particulate matter, Dry eye syndromes, Cornea, Retina, Inflammation

PS-A-020

Inhibition of monosodium urate-induced NLRP3 inflammasome activation through NOX3/4-dependent mitochondrial oxidative stress in RAW 264.7 and bone marrow-derived macrophages by diallyl trisulfide

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Monosodium urate (MSU) crystals or uric acid are associated with inflammatory disorders such as osteoarthritis and atherosclerosis. MSU-associated inflammatory diseases are triggered by various factors including NOD-like receptor protein 3 (NLRP3) inflammasome that can promote the secretion of proinflammatory cytokine interleukin (IL)-1β. Diallyl trisulfide (DATS), an organic polysulfide component of garlic, is well-known to possess effective anti-inflammatory effects. However, the underlying molecular mechanism of its anti-inflammatory effect has not been completely understood yet. The objective of the current study was to investigate anti-inflammasome effects of DATS and its action mechanisms in MSU-stimulated RAW 264.7 and bone marrow-derived macrophages. The effect of DATS on inflammasome in MSU-stimulated macrophage was measured. The concentrations of IL-1β were analyzed with enzyme-linked immunosorbent assay. The MSU-induced mitochondrial damage and reactive oxygen species (ROS) production were detected by fluorescence microscope and flow cytometry. The protein expressions of NLRP3 signaling molecules, NADPH oxidase (NOX) 3/4 were assessed with Western blotting. DATS suppressed MSU-induced release of IL-1β and caspase-1 accompanied by decreased inflammasome protein complex formation comprised of NLRP3, ASC, and caspase-1 in RAW 264.7 and bone marrow-derived macrophages. In addition, MSU treatment decreased mitochondrial membrane potential and increased mitochondrial ROS generation. These mitochondria damaging effects were significantly alleviated by DATS. Microarray analysis revealed that MSU stimulation increased genes expression levels of NOX3/4. However, these upregulated NOX 3/4 were downregulated by DATS. NOX3/4 gene expression changes were confirmed by Western blotting, suggesting that DATS could protect cells from NOX3/4-dependent mitochondrial ROS generation. The current study provides the first mechanistic finding that DATS can alleviate NLRP3 inflammasome by mediating NOX3/4-dependent mitochondrial ROS production in macrophages. This suggests that DATS is a potential effective therapeutic candidate for inflammatory disease caused by NLRP3 inflammasome activation.

Corresponding author : Yung Hyun Choi

Keywords : Diallyl trisulfide, Monosodium urate, NLRP3, Mitochondria, Reactive oxygen species

PS-A-021

Construction and performance evaluation of 3D bio-printed small-caliber artificial blood vessel graft

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Autologous graft has been the only option which has been clinically approved for small-caliber revascularizations. However, autologous grafts have some limitations in quantity and quality, and also have the possibility to cause an invasiveness to patients when harvested. Therefore, development of artificial small-caliber vessels (5<mm) which are maintaining long-term patency without causing restenosis and intimal hyperplasia has been urged. Here, we report on the long-term (until 24 weeks) experimental assessment of our 3D bio-printed biodegradable artificial vessels (diameter : 3.5mm, length : 20mm) fabricated with mixture of polycaprolactone (PCL) and atelocollagen for arterial replacement in canine. Canine underwent both carotid artery replacement with PCL/collagen prosthesis and polytetrafluoroethylene (ePTFE) as control. Ultrasonography, tissue pathology, angiography, and optical coherence tomography (OCT) were applied to evaluate stenosis, patency, inflammatory reaction and biocompatibility. Long term patency was observed without any stenosis at the implanted area. Mild fibrosis and inflammatory reaction was observed, but not critical. Results of the analysis suggest that transplanted vessels were opened without leakage, and the stable performance was maintained without significant error in each part of the tests.

Corresponding author : Sanghyun An, Kyoung Ho Lee

Keywords : 3D bio-printing, Artificial blood vessel, Ultrasonography, Angiography, OCT

PS-A-022

Retinoic acid alleviates the reduction of the ubiquitin-proteasome system due to ischemic brain injury

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Retinoic acid is a major metabolite of vitamin A and exerts beneficial effects including anti-oxidant and anti-inflammatory activities in neurons. The ubiquitin-proteasome system is an important biological system that regulates cell survival. Ubiquitination regulates protein degradation and plays an important role in oxidative stress. Deubiquitinating enzymes cleave ubiquitin from proteins and control ubiquitination-induced degradation. We detected decreases in ubiquitin carboxy-terminal hydrolase L1, ubiquitin thioesterase OTUB1, and proteasome subunit alpha types 1 and 3 in cerebral ischemic damage. In this study, we investigated whether retinoic acid regulates the expression of deubiquitinating enzymes ubiquitin carboxy-terminal hydrolase L1, ubiquitin thioesterase OTUB1, and proteasome subunit alpha types 1 and 3 in cerebral ischemic injury. Right middle cerebral artery occlusion (MCAO) was performed to induce cerebral ischemic damage in male rats. Retinoic acid (5 mg/kg) or vehicle was intraperitoneally injected every day from 4 days before surgery. Neurological behavioral tests were performed 24 h after MCAO, and right cerebral cortical tissues were collected. MCAO damage caused neurological behavioral dysfunction, and retinoic acid alleviated these deficits. The identified proteins decreased in MCAO animals with vehicle, while retinoic acid treatment attenuated these decreases. The results of proteomic study were confirmed by a reverse transcription-PCR technique. Expressions of ubiquitin carboxy-terminal hydrolase L1, ubiquitin thioesterase OTUB1, and proteasome subunit alpha types 1 and 3 were decreased in MCAO animals treated with vehicle. Retinoic acid treatment alleviated these MCAO-induced reductions. The ubiquitin-proteasome system plays an essential role in maintaining cell function and preserving cell shape against ischemic damage. These findings suggest that retinoic acid regulates ubiquitin- and proteasome-related proteins including ubiquitin carboxy-terminal hydrolase L1, ubiquitin thioesterase OTUB1, and proteasome subunit alpha types 1 and 3 in a brain ischemia model. Changes in these proteins are involved in the neuroprotective effects of retinoic acid. This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (NRF-2021R1F1A105878711).

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Keywords : Cerebral ischemia, Neuroprotection, Retinoic acid, Ubiquitin-proteasome system

PS-A-023

Quercetin prevents glutamate toxicity-induced neuronal cell damage by regulating parvalbumin expression

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Glutamate is the main excitatory neurotransmitter. Excessive glutamate causes excitatory toxicity and increases intracellular calcium, leading to neuronal death. Parvalbumin is a calcium-binding protein that regulates calcium homeostasis. Quercetin is a polyphenol found in plant and has neuroprotective effects against neurodegenerative diseases. We investigated whether quercetin regulates apoptosis by modulating parvalbumin expression in glutamate induced neuronal damage. Glutamate was treated in hippocampal-derived cell line, and quercetin or vehicle was treated 1 h before glutamate exposure. Cells were collected for experimental procedure 24 h after glutamate treatment and intracellular calcium concentration and parvalbumin expression were examined. Parvalbumin small interfering RNA (siRNA) transfection was performed to detect the relation between parvalbumin and apoptosis. Glutamate reduced cell viability and increased intracellular calcium concentration, while quercetin preserved calcium concentration and neuronal damage. Moreover, glutamate reduced parvalbumin expression and quercetin alleviated this reduction. Glutamate increased caspase-3 expression, and quercetin attenuated this increase in both parvalbumin siRNA transfected and non-transfected cells. The alleviative effect of quercetin was statistically significant in non-transfected cells. Moreover, glutamate decreased bcl-2 and increased bax expressions, while quercetin alleviated these changes. The alleviative effect of quercetin in bcl-2 family protein expression was more remarkable in non-transfected cells. These results demonstrate that parvalbumin contributes to the maintainace of intracellular calcium concentration and the prevention of apoptosis, and quercetin modulates parvalbumin expression in glutamate-exposed cells. Thus, these findings suggest that quercetin performs neuroprotective function against glutamate toxicity by regulating parvalbumin expression. This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (NRF-2021R1F1A105878711).

Corresponding author : Phil-Ok Koh

Keywords : Glutamate excitotoxicity, Neuroprotection, Parvalbumin, Quercetin

PS-A-024

The roles of enteric nervous system in radiation-induced enteropathy

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Radiation-induced enteropathy is progressive disease of the intestines that occurs after abdominal or pelvic radiation therapy as well as the event of nuclear accidents or radiological terrorism. Exposure to a high dose of radiation cause severe damage of gastrointestinal (GI) epithelium with depletion of the pool of intestinal stem cells. Enteric nervous system (ENS) is comprised of neurons and supportive glial cells present in two major plexuses (the myenteric plexus and the submucosal plexus). ENS was first reported for its major role in intestinal motility, but is now known to be responsible for many aspects of intestinal physiologic functions to maintain intestinal homeostasis. to stress challenge. Here, we explored the roles of ENS for the management of radiation-induced enteropathy. We identified that radiation-induced enteropathy were characterized by a reduced number of enteric neurons defining neuropathy and delayed GI motility. We treated prucalopride, a well-known 5-HT4 agonist and prokinetics, and GR113808, a 5-HT4R antagonist, in radiation-induced enteropathy model and performed GI transit assay. Treatment of prucalopride resulted in prevention of delayed intestinal motility and significantly increased the number of peripherin, HuC/D, and nNOS-positive enteric neurons in irradiated (IR) mouse. Radiation-induced histological damage in the ileum were improved in prucalopride-treated IR mice. Otherwise, benzalkonium chloride (BAC) treatment destroyed myenteric plexus and BAC-treated IR group showed more severe histological damage compared with IR group. In BAC-treated IR group, the inflammatory cytokines and chemokine expression did not revealed difference compared with IR group, but stem cell markers were significantly decreased compared with IR group. Taken together, enteric neuron contributes to the epithelial regeneration by regulating stem cell niche. These findings suggested that protection of ENS is a novel therapeutic target for treatment of radiation-induced GI damage.

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Keywords : Enteric nervous system, Radiation-induced enteropathy, Stem cell

PS-A-025

The effect of functional foods containing collagen on osteoarthritis rat models

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This study is about the evaluation of the efficacy of the functional foods based on low molecular collagen. As a methodology, we induced osteoarthritis with using Monosodium iodoacetate on the SD rat as an animal model.
We made five groups such as a normal group, experimental control group, *L. plantarum* group, collagen group, and YB-Exp group, and each group had twelve heads. We induced osteoarthritis on the groups except the normal group and conducted the oral administration to all five groups for five weeks every day. To evaluate the effect of protecting and recovering from joint cartilage damage caused by osteoarthritis, we used Micro CT, H&E staining, and Safranin-O staining.
As the result of the Micro-CT, the experimental control group and *L. plantarum* group showed the increase of bone erosion and destruction, whereas the Collagen group and YB-Exp group showed a decrease in the amount of cartilage and severe bone erosion and destruction
As the result of the H&E staining and Safranin-O staining, and histopathological scores from those stainings on the histopathological examination, the experimental control group and *L. Plantarum* group showed loss of synovial membrane and bone tissue, infiltration of inflammatory cells, and loss of proteoglycan. However, the YB-Exp group showed recovering up to the similar level to the normal group on the shape of the synovial membrane and bone tissue. Our goal was to bring the positive result on the YB-Exp group.
In this research, it was confirmed that the functional foods based on the low molecular collagen had a positive effect on osteoarthritis by with restoring articular cartilage and anti-inflammatory caused by the osteoarthritis.
According to the result of our research, if the YB-Exp is more developed, it would be a good supplement for dogs to prevent and recover from osteoarthritis.

Corresponding author : Ok jin Kim

Keywords : Osteoarthritis, Monosodium iodoacetate(MIA), Collagen, Functional Foods

PS-A-026

CD47;Rag2;IL2rg triple KO mice preconditioning with busulfan injection could be a noble platform for generating hematopoietic stem cells engrafted humanized mice

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Recently, humanized immune system mice (HIS mice) have been widely used for the study of various human diseases. hCD34^{+} Hematopoietic stem cells (HSCs) engrafted HIS mice were generated by HSCs transplantation after immune suppression, and this model have several limitations such as insufficient engraftment of human immune cells and development of graft versus host disease (GVHD). To overcome the limitations, we generated CD47;Rag2;IL2rg triple KO mice (TKO) using NOD-Rag2^{null} IL2rg^{null} mice (DKO) and compared the efficacy of HSCs transplantation. HSCs was injected via tail veins to 4-week female DKO and TKO mice after myelosuppression by total body irradiation (TBI) or busulfan (BSF) injection. The survival rate was 50.0-71.4% up to 48 weeks, and weight loss was observed after 40 weeks. Clinical symptoms such as anemia, cachexia, hyper-keratinization, and alopecia were observed. In flow cytometry results, hCD45^{+} cells were observed from 8 weeks after HSCs administration, and levels of 20% or more were confirmed until 40 weeks in all groups. BSF-treated groups showed higher hCD45^{+} cells engraftment than TBI groups, and all TKO groups were significantly higher than the DKO group. Transplanted human immune cells were initially CD19^{+} B cells, but gradually decreased, and on the contrary, CD3^{+} T cells increased over time. In histopathology, inflammatory cell aggregation in various organs, and epidermal hyperplasia of skin were observed, and there was no significant difference between experimental groups. In conclusion, generating HSCs engrafted HIS mice, BSF injection was more efficient pre-conditioning method than TBI. Human immune cells were more efficiently transplanted in TKO mice than DKO, and GVHD symptoms were minimal except for the skin. Considering these results, it is expected that HIS mice transplanted with HSC preconditioned with BSF using TKO mice could be a useful tool for human disease research.

Corresponding author : Seung-Ho Heo

Keywords : Busulfan, CD47, Hematopoietic stem cell, Humanized mice, Total body irradiation

PS-A-027

Embryotoxic and teratogenic effects of Scolopendra water extract in mice

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Scolopendra have been used in oriental medicine including Korea and China. Although Scolopendra water extracts (SWE) have been reported to be effective in neuroinflammatory disease and neuropathic pain. There are no reported about the effects on fetus when pregnant women used this oriental medicine. In this study we investigated to effect on fetal toxicity of oral exposure to SWE in pregnant mice. SWE was oral administered to pregnant ICR mice until Gestation days 18 (GD) at dosage levels in 100, 500, 1000 mg/kg/day and sacrificed the mice on GD18. We examined the fetal mortality and morphological examinations. This study showed that SWE groups are not different between control group in the fetal mortality. In additions the results of the morphological examinations showed no different between this groups. Oral exposure to SWE during pregnancy didn't affect critical toxicities in the fetal mortality and morphological examinations. Based on these findings, we concluded that oral exposure of mice with SWE didn't significant in toxicity. This study evaluates the risk of fetuses during oral exposure to SWE during pregnancy.

Corresponding author : Joong-Sun Kim

Keywords : Scolopendra water extracts (SWE), Embryotoxicity, Teratogenic effect, Pregnancy mice

PS-A-028

Olaparib, a selective PARP-1 inhibitor, aggravates radiation induced intestinal injury

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Olaparib is a PARP inhibitor and this therapeutic application has been expanded to several other diseases, including cancer. Notably, a treatment strategy combining the use of olaparib and radiation therapy has been employed for improving the control of local cancers. In this study, we examined the effect of olaparib on irradiation-induced intestinal damage. For *in vitro* tests, olaparib treatment before irradiation exposure to IEC-6 increased cytotoxicity and the loss of cell viability (5, 10 and 15 Gy). And olaparib treatment also elevated radiation-induced PARP-1, cleavage of caspase-3 and γH2ax in IEC-6. In vivo test, the mice were divided into the groups; 1) sham-irradiated control group, 2) Olaparib -treated group, 3) irradiated group, and 4) olaparib-treated irradiation group. Mice were orally ad-ministered olaparib at a dose of 25 mg/kg in a 0.1 mL volume, daily for 4 days after irradiation exposure (10 and 15 Gy). Then, histological examinations of the jejunal villous height, crypt survival, and crypt size were performed. The jejunal villi height and the crypt survival were reduced in the irradiation group compared with the sham-irradiated group. Olaparib treatment in irradiation mice even more decreased those indicators. Crypt size was increased in the radiation group compared to the sham-irradiated control group, whereas the size was decreased in the olaparib treated irradiation group compared with the group exposed to the radiation injury. In this study, we confirmed that olaparib treatment after irradiation aggravated the irradiation-induced intestinal damage. These results suggest that a caution need to be administered when olaparib treatment is performed in combination with radiation therapy for cancer treatment.

Corresponding author : Joong-Sun Kim

Keywords : PARP-1, Radiation, Intestine, Olaparib, Mice

PS-A-029

Expression of ATG9A and ATG9B in mouse oocytes and reproductive tissues

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Autophagy is an intracellular catabolic process governed by sequential actions of proteins encoded by *autophagy-related* (*Atg)* genes and others. ATG9 is the only transmembrane protein among ATG proteins and is thought to be involved in translocating phospholipids to autophagosomes during the initial stages of autophagy. ATG9 localizes to various membranous structures in mammalian cells, suggesting more complex roles than the yeast ortholog. In mammals, two isoforms of ATG9 have been reported as ATG9A and ATG9B. These two isoforms of ATG9 seems to exhibit differential expression pattern: ATG9A was shown to be expressed in all tissues, while ATG9B expression seems to be limited to reproductive and endocrine tissue such as placenta, pituitary, and uterus, suggesting distinct roles in autophagy. In this investigation, we examined expression of ATG9A and ATG9B in specific reproductive tissues such as oocytes, granulosa cells, and pregnant uterus in mice. RT-PCR, western blotting, and immunofluorescence staining were performed to examine the spatiotemporal expression of these two factors. Mouse oocytes were collected from PMSG-primed mice at germinal vesicle (GV) stage and PMSG- and hCG-injected mice at polar body (PB) stage. Uterine tissue samples were collected from pregnant mice at day 1, 4, 6, and 8 of pregnancy. We performed RT-PCR for GV and MII phase of oocytes using ATG9A and ATG9B primers. By RT-PCR, we confirmed that both germinal vesicle (GV) and polar body (PB) stage oocytes express *Atg9a* and *Atg9b*. In pregnant mouse uteri, ATG9A is detected in all days of pregnancy. Immunofluorescence staining of ATG9A in granulosa cells and oocytes showed that ATG9A exhibits puncta-like patterns in the cytoplasm. Collectively, the results show that both ATG9A and ATG9B are present in oocytes, ovary, and uterus and may be involved in autophagy during oocyte maturation and pregnancy.

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Corresponding author : Hyunjung J. Lim

Keywords : Autophagy, ATG9, Uterus, Ovary, Oocyte

PS-A-030

Effect of Lactobacillus fermentum BCC-LF-01 on alveolar bone loss in ligature-induced periodontitis mice

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Periodontitis is an inflammation disease, it mainly caused by anaerobic bacteria in the mouth. It occurs loss of alveolar bone that supports teeth due to inflammation of the periodontium. Recently, it is reported that periodontitis could worsen systemic diseases, including cardiovascular disease and cerebrovascular disease. Therefore, it is important to prevent and treat periodontitis. In a previous study, about 2,000 strains were isolated from various foods and human materials. And *Lactobacillus fermentum* BCC-LF-01 (BCC-LF-01), multifunctional probiotics with antimicrobial and anti-inflammatory properties *in vitro* study, was isolated. The purpose of this study is to investigate whether BCC-LF-01 could reduce alveolar bone loss by periodontitis. For 14 days, BCC-LF-01 ($5x10^8$ CFU) was administered twice a day in oral cavity of ligature-induced periodontitis (LIP) mice. After that the distance between cemento-enamel junction (CEJ) and alveolar bone crest (ABC) was measured using micro-computed tomography (micro-CT). As a result, it was showed that alveolar bone loss was reduce in mice administered with BCC-LF-01. Therefore, this result is suggested that BCC-LF-01 could inhibition alveolar bone loss by periodontitis.

Corresponding author : Ki-Yeon Yoo

Keywords : Periodontitis, Alveolar bone, *Lactobacillus fermentum*, BCC-LF-01, Microtomography

PS-A-031

Indoxyl sulfate, a uremic toxin, induces trained immunity of monocytes through AhR-dependent arachidonic acid pathway

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Trained immunity is a long-term functional reprogramming of innate immune cells exposed to infectious or sterile insults, which causes altered responses towards a secondary challenge. Indoxyl sulfate (IS) is a uremic toxin associated with immune dysfunction in chronic kidney diseases. Despite its role as a potent stimulus on monocytes and macrophages, the impact of IS on the induction of trained immunity has not been explored. We found that in human monocytes, IS induced trained immunity *via* epigenetic and metabolic reprogramming, typical features of trained immunity, showing a hyperactivation such as an augmented TNF-α and IL-6 production in response to LPS. Mechanistically, the aryl hydrocarbon receptor (AhR), an intracellular sensor of IS, contributed to IS-trained immunity by an enhanced expression of arachidonic acid (AA) metabolism-related genes such as ALOX5 and ALOX5AP. Inhibition of AhR during IS training suppressed the induction of IS-trained immunity. We confirmed that monocyte and macrophages of the patients with end-stage renal disease (ESRD) had a higher ALOX5 expression than healthy controls (HC). Furthermore, HC-derived monocytes trained with a uremic serum of ESRD patients led to increased production of TNF-α and IL-6 upon LPS re-stimulation. Consistently, mice trained with IS and their splenic myeloid cells had increased production of TNF-α after *in vivo* and *ex vivo* LPS stimulation, respectively than control mice. These results provide insight into the role of IS in the induction of trained immunity, which is critical in inflammatory immune responses in patients with CKD and may be a potential therapeutic target for them.

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Keywords : Indoxyl sulfate, Chronic kidney disease, Trained immunity, Aryl hydrocarbon receptor, Epigenetic and metabolic reprogramming

PS-A-032

Hepatoprotective effects of catechin in carbon tetrachloride-induced hepatic damage in rats

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The hepatoprotective activities of catechin were investigated under carbon tetrachloride -induced hepatotoxicity in rats. Catechin (50 and 100 mg/kg) was orally administered to mice for 7 days, after which liver injury was induced by CCl_4 (1.5 ml/kg, i.p.). The vehicle and positive controls consisted of phosphate-buffered saline and silymarin (100 mg/kg), respectively. The treatment of catechin significantly suppressed the increase of alanine aminotransferase and aspartate aminotransferase in the sera of CCl_4 injured rats, and restored the decreased levels of anti-oxidant enzymes such as superoxide dismutase, catalase, while reducing lipid accumulation and, concurrently, the expression of genes involved in lipid synthesis, including peroxisome proliferator-activated receptor γ and adipocyte protein-2. Catechin also ameliorated inflammation by down-regulating the expression of the pro-inflammatory mediators, TNF-α, IL-1β and iNOS and up-regulating the Nrf2/HO-1 pathway. Collectively, these findings imply that catechin mitigates CCl_4-induced hepatic injury by increasing anti-oxidative activity, down-regulating pro-inflammatory mediators in the liver. (Acknowledgement: This work was supported by the National Research Foundation of Korea: Grant number NRF-2021M3H9A1097596)

Corresponding author : Meejung Ahn

Keywords : Carbon tetrachloride, Catechin, Inflammatory mediators, Rat model

PS-A-033

Resveratrol alleviates the symptoms of experimental autoimmune neuritis

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The present study evaluated the therapeutic effects of resveratrol on experimental autoimmune neuritis (EAN), which is a widely used for the experimental studies on human peripheral demyelinating diseases including Guillain-Barré syndrome (GBS). EAN was induced in Lewis rats by immunization with a neurogenic peptide homologous to amino acids 53–78 of bovine myelin P2 protein. The rats were checked daily for clinical symptoms, such as paralysis, and the levels of inflammatory mediators were analyzed using PCR, western blot analyses, and immunohistochemistry. The daily oral administration of resveratrol to EAN-induced rats significantly reduced the severity of paralysis. Additionally, histopathological examinations confirmed that resveratrol mitigated inflammation in the sciatic nerves after EAN induction, suppressed the infiltration of CD68-positive inflammatory cells, and reduced the production of various pro-inflammatory cytokines, including interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, as well as inducible nitric oxide synthase (iNOS). Furthermore, the resveratrol-treated group exhibited a decrease in the number of iNOS-positive inflammatory cells compared to the vehicle-treated group. Taken together, the present results suggest that resveratrol alleviated neuro-inflammation in the sciatic nerves of EAN-induced rats, as well as by systemically suppressing the circulating pro-inflammatory factors. (Acknowledgement: This work was supported by the National Research Foundation of Korea: Grant number NRF-2017R1A2B4012478)

Corresponding author : Meejung Ahn

Keywords : Experimental autoimmune neuritis, Inflammatory mediators, Resveratrol, Peripheral nerve

PS-A-034

The role of leucine influx and its catabolism for regulating Th17 responses of human CD4$^+$ T cells

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Branched-chain amino acids (BCAAs) such as leucine (Leu), Isoleucine, and valine, are essential amino acids that play a regulatory role in immune responses by modulating metabolic rewiring. However, the molecular mechanism underlying this phenomenon remains unclear. Here, we found that in human CD4$^+$ T cells, TCR stimulation predominantly induced the expression of BCATc, a cytosolic BCAA catabolic enzyme, and the expression of SLC7A5, a major transporter of BCAAs. The uptake of ^{3}H-labeled Leu and treatment with chemical inhibitors of SLC7A5 and BCATc indicate that SLC7A5-mediated Leu influx and BCATc-mediated Leu catabolism are closely involved in human Th17 responses. RNA-Seq data analysis indicated that the expression of cytosolic Leu catabolism-related gene for the synthesis of β-hydroxy-β-methyl butyrate (HMB) was significantly increased in TCR-activated CD4$^+$ T cells, implying an important role of BCATc-mediated leucine metabolite for IL-17 production. In CD4$^+$ T cells, HMB supplementation recovered the suppressed production of IL-17 by BCATc inhibitor. Mechanistically, HMB contributes to the regulation of HIF1α, which is a major transcription factor for IL-17 production *via* enhanced mRNA expression of HIF1α. Our finding was further supported by the finding in which the treatment of L-β-homo-leucine (LβhL), a Leu-specific analogue that is a competitive inhibitor of BCAT, significantly reduces IL-17 production by TCR-activated human CD4$^+$ T cells. *In vivo* EAE model demonstrated that the inhibition of BCATc-mediated Leu catabolism by BCATc inhibitor and LβhL treatment ameliorates the severity of EAE by a decrease of HIF1α expression and IL-17 production in spinal cord mononuclear cells. Our findings suggest the possible role of SLC7A5-mediated Leu influx and BCATc-mediated Leu catabolism in modulating human CD4$^+$ T-cell responses, especially IL-17 production *via* the regulation of HIF1α, and this mechanism might be related to various inflammatory conditions.

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Keywords : SLC7A5, Leucine, BCATc, IL-17, HIF1α

PS-A-035

Different anti-tumor immune responses of fractionated high-dose radiation applied with intervals of 1- or 5-day in FSaII-bearing C3H mice

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High-dose radiation could induce anti-tumor immunity by increasing necrosis, which leads to secrete antigens that stimulate dendritic cells (DCs), which play a role in T cell priming. The activated T cells could increase immunogenic tumor cell death and initiate the abscopal effect by reducing metastatic cancer. However, the high-dose radiation could also induce immunosuppressive responses at the same time by upregulating immune checkpoint proteins and immune suppressive cells such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophage (TAM) in the tumor microenvironment. Therefore, the anti-tumor effect will be increased when these immunosuppressive effects are lowered and the immune response is increased. Although some studies have investigated appropriate fraction studies that include the fractionation number and total dose, the optimized fractional interval radiotherapy (RT) in clinical setting have not yet been performed. Here, we found that the 5-day interval RT was more effective for anti-tumor immunity in FSaII tumors. In the immunocompetent C3H mice, the irradiation with 20 GyX2-fractions were applied with 1- or 5-day intervals, and the 5-day interval RT was more effective than the 1-day interval in suppressing tumor growth. However, in the immunodeficient Balb/c-nude mice, the difference in tumor growth delay between two groups was insignificant. Therefore, the difference in tumor growth probably due to the difference in immune responses between the two groups. In ELISpot assay, the antigen-specific T cell responses were increased in 5-day interval RT than 1-day interval. In addition, we observed significant upregulation in Granzyme B-positive T cells. To investigate the abscopal effect, we evaluated the number of metastatic nodules in the lung and observed that the 5-day interval group showed a lower lung metastasis than that of the 1-day interval. Therefore, our results showed that greater induction of anti-tumor immune response was observed in 5-day interval RT compared to 1-day interval.

Corresponding author : Mi Sook Kim

Keywords : High-dose radiation, Anti-tumor immune response, Murine fibrosarcoma, C3H mouse, Fractionated radiation therapy

PS-A-036

The differentiation of epidermis of minipig skin during pregnancy

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Epidermis layer of skin is highly keratinized so it protects body from toxic, mechanical insults and water loss from evaporating. However, the skin is not keratinized from the embryonic stage because bi-directional exchange between amniotic fluid (AF) and fetal extracellular component should occur in the early stage of pregnancy. In human, the pH and osmolarity between amniotic fluid and fetal extracellular matrix is similar until 12-20 weeks of gestation, at which keratinization of skin occurs. Minipig is one of the useful animals in studying embryology for its high similarity to human. In spite of numerous embryologic researches using minipig, the time point at which keratinization occurs in minipig has not been confirmed yet.
Here, we designed an experiment to discover when the keratinization occurs in minipig. We prepared skin of minipig from 8, 10, 12, 14-week gestation aged minipig and 30 postnatal date (PND 30) minipig. Every skin of minipig was stained H&E and Masson trichrome stain. In order to find when the differentiation of epidermis is fully complete, we also immunostained skin with involucrin.
As a result, the hair bulb starts to appear from 12-week aged minipig and thick keratinized layer starts to appear from 14-week gestation aged minipig. Interestingly, involucrin, which is observed in stratum corneum, doesn't appear until PND30 aged minipig.
The results shows that the keratinization of minipig skin occurs between 12–14-week gestational aged minipig but the differentiation of minipig skin is not finished until PND30 aged minipig. The study infers that the skin is still developing after the parturition. Further study regarding differentiation of epidermis layer is required.

Corresponding author : Mi-Jin Yang

Keywords : Minipig, Skin, Differentiation, Keratinization

PS-A-037

An octopus-derived peptide with antidiuretic activity in rats

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Discovering new drug candidates with high efficacy and few side effects is a major challenge in new drug development. The two evolutionarily related peptides oxytocin (OXT) and arginine vasopressin (AVP) are known to be associated with a variety of physiological and psychological processes via the association of OXT with three types of AVP receptors. Over decades, many synthetic analogs of these peptides have been designed and tested for therapeutic applications; however, only a few studies of their natural analogs have been performed. In this study, we investigated the bioactivity and usefulness of two natural OXT/AVP analogs that originate from the marine invertebrate *Octopus vulgaris*, named octopressin (OTP) and cephalotocin (CPT). By measuring the intracellular Ca^{2+} or cyclic AMP increase in each OXT/AVP receptor subtype–overexpressing cell, we found that CPT, but not OTP, acts as a selective agonist of human AVP type 1b and 2 receptors. This behavior is reminiscent of desmopressin, the most widely prescribed antidiuretic drug in the world. Similar to the case for desmopressin, a single intravenous tail injection of CPT into Sprague-Dawley rats reduced urine output and increased urinary osmolality. In conclusion, we suggest that CPT has a significant antidiuretic effect and that CPT might be beneficial for treating urological conditions such as nocturia, enuresis, and diabetes insipidus. [Marine Drugs 2022, 20(5), 328]

Corresponding author : Seonmi Jo, Dong Ho Woo

Keywords : Antidiuretic, Cephalotocin, Octopus, Vasopressin, Rat

PS-A-038

Evaluation of antitumor activity of N3095 as a novel orally active pyruvate dehydrogenase kinase inhibitor in mice of colorectal cancer

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Background: Discovery of new therapeutic strategies are urgently required to overcome colorectal cancer. Recently, pyruvate dehydrogenase kinase (PDK) has been demonstrated to be reasonable target against colorectal cancer. Herein, we identified N3095 as a highly potent and orally available PDK4 inhibitor for novel therapeutic agents of colorectal cancer.
Experimental Design: Phosphorylated PDHE1α levels as a substrate of PDK4 were determined in normal tissues and colorectal cancer tissues using western blotting analysis. The effects of N3095 on cell proliferation, cell cycle, and apoptosis was examined. Western blotting analysis was conducted to determine the change of cell cycle- and apoptosis-related genes after N3095 treatment. Status of phosphorylated PDHE1α, phosphorylated and acetylated p53 was examined in N3095-treated cells. *In vivo* therapeutic effects of N3095 were investigated in colorectal tumor-bearing mice by tumor volume measurement, followed by TUNEL assay.
Results: Western blotting analysis demonstrated higher expression of phosphorylated PDHE1α in colorectal tissues than normal colorectal tissues. Treatment with N3095 exhibited anti-proliferation effects in colorectal cancer cells but not in normal cells. FACS analysis showed dose-dependent induction of apoptosis and G2/M cell cycle arrest in N3095-treated colorectal cancer cells. Consistent with these findings, p21, BAX, cleaved caspase-3, and cleaved PARP was upregulated in N3095-treated cells with down-regulation of CDK4, cyclin D1, and Bcl-xl. N3095 effectively decreased the phosphorylation of PDHE1α with up-regulation of acetylated and phosphorylated p53. Importantly, oral administration of N3095 led to antitumor effects in a dose-dependent manner. TUNEL analysis revealed higher apoptotic lesion in N3095-treated tumors than vehicle-treated tumors.
Conclusions: N3095 shows potential clinical applicability for treatment of colorectal cancer.

Corresponding author : Yong Hyun Jeon

Keywords : Pyruvate dehydrogenase kinase 4, Pyruvate dehydrogenase E1α (PDHE1α), Colorectal cancer

PS-A-039

Peripheral nerve-derived stem cell spheroids induce functional recovery and repair after spinal cord injury in rodent

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Stem cell therapy is one of the most promising candidate treatments for spinal cord injury. Research has shown optimistic results for this therapy; however, clinical limitations remain such as poor viability, engraftment, and differentiation. Here in this study, we have isolated novel peripheral nerve-derived stem cells (PNSCs) from adult peripheral nerves. PNSCs expressed neural-crest specific markers and showed multi-lineage differentiation potential into Schwann cells, neuroglia, neurons, and mesodermal cells. In addition, PNSCs showed therapeutic potential by releasing the neurotrophic factors, including glial cell-line-derived neurotrophic factor, insulin-like growth factor, nerve growth factor, and neurotrophin-3. Also it has shown that PNSCs abilities were enhanced by their development into spheroid which secreted neurotrophic factors several times more than non-spheroid PNSCs and expressed several types of extracellular matrix. Also, in an animal spinal cord injury (SCI) model, these PNSC spheroids induced functional recovery and neuronal regeneration. These PNSC spheroids also reduced the neuropathic pain which accompanies spinal cord injury (SCI) after remyelination. These PNSC spheroids may represent a new therapeutic approach for patients suffering from spinal cord injury (SCI).

Corresponding author : Young-Il Yang, Keung-Nyun Kim

Keywords : Funtional recovery, Spinal cord injury, Peripheral nerve-derived stem cells (PNSCs), Rodent

PS-A-040

Developing an ADHD animal model by inducing neuroendocrine dysregulation during the prenatal period

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The high level of blood cortisol is the key factor to identify major depressive disorder (MDD) which is mediated with the abnormal modulation of brain-derived neurotrophic factor (BNDF) in mammalian brains. However, it is not well known if and how the elevation of cortisol level during prenatal period affects brain functions and induces psychiatric disorders such as MDD or attention deficit hyperactivity disorder (ADHD) after birth. For this issue, we constantly elevated the cortisol level during the prenatal period by a injection of corticosterone (20 mg/kg) per day to maternal rats for 21 consecutive pregnant days until delivery. This procedure critically elevated cortisol level in both maternal and pre- and postnatal pups. After birth, we first electrophysiologically tested synaptic plasticity in hippocampal CA1 neurons of cortisol pups (Corti.Pups, p14~21 days), and then compared to normal pups (Nor.Pups, saline injected for 21 days). Behavioral tests to observe cortisol effects in brain functions were performed by hiring a forced swim test (FST), open field moving test (OFT) and Morris water maze test (MWT) after weaning at the postnatal 21th day. In results, Corti.Pups showed significantly different behavioral patterns; 1) In FST, immobility time of CortiPups was critically shorter than that of NorPups, showing anxiety-mediated hyperactivity. 2) In OFT, the moving distance significantly increased in CortiPups. 3) In MWT, CortiPups showed the critical impairment of learning and memory functions. These behavioral patterns of Corti.Pups were definitely similar to those shown in other ADHD models. In the electrophysiological and molecular studies, long-term potentiation (LTP) of EPSC was impaired in hippocampal CA1 neurons, showing the diminishment of late-phase potentiation and PSD95-mediated signaling was significantly downregulated in prefrontal cortex and hippocampus of Corti.Pups. These results mean that synaptic plasticity may be affected by high cortisol, indicating incomplete brain development in Corti.Pups. These results suggest that neuroendocrine dysregulation during the prenatal period may affect learning and memory functions in developmental brains and consequently induce ADHD-like behaviors in rat pups (GRANT NRF 2022R111A3063177).

Corresponding author : Sung-Cherl JUNG

Keywords : Cortisol, Major depressive disorder, Attention deficit hyperactivity disorder, Neuroendocrine dysregulation, Long-term potentiation

PS-A-041

Anti-inflammatory and anti-obesity effects of Pachydictyon coriaceum extract

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The inhibitory effect of Pachydictyon coriaceum extract (PC-WE) on high-fat diet-induced obesity and LPS-induced inflammatory responses was investigated in mice and RAW 264.7 macrophages, respectively. PC-WE was prepared by extracting in hot water (75℃) for 12 hr. For induction of obesity, ICR mice were given high fat diet (HFD) for 4 weeks. Mice were orally administered with the indicated PC-WE every day for 4 weeks, and the body weight was measured to evaluate the anti-obesity effect. Inflammatory responses of RAW 264.7 cells was produced by LPS (100 ng/ml) stimulation for 24 hr. Anti-inflammatory effect of PC-WE was examined by determining the level of inflammatory mediators such as NO, TNF-α and IL-6 using ELISA kits. Oral administration of PC-WE significantly reduced the body weight of the mice compared to HFD-fed mice. Administration of PC-WE also significantly reduced the accumulation of abdomen fat, and the level of serological factors related obesity and hyperlipidimia such as total cholesterol, triglyceride, and glucose. Treatment with PC-WE significantly inhibited the production of NO, TNF-α and IL-6 produced from LPS-stimulated RAW 264.7 macrophages. These results indicate that PC-WE is a promising candidate applicable to the development of anti-obesity and anti-inflammatory nutritional foods and drugs. This research was a part of the project titled 'Bioactive material for algae-based bio-health care substantiation (Project No. 20210656)', funded by the Ministry of Oceans and Fisheries, Korea.

Corresponding author : Yung Choon Yoo

Keywords : Pachydictyon coriaceum, Obesity, Hyperlipidemia, Inflammation, Functional food

PS-A-042

Inhibitory effects of Endarachne binghamiae extract on inflammation and obesity

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In this study, the anti-inflammatory and anti-obesity effect of Endarachne binghamiae extract (EB-WE) was investigated using LPS-induced RAW 264.7 cells and high-fat diet (HFD)-fed mice respectively. PC-WE was prepared by extracting in hot water (75℃) for 12 hr. Treatment of EB-WE significantly inhibited the production of inflammatiory mediatore such as nitric oxide (NO), tumor-necrosis factor-alpha (TNF-a) and interleukin-6 (IL-6) from lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. In addition, treatment of EB-WE effectively inhibited the differentiation of 3T3-L1 pre-adipocytic cells. In animal experiment for HFD-induced obesity, administration of EB-WE significantly suppressed the gain of body weight of HFD-fed ICR mice. Administration of EB-WE also significantly reduced the accumulation of fat in abdomen and epididymis. The assay of blood lipids revealed that EB-WE-fed mice showed a significant reduction of the level of total cholesterol (T-CHO) and triglyceride (TG). Furthermore, administration of EB-WE markedly lowered the level of glutamic oxaloacetic transaminase (GOT), glutamlc pyruvic transamlnase (GPT), creatinine (CRE) and blood urea nitrogen (BUN), suggesting that EB-WE ameliorated hepatotoxicity and nephrotoxicity induced by obesity in mice. These results indicate that EB-WE is a promising candidate applicable to the development of anti-obesity and anti-inflammatory nutritional foods and drugs. This research was a part of the project titled 'Bioactive material for algae-based bio-health care substantiation (Project No. 20210656)', funded by the Ministry of Oceans and Fisheries, Korea.

Corresponding author : Yung Choon Yoo

Keywords : Endarachne binghamiae, Obesity, Hyperlipidemia, Inflammation, Functional food

PS-A-043

Effects of Sargassum horneri extract on LPS-induced inflammation in RAW 264.7 cells and high fat diet-induced obesity in mice

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In this study, the anti-inflammatory and anti-obesity effect of Sargassum horneri extract (SH-WE) was investigated using LPS-induced RAW 264.7 cells and high-fat diet (HFD)-fed mice respectively. SH-WE was prepared by extracting in hot water (75℃) for 12 hr. Treatment of SH-WE significantly inhibited the production of inflammatiory mediatore such as nitric oxide (NO), tumor-necrosis factor-alpha (TNF-a) and interleukin-6 (IL-6) from lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. In addition, treatment of SH-WE effectively inhibited the differentiation of 3T3-L1 pre-adipocytic cells. In animal experiment for HFD-induced obesity, administration of SH-WE significantly suppressed the gain of body weight of HFD-fed ICR mice. Administration of SH-WE also significantly reduced the accumulation of fat in abdomen and epididymis. The assay of blood lipids revealed that SH-WE-fed mice showed a significant reduction of the level of total cholesterol (T-CHO) and triglyceride (TG). In an animal experiment for non-alcoholic fatty liver disease (NAFLD), administration of SH-WE significantly inhibited the increase of body weight gain liver weight, and restored hepatotoxic effect induced by HFD feeding in mice.

These results indicate that SH-WE is a promising candidate applicable to the development of anti-obesity and anti-inflammatory nutritional foods and drugs. This research was a part of the project titled 'Bioactive material for algae-based bio-health care substantiation (Project No. 20210656)', funded by the Ministry of Oceans and Fisheries, Korea.

Corresponding author : Yung Choon Yoo

Keywords : Sargassum horneri, Obesity, Hyperlipidemia, Inflammation, Functional food

PS-A-044

Tumor-derived noncanonical Notch ligand DLK1 attenuates tumor growth by regulating macrophages

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Despite recent advances in the immune checkpoint inhibitors, it remains elusive how tumor cells acquire an ability to reshape a tumor immune microenvironment (TIME). We have performed a comparative transcriptomic analysis using melanomas derived from mice transplanted with B16F1 cells manipulated to accelerate tumor growth and identified delta-like noncanonical Notch ligand 1 (DLK1) as a putative tumor intrinsic immune regulator. Forced expression of DLK1 in B16 melanoma suppressed tumor growth along with a marked change in the immunophenotype within tumor tissues without affecting proliferation and stemness of tumor cells. Moreover, ectopic expression of DLK1 also attenuated melanoma growth in *RAG1*$^{-/-}$ mice lacking mature T & B cells, implying an impact of DLK1 on innate immune populations including macrophages. In vitro treatment with soluble DLK1 enhanced secretion of TNF-α and IFN-γ from macrophages while it did not affect induction of type 1 T cells. We are in progress to investigate a mechanism by which tumor modulate the expression of DLK1 and how DLK1 regulates tumor associated macrophages within the TIME. Collectively, our experimental data suggest DLK1 as a pivotal tumor derived immunomodulator and provide a novel therapeutic venue for cancer treatment by reshaping the tumor immune microenvironment.

Corresponding author : Keunwook Lee

Keywords : Delta-like noncanonical Notch ligand 1, Tumor immune microenvironment, Tumor associated macrophages

PS-A-045

Dohongsamul-tang ameliorated cardiac function through calcineurin/ NFATc4 signaling pathway in TAC-induced left ventricular hypertrophy rat

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In cardiovascular disease, cardiac hypertrophy refers to a change in the heart structure's shape due to pressure overload in the heart. It is known that the increase in myofibrils causes thickening of the heart and causes high blood pressure. Therefore, suppressing cardiac hypertrophy may be a major factor in lowering cardiovascular disease morbidity, mortality and heart failure. Dohongsammul-tang (DH) is Korean traditional herbal medicine, which was used for symptoms caused by extravasated blood, and it is known that it protects cardiovascular diseases and promotes blood circulation due to the effect of activating blood circulation to dispel blood stasis. Therefore, this study was performed to verify the pharmacological effect of DH on improving cardiovascular disorders and also to demonstrate the mutual improvement effect on renal function. In this study, the transverse aortic arch connected to the left ventricle was sutured with a blunt needle and the needle was immediately removed to induce cardiac hypertrophy due to pressure overload. To verify the effect of the drug from 1 week after surgery, 10 mg/kg/day PRO and 100, 200 mg/kg/day DH were administered orally for a total of 10 weeks using zonde at the same time. As a result, the left ventricle weight was significantly increased in the TAC group compared to the sham group, so it was judged that TAC induced cardiac hypertrophy. Cardiac function was attenuated by DH treatment groups, which was deteriorated in TAC group in echocardiographic data, such as ejection fraction (EF) and fractional shortening (FS). In the left ventricle pressure-volume curve graph, the pressure and volume tended to increase in the TAC group compared to the sham group, and the pressure and volume tended to decrease in the DH-treated group. In addition, as a result of analyzing the function of the heart measured in the left ventricle, the stroke work (SW), the EF, the cardiac output (CO), and the single stroke of the heart (stroke volume, SV) were significantly changed in the TAC group compared to the sham group, and the function was significantly restored in the DH-treated group. According to analyzing the cardiovascular function through a pressure-volume curve experiment in the left ventricle, systolic blood pressure and morphological data, cardiovascular function was attenuated by treatment of DH, which was aggravated by TAC. Collagen I and collagen III through TGF-β/smad2 signaling pathway were downregulated in DH treatment, which were increased in the TAC-induced group. DH treatment showed the significant improvement effect in lactate dehydrogenase (LDH) and (CK-MB), which are the hematological biomarkers of cardiac hypertrophy. Renal function-related biomarkers including concentration of blood creatinine, blood urea nitrogen (BUN) and Neutrophil gelatinase-associated lipocalin (NGAL) were improved in the DH treatment group, which were increased by TAC-induced cardiac hypertrophy. Taken together, DH treatment inhibited the cardiac remodeling due to pressure overload in the TAC-induced cardiac hypertrophy model, and this effect is thought to be manifested by improving the functional and morphological changes through the Calcineruin/ NFATc4 and TGF-β/smad2 signaling pathway.

Corresponding author : Ho Sub Lee, Dae Gill Kang

Keywords : Dohongsamula-tang (DH), Transverse aortic contraction (TAC), Cardiac hypertrophy, Cardiac fibrosis, Cardiovascular function

PS-A-046

Adipose stem cell-derived exosomes in combination with Hyaluronic acid for soft tissue augmentation: *in-vivo* study

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Background: We performed an animal study to identify the effect of exosome on the longevity of hyaluronic acid filler in the subcutaneous fat layer.
Methods: Filler injection was performed on twenty-three C57B6/J mice. Local infiltration of 200 μL Hyaluronic acid filler (Monalisa Mild, Genoss, Korea) and Hyaluronic acid filler mixed with exosome of human adipose tissue-derived stem cells (Baobab healthcare, Korea) were performed to left inguinal fat pad. At 4, 8, and 12 weeks, four C57B6/J mice were killed and fat tissue was obtained to measure the weight and perform H&E staining in each groups (HA group, n=15; exoHA group, n=16).
Results: The weight of tissue was not significantly different between the groups by time point. However, exoHA group demonstrated less inflammatory reaction compared with HA group at each time points. Furthermore, capsule formation around HA filler was significantly reduced in exoHA groups at 12 weeks.
Conclusion: Our findings show that exosome can reduce inflammatory reaction upon HA filler injection at early time point and capsular formation at late time point. This result suggests that the addition of exosome to HA filler can reduce complications of HA filler, such as inflammation and lump formation.

Corresponding author : Woonhyeok Jeong

Keywords : Hyaluronic acid filler, Exosome, Adipose tissue-derived stem cell

PS-A-047

Encapsulation of metformin within alginate shell-microcapsule with a thin oil layer ameliorates inflammatory bowel disease and improves gut microbiome

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Metformin, a biguanide class hypoglycemic agent, has been widely used to treat a type 2 diabetes. Recently, metformin was shown to decrease the incidence of inflammatory bowel disease (IBD) in patients with the diabetes, but their low and variable bioavailability in the colon is a major factor limiting the clinical potential of metformin treatment. We develop the alginate hydrogel microcapsules enable intestine-targeted oral delivery of metformin in a highly efficient manner. The thin oily layer of alginate shell microcapsules containing metformin (MC) prevents direct exposure to acidic conditions in the stomach, and the alginate shell degrade during passage through the intestine, allowing for efficient delivery of metformin. MC is shown to lower the levels of disease activity index, the intestinal epithelial injury and the infiltration of macrophages responsible for the secretion of pro-inflammatory factors during the pathological process of IBD in mouse treated with dextran sulfate sodium. In addition, MC improves the dysbiosis of specific bacterial genera, including *Bacteroides vulgatus, Lactobacillus (L.) gasseri, L.reuteri* and *L.intestinalis* indicating optimization of the abundance and composition of microorganisms. Hence, these results suggest that encapsulation of metformin within alginate shell-microcapsule with a thin oil layer a potential delivery system of metformin for IBD treatment.

Corresponding author : Chang-Hyung Choi, Sei-Jung Lee

Keywords : Metformin, Alginate shell-microcapsule, Inflammatory bowel disease, Microbiome

PS-A-048

Oral delivery of pentoxifylline loaded microcapsules prevents inflammatory bowel disease and improves gut microbiome

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Pentoxifylline (PTX) is a methylxanthine derivative and has been reported to inhibit the production of tumor necrosis factor-α and to ameliorate the inflammatory diseases, but their poor delivery to an inflamed colon is one of the major factors limiting its oral delivery. We report a simple and rapid microfluidic approach to produce multicompartmental core-shell hydrogel microcapsules with a thin interstitial oil layer. We utilize triple emulsion drop as capsule templates to form multicompartmental hydrogel microcapsules consisting of polyacrylic acid-functionalized PEG shell compartment and bare PEG core compartments via photopolymerization. The interstitial oil layer between two hydrogel compartments play a critical role in protecting the encapsulated PTX from harsh conditions while they pass through the stomach. The PTX microcapsules (PC) ameliorates the pathological process of inflammatory bowel disease (IBD) and lowers the disease activity index scores in ICR mice treated with dextran sulfate sodium. PC is shown to repair the tissue damage, decrease the colonic macrophage infiltration responsible for the secretion of pro-inflammatory factors, and effectively mitigate the severity of IBD. Moreover, a gut microbiota analysis reveals that PC ameliorates the dysbacteriosis of specific bacterial genera, including *Bacteroides acidifaciens* and *PAC001809_s*, implying optimization of the gut microorganism's composition and abundance. Thus, we suggest that PC is a potential delivery system of PTX for IBD treatment.

Corresponding author : Chang-Hyung Choi, Sei-Jung Lee

Keywords : Pentoxifylline, PEG shell microcapsules, Inflammatory bowel disease, Microbiome

PS-A-049

Association of haptoglobin phenotype with neurological and cognitive outcome in patients with subarachnoid hemorrhage

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Background: To evaluate the association between haptoglobin (Hp) phenotype and neurological and cognitive outcomes in multiple patients with subarachnoid hemorrhage (SAH).
Method: This preliminary multicenter study enrolled patients with aneurysm SAH between May 2015 and September 2020. The Hp phenotype was identified through Western blot. The relative intensity of α1 in people with Hp2-1 was compared with that of albumin. We used multivariate logistic analysis and Cox proportional-hazard regression to identify risk factors for 6-month and long-term outcomes, respectively.
Result: A total of 336 patients were analyzed, including phenotypic Hp1-1 (n = 31, 9.2%), Hp2-1 (n = 126, 37.5%), and Hp2-2 (n = 179, 53.3%). The Hp phenotype was closely related with 6-month outcome ($p < 0.001$) and cognitive function ($p < 0.013$), and long-term outcome ($p < 0.002$) and cognitive function ($p < 0.001$). Compared with Hp1-1 as the reference value, Hp2-2 showed a significantly increased the risk in 6-month poor outcome (OR: 7.868, 95% CI: 1.764–35.093) and cognitive impairment (OR: 8.056, 95% CI: 1.020–63.616), and long-term poor outcome (HR: 5.802, 95.75–40.95), and cognitive impairment (HR: 7.434, 95% CI: 2.264–24.409). Long-term cognitive impairment based on the Hp phenotype was significantly higher in patients female gender ($p < 0.001$) and under 65 year of age ($p < 0.001$). A lower relative α1/albumin intensity (OR: 0.010, 95% CI: 0.000–0.522) was associated with poor outcome at 6 months but not cognitive impairment in patients with SAH expressing Hp2-1
Conclusion: Hp2-2 increased the risk of poor neurological outcomes and cognitive impairment compared to Hp1-1. In the case of Hp2-1, higher relative α1 intensity was associated with favorable outcome for 6 months.

Corresponding author : Jin Pyeong Jeon

Keywords : Subarachnoid hemorrhage, Haptoglobin, Cognition, Outcome, Intracranial aneurysm

PS-A-050

The combination of prebiotics with probiotic complex affected differently intestinal hydrolase activity, microbial population and immunological biomarkers in SD rats fed an AIN-diet

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Gastrointestinal microbiota plays crucial roles in the host's health. However, probiotics (PRO) did not always have a positive benefit on the host, depending on microbial strains, and the physiochemical properties of prebiotics (PRE), indicating that the properties of PRE in combination with PRO might have different effects on the gut ecology. The aim of study was to assess the effects of insoluble or soluble PRE with PRO on intestinal hydrolase, the fecal microbes, and immunological biomarkers in rats fed an AIN-93G diet. Forty, 8-wk-old SD rats were assigned to 4 groups with 10 replicates in each; cellulose (CELL), cellulose+probiotics (CELPRO), oatmeal (OATS), and oatmeal+probiotics (OATPRO) groups. After 4-wk feeding trial, rats were treated with saline or LPS(1 mg/kg) to examine the alleviating effects of PRO and PRE on immunological responses. There was a significant ($P<0.05$) decrease in feed intake of rats fed the oatmeal diet without affecting growth performance. Blood triglyceride was decreased ($P<0.05$) in rats fed the oatmeal, and aspartate aminotransferase (AST) was decreased ($P<0.05$) in rats fed the PRO supplemented diet. Intestinal maltase, sucrose, and lactase activities were higher ($P<0.05$) in rats fed PRO compared with rats not fed PRO. Rats fed the oatmeal showed a significant increase ($p<0.01$) in the colony forming units (CFU) of Lactobacillus plantarum, Bacillus subtilis, and Saccharomyces cerevisiae compared with those fed cellulose. LPS-treated rats fed PRO showed a significant increase ($p<0.01$) in blood secretory immunoglobulin A compared with those not fed PRO. The LPS-treated rats fed PRO resulted in decreased ($p<0.05$) blood IL-6 compared with those not fed PRO, indicating that dietary PRO alleviated inflammatory responses. Dietary oatmeal increased fecal microbes, and PRO supplement resulted in increased intestinal hydrolase and immune functions of the host, suggesting that soluble PRE with supplemented with PRO could be a more bioactive combination of synbiotics.

Corresponding author : InSurk Jang

Keywords : Prebiotics, Probiotics, Intestinal hydrolase, Microbiota, Immunity

PS-A-051

Mild traumatic brain injury and subsequent acute pulmonary inflammatory response

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Objective: Although the effects of moderate to severe traumatic brain injury (TBI) on acute lung injury are well known, the relevance of mild TBI is not well known. In this study, fluctuations in inflammatory markers and histological changes in the lungs were evaluated to determine whether acute pulmonary inflammatory response occurs after mild TBI
Methods: A mild TBI mouse model (n=24) was induced via open head injury using a stereotactic impactor. The brain and lungs were examined at 6 hours, 24 hours, and 72 hours after mild TBI, and compared with a sham-operated model (n=24). Fluoro-Jade B (FJB) staining and Hematoxylin and Eosin Y (H&E) staining were performed to evaluate each cerebral neuronal degeneration and lung histological structure. quantitative real-time polymerase chain reaction (qRT-PCR) was performed to measure inflammatory cytokines
Results: Increased neuronal degeneration and mRNA expression of IL-6, TNF-α, IL-10 and TGF-βin the brain were observed after mild TBI. IL-6, TNF-α, and TGF-βof mild TBI were significant difference at 24 hours after injury and more pronounced at 72 hours compared with the sham-operated mice. Mild TBI induced acute pulmonary interstitial edema with cell infiltration and alveolar morphological changes in the lungs. In particular, a significant of infiltration of mast cells were observed. Among inflammatory cytokines, TNF-α was significantly increased in the lung at 6 hours, but there was no significant difference at 24 hours and 72 hours after injury
Conclusions: Mild TBI induced acute pulmonary interstitial inflammation and alveolar structiral changes. It has the potential to worsen the patient's prognosis. Further studies on the clinical implications of this associaltion are needed.

Corresponding author : Jin Pyeong Jeon

Keywords : Traumatic brain injury, Acute pulmonary injury, Inflammation

PS-A-052

Involvement of lipocalin-2 in experimental autoimmune uveitis

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Lipocalin-2, a siderophore-binding protein, is an antimicrobial with both proinflammatory and antiinflammatory activities. Lipocalin-2 is also involved in glial activation, matrix metalloproteinase stabilization, and cellular iron flux, which play roles in autoimmune diseases. The present study was performed to determine the expression of lipocalin-2 in the eyes of interphotoreceptor binding protein-induced experimental autoimmune uveitis (EAU) Lewis rats. A significantly elevated serum lipocalin-2 level was also detected in EAU rats by ELISA. Western blotting analysis showed that lipocalin-2 was significantly upregulated in the eyes of EAU rats. Lipocalin-2 immunostaining was detected predominantly in activated glial cells, including glial fibrillary acidic protein (GFAP)-positive astrocytes, glutamine synthase-positive Müller cells, and ionized calcium binding adaptor molecule 1 (Iba1)-positive microglia and macrophages in EAU rats. Taken together, the results presented here show that lipocalin-2 can serve as an early biomarker of the pathogenesis of EAU in rat models.

Corresponding author : Taekyun Shin

Keywords : Experimental autoimmune uveitis, Lipocalin-2, Retina

PS-A-053

Regulation of γc expression via IL-4 signaling in regulatory T cells

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Regulatory T (Treg) cells are critical in maintaining immune tolerance and homeostasis of the immune system. Interleukin-2 (IL-2) receptor signaling is essential for the Treg function and maintenance of forkhead box P3 (Foxp3) expression. Optimal IL-2 signaling is transduced through the IL-2 receptor complex including IL-2Rα, IL-2Rβ and common γ chain(γc). γc is a key component of the receptors for γc cytokines, including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 which are critical for the development and homeostasis of T cells. However, the role and expression of γc in cytokine signaling have not been fully defined. Recently, we reported that γc expression was dynamically regulated during development and activation of T cells and involved in regulation of γc cytokine signaling. Based on previous studies, we specifically evaluated the regulatory mechanism of γc expression by IL-4 signaling in Treg cells. First, we found that only IL-4 of γc cytokines significantly upregulated the γc expression in T cells, but not in Treg cells despite of induction of STAT6 phosphorylation upon IL-4 stimulation. Since GATA-binding protein 3 (GATA3) among factors that are upregulated by IL-4 signaling, we examined the role of GATA3 on regulation of γc expression in IL-4-primed T cells. Using GATA3-specific inhibitor, we found that the expression of γc was not enhanced by IL-4 in the presence of GATA3 inhibitor. Thus, we hypothesized that GATA3 function in Treg cells would be inhibited and Foxp3 would be involved in the inhibition. Relationship data between GATA3 and Foxp3 upon IL-4 stimulation indicates that GATA3-Foxp3 interaction affects upregulation of γc expression in Treg cells. Although further studies are required, our study provides novel insights for the regulatory mechanism of γc expression by IL-4, with proving the regulatory role of GATA3 in Treg cells.

Corresponding author : Changwan Hong

Keywords : Interleukin-4 (IL-4), Common γ chain (γc), Forkhead box P3 (FOXP3), GATA-binding protein 3 (GATA3), Regulatory T cells

PS-A-054

IL-4-induced forkhead box protein O regulates γc expression in T cells

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The common γ chain (γc) is necessary to signal γc cytokines, including Interleukin (IL)-2, -4, -7, -9, -15, and –21, and is critical in the proliferation, differentiation, and survival of T cells. γc deficiency leads to X-linked severe combined immunodeficiency (X-SCID), characterized by a complete absence of T and natural killer cells. Since γc cytokine signaling had been thought to be regulated by cytokine-specific receptor subunits, the regulatory mechanism of γc expression has not been identified, although γc is an indispensable receptor in the immune system. Here, we showed that only IL-4 of γc cytokines significantly upregulated the γc expression in lymph node (LN) T cells. First, to determine the pathway responsible for upregulation of γc by IL-4, we stimulated LNT cells with IL-4 and investigated the expression of major transcription factors (TFs) related to IL-4 signaling pathways such as STAT6, STAT5, AKT, and Foxo. Next, we investigated their roles in the upregulation of γc by IL-4 through their specific inhibitors. Interestingly, STAT6 and Foxo-specific inhibitors suppressed the upregulation of γc by IL-4. We further examined loss-of-function of STAT6 and Foxo with STAT6-deficient (STAT6-/-) and T cell-specific Foxo-deficient (CD4creFoxof/f) mice. In STAT6-/- LNT cells, the expression of γc was still upregulated by IL-4. In contrast, the expression of γc was not significantly enhanced by IL-4 in Foxo-deficient T cells compared with WT T cells. These data show that the Foxo-dependent transcriptional program by IL-4 signaling is essential for regulating the γc expression. In conclusion, the current study has demonstrated that γc expression is upregulated at the transcriptional levels upon IL-4 stimulation and that Foxo TFs are essentially involved in the upregulation of γc gene expression. Identification of the regulatory mechanism of γc expression provides further insight of γc family cytokine responsiveness and its beneficial effects in autoimmune diseases and cancers.

Corresponding author : Changwan Hong

Keywords : T cells, Common γ chain (γc), Interleukin-4 (IL-4), Signal transducer and activator of transcription 6 (STAT6), Forkhead box protein O (Foxo)

PS-A-055

NKP46$^+$NK1.1$^+$ cells derived from definitive hemogenic endothelium of mouse aorta gonad mesonephros were markedly increased by 3-Deazaneplanocin A hydrochloride

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Hemogenic endothelium (HE) is a specialized subset of endothelial cells which can generate both all blood lineage cells and endothelial lineage cells in aorta gonad mesonephros (AGM) of fetal embryo. A hematopoietic lineage cells via HE can exhibit different capability to differentiate into myeloid and lymphoid lineage cells depends on media culture condition. Here, we investigated whether the frequency of NK cells and maturity were affected by 3-Deazaneplanocin A hydrochloride (3-DAH). To end this, fetus embryos (C57BL/6) at day 10.5 were acquired and separately isolated AGM and livers. To evaluate cell activity, FACS analysis and H&E staining were performed using differentiated NK cells according to media condition at day 6 and 9. HE from AGM can abundantly produce all hematopoietic lineage cells including erythroblast, megakaryocyte, and white blood cells. The result showed that the frequency of NK cells was increased in APEL2 based media, compared to DMEM/F12 based media and no significant differences of the frequency showed by 3-DAH. However, Ly49$^+$NK1.1$^+$ cells involving in maturation and NKP46$^+$NK1.1$^+$ cells with natural cytotoxicity receptor were significantly increased from day 4 of differentiation under exposure of 3-DAH, reaching a peak at day 9 (In CD3$^-$Ly49$^+$NK1.1$^+$ cells at day 4, 3-DAH$^+$ group, 13.7 ± 3.5, 3-DAH$^-$ group, 0.2 ± 0.0; In CD3$^-$NKP46$^+$NK1.1$^+$ cells, 3-DAH$^+$ group, 17.7 ± 4.0, 3-DAH- group, 1.6 ± 0.2) (In CD3$^-$Ly49$^+$NK1.1$^+$ cells at day 9, 3-DAH$^+$ group, 12.0 ± 2.8, 3-DAH$^-$ group, 0.3 ± 0.1; In CD3$^-$NKP46$^+$NK1.1$^+$ cells, 3-DAH$^+$ group, 14.7 ± 10.3, 3-DAH$^-$ group, 2.0 ± 0.2), suggesting NK cell activation. Based on this data, we will use 3-DAH and further develop the protocol for NK derived from pluripotent stem cells, which can provide to the basic scientific clue that will make it possible to apply clinical trials in the future.

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Corresponding author : Ji Yoon Lee

Keywords : Hemogenic endothelium, Murine aorta gonad mesonephros, Hematopoiesis, NK cell

PS-A-056

Protective effects of an aqueous extract of *Protaetia brevitarsis seulensis* larvae against radiation-induced testicular injury in mice

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The larvae of *Protaetia brevitarsis seulensis* have been used as a food ingredient and are known for their nutritional value and anti-inflammatory properties. However, whether *P. brevitarsis seulensis* larvae demonstrate protective effects against radiation-induced testicular injury has not been investigated. In this study, the protective effects of an aqueous extract of *P. brevitarsis seulensis* larvae (PBE) against radiation-induced testicular injury were tested. Male C57BL/6 mice were administered PBE (5 or 10 mg/kg) orally for 14 days before exposure to focal pelvic irradiation. Histopathological examinations were conducted at 8 h and 30 d after radiation exposure. PBE pretreatment reduced the radiation-induced apoptosis of germ cells at 8 h after irradiation and significantly increased testis and epididymis weights relative to those of the irradiated control mice at 30 days. PBE protected against histopathological damage and decreased the radiation-induced effects on the epithelium height and seminiferous tubule diameter. Furthermore, the extract ameliorated the radiation-induced morphological abnormalities of sperm cells and improved their motility. It also prevented a decrease in the epididymal sperm count caused by irradiation. Moreover, the extract alleviated the generation of reactive oxygen species, and its antioxidative activity increased in a dose-dependent manner. Among the six major compounds isolated from PBE, benzoic acid and uridine showed the highest antioxidant activities. These results suggest that PBE protects against radiation-induced testicular injury via its antioxidative properties. Thus, it has potential clinical applicability as a neoadjuvant therapy for the prevention of testicular damage caused by cancer radiotherapy.

Corresponding author : Joong-Sun Kim

Keywords : *Protaetia brevitarsis seulensis*, Radiation, Testis, Sperm, Oxidative stress

PS-A-057

Effects of Sparganii Rhizoma on osteoclast formation and osteoblast differentiation and on an OVX-induced bone loss model

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Postmenopausal osteoporosis is caused by an imbalance between osteoclasts and osteoblasts and causes severe bone loss. Osteoporotic medicines are classified into bone resorption inhibitors and bone formation promoters according to the mechanism of action. Long-term use of bisphosphonate and selective estrogen receptor modulators (SERMs) can cause severe side effects in postmenopausal osteoporosis patients. Therefore, it is important to find alternative natural products that reduce osteoclast activity and increase osteoblast formation. Sparganii Rhizoma (SR) is the dried tuberous rhizome of Sparganium stoloniferum Buchanan-Hamilton and is called “samreung” in Korea. However, to date, the effect of SR on osteoclast differentiation and the ovariectomized (OVX)-induced bone loss model has not been reported. In vitro, tartrate-resistant acid phosphatase (TRAP) staining, western blots, RT-PCR and other methods were used to examine the effect of SR on osteoclast differentiation and osteoblasts. In vivo, we confirmed the effect of SR in a model of OVX-induced postmenopausal osteoporosis. SR inhibited osteoclast differentiation and decreased the expression of TNF receptor-associated factor 6 (TRAF6), nuclear factor of activated T cells 1 (NFATc1) and c-Fos pathway. In addition, SR stimulates osteoblast differentiation and increased protein expression of the bone morphogenetic protein 2 (BMP-2)/SMAD signaling pathway. Moreover, SR protected against bone loss in OVX-induced rats. Our results appear to advance our knowledge of SR and successfully demonstrate its potential role as a osteoclastogenesis-inhibiting and osteogenesis-promoting herbal medicine for the treatment of postmenopausal osteoporosis.

Corresponding author : Youngjoo Sohn, Hyuk-Sang Jung

Keywords : Sparganii rhizoma, Bone remodeling, Osteoclast, Osteoblast, Ovariectomized

PS-A-058

Design and evaluation of an AI model for automated tail vein administrator in rodent models

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Drug injection via the tail vein is an essential biological tool for animal experiments modeled on rodents such as rat and mouse. However, since tail vein administration is done by humans, it takes a lot of time and cost for an unskilled person to obtain accurate experimental results. In response to these problems, we propose a Vision machine learning technology of a Vessel-constraint network (U-Net) that utilizes tail vein information with infrared (730nm, 840nm) light emitting diodes. The AI model was designed by training a venous vascular imaging library of rodent tails using a U-Net model. U-net is a powerful tool for biomedical image segmentation, such as recognizing blood vessels in tissue via Convolutional Neural Networks (CNNs). It is a high-precision V/T(vessel/tissue) classification model based on data fusion. Based on the learned U-Net model, we introduced an automatic administrator that determines the ideal position of the needle. This administrator recognizes the image of the tail of a rat with a camera and determines the location of the venous blood vessels based on the learned AI model for administration. It is composed of a collaborative robot that moves the needle to the correct insertion position and an animal movement control device with anesthetic device attached. In the administration experiment using rats, the positional accuracy of repeated injections improved dramatically, and the injection error range was within 0.01mm. These results indicate that U-Net-based AI technology is a cost-effective and convenient method for unskilled researchers to easily administer tail vein injection to rodents without special skills, and can reduce the stress and sacrifice of experimental animals.

Corresponding author : Sung Gurl Park

Keywords : Tail vein, Injector, Automated robot, Vision machine learning, Rat

PS-A-059

Metabolites analysis and pharmacokinetic studies of [^{18}F]FP-CIT in preclinical models

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Various radiopharmaceuticals are being developed to diagnose Parkinson's disease with several targeting molecules (eg. TSPO, mGluR5, etc). In this study, the metabolites and pharmacokinetic properties of [^{18}F]FP-CIT (PDVUE®) that targeting the dopamine transporter were analysed in animal models.
C57BL/6 mice were injected [^{18}F]FP-CIT and sacrificed for biodistribution and excretion studies. Metabolites of FP-CIT were studied with SD rats that injected cold authentic form. Harvested samples (Blood, brain, liver and urine) were analysed using HPLC. To evaluate uptake efficacy at the striatum, PET/CT imaging of [^{18}F]FP-CIT was performed on 6-OHDA induced model.
Rapid absorption and distribution of [^{18}F]FP-CIT was observed in the brain, especially 23.50 ± 12.46 % ID/g in striatum 1 min post i.v. injection. The radioactivity of [^{18}F]FP-CIT in brain was rapidly excreted and decreased by passive diffusion. After 360 min, it was measured that 21.46 ± 9.53% of injected radioactivity was excreted by urine and feces. The metabolites of the FP-CIT cold compound were Nor-β-CIT, β-CIT Acid, and Nor-β-CIT Acid, and it was confirmed that there was no difference from the major metabolites of FP-CIT in the previous studies. In the 6-OHDA model, [^{18}F]FP-CIT showed a significant difference uptake at lesion striatum sites compared with intact sites.
The pharmacokinetic properties of normal rodents and the results of the contrast uptake at the striatum of 6-OHDA model indicate that the targeting affinity of [^{18}F]FP-CIT to the dopamine transporter, with the similar metabolites thatenhanced high specific radioactivity. As a result of this studies, [^{18}F]FP-CIT has similar metabolites to [^{123}I]FP-CIT, but it is possible to obtain higher quality images due to structural features and low toxicity due to rapid clearance.

Corresponding author : Yong Jin Lee

Keywords : Parkinson's disease, [^{18}F]FP-CIT, PET/CT, Pharmacokinetic study

PS-A-060

Albiflorin promotes osteoblast differentiation and healing of rat femoral fractures through enhancing BMP-2/Smad and Wnt/β-Catenin signaling

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Fracture healing is related to osteogenic differentiation and mineralization. Recently, due to the unwanted side effects and clinical limitations of existing treatments, various natural product-based chemical studies have been actively conducted. Albiflorin is a major ingredient in *Paeonia lactiflora*, and this study investigated its ability to promote osteogenic differentiation and fracture healing. To demonstrate the effects of albiflorin on osteoblast differentiation and calcified nodules, alizarin red S staining and von Kossa staining were used in MC3T3-E1 cells. In addition, BMP-2/Smad and Wnt/β-catenin mechanisms known as osteoblast differentiation mechanisms were analyzed through RT-PCR and western blot. To investigate the effects of albiflorin on fracture healing, fractures were induced using a chainsaw in the femur of Sprague Dawley rats, and then albiflorin was intraperitoneally administered. After 1, 2, and 3 weeks, bone microstructure was analyzed using micro-CT. In addition, histological analysis was performed by staining the fractured tissue, and the expression of osteogenic markers in serum was measured. The results demonstrated that albiflorin promoted osteoblastogenesis and the expression of RUNX2 by activating BMP-2/Smad and Wnt/β-catenin signaling in MC3T3-E1 cells. In addition, albiflorin upregulated the expression of various osteogenic genes, such as alkaline phosphatase, OCN, bone sialoprotein, OPN and OSN. In the femur fracture model, micro-CT analysis showed that albiflorin played a positive role in the formation of callus in the early stage of fracture recovery, and histological examination proved to induce the expression of osteogenic genes in femur tissue. In addition, the expression of bone-related genes in serum was also increased. This suggests that albiflorin promotes osteogenesis, bone calcification and bone formation, thereby promoting the healing of fractures in rats.

Corresponding author : Hyuk-Sang Jung, Youngjoo Sohn

Keywords : Paeonia lactiflora, albiflorin, Osteoblast, Bone morphogenetic protein 2, Runt-related transcription factor 2

PS-A-061

Effects of chloroform fraction of *Fritillariae Thunbergii* Bulbus on atopic symptoms in a DNCB-induced atopic dermatitis-like skin lesion model and in vitro models

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Fritillariae thunbergii Bulbus (FT) has traditionally been used to treat symptoms such as purulent pneumonia. Efficacy of FT for atopic dermatitis (AD) has not yet been proven. The anti-inflammatory effects of FT-Et and FT-Cl on AD was observed using 1-chloro-2,4-dinitrobenzene (DNCB)-induced AD-like skin lesion model in vivo, and HaCaT and RBL2H3 cells in vitro. In vivo, FT was topically applied to the skin lesion for 35 days. Epidermis and dermis thickness, scratching behavior, infiltration of inflammatory cells, skin barrier proteins, serum total IgE, and cytokines levels in skin lysates were measured. In vitro, TARC, MDC, and IL-4 were analyzed using ELISA in HaCaT cells. β-hexosaminidase and IL-4 were measured in RBL2H3 cells. The expression of skin barrier-related proteins such as filaggrin, loricrin, involucrin, and aquaporin-3 was measured by PCR. Phosphorylation of MAPKs was analyzed using western blot technique. In the AD-like skin lesion model, FT-Cl significantly reduced ear swelling, scratching behavior, SCORAD index, epidermal thickness, infiltration of inflammatory cells, loss of skin barrier proteins, inflammatory cytokines levels in skin lysates, and serum total IgE. FT-Et inhibited the infiltration of mast cells and CD8+ cells and decreased the loss of skin barrier proteins. In TNF-α/IFN-γ-stimulated HaCaT cells, FT-Cl inhibited TRAC, MDC, and IL-4 expression and upregulated the expression of skin barrier-related proteins, whereas FT-Et inhibited the expression of TRAC and MDC and increased the expression of skin barrier-related proteins at high concentrations. In RBL2H3, FT-Cl downregulated β-hexosaminidase and IL-4 expression. In addition, FT-Cl inhibited the phosphorylation of ERK and p-38 in HaCaT and RBL2H3 cells. Collectively, FT-Cl showed better effect than FT-Et in vivo and in vitro. These results suggest that a specific component present in FT-Cl acted against AD. Future research should focus on the analysis of components contained in FT-Cl and the anti-inflammatory effects of the active ingredient.

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Keywords : Fritillariae thunbergii Bulbus, DNCB, Atopic dermatitis-like skin lesion, HaCaT, RBL2H3

PS-A-062

Association between sex-biased Cux2 expression and pancreatic cell damage in type 2 diabetic mice

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Type 2 diabetes mellitus is a metabolic disease characterized by hyperglycemia. The prevalence of type 2 diabetes in men is higher than that in women at the same age. Thus, gender is one of the important key factor on metabolic function. This study aimed to elucidate the association between Cux2 gene and pancreatic cell damage in type 2 diabetic mice. Type 2 diabetes was induced by administration of low-dose streptozotocin (STZ, 40mg/kg 5times every other day). Fasting blood glucose was highest in male (M-STZ) followed by ovariectomized female (FOVX-STZ) and female (F-STZ). STZ groups showed impaired glucose tolerance after 4 and 8 weeks of STZ treatment. Beta cell function was significantly decreased in M-STZ and FOVX-STZ compared to each control groups after 4 weeks of STZ administration, whereas insulin sensitivity was significantly decreased in F-STZ and FOVX-STZ. The size of pancreatic islets in STZ groups decreased significantly than control groups at 8 weeks after STZ treatment. At 4 and 8 weeks, the intensities of glucagon and somatostatin were significantly higher in M-STZ and FOVX-STZ compared to controls, indicating that pancreatic cell rearrangement occurred. The Cux2 showed female specific expression and co-localized in glucagon cells. The expression of Cux2 in F-STZ and FOVX-STZ groups increased significantly than control groups at 4 and 8 weeks. These results suggest that female mouse is more resistance to STZ though beta cell repair by Cux2.

Corresponding author : Jun Gyo Suh

Keywords : Type 2 diabetes, Gender difference, Cux2, Insulin resistance, Beta cell function

PS-A-063

Phenotyping of fecal microbiota of Korean wild mouse (KWM/Hym)

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Laboratory mice are bred in a strictly controlled environment, but humans are not, so it is difficult to apply the results of various metabolic or immunological studies using mice to humans. In order to overcome this situation, Korean wild mice were captured from Chuncheon (South Korean) and the strain (KWM/Hym) are maintaining in Laboratory Animal Resource Center of Hallym University. Since fecal microbiota plays an important role in the biological function of mice, we analyzed the fecal microbiota from three types of strains, including KWM/Hym, specific pathogenic free KWM (SKWM/Hym) and C57BL/6J (B6). The alpha diversity, which describe the abundance and diversity of microbiota, was significantly greater in B6 than the KWM/Hym. The beta diversity, which describe dissimilarity and distance of each set, indicated a distinct clustering of the fecal microbiota between B6 and SKWM/Hym but not KWM/Hym. When the microbiome of each strain was compared with the human gut microbiota, KWM/Hym showed the most similar microbiome composition to that of humans. When each strain was compared at the phylum and genus levels, *Proteobacteria* was the most dominant phylum, followed by *Tenericutes* and *Verrucomicrobia* in the KWM/Hym, whereas *Actinobacteria* and *Cyanobacteria* were abundant phylum in B6 and SKWM/Hym. At the genus level, *Bacteroides* was the most enriched genus, followed by *LARJ_g* and *Lonicatena* in KWM/Hym. In conclusion, Korean wild mice can be utilized as new biological research resources that can overcome the limitations of the laboratory inbred mice.

Corresponding author : Jun Gyo Suh

Keywords : Korean wild mice (KWM/Hym), Fecal microbiota, *Proteobacteria*, *Actinobacteria*, *Cyanobacteria*

PS-A-064

Neuroprotective effects of Populus tomentiglandulosa on neurotoxicity in an amyloid beta-induced Alzheimer's disease mouse

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Accumulation of amyloid beta (Aβ) in the brain is a common cause of Alzheimer's disease (AD). Populus tomentiglandulosa (PT) is cultivated in Korea, it has several beneficial effects such as antioxidants. In our previous study, administration of ethyl acetate fraction from PT (EFPT) improved cognitive function in $A\beta_{25-35}$-induced AD mouse. In this study, we investigated the neuroprotective mechanisms of EFPT against neurotoxicity in an $A\beta_{25-35}$-induced AD mouse model. We orally administered EFPT at doses of 50 and 100 mg/kg/day for 14 days in an $A\beta_{25-35}$-induced AD mouse. The amyloidogenesis-related proteins such as amyloid precursor protein, beta-secretase, presenilin 1, and presenilin 2 in the brain significantly increased by intracerebroventricular injection of $A\beta_{25-35}$. However, administration of EFPT significantly down-regulated amyloidogenic pathway-related protein expressions, compared with Aβ-induced control group. In addition, the EFPT-administered group down-regulated inflammatory mediators including inducible nitric oxide synthase and cyclooxygenase-2 (COX-2) in the brain of AD mice. In particular, the EFPT-administered group inhibited protein expression of COX-2 in a dose-dependent manner. Furthermore, administration of EFPT inhibited apoptosis-related proteins in the brain of AD mice, compared with Aβ-induced AD mice. In particular, EFPT-administered groups dose-dependently decreased protein expression of poly (ADP-ribose) polymerase in the brain of AD mice. Therefore, this study indicated that the administration of EFPT attenuated neurotoxicity by down-regulation of amyloidogenesis, neuroinflammation, and neuronal apoptosis-related proteins in the brain of $A\beta_{25-35}$-induced AD mice. In conclusion, we suggest that PT would be considered a potential therapeutic material for AD.

Corresponding author : Eun Ju Cho, Hyun Young Kim

Keywords : Amyloid beta, Alzheimer's disease, Populus tomentiglandulosa, Neurotoxicity, Neuroinflammation

PS-A-065

D-Allulose ameliorates hyperglycemia through IRE1α sulfonation-RIDD- sirt1 decay axis in the skeletal muscle

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Aims: The skeletal muscle maintains glucose disposal *via* insulin signaling and glucose transport. The progression of diabetes and insulin resistance is critically influenced by endoplasmic reticulum (ER) stress. d-Allulose, a low-calorie sugar substitute, has shown crucial physiological activities under conditions involving hyperglycemia and insulin resistance. However, the molecular mechanisms of d-allulose in the progression of diabetes have not been fully elucidated. Here, we evaluated the effect of d-allulose on hyperglycemia-associated ER stress responses in human skeletal myoblasts (HSkM) and *db/db* diabetic and high-fat diet-fed mice.
Results: d-allulose effectively controlled glycemic markers such as insulin and hemoglobin A1c (HbA1c), showing anti-diabetic effects by inhibiting the disruption of insulin receptor substrate (IRS)-1 tyrosine phosphorylation and glucose transporter 4 (GLUT4) expression, in which the phosphatidylinositol-3 kinase (PI3K)/protein kinase B (Akt) pathway is involved. The levels of glucose dysmetabolism-based NADPH oxidase, such as NADPH-dependent oxidoreductase (Nox) 4, were highly increased, and their interaction with IRE1α and the resultant sulfonation-regulated IRE1-dependent decay (RIDD)-*Sirt1* decay were also highly increased under diabetic conditions, which were controlled with d-allulose treatment. Skeletal muscle cells grown with a high glucose medium supplemented with d-allulose showed controlled IRE1α sulfonation-RIDD-*Sirt1* decay, in which Nox4 was involved.
Innovation and Conclusion: The study observations indicate that d-allulose contributes to the muscular glucose disposal in the diabetic state where ER-localized Nox4-induced IRE1α sulfonation results in the decay of *Sirt1*, a core factor for controlling glucose metabolism.

Corresponding author : Han-Jung Chae

Keywords : NADPH oxidase, IRE1α, *Sirt1*, Type 2 diabetes

PS-A-066

The desalted Salicornia herbacea water extract prevent osteoporosis by reduced osteoclast differentiation and ROS

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Bone homeostasis is maintained by the balance of osteoclast and osteoblast. When a woman enters menopause, due to lack of estrogen, osteoclast activity is promoted and osteoblast activity is decreased, caused to osteoporosis. Therefore, resolving this imbalance is very important in the treatment of osteoporosis. In this study, we investigated whether desalted Salicornia herbacea water extract can inhibit the differentiation and activity of osteoclasts induced by RANKL. We found that desalted Salicornia herbacea water extract significantly inhibited of osteoclast differentiation in TRAP staining. Also, desalted Salicornia herbacea water extract significantly inhibited of osteoclast bone resorptive activities in bone resorption assay. Desalted Salicornia herbacea water extract was reduced of mRNA expression of NFATc1, DC-stamp, TRAP, required for osteoclast differentiation. Furthermore, Desalted Salicornia herbacea water extract was decreased TRAF6 and PRMT1 protein level in osteoclast. Osteoclasts need to many energies, so mitochondria activity increased. We identified that Desalted Salicornia herbacea water extract inhibition of ROS. Next, we accomplished in vivo using ovariectomized (OVX) mouse to investigating (OVX) mediated bone loss prevention, we found that Desalting Salicornia herbacea water extract prevented OVX mediated bone loss. We also demonstrated that desalting Salicornia herbacea water extract major compounds, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, also significantly inhibited of osteoclast differentiation in trap staining and decreased of mRNA expression of NFATc1. The results indicated that Desalted Salicornia herbacea water extract was prevented osteoporosis by reduced osteoclast differentiation and ROS.

Corresponding author : Jong-Hwan Park

Keywords : Osteoclast, Osteoporosis

PS-A-067

Visualization of interscapular brown adipose tissue (iBAT) with TSPO targeting ligand by Cerenkov luminescence imaging (CLI) in the UCP1 Thermomouse

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Introduction: Uncoupling protein 1 (UCP1) is used as a biomarker of interscapular brown adipose tissue (iBAT), and reporter mice (UCP1-Luc2-tdTomato expressing mouse, Thermomouse) have been established. [^{18}F]FDG-PET is known as the standard imaging method for obtaining iBAT, but higher glucose uptake in the brain or heart interferes with iBAT imaging. Recently, the translocator protein (TSPO) targeting probe such as [^{18}F]fluoromethyl-PBR28-d_2 ([^{18}F]fm-PBR28-d_2) has been suggested to visualize iBAT. Cerenkov luminescence imaging (CLI) is an emerging optical imaging method that captures visible photons emitted by Cerenkov radiation, and it is proposed as an alternative modality for PET. In this research, we aim to compare the between [^{18}F]FDG/or [^{18}F]fm-PBR28-d_2 imaging for iBAT using PET and CLI in UCP1 Thermomouse.
Methods: UCP1 Thermomouse was established by inserting the Luc2-T2A-tdTomato cassette into the initiation codon of the *Ucp1*-coding sequence in exon 1. Bioluminescence imaging (BLI) and CLI was monitored using an IVIS 100 imaging system. PET scans were acquired by a SimPET. Mice were anesthetized with isoflurane and intravenously injected with 11.1 to 14.8 MBq of [^{18}F] FDG or [^{18}F]fm-PBR28-d_2.
Results: Bioluminescence or fluorescence signals were observed in iBAT of Thermomouse. Higher expressions of UCP1 and TSPO were observed in iBAT. [^{18}F]FDG-PET/CLI signals were observed not only in iBAT as well as in brain. However, TSPO-PET/CLI signals in iBAT were clearly observed without brain uptake. TSPO-PET/CLI signals were higher in the high UCP1 expression group compared to the low UCP1 expression group, but there was no difference of [^{18}F]FDG-PET/CLI signals between the different UCP1 expression levels. [^{18}F]fm-PBR28-d_2 with higher molar activity enabled better CLI as well as PET. We observed that the TSPO-PET/CLI signals from iBAT were higher in the cold exposed group than in the thermoneutral group. We also observed iBAT activity with TSPO-PET and CLI under various anesthesia conditions.
Conclusions: Our results show that the TSPO targeting probe, [^{18}F]fm-PBR28-d_2, reflects UCP1 expression in iBAT better than [^{18}F]FDG, indicating that TSPO-CLI can be used as an alternative imaging method to replace TSPO-PET for iBAT imaging.

Corresponding author : Hyewon Youn

Keywords : Interscarpular brown adipose tissue (iBAT), Uncoupling protein 1 (UCP1), Translocator protein (TSPO), Positron emission tomography (PET), Cerenkov luminscence imaging (CLI)

PS-A-068

Codonopsis laceolata water extract ameliorates asthma severity by Inducing Th2 Cells' and pulmonary epithelial Cells' apoptosis via NF-κB/COX-2 pathway

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Asthma is an incurable pulmonary disease with several symptoms; abnormal breathing, cough, and sleep apnea to death and the population of asthma patients has been increased in the worldwide. However, there are many adverse effects in current drugs we have tried to develop anti-asthmatic agents from natural products like Codonopsis laceolata. To define the anti-asthmatic effect and the mechanism of Codonopsis laceolata that animal study was conducted using with differential cell count of BALF, serum IgE level, morphological changes in pulmonary system, Th2 cell transcription factor (GATA-3), and apoptotic pathway (NF-kB/COX-2). Codonopsis laceolata significantly suppressed the representative asthmatic changes such as airway remodeling, mucous hypersecretion, epithelial hyperplasia, and inflammatory cells infiltration in the respiratory system. It suppressed the levels of GATA-3, IL-4, and IL-13. The down-regulation of Th2-related factors such as GATA-3, IL-4, and IL-13 results from the stimulated apoptosis of Th2 cells and epithelial cells via decreasing the levels of NF-kB and COX-2. We concluded that Codonopsis laceolata might be a promising anti-asthmatic drug.

Corresponding author : Seung-Sik Cho, Dae-Hun Park

Keywords : Codonopsis laceolata, Apoptosis, NF-κB/COX-2 pathway, Asthma severity

PS-A-069

Usage of natural volatile organic compounds as biological modulators of disease

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Plants produce a wide variety of natural volatile organic compounds (NVOCs), many of which are unique to each species. These compounds serve many purposes, such as fending off herbivores and adapting to changes in temperature and water supply. Interestingly, although NVOCs are synthesized to deter herbivores, many of these compounds have been found to possess several therapeutic qualities, such as promoting nerve stability, enhancing sleep, and suppressing hyperresponsiveness, in addition to acting as antioxidants and anti-inflammatory agents. Therefore, many NVOCs are promising drug candidates for disease treatment and prevention. Given their volatile nature, these compounds can be administered to patients through inhalation, which is often more comfortable and convenient than other administration routes. However, the development of NVOC-based drug candidates requires a careful evaluation of the molecular mechanisms that drive their therapeutic properties to avoid potential adverse effects. Furthermore, even compounds that appear generally safe might have toxic effects depending on their dose, and therefore their toxicological assessment is also critical. In order to enhance the usage of NVOCs this short review focuses not only on the biological activities and therapeutic mode of action of representative NVOCs but also their toxic effects.

Corresponding author : Min-Jae Lee, Dae-Hun Park

Keywords : Natural volatile organic compound (NVOC), Biological modulator, Safety

PS-A-070

Transcranial alternating current stimulation exerts symptom relieving effects in the brain of a rat transient MCAo model

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Ischemic strokes occur when the blood supply to part of the brain is interrupted or reduced, preventing brain tissue from getting oxygen and nutrients. Brain cells begin to die in minutes. Stroke is a medical emergency, and prompt treatment is crucial. Also, early action can reduce brain damages and other complications.
Transcranial Alternating Current Stimulation (tACS) is a device that applies a low-intensity sinusoidal electrical current to the brain through electrodes on the scalp. The technique can be painless and is thought to boost the brain's own oscillations, which can be used to treat ischemic disease or enhance brain function.
Therefore, we conducted transcranial alternating current stimulation(tACS) in a rat middle cerebral artery occlusion (MCAo) model for stroke to study the effect of transcranial alternating current stimulation(tACS) on relieving the course of ischemic stroke.
Experiments were conducted with MCAo+tACS group, MCAo+Sham group, Control + tACS group, and Control + Sham group. After MCAo surgery, stimulation intensity of 1.0 to 2.0 mA, a frequency of 10 Hz for 20 min were applied. To evaluate the ischemic area in the brain, 2,3,5- triphenyl tetrazolium chloride reaction test was performed and behavioral tests were also confirmed the symptomatic relief in ischemic stroke model using transcranial alternating current stimulation (tACS).

Corresponding author : Woori Jo, KilSoo Kim

Keywords : MCAo, Ischemic stroke, Transcranial Alternating Current Stimulation (tACS)

PS-A-071

Characterization of rabbit diabetic mellitus model using alloxan

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Diabetes mellitus is a chronic disease with hyperglycemia and complications followed by hyperglycemia. Rabbit is a suitable for researching diabetic complications because it has a human-like lipid profile than those of rodents. Streptozotocin (STZ) and alloxan (AL) are used in producing animal models of diabetes, for their toxicity to pancreatic beta cells. We compared the ability of STZ and AL to induce DM in rabbits. Six male New Zealand White (NZW) rabbits were divided into three groups, STZ group, STZ-AL group, and AL group, with 2 rabbits each. STZ group were administrated 65 mg/kg of STZ through Intravenous (IV) route, and 17 days after the first STZ administration, 100 mg/kg of STZ were IV injected. STZ-AL group were injected 50 mg/kg of AL through IV 20 days after the second STZ administration. STZ administration methods were the same in the STZ group and STZ-AL group. AL group were injected 50 mg/kg of AL only through IV. One rabbit did not show hyperglycemia after first AL administration and the rabbit was injected second 50 mg/kg of AL through IV 6 days after first AL injection. Blood glucose levels were measured by Accu-Check® performa and higher than 200mg/dl is considered hyperglycemia. In the STZ group and STZ-AL group, the first administration of STZ failed to induce hyperglycemia at any period, and the second administration of STZ induced short-term hyperglycemia in one rabbit. In the STZ-AL group, stable hyperglycemia was induced after a single dose of AL administration. In the AL group, one rabbit was induced stable hyperglycemia after a single dose of AL administration. Another rabbit did not show hyperglycemia in the first AL administration, but the second AL administration induced stable hyperglycemia. This result suggests that AL is an appropriate choice for producing diabetes models in rabbits, compared to STZ.

Corresponding author : Woori Jo, KilSoo Kim

Keywords : Diabetes, STZ, Alloxan, Rabbit, Hyperglycemia

PS-A-072

HPVSC-derived cyclophilin-A can recover ovarian function in mice with cyclophosphamide-induced premature ovarian failure

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Primary Ovarian Failure (POF) is the depletion or dysfunction of ovarian follicles with cessation of menses before the age of 40. This could be caused by ovarian damage and chemotherapy. Cyclophosphamide (CP), widely used in women's cancer treatment, provokes difficulties in endocrine and ovarian functions, producing a menopause-like condition after chemotherapy. Previously we demonstrated that cyclophilin-A (CYP-A), secreted from human perivascular stem cells (hPVSCs), restores the function of the damaged uterus. Thus, this study aimed to examine whether CYP-A could rescue POF-like ovarian impairment in mice that received CP.

Nine-week-old female mice (C57BL/6) were randomly divided into saline, CP, CP+hPVSCs, and CP+CYP-A groups. Single injection of CP (240 mg/kg) was intraperitoneally administrated to mice. hPVSC ($1x10^6$) or CYP-A (10 ng) were delivered weekly three times from a week after CP injection. Three weeks later, ovaries were collected, sectioned, and then stained with H&E for follicle counts. PMSG followed by hCG with a 48h interval was given to the mice for ovulation induction. Ovulated oocytes were fertilized in vitro and cultured up to the blastocyst stage. The rates of fertilization and blastocyst formation and ICM/TE ratio in blastocysts by OCT4 immunostaining were examined in all groups.

CP significantly depleted not only the total number but also the number of follicles in all stages of follicle development. CYP-A and PVSCs effectively recovered both numbers in the ovary ($p<0.01$). CP significantly reduced the fertilization rate, but PVSCs and CYP-A tended to restore it in the CP group. Whereas blastocyst development was comparable between all groups, CYP-A significantly increased ICM/TE ratio that was reduced in the blastocyst from CP mice ($p<0.001$). Collectively, CYP-A secreted from PVSCs provides beneficial effects to restoring impaired ovarian function in CP mice.

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Keywords : Premature ovarian failure, Cyclophilin A, Follicle development, In vitro fertilization, Developmental competence

PS-A-073

LncRNA *PTPRE-AS1* expression is significantly increased in IL-4 induced M2 macrophages

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Macrophages display a series of continuous functional states from classically activated M1 (pro-inflammatory) to alternatively activated M2 (anti-inflammatory). Macrophage polarization toward M2 plays an essential role in tissue repair and wound healing following inflammation. Long non-coding RNAs (lncRNAs), longer than 200 nucleotides, are not normally translated into proteins. The critical roles of lncRNAs in regulating the inflammatory responses of macrophages have been discovered. Thus, this study examined expression profiles of lncRNA *PTPRE-AS1* in mouse tissues and in bone marrow-derived macrophages (BMDM) and RAW 264.7 cells, a mouse macrophage cell line, under various conditions that influence macrophage polarization. Tissues were collected from 7-week-old C57BL/6 mice to examine expression profiles of lncRNA *PTPRE-AS1*. To obtain BMDM, bone marrow was extracted from the femur and red blood cells were lysed. After culturing the cells from bone marrow for 7 days in a medium containing M-CSF (50 ng/ml), the efficiency of macrophage differentiation was evaluated by FACS analyses. BMDM and RAW 264.7 cells were seeded in 12 wells (2 x 10^5/well) and treated with IL-4 (20 ng/ml) for 24 h to induce M2 polarization. Cells were harvested at various time points (1, 2, 6, and 24 h) for RNA preparation to perform RT-PCR. RT-PCR results showed that lncRNA *PTPRE-AS1* is highly expressed only in the thymus, but not in other tissues examined. FACS analyses showed that our BMDM are 98.41% F4/80+ and 99.3% CD11b. IL-4 treatment significantly increased the expression levels of M2 markers, such as Socs1 and Mrc1 in both cells. The expression levels of lncRNA *PTPRE-AS1* were considerably elevated by IL-4 treatment in both cells in a time-dependent manner. Interestingly, IL-4 induced expression of lncRNA *PTPRE-AS1* in BMDM was significantly higher than that in RAW264.7 cells. Functional studies for lncRNA *PTPRE-AS1* are further warranted to understand its major actions during macrophage polarization.

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Keywords : Long non-coding RNA, Macrophage, M2 polarization, *PTPRE-AS1*

PS-A-074

Inhibitory effects of endarachne binghamiae extract on inflammation and obesity

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In this study, the anti-inflammatory and anti-obesity effect of Endarachne binghamiae extract (EB-WE) was investigated using LPS-induced RAW 264.7 cells and high-fat diet (HFD)-fed mice respectively. PC-WE was prepared by extracting in hot water (75°C) for 12 hr. Treatment of EB-WE significantly inhibited the production of inflammatiory mediatore such as nitric oxide (NO), tumor-necrosis factor-alpha (TNF-a) and interleukin-6 (IL-6) from lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. In addition, treatment of EB-WE effectively inhibited the differentiation of 3T3-L1 pre-adipocytic cells. In animal experiment for HFD-induced obesity, administration of EB-WE significantly suppressed the gain of body weight of HFD-fed ICR mice. Administration of EB-WE also significantly reduced the accumulation of fat in abdomen and epididymis. The assay of blood lipids revealed that EB-WE-fed mice showed a significant reduction of the level of total cholesterol (T-CHO) and triglyceride (TG). Furthermore, administration of EB-WE markedly lowered the level of glutamic oxaloacetic transaminase (GOT), glutamlc pyruvic transamlnase (GPT), creatinine (CRE) and blood urea nitrogen (BUN), suggesting that EB-WE ameliorated hepatotoxicity and nephrotoxicity induced by obesity in mice. These results indicate that EB-WE is a promising candidate applicable to the development of anti-obesity and anti-inflammatory nutritional foods and drugs. This research was a part of the project titled 'Bioactive material for algae-based bio-health care substantiation (Project No. 20210656)', funded by the Ministry of Oceans and Fisheries, Korea.

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Keywords : Endarachne binghamiae, Obesity, Hyperlipidemia, Inflammation, Functional food

PS-A-075

Melatonin significantly down-regulates long non-coding RNA *Cox2* in M1 macrophages stimulated with LPS

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Macrophages show a wide spectrum of plasticity from classically activated M1 (pro-inflammatory) to alternatively activated M2 (anti-inflammatory), depending on the microenvironment. Chronic inflammation could occur partly due to a high M1:M2 macrophage ratio. Melatonin, a hormone released by the pineal gland, promotes M2 polarization. Long noncoding RNAs (lncRNAs), more than 200 nucleotides, that do not translate proteins play important roles in various biological processes and diseases at multiple levels, including transcriptional, post-transcriptional, and chromatin modifications. LncRNA *Cox2* was reported to polarize macrophages toward M1 phenotypes. Thus, we examined whether melatonin regulates expression profiles of lncRNA *Cox2* in macrophages during the conversion of macrophage polarization.

Various tissues were collected from 8-week-old C57BL/6 mice to examine expression profiles of lncRNA *Cox2* in mice. RAW 264.7 cells, murine macrophage cell line, were stimulated with lipopolysaccharide (LPS) (100 ng/ml) or LPS+melatonin (230 ug/ml) for 12 h otherwise indicated. Total RNA and proteins were harvested from RAW 264.7 cells treated with LPS or LPS+melatonin for RT-PCR and Western blotting, respectively.

LPS significantly increased expression levels of M1 macrophage markers, such as *Il-1b, Cox2, Il-6, Tnf-a, Inos*, and *Socs3* in RAW264.7 cells. However, melatonin considerably reduced mRNA expression levels of the M1 markers and increased those of M2 markers, such as *Scocs1* and *Mrc1* in RAW264.7 cells treated with LPS. Western blotting for polarization markers reinforced that LPS induces M1 macrophage polarization and melatonin converts LPS-induced M1 macrophages to M2. LncRNA *Cox2* was highly expressed in the kidney and spleen among the tissues examined. RT-PCR and real-time RT-PCR showed that LncRNA *Cox2* expression is gradually increased by LPS treatment in a time-dependent manner. Interestingly, melatonin dampened the expression of LncRNA *Cox2* which is significantly increased in RAW264.7 cells by LPS. These results suggest that LncRNA *Cox2* may function during the conversion of macrophage polarization.

Corresponding author : Haengseok Song

Keywords : Macrophage, Macrophage Polarization, LncRNA *Cox2*, Inflammation

PS-A-076

Cardiovascular hemodynamic evaluation using PV loop analysis in rats

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Acute myocardial infarction (AMI) is one of the leading causes of increased cardiovascular morbidity and mortality worldwide. In the course of new drug development of cardiovascular disease, the characterization of animal disease model and evaluation methods is important to reflect the course of cardiovascular disease in human. Echocardiography and cardiac MRI are the two major non-PV modalities used for assessing cardiac function in animal model. Cardiac pressure-volume(PV) loop analysis is the reference standard for studying the cardiovascular implications of clinical perturbations and is a benchmark for comparisons with noninvasive alternatives including echocardiography, cardiac magnetic resonance imaging(MRI). In this study, to acquire hemodynamic and contractile data directly from the animal heart, PV catheters were used. Similar to echocardiography and MRI, PV catheters are possible to assess end-diastolic volumes and end-systolic volumes, and from these results, we can calculate the stroke volume, ejection fraction, and cardiac output, and also hemodynamic parameters associated with the contractility including stroke work, Tau, dP/dt Max and Min, contraction time, and relaxation time. Furthermore, measuring load-independent parameters of contractility is one of the main advantages of PV loop analysis. Preload reduction techniques called inferior vena cava (IVC) occlusion is the gold-standard method for acquiring load-independent measures of contractility. Preload is the amount of stretch that a left ventricle experiences prior to a contraction. In summary, cardiovascular hemodynamic evaluation using PV loop analysis can be further utilized in preclinical studies of cardiovascular drug candidate development.

Corresponding author : Woori Jo, KilSoo Kim

Keywords : Cardiovascular disease, Hemodynamic parameter, PV loop anlaysis

PS-A-077

Anti-obesity effects of *Lactobacillus paracasei* subsp. *paracasei*, L. casei 431 on high fat diet-induced obese rats

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Obesity is a chronic disease associated with inflammatory reactions and it causes several metabolic syndromes. In addition, its severity is increasing worldwide and has become an epidemic.
Recently, it has become clear that changes in the composition of the gastrointestinal microbiota can contribute to the development of metabolic disease, and numerous studies have shown the effect of probiotics on this disease. *Lactobacillus paracasei* subsp. *paracasei*, L. casei 431 (L. casei 431) is a strain that is isolated from infant feces and is associated with the regulation of the host's immune system and is observed to play a functional role in immunity and intestinal health. However, the effect of L. casei 431 on obesity is not yet known, so the effect of anti-obesity was investigated in this experiment.
High-fat diet induced obese rats were treated L. casei 431 for 10 weeks. All rats were measured body fat mass every two weeks and it was observed to be significantly suppressed the increase of body fat mass dose-dependently. In addition, epididymal fat and retroperitoneal fat were also reduced in L. casei 431 administered groups. Comparison of liver and epididymal adipose tissues with hematoxylin and eosin staining, it was confirmed that the L. casei 431 treated groups reduced the overall number of lipid droplets and the size of adipocyte.
A comprehensive analysis of these results indicated that L. casei 431 activates body fat decomposition in rats that induce obesity using a high-fat formula. This suggests that L. casei 431 can be an alternative solution to prevent obesity and related diseases.

Corresponding author : Yun Jeong Shin

Keywords : Anti obesity, Probiotics, *Lactobacillus paracasei*, High fat diet

PS-A-078

Effect of glycemic variability on infarct volume in ischemic stroke model

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Diabetes mellitus(DM) is a widely known risk factor for cardio-cerebrovascular disease. Chronic hyperglycemia(CH) is a major risk factor for the development of complications in DM. However, recent clinical data indicate that glycemic variability(GV) is associated with increased risk of hypoglycemia, microvascular and macrovascular complications in patients with diabetes, independently of glycated hemoglobin level. In most study, high GV was associated with a noticeable increase in risk for poor prognosis of cardiovascular disease(CVD). Even in patients without diabetes, GV was associated with a greater risk of CVD. However, there is still a lack of study on the effects of GV on cerebrovascular diseases and other brain diseases. This study shows that GV is associated with ischemic cerebrovascular disease. Experiments were conducted in three groups: GV, CH, and Control(C). Male 8–12 week old C57BL/6J mice were used for the experiments. Diabetes was induced by administering streptozotocin intraperitoneally during the fasting state and consecutively for 5 days. Then, the diabetic animals were divided into two groups : GV, CH. GV was treated with a subcutaneous injection of insulin glargine, plus two times of 0.35ml of 33 % glucose solution by gavage daily for 30 days. CH received insulin glargine, plus two times of 0.35ml of saline solution for 30 days. Control group(C; Untreated normoglycaemic) was also received two times of 0.35ml of saline solution for 30 days. For 30 days, the glycaemic values of each mouse were measured in tail vein blood by the glucometer at 7 times one day. After 30 days, ischemic stroke was induced in GV, CH and C mice by middle cerebral artery occlusion(MCAO). At 1 day after surgery, mice were sacrificed and remove the pancreas and brain. After that, the islets of Langerhans was observed through insulin and glucagon IF staining in pancreas, and the volume of cerebral infarction was confirmed through TTC staining and FJB staining with brain. As a result, cerebral infarction volume was observed to be larger in the GV group than in the CH and C groups.

Corresponding author : Sang Hwa Lee

Keywords : Diabetes mellitus(DM), Glycemic variability(GV), Ischemic stroke, MCAO

PS-A-079

6-Shogaol suppresses osteoclastogenesis and alveolar bone resorption in mice with ligature-induced periodontitis

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The connective tissues around teeth are destroyed by the inflammatory condition known as periodontitis. Osteoclasts are primarily responsible for the resorption of alveolar bones during the progression of periodontitis, which leads to tooth loss if not appropriately managed. Thus, the treatment of patients with periodontitis will considerably enhance the development of efficient anti-resorptive medicines. We propose an inhibitory impact of 6-shogaol, a component of ginger, on osteoclast development and bone resorption in the current investigation. Mouse bone marrow cells were cultured in the presence of macrophage-colony stimulating factor and receptor activator of nuclear factor-κB ligand (RANKL) to investigate the effect of 6-shogaol on osteoclast differentiation and intracellular signaling pathways. 6-shogaol significantly reduced osteoclast differentiation, actin ring formation, and resorption. In the presence of 6-shogaol, osteoclast signaling including the RANKL-induced activation of mitogen-activated protein kinases, Ca^{2+} oscillation, generation of reactive oxygen species, and nuclear factor of activated T-cells, cytoplasmic 1 nuclear translocation was significantly inhibited in vitro. Furthermore, a ligature-induced periodontitis model in mice was used to determine the role of 6-shogaol in vivo. As a result, 6-shogaol treatment reduced osteoclastgenesis and alveolar bone resorption caused by ligation. Additionally, after shogaol injection, the ligature-induced number of neutrophils and macrophages or the expression of interleukin-1 and tumor necrosis factor were significantly reduced in the periodontal tissues. These findings support 6-shogaol's anti-osteoclastogenic properties and raise the prospect of using it as an anti-resorptive tactic in periodontitis.

Corresponding author : Eun-Kyong Kim, Myoung-Ok Kim

Keywords : 6-Shogaol, Periodontitis, Osteoclast, Alveolar bone resorption

PS-A-080

Anti-inflammatory effect of Stachys affinis extract on DSS-induced colitis in mice

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Ulcerative colitis (UC) is one of inflammatory chronic intestinal diseases with pathological characteristics, including the overproduction of inflammatory cytokines and mediators. Stachys affinis (SA) is an important food and traditional medicine in Korea and China, used to improve the memory of patients with senile dementia or cardiovascular diseases. It has been resported that it has anti-inflammatory, antitoxic and antibacterial effects. This study is to evaluate the anti-inflammatory effect of SA extract (SAE) in dextran sulfate sodium (DSS)-induced colitis mice. Five-week-old male Balb/c mice were randomized and divided into five groups. Four groups, excluding the normal group, were orally treated with either distilled water or SAE (150, 300 and 500 mg/kg) for 21 days, and acute colitis was induced during the last 5 days by 3% DSS in the drinking water. SAE improved colitis symptoms, including body weight loss, colon length shortening, disease activity index and colon mucosal damage. SAE also reduced MPO activity and the serum levels of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) in the SAE-treated groups. Histological findings showed that SAE suppressed edema, mucosal damage and the loss of crypts in DSS-induced colitis mice. SAE may improve DSS-induced colitis at least in part by suppressing the pro-inflamatory cytokines. These results suggest that SAE may exert anti-inflamatory effect at least in part by reducing the production of pro-inflamatory cytokines and chemokines in DSS-induced colitis mice.

Corresponding author : Yun-Seong Lee

Keywords : Stachys affinis, Anti-inflammation, Cytokine, Chemokine, Colitis

PS-A-081

Effect of grain mixture powder with sword bean on loperamide-induced constipation in C57BL/6 mice

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This study was to evaluate the effect of improving grain mixture powder with sword bean on loperamide-induced constipation in a mouse model. The experimental animals were classified into four groups, and the group composition was classified by administration concentration as a normal group (CON), a control group (LOP), a grain mixture powder with sword bean water extract 300 mg/kg (MPE300), and a grain mixture powder with sword bean water extract 500 mg/kg(MPE500). After administering the C57BL/6 mouse for 28 days, loperamide (2 mg/kg) was dissolved in 0.9% saline from 4 days before the autopsy, and oral administration (9 am and 6 pm) was performed twice a day to induce constipation. As a result of measuring the number of variables, it was found that the number of feces in the LOP was remarkably small, and in the case of the sample administration group, a large number of feces were discharged within a short period of time. As a result of measuring the total time taken to defecate after 12 hours of fasting on the third day of constipation induction by the rate of movement of the digestive tract of the diet, the LOP was 30.31 ± 7.71%, and the grain mixture powder with sword bean extract (MPE) administration group was significantly reduced to 45.26 ± 4.24% and 52.60 ± 5.35%, respectively. As a result of measuring the number of sides, the CON confirmed the number of residual variables of 1.4 ± 0.5 and the LOP showed a significant difference of 5.8 ± 0.8.The cause of constipation due to loperamide was confirmed once again, and the results showed that MPE can be effective for the prevention of constipation.

Corresponding author : Yun-Seong Lee

Keywords : Sword bean, Loperamide, Constipation, Grain mixture powder, Smooth bowel movement

PS-A-082

Costunolide suppresses colorectal cancer cells growth and induces apoptosis *in vitro* and *in vivo*

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Colorectal cancer (CRC) is a widespread condition that is the third greatest cause of cancer-related death in people worldwide. Sesquiterpene lactone costunolide (CTD), a naturally occurring molecule, has been shown to possess anticancer effects. In CRC, the substance's particular target and regulatory mechanism have yet to be discovered. In this study, we demonstrated that CTD restricted the growth of CRC cells both in vitro and in vivo. Here the cell proliferation assays, migration, and invasion, propidium iodide, and annexin V-staining analyses were used to assess the effects of CTD on colon cancer cell growth in vitro. Western blot assays were used to examine the underlying processes of CTD. Cell-derived tumor xenografts (CDX) in nude mice and immunohistochemistry were used to assess anti-tumor effects of CTD in vivo. CTD inhibited the proliferation, anchorage-independent colony development, and epithelial-mesenchymal transformation (EMT) of HCT-15, HCT-116, and DLD1 CRC cells. Additionally, the CTD induced cell apoptosis and G2/M phase cell cycle arrest. The CTD reduced the volume of CDX tumors without causing body weight reduction. This study investigated the efficacy of the newly discovered AKT inhibitor CTD in the prevention or treatment of colon cancer which provides a reliable fundamental for the preclinical study of CTD.

Corresponding author : Myoung Ok Kim

Keywords : Colon cancer, Costunolide (CTD), Apoptosis, Xenograft model

PS-A-083

In vitro and In vivo costunolide inhibits the growth of colorectal cancer cells and induces apoptosis

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Colorectal cancer (CRC), which is also very common, is the third most common cause of cancer-related death in humans worldwide. The current standard of care for CRC is a combination of fluorouracil and oxaliplatin. Furthermore, clinical trials have shown that combining oxaliplatin with FU or capecitabine is beneficial. However, it is unknown whether all CRC patients benefit from adjuvant chemotherapy, which would attest to its associated toxicity, inconvenience, and cost. The development of targeted therapeutics for CRC has not proceeded as quickly or smoothly as anticipated over the last few decades. Medication targeting VEGF and EGFR has already been used in clinical trials. Such drugs should be studied further. A serine/threonine kinase known as Protein Kinase B (PKB), AKT prevents apoptosis, regulates glycogen metabolism, and contributes to cancer development. The overexpression of phosphorylated AKT in malignant tumors can be targeted for treatment. Previous research has shown that the AKT-MDM2-p53 signaling pathway is associated with the onset and progression of many cancers, including colorectal cancer, and has a significant impact on cell death. AKT-mediated MDM2 phosphorylation has been shown to promote apoptosis in p53-deficient colon cancer cells by activating p73 and E2F1. However, the AKT-MDM2-p53 pathway in CRC cells has not been fully elucidated. MDM2 is a great substrate for AKT and acts as a link between it and p53. As a result, inhibiting MDM2 phosphorylation in cancer treatments may aid p53 function. AKT/mTOR can be inhibited by p53 activation via an AMP kinase (AMPK)-mediated mechanism or by PTEN upregulation, resulting in anticancer activity. MDM2 can, on the other hand, be controlled by p53-induced negative feedback via AKT suppression. As a result, the AKT-MDM2-p53 pathway is critical in cancer development, and targeting AKT may be the most effective strategy.

Corresponding author : Myoung Ok Kim

Keywords : Colon cancer, Costunolide (CTD), AKT, Ubiquitination, Xenograft model

PS-A-084

Targeting protein kinase B pathway with costunolide induces apoptosis of oral cancer cells *in vitro and in vivo*

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Oral cancer (OC) is one of the most common malignant tumors in the world, with squamous cell carcinoma accounting for approximately 90% among all oral and oropharyngeal malignancies. The prognosis for patients with oral cancer remains poor due to a lack of effective chemotherapeutic agents. A natural sesquiterpene lactone, Costunolide (CTD), showed anticancer and bioactive properties that have been demonstrated in a variety of cancers. However, its impact on oral cancer is unknown. This study investigated the anticancer potential and underlying mechanisms of CTD in OC in vivo and in vitro. The antigrowth effects of CTD on OC cells were investigated using cell viability and anchorage-independent colony formation assays; cell cycle and apoptosis were investigated utilizing flow cytometry and confirmed using immunoblotting. The results showed that treating oral cancer cells with CTD resulted in significant inhibition of cell proliferation, anchorage-independent colony formation and induction of G2 phase cell cycle arrest and apoptosis; In terms of mechanism, CTD bound to AKT directly via binding assay and repressed AKT activities via kinase assay, thereby downregulating AKT downstream. Notably, in an in vivo mouse model, CTD strongly suppresses cell-derived xenograft OC tumor growth and showed no significant toxicity of CTD to mice. Finally, our findings suggested that costunolide may slow the progression of OC and may be a novel AKT inhibitor.

Corresponding author : Ki-Rim Kim, Myoung Ok Kim

Keywords : Costunolide, Oral cancer, Protein kinase B pathway

PS-A-085

Effect of oocyte maturation, *in vitro* embryo development by ovum pick-up of follicle-stimulating hormone-treated Hanwoo (Bos taurus coreanae)

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Premium quality cattle production is being studied around the world, so we must be developed to efficiently produce oocytes using premium cattle. Ovum pick-up (OPU) is the most commonly used oocyte collection method. And genetic progression can be accelerated rapidly through the maternal lineage of cattle. The FSH mild stimulation system used results in embryos with greater capacity for embryonic development and consequently more resistance to vitrification. This study was to compare oocytes and embryos using an FSH synchronization and mild stimulation system to vitrification and warming by evaluating the development and quality of blastocysts after oocyte maturation, in vitro fertilization (IVF), and in vitro culture (IVC). Oocytes were collected from the control group and the Follicle-stimulating hormone (FSH)-treated Hanwoo females, respectively. Reduced doses of FSH (36, 36, 24, 24 mg 12-hour intervals), regulated internal drug release, estrogen and prostaglandin were used for synchronization and weak stimulation. In addition, FSH treatment reduced time and concentration compared to previous studies. In vitro blastocysts were produced by in vitro maturation, fertilization, and culture. As a result, it was confirmed that the blastocyst rate was higher in the FSH group to compared the control group. In addition, after vitrification and warming of the in vitro culture, the embryo produced increased to a higher proportion and grade in the FSH group. This study demonstrated that FSH improves the efficiency of the production, freezing, and preservation of embryos during the OPU-in vitro embryo development.

Corresponding author : Myoung Ok Kim, Junkoo Yi

Keywords : Ovum pick-up(OPU), Follicle-stimulating hormone (FSH), Oocyte, Embryo, Vitrification

PS-A-086

Agrimonia eupatoria alleviates hepatic fat accumulation and inflammation in the CDAHFD-diet-induced murine NASH model

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Non-alcoholic steatohepatitis (NASH) is a pathological condition that causes inflammation and accumulation of fat and fibrotic components without consuming alcohol, and further progresses to cirrhosis, and hepatocellular carcinoma. NASH is estimated to affect between 3%~5% of the world's population, but currently, there are no effective treatments for NASH approved. Agrimonia eupatoria (AE) has been used in traditional medicine for the treatment of anti-inflammatory-related diseases. In the present study, we demonstrated the protective effect of AE in CDAHFD (choline-deficient, L-amino acid defined, high-fat diet with 0.1% methionine) diet-induced murine NASH model. C57BL/6 mice were fed a CDAHFD diet for 12 weeks and were orally administered with AE extracts (25, 50, and 100mg/kg/day). Silymarin(100mg/kg/day), and luteolin-7-O-glucuronide (20mg/kg/day) were treated as positive control materials. These results indicate that AE treatment led to the reduction of fat accumulation and inflammatory lesion in the liver on histopathological analysis, and suppressed the plasma levels of alanine aminotransferase (ALT), and aspartate aminotransferase (AST), ameliorating hepatic injury. Also, AE extract significantly decreased the hepatic mRNA and protein expression on the pro-inflammatory cytokines, reactive oxygen species (ROS)-generating enzymes, and oxidative stress targeting of nuclear factor E2-related factor 2 (NRF2). Furthermore, in the case of fibrosis, the expression of fibrogenic marker such as α-smooth muscle actin (SMA) was alleviated in the liver of AE treated groups. In conclusion, our findings show that AE has a possibility as a promising therapeutic agent in preventing NASH.

Corresponding author : Sang-Joon Park

Keywords : Agrimonia eupatoria, Non-alcoholic steatohepatitis, Inflammation, Oxidative stress, Fibrosis, Therapeutic agent

PS-A-087

The anti-obesity effect of Lactobacillus plantarum strain BK21 postbiotics isolated from Kimchi on high-carbohydrate-diet induced obesity in mice

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Postbiotics from Lactobacillus plantarum has been repoted to improved growth performance, nutrient utilization, immune status and gut health in livestock. However, there is no information on the anti-obesity effect of BK21 postbiotics. In this study, we investigated that the anti-obesity effects of postbiotic-metabolites from L. plantarum BK21 was evaluated in FFAs (free-fatty acids)-induced HepG2 cells and high-carbohydrate-diet (HCD) induced obesity model in mice. The pretreatment of BK21 postbiotics showed inhibition of FFAs-induced lipid accumulation on Oil Red O staining and reduction of intracellular triglyceride concentration within HepG2 cell, and reduced significantly mRNA expression of CPT1a, FAS (fatty acid synthesis), C/EBPα, and SREBP-1c. In HCD-induced murine obesity model, BK-21 postbiotics-treated groups were significantly lower in body and white adipose tissue weight compared to the HCD group. BK-021 postbiotics-treated groups also reduced lipid accumulation in the liver and size of adipocyte within the adipose tissue on histological analysis, and decreased alanine aminotransferase (ALT) and aspartate aminotransferase (AST), triglyceride, total cholesterol, and low-density lipoprotein (LDL) levels in plasma. In addition, the hepatic mRNA expression of CPT1a, C/EBPα, SREBP-1c, and PPARγ was downregulated significantly in BK-21 postbiotics-treated groups compared to HCD group. Conclusively, our results indicate that BK-21 postbiotics have effectively anti-obesity function as an inhibition of lipid accumulation.

Corresponding author : Sang-Joon Park

Keywords : Lactobacillus plantarum strain BK21, Postbiotics, Anti-obesity, Lipid accumulation

PS-A-088

Characterization of regucalcin as a novel tubulin deacetylase

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Regucalcin (RGN) plays an important role in regulating intracellular calcium homeostasis and as a regulatory protein in cell signaling system. Reversible tubulin acetylation is one of the major post-translational modification in controlling the stability and function of microtubules. In this study, we report that RGN has a novel function as a microtubule-associated protein that deacetylates α-tubulin both in vitro and in vivo. RGN was predominantly localized in the cytoplasm, colocalized with the microtubule network via immunofluorescence, and interacted with α-tubulin and acetylated-tubulin, respectively. Especially C-terminal residue of RGN is responsible for interaction with α-tubulin. The overexpression of RGN showed hypoacetylation of α-tubulin, while knockdown of RGN via shRNA resulted in tubulin hyperacetylation. Furthermore, the expression of acetylated α-tubulin showed the opposite patterns in the various organs of RGN transgenic or knockout mice. To investigate the correlation with representative tubulin deacetylases, we conducted whether RGN colocalizes or interacts with SIRT2 and HDAC6, respectively or with complex. Interestingly, we found that RGN forms a complex with SIRT2 and HDAC6 within the microtubule. In conclusion, RGN may play an important role in the regulation of intracellular calcium homeostasis and multiple transcription network as a tubulin deacetylase or microtubule-associated protein.

Corresponding author : Sang-Joon Park

Keywords : Regucalcin, Tubulin deacetylase, Microtubule-associated protein, SIRT2, HDAC6

PS-A-089

Conjugation of pheophorbide A and SN38 with hyaluronan nanoparticles for photodynamic- and cascadic chemotherapy in cancer stem-like ovarian cancer

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Ovarian cancer (OC), the seventh most common cancer, is still a major cancer with a high mortality rate. However, to date, there is a critical requirement to optimize the presently applicable treatments and develop novel therapeutic treatments. Therefore, here we have designed photo-triggered reactive oxygen species (ROS) generating pheophorbide A and ROS cleavable thioketal-SN38 conjugated with hyaluronan-cholesterol nanoparticles (PheoA-SN38-HC NPs). In this present study, we have discovered the combination therapeutic effects of PheoA-SN38-HC NPs in HEY-T30 human oc model. Clinical Proteomic Tumor Analysis Consortium (CPTAC) data have showed that the expression of cancer stem cell (CSC) markers (CD44, ALDH1A1, and CD117) is highly correlated with poor clinical outcomes in OC patients. We have proved that HEY-T30 cells overexpress CSC markers and is noticeably more invasive than other cancer cells. Flow cytometry (FACS) and microscopic analysis revealed the active targeting property of PheoA-SN38-HC NPs to CD44+ in HEY-T30 cells. Furthermore, in HEY-T30 cells, PheoA-SN38-HC NPs has apparently demonstrated the combination therapeutic effects in vitro and in vivo xenograft mouse model. Notably, the paracrine cytotoxic effect of SN38 possibly compensates the locoregional therapeutic limitation of photodynamic therapy. Thus, PheoA-SN38-HC NPs with photodynamic and ROS-cleavable SN38 therapy clearly demonstrated advanced CD44 positive cancer targeting efficiency and anticancer effects. Thereby, these results suggests that CD44 can be applied for pan-CSC.

Corresponding author : Su-Geun Yang

Keywords : Hyaluronan-cholesterol conjugate, Combination chemotherapy, Photodynamic therapy, SN38, Hyaluronan nanoparticles

PS-A-090

Visualization of a novel human monoclonal antibody against Claudin-3 using human ovarian cancer-bearing mice

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Claudin-3 (CLDN3), a tight junction protein, regulates cell-to-cell interactions in epithelial or endothelial cell sheets. During tumorigenesis, epithelial cells are transformed and tumor cells proliferate through out-of-plane division, resulting in external exposure of CLDN3. This alteration of CLDN3 expression is associated with cancer progression, correlating with malignancy in various carcinomas. Since CLDN3 is particularly overexpressed in most ovarian cancers and used as an effective diagnostic marker, we tested the possibility of using a CLDN3-specific antibody as a novel imaging probe. The labeling efficiency of NOTA-^{111}In or antibody-NOTA-^{111}In was 98.52% or 100%, respectively. FNR648 labeled CLDN3 antibody was bound to the cell surface of OVCAR-3 at 83.4% and to U87MG at 5.7%, respectively. In OVCAR-3 tumor xenografted mice, mice injected with CLDN3 antibody showed 2.5-fold higher tumor uptake (20.4 ± 7.4% ID/g) than mice injected with human IgG (8.8 ± 2.6% ID/g) at 24 hour p.i. The fluorescence signal of CLDN3 antibody peaked at 24 hour p.i. Since the specific binding of CLDN3 antibodies to OVCAR-3 tumors has been validated in a mouse model and diagnostic radionuclides can be replaced with therapeutic radionuclides, this human monoclonal antibody could be used as a useful theranostic probe.

Corresponding author : Hyewon Youn

Keywords : Human monoclonal antibody, Ovarian cancer-bearing mice, Claudin-3, Dual imaging

PS-A-091

Metabolic index using Glucose-Thymidine Ratio (GTR) as a potential imaging biomarker to assess response to immune checkpoint inhibitor therapy in mouse melanoma model

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Immune checkpoint inhibitors (ICI) are widely used for cancer immunotherapy, requiring effective methods for response monitoring. In this study, ^{18}F-FDG and ^{18}F-fluorothymidine (FLT) uptake by tumors and their changes with ICI treatment were evaluated as potential imaging biomarkers in an animal model. In the treatment group, tumor growth was effectively inhibited (volume 464 ± 300 *vs*. 182 ± 128 mm^3, P = 0.0049), and higher proportions of immune cells were observed. In the early phase, ^{18}F-FDG uptake was higher in the treated group (P = 0.0089), whereas ^{18}F-FLT uptake was not different. In the late phase, ^{18}F-FDG uptake was not different in both groups. However, ^{18}F-FLT uptake of the control group was markedly increased and was higher than the treated group (P < 0.0001). GTR was consistently higher in the treated group in early (P = 0.0035) and late (P < 0.0001) phases. In both phases, the group with a GTR ratio of 1.5 or more was divided into high GTR, and those with a GTR ratio of 1.5 or less were divided into low GTR. In the case of the high GTR group, the difference in tumor size at the end point compared to the baseline was 85.08 ± 49.24 mm^3. In the case of the low GTR group, the difference in tumor size after ICI treatment was 173.88 ± 103.25 mm^3. In this study, we evaluated glucose and nucleic acid metabolisms of tumors and their alterations following ICI treatment by using ^{18}F-FDG and ^{18}F-FLT PET. GTR, an index for glucose metabolism relative to nucleic acid metabolism, was consistently higher in the treatment group in both phases. It is expected to serve as a potentially effective imaging biomarker for monitoring ICI treatment.

Corresponding author : Jin Chul Paeng, Hyewon Youn

Keywords : Metabolic index, Glucose-thymidine ratio, Immune checkpoint inhibitor therapy, Mouse melanoma model, Nuclear medicine imaging

PS-A-092

Immunomodulatory role of fermented garlic extract on macrophage cell proliferation, cytokine secretion and natural killer cell cytotoxicity

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Fermented garlic extract is popular due to its powerful immune boosting and anti-tumor activity than fresh garlic extract, which can be attributed to the enhanced number of polyphenols and S-allyl cysteine during fermentation. Using a mouse macrophage cell line, RAW264.7 and peritoneal macrophages obtained from Balb/c mice, we evaluated the immune-boosting characteristics of fermented garlic extract (FGE), which was generated by fermenting garlic with *Bacillus subtilis* for 3-15 days at 20-40°C. Nitric oxide (NO) and pro-inflammatory cytokine levels were measured in RAW264.7 cells and peritoneal macrophages with or without FGE (0.016, 0.08, 0.4, 2 and 10 mg/mL), GE (0.016, 0.08, 0.4, 2 and 10 mg/mL), and lipopolysaccharide (LPS) (1 μg/mL) treatment respectively. NK cell in mouse splenocytes were also assayed with regard to their lytic activity against YAC-1 cells. FG treatment dramatically accelerated cell proliferation and further, induced macrophage activation by raising NO, tumor necrosis factor (TNF-α), interleukin-12 (IL-12), and interleukin-6 (IL-6) levels in RAW264.7 cells and murine peritoneal macrophages. In contrast to FGE, GE promoted RAW264.7 cell proliferation at lower dosages and toxicity at higher doses, whereas NO was not produced at any concentrations studied. In addition, FGE at lower dosage induced the significant enhancement of NK cell's activity against tumor cells (YAC-1). Collectively, these findings suggest that FGE has potent innate immuno-modulatory and anti-cancer activity. Therefore we anticipate that FGE can be employed as an alternative immunostimulator.

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Keywords : Fermented garlic extract, Macrophage, Natural killer cell, Balb/c mice, Nitric oxide

PS-A-093

Comparison of the characteristcs between porcine bone marrow-derived Mesenchymal stem cells and peripheral blood mononuclear cells for transplantation therapies

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Mesenchymal stem cells (MSCs) have been isolated from diverse organs and are under investigation for regulating transplantation. Although the MSCs of various mammals has been studied, porcine MSCs are still poorly understood. To investigate characteristcs of porcine bone marrow-derived MSCs, we compared expression level of CD73, CD90 and CD105 known as human MSC markers through qPCR, flow cytometry and immunocytochemistry in MSCs, bone marrow-attached cells (BMACs), peripheral blood mononuclear cells (PBMCs) and porcine kidney epithelial cell line (PK(15)). As a result, CD73, CD90 and CD105 were upregulated in MSCs and BMACs except for PK(15) cells. In contrast to MSCs and MBACs, there was little expression of immune checkpoint CD73 in PBMCs. To determine the heterogeneity between PBMC and MSC or BMAC, we performed RNA sequencing. Comparing the RNA expression patterns, there was up-regulated and down-regulated to 46.3% and 49.8% of common genes, respectively in BMAC and MSC compared to PBMC. In analysis of KEGG pathway, the genes related to inducing allograft rejection were down-regulated in MSCs. Therefore, These findings confirm that porcine MSCs having identical characteristcs of human MSCs and regulation of immune response would be a useful tool to overcome transplantation rejection.

Corresponding author : Jeong Ho Hwang

Keywords : Pig, Mesenchymal stem cell, Transplantation

PS-A-094

Hydrogen peroxide regulates gonadotropin-releasing hormone neurons excitability in postnatal and concentration-dependent manner in mice

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Gonadotropin-releasing hormone (GnRH)-secreting neurons are the critical component of the reproductive axis in all mammals. Reactive oxygen species (ROS) are known to act as signaling molecules in several neuronal populations across the brain under physiological conditions. In addition, ROS have been demonstrated to disrupt reproductive function, decrease gonadal hormones and interfere with cross-talk between the hypothalamic-pituitary-gonadal axis and other endocrine axes, thus affecting fertility. However, ROS effect on the GnRH neuron excitability remains unknown. Thus, we aimed to determine the effect of hydrogen peroxide (H_2O_2), an ROS source, on GnRH neuron physiology. We used whole-cell patch-clamp techniques for brain slices to determine the effect of H_2O_2 on GnRH neuron excitability. H_2O_2 exposure induced variegated response on GnRH neurons across postnatal development and the estrous cycle. H_2O_2-induced membrane depolarization in the majority of GnRH neurons from immature mice and hyperpolarization in the majority of GnRH neurons from adults. In addition, female GnRH neurons demonstrated estrous cycle-dependent variation in response to H_2O_2 exposure. Similarly, H_2O_2-induced membrane polarization was concentration-dependent, with depolarization at low concentrations and hyperpolarization at high concentrations. Next, inhibiting the antioxidant system, glutathione peroxidase with mercaptosuccinic acid resulted in a cessation of spontaneous activities in most GnRH neurons. These findings suggest that H_2O_2 effects on GnRH neurons are age, estrus cycle, and concentration-dependent. Furthermore, these results imply that adult GnRH neurons are more vulnerable to ROS, which may potentially influence gonadotropin release from the hypothalamus.

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Corresponding author : Dong-Hyu Cho, Seong-Kyu Han

Keywords : Hydrogen peroxide, Hypothalamus, Gonadotropin-releasing hormone neurons, Patch-clamp, Reactive oxygen species

PS-A-095

GABA- and glycine-mimetic responses of honokiol on the substantia gelatinosa neurons of the trigeminal subnucleus caudalis in mice

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Substantia gelatinosa (SG) neurons of the trigeminal subnucleus caudalis (Vc), with the abundance of inhibitory neurotransmitters like gamma-aminobutyric acid (GABA) and glycine, are recognized as initial synaptic sites for the regulation of orofacial nociceptive stimuli. Honokiol, a major bioactive constituent extracted from *Magnolia officinalis* bark, has been exploited in traditional remedies with multiple effects on humans such as antianxiety, antidepression and antinociception. However, the antinociceptive mechanism of this compound on the SG neurons of the Vc still remains fully elusive. We used patch-clamp technique to investigate if honokiol might evoke various responses on the SG neurons of the Vc in mice. The administration of honokiol induced inward currents that were noticeably suppressed in the presence of picrotoxin, a GABAA receptor antagonist. We also found that the honokiol perfusion not only exerted an additive effect with glycine-mediated inward currents, but also potentiated GABA-induced inward currents in the majority of the SG neurons.

The present work reveals that the inhibitory synaptic activities of honokiol may be provoked via the modulation of glycine and GABA neurotransmission on the SG of the Vc and to be further, honokiol may be appreciated as a potential therapeutic target for orofacial pain.

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Corresponding author : Dong-Hyu Cho, Seong-Kyu Han

Keywords : Substantia gelatinosa, Patch-clamp, GABAA receptor, Glycine receptor, Orofacial pain

PS-A-096

Visual phenotyping analysis of korean wild mouse KWM/Hym

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Laboratory inbred mice are widely and commonly used in biomedical research, however, inbred mice have been genetically isolated from their free-living relatives for many years such that laboratory strains capture only a small part of the genetic variation present in wild populations. Although the use of laboratory mice has been productive in research, this animal model might has been affected by selective pressures of domestication to no longer represent a naturally adapted system. In last few decades, numerous efforts have been made to develop the better mouse model to overcome the limitations of laboratory inbred mouse models.

As part of these efforts, in Korea, Hallym university research team has been developing inbred strain of Korean wild mouse KWM/Hym*. We aimed to determine the visual phenotype of this unique inbred strain of KWM/Hym mice, and consider whether they are suitable for visual experiment. To analyze the visual phenotype of this mice, we determined the function of photoreceptor cells, and measured intraocular pressure, thickness of cornea and retinal layers of KWM/Hym mice.

**KWM/Hym: inbred strain of Korean wild mouse KWM/Hym by Hallym university research team*

Corresponding author : KyungYul Seo

Keywords : Korean wild mouse, KWM/Hym, Visual phenotyping

PS-A-097

Coenzyme Q10-Micelles complex ameliorates osteoarthritis by targeting for RIP3 and p-MLKL regulated necroptosis

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Background: Osteoarthritis (OA) is the most common degenerative joint disease and is characterized by breakdown of joint cartilage. Coenzyme Q10 (CoQ10) exerts diverse biological effects on bone and cartilage; observational studies have suggested that CoQ10 may slow OA progression and inflammation. However, any effect of CoQ10 on OA remains unclear. Here, we investigated the therapeutic utility of CoQ10-micelles.
Methods: Seven-week-old male Wistar rats were injected with monosodium iodoacetate (MIA) to induce OA. CoQ10-micelles were administered orally to MIA-induced OA rats; celecoxib served as the positive control. Pain, tissue destruction, and inflammation were measured. The expression levels of catabolic and inflammatory cell death markers were assayed in CoQ10-micelle-treated chondrocytes.
Results: Oral supplementation with CoQ10-micelles attenuated OA symptoms remarkably, including pain, tissue destruction, and inflammation. The expression levels of the inflammatory cytokines IL-1β, IL-6, and MMP-13, and of the inflammatory cell death markers RIP1, RIP3, and pMLKL in synovial tissues were significantly reduced by CoQ10-micelle supplementation, suggesting that CoQ10-micelles might attenuate the synovitis of OA. CoQ10-micelle addition to cultured OA chondrocytes reduced the expression levels of catabolic and inflammatory cell death markers.
Conclusions: CoQ10-micelles might usefully treat OA.

Corresponding author : Mi-La Cho

Keywords : Osteoarthritis, Inflammation, Inflammatory cell death, Coenzyme Q10 (CoQ10)

PS-A-098

Age- and sex-relative effect of naringenin on substantia gelatinosa neurons of trigeminal subnucleus caudalis in immature mice

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Naringenin (4,5,7-thrihydroxyflavanone), a type of bioflavonoid, is known to possess various biological effects in the central nervous system. The substantia gelatinosa (SG) of the trigeminal subnucleus caudalis (Vc) is admitted as a pivotal site of integrating and modulating afferent fibers carrying the orofacial nociceptive information. However, there have been no reports of the effects of naringenin according to age and sex on the orofacial nociceptive site. In this study, the technique of whole-cell patch-clamp was used to investigate the role of naringenin and its mechanism of action on SG neurons of the Vc in immature mice. Under the high chloride pipette solution, naringenin tended to induce inward currents in a concentration-dependent manner. Membrane current changes and area under the curve (AUC) induced by GABA/muscimol in the presence or absence of naringenin were relatively compared, and whether these responses differ according to age or sex was evaluated. Furthermore, amplitude and AUC were increased by co-administration of GABA and naringenin compared to GABA alone. In addition, naringenin with muscimol increased amplitude and AUC compared to muscimol alone. Interestingly, changes in amplitude and AUC of GABA- or muscimol-induced currents by naringenin showed similar decline patterns with age, and the effect of naringenin according to sex was significantly increased in females compared to males. These results show that naringenin acts on SG in the Vc and is involved in the regulation of GABAA receptor activity. Moreover, these results suggest that naringenin may affect pain control according to the change in the expression of GABAA receptor subunits by age/sex. Therefore, naringenin contributes at least in part to orofacial nociceptive modulation and may be a promising target for developing age- and sex-specific therapeutics in the orofacial pain treatment.
This research was supported by National University Development Project at Jeonbuk National University in 2021.

Corresponding author : Seong-Kyu Han

Keywords : Substantia gelatinosa, Orofacial nociception, GABA, Muscimol, GABAA receptor

PS-B-001

Schisandrin C interact with V225A and V288Y on serotonin receptor

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Schisandrin C (Sch C) is one of the main components of Schisandra chinensis (Schisandra). Since olden times, Schisandra has been used as a traditional herbal medicine in Asia. Recent studies have shown that Schisandra is effective against irritable bowel syndrome (IBS) in an animal study. There is a research result that Schisandra affects IBS through the 5-HT3A pathway in the IBS rat model. However, fundamental research on which a substance of Schisandra interacts with 5-HT3A receptor to treat IBS is unknown. We hypothesized that a component of Schisandra would bind to the 5-HT3A receptor. We found Sch C via a screening work using two electrode-voltage clamps (TEVC). Thus, we aimed to elucidate the neuropharmacological actions between Sch C and the 5-HT3A receptor in molecular and cell levels. We found these described below. Co-treatment with Sch C inhibited I5-HT in a reversible manner, concentrate-dependent, like-competition, voltage-independent, and IC50 values of Sch C. Besides, the main binding positions of Sch C identified through 3D modeling and point mutation are V225A and V288Y on 5-HT3A receptor. This is fundamental evidence of the effect of Schisandra in the previous study. Thus, we suggest the potential that Sch C may treat IBS in a way that suppresses excessive neuronal serotonin signaling in the synapse of sensory neurons and enterochromaffin (EC) cells. In conclusion, this paper identified the mechanism of interaction between Sch C and 5-HT3A receptor, and demonstrated Sch C is a novel antagonist.

Corresponding author : Junho Lee

Keywords : Bowel syndrome, 5-HT3A receptor

PS-B-002

Binding sites D177 and F199 are the major binding sites of kaempferol on human 5-HT3A receptors

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Monoamine serotonin is a major neurotransmitter that acts on a wide range of central nervous system and peripheral nervous system functions and is known to have a role in various process-es. Recently, it has been found that 5-HT is involved in cognitive and memory functions through interaction with cholinergic pathways. The natural flavonoid kaempferol (KAE) extracted from Cudrania tricuspidata is a secondary metabolite of the plant. Recently studies have confirmed that KAE possesses a neuroprotective effect because of its strong antioxidant activity. It has been con-firmed that KAE is involved in the serotonergic pathway through an in vivo test. However, these results need to be confirmed at the molecular level, because the exact mechanism that is involved in such effects of KAE has not yet been elucidated. Therefore, the objective of this study is to confirm the interaction of KAE with 5-HT3A through electrophysiological studies at the mo-lecular level using KAE extracted from Cudrania tricuspidata. The natural flavonoid kaempferol (KAE) extracted from Cudrania tricuspidata is a secondary metabolite of the plant. This study confirmed the interaction between 5-HT3A and KAE at the molecular level and KAE possesses a neuroprotective effect. KAE inhibited 5-HT3A receptors in a concentra-tion-dependent and voltage-independent manner. Site-directed mutagenesis and molecu-lar-docking studies confirmed that the binding sites D177 and F199 are the major binding sites of human 5-HT3A receptors of KAE.

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Keywords : Kaempferol, Neuroprotective

PS-B-003

Study of parasite pheromone receptor and gating mechanism to receptors of neurotransmitters

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The olfactory nervous system recognizes and distinguishes many different chemicals in the general living environment. Insects have evolved a group of odorant-gated ion channels composed of highly-developed olfactory receptors capable of distinguishing and distribution between various chemicals with symbolic or evasive specificities. Recently, aphid genomes related to olfaction, including olfactory receptors and proteins, have been identified and olfactory receptors have been reported that are differentially differentiated from Drosophila. The genome of the olfactory receptor has a very conservative sequence and a systematic signaling system. A representative receptor, odorant-gated ion channels comprised of a highly conserved co-receptor (Orco) has a homotetramer channel structure with four subunits arranged symmetrically around the central hole. It has a very similar structure to the 7-transmembrane receptor present in the human body and has a very similar structural form and gating mechanism to receptors of neurotransmitters. In this study, whole cell voltage clamp recording was performed with cell expression system of OR65 gene, which is a subtype of olfactory receptor isolated from Drosophila. After the successful expression of this receptor, microbial culture extract of microorganism, a harmful insect inducer, was used to investigate whether olfactory receptor activity was regulated. The activity of the receptor was confirmed in the recording media diluted 10,000 times with the microbial culture extract. Therefore, it is possible to identify attractant or repellent substance using the olfactory receptor activity regulating system of insects. Through this study, new attractant shows the attracting phenomenon by activating insect receptor OR65, The results of the scientific analysis of the performance of the extracts are presented.

Corresponding author : Junho Lee

Keywords : Parasite pheromone, Olfactory nervous system

PS-B-004

Transferability, and within/between-laboratory validation studies of improved in chemico alternative assay, spectro-DPRA, for skin sensitization test

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Spectro-DPRA is the modified spectrophotometric method by assessing a substance's reaction to two model peptides, which can be a better method to improve some limitations of Direct peptide reactivity assay (DPRA) adopted in the OECD guideline as an alternative method for skin sensitization test.

Within (12 chemicals) and between (20 chemicals) laboratory reproducibility for Spectro-DPRA were conducted followed by a transferability (5 chemicals) and proficiency (10 chemicals), at three laboratories (AP, KTR, and ChemOn) based on GLP principles. All laboratories passed a transferability and proficiency test, and showed more than 80% of concordance compared to the standard operating procedure (SOP), for the within and between laboratory reproducibility evaluation. These results indicated that the Spectro-DPRA is believed to be a competitive approach that can enter the ITS pipeline for skin sensitization tests, and it can be suggested as a novel, internationally recognized method.

Corresponding author : Bae-Hwan Kim

Keywords : Alternative method, Skin sensitization, Spectro-DPRA, in chemico assay, Within/Between-laboratory validation

PS-B-005

Samhwangsasim-tang improves cognitive function through BDNF-mediated pathway in scopolamine-induced mouse model

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Alzheimer's disease is one of neurodegenerative diseases, which are characterized by cognitive dysfunction and neuronal cell death. Samhwangsasim-tang (SST) is a traditional medicine used to treat hypertension and arteriosclerosis. Additionally, due to the effects of its constituent herbs, SST is considered effective for memory-related disorders. We investigated the effects in a mouse model of scopolamine-induced cognitive dysfunction. C57BL/6J mice were administered with 150 and 300 mg/kg SST once daily for 7 days and then intraperitoneally injected with 1 mg/kg scopolamine for 7 days to induce cognitive impairment. We then measured cognitive behavior using a novel object recognition test (NORT) and passive avoidance test (PAT) and analyzed the histological and protein changes. Our results showed that SST administration improved cognitive impairment, similar to donepezil treatment, in NORT and PAT. SST and donepezil decreased neuronal cell death and apoptosis, and acetylcholine levels were increased in the scopolamine-treated hippocampus. Additionally, SST promoted CREB phosphorylation and BDNF maturation while reducing JNK and P75NTR activation; in contrast, donepezil did not alter levels of these proteins in the scopolamine-treated mouse hippocampus. Our results suggest that SST has neuroprotective effects to attenuate neuronal cell death and oxidative stress through CREB/JNK signaling via BDNF activation. SST may regulate endogenous survival factors in the hippocampus, which may be a safe and potential clinical treatment for cognitive impairment in AD.

Corresponding author : Younghoon Go

Keywords : Cognition, Samhwangsasim-tang, Scopolamine, BDNF, Neuroprotection

PS-B-006

No apparent cellular immunotoxicity in mice subchronically exposed to polyethylene or polytetrafluorethylene microplastics through gastric intubation

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Microplastics(MPs) have been recently recognized as a global environmental threat and its exposure as a risk factor to human health. Health effects through MPs exposure have been recently reported, especially through oral route of exposure. The present study was designed to evaluate the immunotoxicity of polyethylene(PE) or polyteteafluorethylene(PTFE) MPs exposed for 4 wks through gastric intubation. PE (6 or 31 average μm diameter) or PTFE (6 or 27 average μm diameter) MPs were administered at 500, 1000, or 2000mg/kg bw/day to 6 wk old 4 mice for each sex per group. No significant differences were observed on the major immune cell proportions, including thymic CD4(+), CD8(+), CD4/CD8 double(+) T lymphocyte, and splenic helper T cell, cytotoxic T cell, and B cell, among the groups. Serum IgG2a/IgG1 ratio was apparently lowered in the female administered with 31μm-2000mg PE MPs than the female administered with 31μm-500mg PE MPs and the control group. The ratio was also lowered in the male administered with 27μm-500mg PTFE MPs than the male administered with 6μm-2000mg PTFE MPs and the vehicle control. Ratio of IFN-γ/IL-4 in the culture supernatants from activated splenocyte cells was significantly lower in the male administered with 31μm-500 or 1000 mg PE MPs than the male administered with 31μm-2000mg PE MPs. The IFNγ/IL-4 ratio was also significantly lower in the female administered with 27μm-2000mg PTFE MPs than the female administered with 27μm-500mg PTFE MPs and the vehicle control. No alteration was observed with production of TNFα. The present study implies that cellular or humoral immunity could be altered depending on size, dose, or type of MPs in mice subchronically exposed to MPs through gastric intubation. [supported by Korea Environmental Industry & Technology Institute (Grant No.2020003120002) and by the Ministry of Environment-Educational training program for the hazards and risk of chemical substances]

Corresponding author : Yong Heo

Keywords : Polyethylene, Polytetrafluorethylene, Microplastics, Immunotoxicity, Subchronic intragastric administration

PS-B-007

Monitoring of maternal antibody for 8th industrialization line Korean Native Chicken in Poultry Research Institute during 4 weeks

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Egg transmission and effect of vaccine inoculation was investigated through analysis of maternal antibody change for 8th industrialization line Korean Native Chicken in Poultry Research Institute(PRI) during 4 weeks. Antibody of 16 diseases was examined by ELISA and HI test in serum samples of 30 (0 week), 5 (1 week), and 10 (2-4 week) chicks. Vaccines of 10 diseases were inoculated with SC, IM, drinking water administration among vaccine program in PRI. Positive rate and titer of maternal antibody against salmonella galinarum (SG), Salmonella pullorum (SP), Avian Luekosis virus (ALV), Mycoplasma synoviae (MS), Reticuloendotheliosis virus (REV) for which vaccines were not inoculated was 0%, 0 respectively. Mean of antibody titer for Fowl Adenovirus (FAdV) was 2.1, 0.2, 0.2, 0, 0 respectively in 0, 1, 2, 3, 4 weeks. Maternal transfer rate was 60.3-98.6% In maternal antibody other 10 disease and positive rate was 33-100% in 0 week and 0-70% in 4 weeks. Antibody titer decreased gradually with the passing of time and antibody titer was 0-1 of 10 items with vaccine inoculation in 4 weeks. Therefor it is considered that these were maternal antibody not against antigen (disease) in chicks. Appearance of FAdV antibody was considered that ELISA specificity used for this study tended to be lower than others. FAdV antibody titer was low and decreased gradually, therefor it is expected not to be egg transmission. As a result, there was no disease by egg transmission in 8th chicks in PRI. Vaccine program of PRI was considered to be proper in Korean Native chickens and contribute to prevention of disease in young chicks by transfer of maternal antibody

Corresponding author : Are-Sun You

Keywords : Maternal antibody, Egg transmission, Vaccine, Disease

PS-B-008

Novel role of *Dipterocarpus tuberculatus* as a stimulator of focal cell adhesion through the regulation of MLC2/FAK/Akt signaling pathway

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Enhancement of focal cell adhesion via the promotion of tissue integration around an implant is considered an essential factor for successful implantation. During this process, cell adhesion to biomaterials can be affected by the chemistry and topography of the material's surface, and various cellular responses, including cell proliferation, cell migration, cell morphology, cell survival, and gene expression, are altered by the interaction between cells and implant materials.

To investigate a novel function of *Dipterocarpus tuberculatus* on focal cell adhesion stimulation, alterations to the regulation of focal cell adhesion-related factors were analyzed in NHDF cells and a calvarial defect rat model after treatment with methanol extracts of *D. tuberculatus* (MED). MED contained gallic acid, caffeic acid, ellagic acid and naringenin induced the increase of the focal cell adhesion ability in NHDF cells, while the expression levels of integrin and E-cadherin were enhanced in the MED-treated group. Also, MED treatment activated focal adhesion kinase (FAK)/myosin light chain (MLC) and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathways within the integrin downstream signaling process. Furthermore, adhesion stimulating effects of MED observed in NHDF cells were subsequently validated in the calvarial defect rat model implanted with MED-coated titanium plate (MEDTiP) during regeneration of calvarial bone. The results of the present study provide novel evidence that MED may stimulate focal cell adhesion in NHDF cells and a calvarial defect rat model.

Corresponding author : Dae Youn Hwang

Keywords : *Dipterocarpus tuberculatus*, Focal cell adhesion, Integrin, FAK, PI3K

PS-B-009

Analysis of methenamine residues in horse muscle by targeted sampling plan in 2021

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Methenamine is a kind of medicine, which is used for the therapy of urinary tract infection and nephropathy. Formaldehyde that derived from methenamine under acidic urine condition has antibacterial activity. However, formaldehyde has carcinogenicity, acute and chronic toxicity. Methenamine is not approved for use as food additive in several countries. Methenamine is administered with MRL of 0.01 mg/kg in muscle of cow, pig, chicken. However, it was prohibited for use in horse production in South Korea. Animal and Plant Quarantine Agency examined this medicine which is suspected to be used by horse farm as a part of targeted or exploratory sampling plans in 2021. Horse muscle was collected at 127 farms in the country and analyzed using modified Food Code methodology and LC-MS/MS. The 2 g of horse muscle was extracted with acetonitrile and cleaned up with hexane and PSA. The separation of compound was conducted using Waters XBridge HILIC (2.1 mm × 100 mm, 3.5 μm) column at 35℃ with 0.2 mL/min flow rate. Mobile phase consisted of 10 mM ammonium formate in water (solvent A) and acetonitrile (solvent B). LC-MS/MS with multiple reaction monitoring was optimized for methenamine with quantitative ion pair 141.1>112.05 at positive mode. The calibration curves in the matrix showed a linear relationship in the range of 1.5 ~ 200 μg/L for methenamine, and the correlation coefficients (R^2) were over 0.998. The limit of detection (LOD) and limit of quantification (LOQ) were 1.41 ppb and 4.28 ppb, respectively. The average recovery was 94.66% at spiked level of 20 μg/kg. The intra-day precision ranged from 4.1 to 10.08%, and the inter-day precision was 6.55%. As a result of residual analysis, methenamine was not detected in any samples. According to the targeted sampling plan & analysis conducted in 2021, it was confirmed the 127 horse muscle samples from 6 provinces were free from contamination of methenamine. The continuous monitoring must be performed to ensure food safety for consumer's health.

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Keywords : Methenamine, Residues, Horse muscle, LC-MS/MS, HILIC

PS-B-010

Optimized characterization of NK cell-derived nano-vesicles as a new mRNA delivery system

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Nanocarriers as biomolecule drug delivery systems have been recently developed. Given the compactable stability, specific delivery, and reasonable efficacy, there are several bottlenecks in the satisfaction of successful clinical trials. Here, we report on the development of a highly efficacious mRNA vaccine, GIBSL that is composed of sequence-modified mRNA encoding the standard protein packaged into NK cell-derived nano-vesicles. Based on the flow cytometry of intracellular vesicles using sucrose gradient ultracentrifugation, we provide a novel and fast approach for qualification and characterization of intracellular vesicles isolated from NK cells suitable for double labeling signals by using flow cytometry and transmission electron microscopy (TEM). Morphological and qualified analyses were performed with nanoparticle tracking analysis, TEM, and a conventional flow cytometer dedicated to detecting sub-micrometer particles. Additionally, we injected and confirmed targeted nucleic acids into the isolated NK cell-derived nano-vesicles, providing the application of a new delivery mediator to carry mRNA candidates. Together, these results suggest the possibility of GIBSL as a new mRNA vaccine carrier that is widely used in pandemics like SARS-CoV2.

Corresponding author : Tae Gyun Kim

Keywords : MRNA vaccine, Nanovesicle, Intracellular vesicle, Nanocarrier, Drug delivery system

PS-B-011

Targeting tumor-intrinsic PD-L1 suppresses the progression and aggressiveness of head and neck cancer cells by inhibiting the GSK3β-dependent Snail degradation

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Background: Programmed death-ligand 1 (PD-L1) is an immune checkpoint protein that allows cells to evade the T-cell-mediated immune responses. We, herein, uncover a tumor-intrinsic mechanism of PD-L1 that is responsible for the progression and aggressiveness of head and neck cancer (HNC) cells and reveal that the extracts of a brown alga can target the tumor-intrinsic signaling pathway of PD-L1.
Methods: Based on an in silico analysis examining the expression and overall survival probability of PD-L1 in HNC, the biological function of PD-L1 on the cell proliferation of HNC was examined by metabolic activity, clonogenic, and in vivo tumorigenicity assays. The clinical importance of PD-L1 expression on the prognosis of HNC patients was analyzed by immunohistochemical staining. To confirm the oncogenic role of PD-L1 in the aggressiveness of HNC in vitro, we conducted wound healing, transwell migration, and invasion assays. The relationship between PD-L1 and the epithelial-mesenchymal transition (EMT) of HNC cells was confirmed via Western blotting, real-time PCR, and immunofluorescence staining.
Results: Through our in silico approach, we found that PD-L1 was upregulated in HNC and was correlated to an unfavorable clinical outcome in HNC patients. Functional studies revealed that PD-L1 was crucial for promoting tumor growth, both in vitro and in vivo. A high expression of PD-L1 was closely correlated with lymph node metastasis of oral squamous cell carcinoma. PD-L1 has facilitated the cytoskeletal reorganization, motility, and invasiveness of HNC cells. In addition, PD-L1 enhanced the EMT of HNC cells by regulating the Snail/vimentin axis. Consistently, we discovered that the methanol extract of *Ishige okamurae* (MEIO) suppressed the PD-L1/Snail/vimentin axis, thereby inhibiting the aggressiveness of HNC cells. The inhibition of PD-L1 that was induced by PD-L1-silencing or by an MEIO treatment led to Snail degradation through a glycogen synthase kinase 3 beta (GSK3β)-dependent mechanism.
Conclusion: The tumor-intrinsic function of PD-L1 on the progression and aggressiveness of HNC could be attributed to the regulation of the GSK3β/Snail/vimentin axis. Therefore, the discovery of a MEIO targeting the tumor-intrinsic function of PD-L1 may prove particularly valuable for the development of novel and effective anti-cancer drug candidates for HNCs overexpressing PD-L1.
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Corresponding author : Sung-Dae Cho

Keywords : PD-L1, *Ishige okamurae*, Head and neck cancer, Metastasis, Snail degradation

PS-B-012

The antitumor effect of Genipin in human oral squamous cell carcinoma

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Background/Objective: Previous studies have reported that Genipin exhibits cytotoxicity against several cancers. However, the detailed mechanism accounting for the antitumor effect of Genipin has not been fully defined in human oral squamous cell carcinoma (OSCC) yet. The aim of the present study is to investigate the molecular mechanism underlying Genipin-induced cell death in human OSCC.
Methods: Cell counting kit-8 and colony formation assays were used to confirm the cytotoxicity effect of Genipin on human OSCC cell lines. Several apoptotic assays, including 4′,6-diamidino-2-phenylindole staining and flow cytometry, were used to determine Genipin-induced apoptosis. In addition, immunocytochemistry and nuclear/cytoplasmic extraction experiments were performed to detect the phosphorylation of STAT3 at tyrosine705 residue (p-STAT3Tyr705) and its nuclear trafficking. Furthermore, an underlying mechanism of Genipin was confirmed using western blot assay and reverse transcription-polymerase chain reaction in human OSCC cell lines.
Results: We observed the inhibitory effect of Genipin on the proliferation of human OSCC cell lines. Genipin induced apoptosis evidenced by the increase in the expression of cleaved-caspase 3 and -poly (ADP-ribose) polymerase. Mechanistic investigation showed that Genipin abrogated STAT3 activity, leading to the downregulation of Survivin and myeloid cell leukemia-1 (Mcl-1) expression at the transcriptional and post-translational levels. In addition, a mechanism of Genipin resulting in the reduced Mcl-1 expression was partly related to proteasomal degradation, which was shown in a cell context-dependent manner.
Conclusion: Our findings raise of possibility that Genipin could be a promising drug candidate and be capable of worthwhile application for human OSCC patients with aberrant expression of STAT3.
Acknowledgements: This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science ICT & Future Planning [2021R1A2C1006470]

Corresponding author : Kyoung-Ok Hong

Keywords : Signal transduction and activator of transcription 3, Oral squamous cell carcinoma, Apoptosis, Genipin, Mcl-1

PS-B-013

Artificial intelligence-based assessment of dependence with cocaine self-administered marmosets data

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Backgrounds: Animal models have played a crucial role in dependence assessment. The non-human primates (NHPs) experiment is regarded as a unique model in neuropharmacological fields. It is because their brains are similar to complicated humans' and they are appropriate for long-term experiment design. However, animal ethics and regulations like 3R encourage researchers to find effective approaches using the minimum number of NHPs. Nowadays, artificial intelligence (AI) is adopted to solve pattern-recognition, classification, and segmentation problems. This study aimed to develop a machine learning (ML)-based classification model for predicting the dependence stage and deep learning-based automatic striatal dopamine transporter (DAT) identification algorithm.
Methods: The data was collected from cocaine self-administered (SA) marmosets and additional toxic dose schedules. First, for classification, dataset consisting of cocaine SA records was divided into several groups by similar dependence patterns with principal component analysis (PCA). And then support vector machine (SVM), a multiclass classification model, was conducted. Second, for striatal DAT segmentation, [F18]FP-CIT PET images were obtained and the U-net, developed for medical image segmentation, was applied.
Results: The cocaine SA dataset PCA algorithm extracted 2 principal components (PC) from the previous dataset, and they explained 97.73% of the variance of previous features. SVM showed classification performance over 0.93 of accuracy for each label. For SD analysis, features were extracted into 2 principal components using PCA. The result of multi-label classification with support vector model (SVM), accuracy was over 0.93 for each label. The results showed that U-NET-based segmentation for striatal DAT achieved 0.96 accuracy, 0.84 IoU, and 0.91 DICE.
Conclusion: The different pattern according to dependence was discovered with machine learning algorithms, and deep learning-based auto identification algorithm for striatal DAT was built.

Corresponding author : Jaesuk Yun

Keywords : Dependence, Self-administration, Artificial intelligence

PS-B-014

The effects of Angelica Gigas Nakai extract on the myelosuppressive mice

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Angelica Gigas Nakai (AGN) is one of the most popular herbs in Korean herbal medicine and is founded in Asian countries including Korea, China, and Japan. The main pharmacological active compounds in AGN are decursin and decursinol angelate from the root of AGN in Korean danggui. It has traditionally been used for therapy of gynecologic disease, anti-inflammation, immunostimulant, and anti-oxidant. However, the hematopoietic effect of AGN was not explored. The present study aimed to investigate the hematopoietic effects of AGN on myelosuppressive mice induced by cyclophosphamide.

We studied the effect of AGN on hematopoiesis in a myelosuppressive mice model. The extract of angelica gigas nakai (AGNEX) was extracted with 70% Ethanol. We confirmed the cytotoxicity of AGNEX on K562 cell line which is a human immortalized myelogenous leukemia cell line, then we moved on to investigate the myelosuppressive mice model. First 3days, cyclophosphamide was injected intraperitoneally to induce myelosuppression after then, AGNEX was injected every day for 10days. To analyze the effect of AGNEX in hematopoiesis, a complete blood count (CBC) analysis was conducted to check the composition change of peripheral blood, and cytokine factors (IL-6 and TNF-a) were measured in serum by ELISA. Bone marrow nucleated cells (BMNCs) and Spleen cells were isolated from mice and used for cell cycle analysis, real-time PCR, and flow cytometry to analyze hematopoiesis factors expression, the cell cycle, and apoptosis. The bone marrow histopathology was processed in femur tissue.

Hematopoiesis factors mRNA and level of cytokines were enhanced in AGNEX treat group. Also, the number of peripheral blood cells was increased after treatment AGNEX. These results suggested that AGNEX facilitates hematopoiesis and has a positive effect to recover myelosuppression.

Corresponding author : Junhong Park

Keywords : Angelica Gigas Nakai, Hematopoiesis, Myelosuppressive

PS-B-015

Administraion of Gastrodia elata blume extract attenuated the acute kidney injury induced by vancomycin in rats

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Vancomycin hydrochloride(VAN), a glycopeptide antibiotic, is associated with several side-effects, including nephrotoxicity. This study investigated the preventive effects of GEB extract on vancomycin-induced acute kidney injury in rats. Five-week-old male Sprague-Dawley rats were randomly divided into the following three groups: control(CON) group, orally administered distilled water(10 mL/kg BW); Vancomycin(VAN) group, orally administered distilled water(10 mL/kg BW); GEB group, orally administered GEB extract(10 mL/kg BW). The treatment period was 14 days. The VAN and GEB groups were intraperitoneally administered VAN(400 mg/kg BW) after oral administration for the last 3-days of the 14-day treatment period. The rats were anesthetized using isoflurane and sacrificed. The kidney weight and the serum levels of blood urea nitrogen and creatinine levels in the GEB group were lower than the VAN group. Histological analysis using hematoxylin & eosin and periodic acid Schiff staining revealed pathological changes in the renal section, such as tubular damage and basement membrane damage of the VAN group, which were mitigated in the renal section of the GEB group. Immunohistochemical analysis revealed that the expression levels of N-acetyl-D-glucosaminidase, myeloperoxidase, and tumor necrosis factor-alpha in the GEB group were decreased when compared with the VAN group. Compared with the VAN group, the number of terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling-positive cells and phosphohistone were lower in the GEB group. Compared with those in the VAN group, the malondialdehyde levels were lower in the GEB group. The levels of total glutathione, an antioxidant, in the GEB group were higher than the VAN group. The findings of this study suggested that GEB extract prevents VAN-induced renal tissue damage by exerting antioxidant and anti-inflammatory effects.

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Corresponding author : Jae-Ho Shin

Keywords : Gastrodia elata Blume, Acute kidney injury, Preventive effect, Anti-inflammation, Anti-oxidation

PS-B-016

Administraion of Gastrodia elata blume extract attenuated the ovalbumin-induced allergic asthma in rats

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Allergic asthma is a chronic lung disease caused by inhalation of external allergens. Symptoms such as dyspnea, cough, wheezing, chest tightness and sputum appear caused. Gastrodia elata blume (GEB) is known to have excellent anti-inflammatory effect and attracting attention as a health functional food. In this study, we investigated the protective effects of GEB extract in allergic asthma model induced by ovalbumin (OVA) in rats. Rats were randomly divided into four groups; ⅰ) negative control group, ⅱ) control (CON) group, ⅲ) OVA group, ⅳ) GEB group. Rats of OVA and GEB groups were sensitized by intraperitoneal injection of 0.3 mg OVA into 1 mL saline containing 30 mg aluminum hydroxide (alum) on every other day for 14 days. The CON group was received 30 mg alum into 1 mL saline. OVA-sensitized rats were challenged with 2% OVA in saline (50 μl) by intranasal inoculation on each side nose from days 29 to 35. The CON group was received only saline. The GEB group was received GEB extract (10 mL/kg, oral) from days 15 to 35 once daily. The CON and OVA groups were received distilled water. All rats were sacrificed 24 h after the last administration. Level of total-IgE was decreased in the GEB group compared to the OVA group. Pulmonary edema, alveolar septa area, infiltration of eosinophils, fibroblast proliferation and collagen in lungs were decreased in the GEB group compared to the OVA group. Bronchoalveolar lavage was performed with 1x PBS 3ml in the lungs. For lung histology stain, grab the left bronchial tubes and performed BALF. In immunohistochemistry, GEB group decreased the expression of interleukin-1 beta, IL-4, IL-5, myeloperoxidase and CD206 compared to the OVA group. In conclusion, GEB extract showed protective effects in allergic asthma rats by reduction of infiltration of inflammatory cells and macrophages.

Acknowledgements: This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No.PJ016773) Rural Development Administration, Republic of Korea.

Corresponding author : Jae-Ho Shin

Keywords : Gastrodia elata Blume, Ovalbumin, Allergic asthma, Protective effect, Anti-inflammation

PS-B-017

Effect of manganese and lead exposure on the expression of serotonin receptors in the stariatum of C57BL/6 mice

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Humans are exposed to Mn and Pb in the workplace and the brain is the major target organ of these metals. Some metals including Mn and Pb are mostly accumulates in the striatum and are known to induce altered expression of neurotransmitters in human brain. Among neurotransmitters, serotonin (5-HT) is a monoamine neurotransmitter in the mammalian CNS and the neurotransmission of serotonin is modulated by 5-HT receptors. In this study, we investigated the expression of genes involved in the serotonergic system in the mouse striatum. We examined the mRNA expression levels of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{3A}, 5-HT_{3B}, 5-HT_{4}, 5-HT_{5A}, 5-HT_{6} and 5-HT_{7} receptors by PCR array. It revealed that the 5-HT_{1b} receptor was likely to be highly expressed in the striatum of Mn+Pb exposed mice, as measured by PCR array. We next performed in Western blot on the striatum to examine the expression of 5-HT_{1B}, 5-HT_{1F}, 5-HT_{2A}, and 5-HT_{2B}. Protein levels of 5-HT_{1B} in Mn+Pb treated groups were significantly increased in the striatum of mice. However, protein levels of 5-HT_{1F}, 5-HT_{2A}, and 5-HT_{2B} in all treated groups did not differ significantly from the control. Our results on the expression of these critical serotonin receptor genes in the striatum provide insight into the toxicogenetics of heavy metals. Furthermore, our findings suggest that targeting the 5-HT_{1B} receptor might have therapeutic potential in neurotoxicity induced by mixed exposure to heavy metals.

Corresponding author : Kisok Kim

Keywords : Heavy metal, Striatum, C57Bl/6, Serotonin receptor

PS-B-018

Maternal DMEP exposure alters neural proliferation and synaptic function in the mice

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Di-methoxyethyl phthalate (DMEP) has been widely used in a variety of products including plastics, medical equipment and associated with neurodevelopmental disease including autism, schizophrenia and intellectual disability. However, the underlying mechanism remain poorly understood. Here, we show that maternal DMEP exposure leads to autism-like behaviors via abnormal brain development in the mice. To investigate the effect of DMEP in brain development, pregnant mice were exposed with either DMEP (10mg/kg) or control orally once a day from E0 to end of breast feeding. We found that Maternal DMEP exposure resulted in disruption of progenitor self-renewal in the developing brain and thereby reduction of neural cell in the cerebral cortex. More importantly, we found that DMEP disrupted normal synaptic formation and function in the cerebral cortex. Finally, maternal DMEP exposure alters hyperactive and anxiety behaviors. Our findings suggest that maternal DMEP exposure leads to abnormal synaptic function and autism-like behaviors.

Corresponding author : Sung-Ae Hyun, Minhan Ka

Keywords : DMEP, Brain development, Phthalate, Maternal exposure, Autism

PS-B-019

Toxicity evaluation of cigarette butts using chironomus riparius

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The purpose of this study is to evaluate the degree of impact on the ecosystem of cigarette butts exposed to the natural environment.
In Korea, more than 10 million cigarette butts are dumped on the street every day. Many cigarette butts containing harmful substances flow into rivers and seas, adversely affecting the ecosystem. The problem is that most cigarettes use plastic filters, and microplastics from discarded cigarette butts are affecting the marine ecosystem as well as our tables.

Chironomus riparius was used to evaluate the toxic effects of exposure. In addition, as a result of the mixed toxicity evaluation of cocobetaine and cocoglucoside daphnia, each EC50 value was evaluated as 41.5ppm, but the actual mixing quality test value was 41ppm. As a result of the mixed toxicity evaluation of Coco Betaine and Decyl-Glucoside, each EC50 value was 38 ppm, but the actual test value of the mixture was 47 ppm. In this study, the results of these complex evaluations are compared. In order to evaluate the complex environmental impact, it is judged that various complex exposure tests are needed in the future.

Corresponding author : Dong Hyun Kim

Keywords : Toxicity, Chironomus riparius, Cigarette butts, Microplastics

PS-B-020

Prediction of skin sensitization potential of silicon dioxide and titanium dioxide nanoparticles through the local lymph node assay: 5-bromo-2-deoxyuridine flow cytometry method

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The development of nanotechnology has encompassed the use of nanoparticles (NPs) in various fields including industry, agriculture, engineering, cosmetics, or medicine. Metal oxide NPs such as silicon dioxide (SiO2) and titanium dioxide (TiO2) are consistently used in cosmetics and dermal products to confer better UV protection, or to make products transparent and aesthetically acceptable. Although used in dermal products, the skin sensitization (SS) potentials of those NPs have not been well investigated. In the present study, we employed local lymph node assay: 5-bromo-2-deoxyuridine flowcytometry method (LLNA:BrdU-FCM) to screen the skin sensitization potential of NPs. LLNA:BrdU-FCM is a modified non-radio isotopic method for screening of SS potential that addressed the activation and proliferation of T-lymphocytes, key event-4, on adverse outcome pathway (AOP) for SS. SiO2 and TiO2 were suspended uniformly in N,N-dimethylformamide and dimethyl sulfoxide, respectively. AOO (acetone: olive oil=4:1) and α-hexyl cinnamaldehyde were used as negative and positive control, respectively. The stimulation index (SI) values of SiO2 were 1.2, 1.3, and 1.6 at 2.5%, 5%, and 10% test concentrations, respectively. The SI values of TiO2 were 0.9, 0.8, and 0.9 at 5%, 10%, and 25% test concentrations, respectively. Since SI $\leq$ 2.7 is considered non-skin sensitizer, both test chemicals were predicted to have no significant skin sensitization potential. Based on the present results, further confirmatory tests addressing other key events of SS AOP should be carried out on SiO2 or TiO2 NPs.
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Corresponding author : Yong Heo

Keywords : LLNA-BrdU-FCM, Skin sensitization, TiO2, SiO2, Nanoparticles

PS-B-021

Electro-acupuncture stimulation of HT7 alleviate sleep deprivation against acute caffeine exposure by regulating BDNF-mediated ER stress in the rat medial septum

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Background: Acupuncture stimulation can protect brain against caffeine-induced sleep deprivation. This study was to investigate whether electroacupuncture stimulation at Shenmen (HT7) alleviates the sleep deprivation by regulating brain-derived neurotrophic factor (BDNF) and endoplasmic reticulum (ER) stress in the medial septum (MS).
Methods and Results: Acute exposure of caffeine (15 mg/kg, i.p.) increased wake time and decreased rapid eye movement (REM) sleep and HT7 stimulation alleviates the wake time and REM sleep. Acute caffeine exposure also increased total distance and decreased immobility time and HT7 stimulation alleviates the behavior changed. The ER stress response protein, glucose-regulated protein 78 (BiP), increased by acute caffeine exposure in the rat MS and HT7 stimulation alleviate BiP expression. Interestingly, HT7 stimulation induced mature Brain-derived neurotrophic factor (mBDNF) and mBDNF phosphorylated receptor, phospho-Tropomyosin receptor kinase B (pTrkB) exposure in the MS. Next experiment was to investigate whether phosphorylated TrkB by HT7 stimulation induce BiP expression in the rat MS. Before electro-acupuncture stimulation at HT7, pTrkB antagonist (ANA-12) treated to caffeine exposed rat. In rat administered ANA-12 to MS, HT7 stimulation did not reduce the expression of BiP.
Conclusion: These finding suggest that HT7 stimulation improve wake, REM sleep dysfunction by regulating BDNF mediated ER stress response in the MS.

Corresponding author : Yeonhee Ryu

Keywords : Sleep, EEG, Caffeine, Acupuncture

PS-B-022

A comparative study on clinical pathology under two different diets in cynomolgus monkeys (*Macaca fascicularis*)

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Background: The cynomolgus monkeys (*Macaca fascicularis*) are most commonly used non-human primate for toxicological studies. Two types of diet for primate (Maintenance diet for non-human primates #6029, Altromin Spezialfutter GmbH & Co., Germany and Certified Primate Diet #5048, PMI Nutrition International, Inc., USA) have been provided to the monkeys in non-clinical studies at the Jeonbuk Branch Institute, Korea Institute of Toxicology (KIT). It is important to establish a baseline for clinical pathology parameters (hematology, clinical chemistry and urine/urine chemistry analysis) compared Altromin diet (#6029) with PMI diet (#5048) and determine the effects of diets on these indices. Therefore, in this study, clinical pathology historical data for non-clinical studies were collected in both sexes of monkeys and analyzed.
Methods: Hematology (RBC, WBC, HCT, MCV, PLT, etc.), clinical chemistry (AST, ALT, ALP, BUN, GGT, etc.) and urine/urine chemistry analysis (potassium, chloride, sodium, etc.) historical data were evaluated and compared in 210 male and 122 female 2- to 4-year-old cynomolgus monkeys that fed Altromin diet (#6029) or PMI diet (#5048) of 61 non-clinical toxicologic studies conducted for past five years (from 2015 to 2019) at the Jeonbuk Branch Institute, KIT.
Results: In hematology, clinical chemistry and urine/urine chemistry analysis values, there were no significant differences between Altromin diet and PMI diet in male and female monkeys.
Conclusions: The significant differences in clinical pathology historical parameters of non-human primate between the PMI diet and Altromin diet were not found.

Corresponding author : Sang-Kyum Kim

Keywords : Cynomolgus monkeys, Clinical pathology, Diet

PS-B-023

Mechanisms of cutaneous neurogenic inflammatory spots expression on referred visceral pain: primary and secondary uterine pain in the adult virgin rat

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Referred pain means that the visceral pain is felt in a part of the body other than the organ that caused the pain. This referred pain is often accompanied by tenderness, hyperalgesia and neurogenic plasma extravasation in the skin areas. Previous studies have demonstrated that uterine inflammation with mustard oil results in neurogenic plasma extravasation in the skin and presented evidence for the trophic changes observed in the area of referred visceral pain, but the central mechanisms of neurogenic inflammatory spots expression and differences in expression patterns according to types of uterine pain are not understood. To explore this, we established a model of primary and secondary uterine pain in the adult virgin rat, plasma extravasation from neurogenic inflammation was examined by intravenously injected Evans blue dye (EBD) to the skin. The expression of the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) measured to analyze physiological-biochemical changes the during neurogenic inflammation in the referred pain area, and mechanical sensitivity at neurogenic spots was determined by the withdrawal response to the probing of von Frey filaments. Primary uterine pain evoked by Estradiol benzoate and oxytocin treatment and 10% mustard oil used to induce the secondary uterine pain. Rats with uterine pain showed abnormal behaviors such as hunching, stretching of the body and increased the number of extravasated EBD on the skin. The mechanical sensitivity, SP and CGRP expression increased at these neurogenic inflammations in the referred pain area occurred during the uterine pain. Our findings suggest that primary and secondary uterine pain increases extravasation from neurogenic inflammation and mechanical sensitivity in specific areas of the skin related to referred pain, and this phenomenon is mediated of SP and CGRP expression.

Corresponding author : Yeonhee Ryu, Hee Young Kim

Keywords : Uterine pain, Neurogenic inflammatory spot, Evans blue, Substance P, CGRP

PS-B-024

Aromadendrin reduces airway inflammation in an experimental mouse of allergic asthma

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It was reported that Aromadendrin (ARO) had anti-inflammatory effects in activated-RAW264.7 macrophages and -T cells. Based on these biological properties, we investigated the protective effect of ARO on allergic asthma (AA) using ovalbumin (OVA)-induced experimental mouse of AA. The results indicated that the significant increase of inflammatory cells (eosinophils/macrophages) and Th2 cytokines [interleukin-4 (IL-4), IL-5 and IL-13] was confirmed in BALF of mice of OVA-induced AA. Results also showed that the level of immunoglobulin E (IgE) was upregulated in serum of OVA-induced AA mice, whereas this trend was effectively decreased by ARO administration. However, the administration of ARO effectively suppressed these levels. The results obtained from histological analysis (Hematoxylin and Eosin staining) also showed that the increased levels of inflammatory cell influx nearby airway and mucus secretion in airway epithelium was significantly decreased by ARO administration. Furthermore, the notable upregulation of inhibitor of nuclear factor kappa beta (NF-kB) and NF-kB p65 phosphorylation in lungs of OVA-exposure mice was significantly downregulated by ARO. Collectively, the results from this study indicate that ARO has protective effect on the OVA-induced airway inflammation in mice by regulating inflammatory cell influx, molecules and NF-kB activation, suggesting that ARO may have potential in the prevention or treatment of AA.

Corresponding author : Sei-Ryang Oh, Jae-Won Lee

Keywords : Allergic asthma, Aromadendrin, Th2 cytokines, IgE, Eosinophil

PS-B-025

Aromadendrin has anti-inflammatory effect on airway inflammation in an experimental animal models of COPD

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Aromadendrin (ARO) has inhibitory effect on generation of inflammatory cytokines in activated-RAW 264.7 macrophages. Thus, we studied the protective effect of ARO on development of airway inflammation in an experimental mouse of chronic obstructive pulmonary disease (COPD). The results showed that the levels of inflammatory cells (neutrophils/macrophages), reactive oxygen species (ROS) and inflammatory cytokines [interleukin-6 (IL-6), IL-1β, tumor necrosis factor-α (TNF-α)]/chemokine [monocyte chemoattractant protein-1 (MCP-1)] are notably elevated in bronchoalveolar lavage fluid (BALF) of cigarette smoke (CS) and lipopolysaccharide (LPS)-induced experimental mice of COPD. However, these trends were effectively suppressed by ARO administration. In addition, it was shown that ARO administration led to reduction the CS and LPS-induced inflammatory cell influx in lung of COPD mice through histological analysis (Hematoxylin and Eosin staining). Results also indicated that ARO effectively suppressed the expression of inducible nitric oxide synthase (iNOS) in lung of CS and LPS-exposure mice. In immunoblotting analysis, the upregulation of inhibitor of nuclear factor kappa beta (NF-κB) p65 phosphorylation in lung tissues of CS and LPS-exposure mice was decreased by ARO. These results reflect that ARO suppresses airway inflammation by regulating inflammatory cell recruitment, inflammatory molecules production and NF-κB activation and thus may be valuable adjuvant in the treatment of COPD.

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Keywords : COPD, airway inflammation, aromadendrin, neutrophil, NF-κB

PS-B-026

Inhibition of liver X receptor attenuates nonalcoholic steatohepatitis by regulating differentiation of monocyte-derived macrophages

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Background: Dysregulation of hepatic fat metabolism and inflammation is associated with non-alcoholic steatohepatitis (NASH). Despite the surge in the prevalence of NASH, there is no approved treatment yet. Liver X receptors (LXRs) are ligand-activated transcription factors of the nuclear receptor superfamily that can stimulate *de novo* lipogenesis. Recently, LXRα has been considered as a crucial factor in the differentiation of bone marrow monocyte-derived macrophages into Kupffer-like cells. Here, we demonstrate that inhibition of LXR by a novel natural compound (NC) alleviated hepatic steatosis and reduced the formation of inflammatory macrophages.
Methods: NASH was induced by 12-week high-fat diet (HFD) feeding with or without NC treatment (50mg/kg) to wild-type (WT) and LXRα knock-out (KO) mice. Time-resolved fluorescence resonance energy transfer (TR-FRET), luciferase reporter assay, and computational modeling were used to evaluate the effects of NCs in LXRα regulation. Isolated primary hepatocytes and bone marrow-derived macrophages were subjected to flow cytometry and qRT-PCR analyses.
Results: Transcriptomics in NASH patients and *in vitro* experiments revealed that hepatic lipogenesis and fat accumulation were significantly related to LXRα-mediated gene expression. Computational prediction of protein-ligand binding and luciferase reporter assay showed that the NC had a high affinity with LXRα, in which S264 residue of LXRα was the major interaction site with NC. In addition, NC suppressed hepatic LXRα activity by allowing the binding of corepressors to LXR response element rather than coactivators as demonstrated by the TR-FRET assay. Moreover, NC inhibited LXRα-mediated differentiation of monocytes into resident-like macrophages, decreasing pro-inflammatory cytokines levels. Accordingly, inhibition of LXRα activity by NC attenuated hepatic steatosis and inflammation in WT mice but not in LXRα KO mice.
Conclusion: Inhibition of LXRα downregulates de novo lipogenesis in hepatocytes and reduces the differentiation of monocytes into inflammatory macrophages, leading to the amelioration of NASH. Therefore, LXRα might be a potential therapeutic target for the intervention of NASH.

Corresponding author : Won-Il Jeong

Keywords : Non-alcoholic steatohepatitis, Liver X receptor α, *De novo* lipogenesis, Monocyte-derived macrophage, Bone marrow-derived macrophage

PS-B-027

Actue toxictiy study of Asparagus officinalis L. root extracts in Sprague Dawley rats

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Asparagus (Asparagus officinalis L.) is a perennial herb with various bioactivities and has been widely used as food and medicine since ancient times. Asparagus root extract (ARE) hanve been proven scientifically for their efficacy as antioxidant and so on. But it cannot be edible as food in Korea. So, the present study is to investigate the acute toxicity of ARE on Sprague Dawley rats using the fixed doses of 300 and 2,000 mg.kg BW. The acute toxicity studies were carried out based on OECD guidelines 423. An acute dose of the test substance was administered by oral route at a dose of 300 mg/kg B.W. (1st, 2nd step) and 2,000 mg/kg B.W. (3rd step, 4th step). Three animals were used for each step and there were 4 steps in total. The animals had been observed for 14 days after administration and mortality, clinical signs, body weight and necropsy findings were recorded. The test substance-related dead animals were not observed during the study period. In clinical signs, the test substance-related clinical signs were not observed in all administration groups. In body weight, the body weight loss observed in some animals of 300 mg/kg B.W. (2nd step) were considered to be adventitious body weight changes by considering to the clinical signs and degree of loss (2.68 %). In necropsy, there were no necropsy findings at all administration groups. No signs of toxicity and no deaths were observed.

Corresponding author : Jae Hee Lee

Keywords : Asparagus officinalis L., Actue toxictiy, Asparagus root extract

PS-B-028

The NLRP3 inflammasome is inhibited in natural products, protecting against acute gout

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Acute gout is caused by the accumulation of urate crystals caused by hyperuricemia and the NLRP3 inflammasome activated to eliminate it. In this study, it is intended to check whether Soyum treatment has an anti-inflammatory effect on acute urate-induced mouse gout. Gout was induced by injectinf urate crystals into the foot of the mouse, and after injection of Soyum into SP3, the foot thickness of the mouse was measured for each time period. MPO activity was measured, caspase-1 and IL-β production were measured by immunological blotting and enzyme-bound immunoadsorption analysis, to determine the activation of the NLRP3 inflammasome. Anti-inflammatory drugs reduced paw thickness in acute gout-induced mice and suppressed caspase-1 and IL-1β production. However in RAW264.7 cells, Soyum did not show the effect of suppressing the production of nitrit, IL-6, and TNF-α. We confirmed that Soyum treatment for urate-induced acute gout in mice suppressed the activity of the NLRP3 inflammasome and was effective in treating gout attacks.

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Corresponding author : Gabsik Yang

Keywords : Gout, Inflammation, NLRP3 inflammasome, Urate crystals, Anti-inflammatory drug

PS-B-029

Natural products protect against acute gout by inhibiting the activation of the NLRP3 inflammasome

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Acute gout, caused by the deposition of urate crystals due to persistent hyperuricemia, is closely related to the NLRP3 inflammasome. In this study, the anti-inflammatory and analgesic effect of the pharmacopuncture treatment for acute gout in mice induced by MSU was confirmed.

Acute gout was induced by injecting MSU into the footpads of mice, and after injecting pharmacopunctur(Zanthoxyli Pericarpium) into the Sameumgyo(SP6), the foot thickness of the mice was measured for each time period. MPO activity was measured, and NLRP3 inflammasome activation was determined by measuring the production of caspase-1 and IL-1β by immunoblotting and ELISA.

As a result of measuring the foot thickness of mice, the foot thickness of the pharmacopuncture group was significantly reduced, and this group showed almost the same effect as the positive group (colchicine). In the pharmacopuncture group, the MPO activity, the expression levels of caspase-1 and IL-1β was significantly reduced. As a result of the RAW264.7 cell experiment, the production of nitrite, IL-6, and TNF-α was significantly reduced in the pharmacopucnture group.

In conclusion, it was confirmed that the pharmacopuncture treatment, for acute gout in mice induced by MSU, had an anti-inflammatory effect. Through follow-up studies, if data on the ingredients, side effects, and optimized administration method of Zanthoxyli Pericarpium pharmacopuncture are accumulated, it is expected that this pharmacopuncture can be used as the first choice treatment for analgesic and anti-inflammatory purposes during acute gout attacks.

* This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number : HF20C0145).

Corresponding author : Gab Sik Yang

Keywords : Gout, NLRP3 Inflammasome, Zanthoxyli Pericarpium, Monosodium Urate crystal, Inflammation

PS-B-030

Ginger and its two active components as novel autophagic and apoptotic mediators in oral squamous cell carcinoma

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Background/Objective: Rhizomes of Zingiber officinale have been reported to display antioxidant, anti-inflammatory, antiulcer, and antitumor properties. In this study, we evaluated the anticancer effect of ethanol extract of Z. officinale rhizomes (ZOE) and identified its active single components in human oral squamous cell carcinoma (OSCC) cell lines.
Methods: After ZOE treatment, cell counting kit-8 assay, trypan blue assay, and soft agar assay were performed in OSCC cell lines to investigate the growth inhibitory activity of ZOE. We also conducted 4′,6-diamidino-2-phenylindole staining, Sub-G1 assay and Annexin V/ propidium iodide double staining to examine apoptosis induction of ZOE on OSCC cell lines. Transmission electron microscope and confocal laser scanning microscope were used to observe the formation of double membraned autophagosomes and autolysosomes. 1-dehydro-6-gingerdione and 8-shogaol, two active components of ZOE, were identified through gas chromatography/mass spectrometry.
Results: ZOE exhibited effective anti-proliferative activity in OSCC cell lines by simultaneously stimulating apoptosis and autophagy. This may be related to endoplasmic reticulum stress induction, as evidenced by the expression of C/EBP homologous. Two active single components, 1-dehydro-6-gingerdione and 8-shogaol, were isolated from ZOE by column chromatography and spectroscopy and exhibited anticancer activity in the same manner as ZOE.
Conclusion: These results suggest that ZOE and its two active components could hinder the proliferation of OSCC cells employing ER stress-associated apoptosis and autophagy.

Corresponding author : Ji-Ae Shin

Keywords : Zingiber officinale, Oral squamous cell carcinoma, Apoptosis, Autophagy

PS-B-031

Development of pcl nasolacrimal stent for the treatment of epiphora

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In general, epiphora was when the lacrimal drainage system is stenotic or obstructed due to various causes such as inflammation, trauma, and obstruction, tears cannot be discharged into the nasal cavity and accumulate in the eyes and then flow out of the eyes. Such epiphora not only causes the inconvenience of always wiping the tears from the eyes, but also causes visual disturbances and dacryocystitis. Therefore, when the lacrimal drainage system is narrowed or blocked as described above, it should be treated early. The purpose of this study was to develop Poly(ε-caprolactone)-based shape memory polymers(SMP) nasolacrimal stent and compared with commercial stent. Since the nasolacrimal stent must be inserted into the nasolacrimal duct and maintained for a certain period of time, safety from inflammatory reactions after transplantation must be ensured. For in vivo experiments, they were implanted in rabbit nasolacrimal duct during 4 months. 11 male rabbits were divided into 2 groups by body weight before stent implantation. Experiment Groups : Implanted commercial silicone stent, Bio lacrimal intubation; group 1(n=5), Implanted developed PCL stent, INNOSELF-L; group 2(n=6). Each group was sacrificed 4 months after implantation, and the results were analyzed through dacryocystography and H&E staining. As a result of in vivo tests, the PCL stent showed no difference compare to commercial stent in body weight, change of internal diameter in nasolacrimal duct and histological analysis for 4 months. In conclusion, it was confirmed that safety against inflammation during implantation in nasolacrimal duct was secured. Also PCL based SMP nasolacrimal stent can be suggested as a superior alternative to the currently used nasolacrimal stents.

Corresponding author : Rae-hyung Rye

Keywords : Epiphora treatment, Nasolacrimal duct diasease, Poly(ε-caprolactone)-based shape memory polymers(SMP) stent, Nasolacrimal stent

PS-B-032

Symptom of collagen induced arthritis in DBA-1J mouse was alleviated by bee venom

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Bee (Apis mellifera L.) venom has been used and investigated for drug and cosmetic ingredient related with its anti-inflammatory, anti-bacterial effect, and anti-aging in last decades. Apitoxin is an injection developed by bee venom and permission to human administration for anti-pyretic, analgesic, anti-inflammatory. Present study was conducted to expand the scope of application of apitoxin to arthritis. We used collagen induced arthritis (CIA) model in DBA-1J mouse. Apitoxin injected intradermally twice a week and we recorded body weight, arthritis score, and hindlimb paw edema once a week until 4 weeks in CIA mouse. Body weight of experimental group were significantly decreased compared with normal group during experimental period. Although arthritis score of all experimental group higher than normal group, Apitoxin high dose treated group significantly ameliorated arthritis score compared with vehicle group in day 14 and 28. Hindlimb paw edema of apitoxin treated group revealed significant reduction of edema level compared with vehicle group. Additionally, inflammatory cytokine such as IL-6, IL-1β and Type 2 collagen IgG were confirmed in articular from CIA mouse at day 28. Both IL-6 and Type 2 collagen IgG were decreased in apitoxin treated group compared with vehicle group significantly. Histopathological analysis of articular tissue also revealed same improved results in apitoxin treated group. Taken together, these results suggest that apitoxin has potential administration to arthritis patients.

Corresponding author : Sang-Ho Seol, Sokho Kim

Keywords : Bee venom, Apitoxin, CIA mouse, Inflammation, Arthritis

PS-B-033

Repeated dose toxicity study in beagle dogs by intrathecal lumbar puncture: a potential non-rodent model for intrathecal drug delivery

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An intrathecal administration is a highly effective route for delivery of neurological disorder drugs do not cross the Blood-Brain Barrier. This route also has an advantage of local delivery to the central nervous system (CNS) with a low load for liver and kidney. An intrathecal lumbar puncture technique in toxicity study is commonly developed in non-human primate, and there are few studies in non-rodent. In this context, dogs are an attractive alternative to cynomolgus monkey, because of their widespread use within the toxicity studies, relatively low cost, and easy to handle for experiments.

To provide toxicity data of non-rodent study with the intrathecal route, this study investigated the toxicity of the intrathecal lumbar puncture administration of the neurological disorder drugs for a 13-week in beagle dogs. Test items were administered monthly to dosing groups (2 or 3 animals/sex/group) at 0, 20 and 60 mg/2 mL. In vehicle groups, clinical signs including unconsumed feed, scratch wound, loss of fur, soft and liquid feces were observed, and there were no abnormal signs in neurological examinations. In test item groups, clinical signs including low body temperature, unconsumed feed, vomiting, eye discharge, eye coloration, scratch wound, loss of fur, skin coloration, soft and liquid feces were observed, and there were abnormal neurological signs in gait, limb, conscious proprioception, pain perception, wheelbarrowing, patellar reflex and anal reflex.

As a results of repeated intrathecal administrations to beagle dogs for a 13-week, there were no adverse effects related to intrathecal lumbar puncture technique, such as reduced/absent patella reflex in the control group following lumbar puncture in previous monkey studies. Collectively, this study could provide a reference to discriminate test-item related and dosing technique related effect by intrathecal route in non-rodent study, and suggest the dog might be a potential animal model for toxicity study of intrathecal drug delivery.

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Corresponding author : Kang-Hyun Han

Keywords : Intrathecal lumbar puncture, Intrathecal drug delivery, Neurological disorder drug, Beagle dog, Repeat dose toxicity study

PS-B-034

Antiepileptic and anxiolytic effects of hinokinin

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The solute carrier 6 (SLC6) transporters, which include the serotonin, dopamine, norepinephrine, GABA, taurine, creatine, as well as amino acid transporters, are associated with a number of human diseases and disorders making this family a critical target for therapeutic development. According to previous studies, a derivative of the lignan cubebin, namely, hinokinin has interaction with neurotransmitter transporters and was suggested as a tool to develop new therapeutic drugs for anxiety that target the DAT, NET, and GAT-1 transporters. In the present study, we evaluated the antiepileptic and anxiolytic effects of hinokinin. For antiepileptic evaluation, we performed pentylenetetrazol (PTZ)-induced seizure test in mice. Anxiolytic effects were performed with elevated plus maze and control diazepam (2 mg/kg i.p.) was used. For all experiments, we used 1 mg/kg or 5 mg/kg hinokinin in C57BL/6J mice. After 30-minute injection of hinokinin seizure score determined via 6 degrees of seizure during 10 minutes. Likewise, after 30-minute injection of hinokinin, we recorded behavior in elevated plus maze for 5 minutes. The results show that hinokinin significantly decreased PTZ-induced seizure. While in elevated plus maze test hinokinin increased time in open arms. Besides, hinokinin showed a dose dependence effect. Taken together, we suggest that hinokinin has antiepileptic and anxiolytic effects.

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Keywords : Transporter, Hinokinin, METH, PTZ, Diazepam

PS-B-035

Evaluation of dependence potential induced by abused drugs with intracranial selfstimulation

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Intracranial self-stimulation (ICSS) is suitable to measure relative drug dependence. Lowering of ICSS thresholds indicates a facilitation of brain stimulation reward, whereas elevations in ICSS thresholds reflect the diminished reward value of the stimulation and thus an anhedonic state. We measured dependence potential of N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide (AKB-48), 3-fluoroethamphetamine (3-FEA), 1-naphthalenyl[4-(pentylox)-1-naphthalenyl]-methanone (CB-13), alpha-pyrrolidinobutiothiophenone (α-PBT), paramethoxymethamphetamine (PMMA), and mg/kg). and α-PBT (10 and 30 mg/kg) treatment showed that the response times were increased at low frequency in comparison with that of saline group, but not in case of CB-13, AKB-48, and PMMA. Maxium threshold alteration (Δmax) of ICSS by drugs is -0.426 at 10 mg/kg for 3-FEA, -0.359 at 10 mg/kg for α-PBT, 0.018 at 0.1 mg/kg for AKB-48, -0.089 at 0.3 mg/kg for CB-13, - 0.106 at 3 mg/kg for PMMA, and -0.246 at 30 mg/kg for larocaine. Based on the ICSS results, the order of reward-related behaviour-evoking potenital to be 3-FEA > α-PBT > larocaine > PMMA > CB-13 > AKB-48. This suggests that ICSS can make a significant contribution to the drug dependence potential comparison evaluation.

Corresponding author : Jaesuk Yun

Keywords : Intracranial self-stimulation, Abused drugs, Dependence potential

PS-B-036

Anti-cancer effects of a novel multi-targets agent, KMU-191 and its electrophysiological safety

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Anti-proliferative effects of a newly developed *N*3-acyl-*N*5-aryl-3,5-diaminoindazole analogue, KMU-191 were previously evaluated in various cancer cells. However, the detailed anti-cancer molecular mechanisms by KMU-191 remained unknown. In this study, anti-cancer mechanisms were investigated regarding the regulation of apoptosis-related genes in KMU-191-mediated apoptosis of human clear cell renal cell carcinoma Caki cells. KMU-191 induced poly ADP-ribose polymerase cleavage and caspase-dependent apoptosis. In addition, KMU-191 induced down-regulation of long form of cellular FADD-like IL-1β-converting enzyme inhibitory protein (c-FLIP (L)) at transcriptional level as well as that of long form of myeloid cell leukemia (Mcl-1 (L)) and B-cell lymphoma-extra large at post-transcriptional level. Furthermore, KMU-191-induced apoptosis was strongly associated with down-regulation of Mcl-1 (L), but in part with c-FLIP (L). On the other hand, KMU-191 induced up-regulation of p53, which was closely related to KMU-191-induced apoptosis. Electrophysiological safety was confirmed by determining the cardiotoxicity of KMU-191 via left ventricular pressure analysis. Taken together, these results suggest that a multi-target small molecule, *N*3-acyl-*N*5-aryl-3,5-diaminoindazole analogue, KMU-191 is a potential anti-cancer agent with electrophysiological safety.

Corresponding author : Jinho Lee, Ki-Suk Kim, Jong-Wook Park

Keywords : KMU-191, Apoptosis, Caki cells, Electrophysiological safety, *N*3-acyl- *N*5-aryl-3, 5-diaminoindazole analogue

PS-B-037

Liver toxicity from PFOS exposure in animal studies: A systematic review and meta-analysis

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Introduction: Perfluorooctanesulfonic acid (PFOS) is a persistent organic compound used in various products such as cooking utensils and cosmetics, but health issue has been raised that it may cause liver toxicity. However, there have been inconsistent results on toxicity study. Therefore, we conducted a systematic review on the association between PFOS exposure and liver toxicity in animal studies.
Method and Results: We searched animal studies related to liver toxicity from PFOS exposure up to May 2022 using three database (PubMed, Embase, Web of science) and found 872 studies excluding duplicates. After screening the title and abstract review, full-text review, 9 studies were finally selected. Of these, two studies with quantitative data were included in the meta-analysis. The meta-analysis was analyzed using the fixed effects model method, and as a result, serum AST was not associated with PFOS exposure (MD: 9.66; 95% CI: -1.40, 20.72; I^2 = 36%; Z = 1.71; P = 0.09).
Conclusion: Our result showed that PFOS exposure was not associated with liver toxicity in animal study. However, due to the small number of studies included in the meta-analysis, more animal studies are needed to conclude the exact impact of PFOS on liver toxicity.
Acknowledgement: This research was supported by a grant (21162MFDS076) from Ministry of Food and Drug Safety in 2022.

Corresponding author : Hae-Jeung Lee

Keywords : Perfluorooctanesulfonic acid, Liver toxicity, Systematic review, Meta-analysis

PS-B-038

Amelioration of tunicamycin-induced liver injury by taurine supplementation in mice

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Non-alcoholic fatty liver disease (NAFLD) is a worldwide chronic liver disease featured with excess of lipid accumulation Especially, non-alcoholic steatohepatitis progressed from simple fatty liver can be exacerbated into very severe liver conditions such as cirrhosis and hepatocellular carcinoma. Whereas several pharmacological approaches have been assessed in clinical trials, there are no approved therapies for NAFLD up to date. Literatures suggested that taurine supplementation alleviates fatty liver, however, the underpinnings of its beneficial effect in mechanism remains obscure. In this study, we investigated the beneficial effects of taurine on fatty liver injury induced by a chemical endoplasmic reticulum (ER) stressor, tunicamycin, under in vivo conditions. 2% taurine in drinking water was supplied to mice for 2 weeks prior to intraperitoneal tunicamycin injection. After 72 hours following the tunicamycin treatment, mice were sacrificed. tunicamycin treatment led to significant augmentation in the activity of ALT and AST as well as hepatic triglyceride level. Of note, all of these levels were alleviated by taurine supplementation. Taurine attenuated protein expression of ER stress markers (i.e. IRE1a, p-IRE1a, ATF6, and CHOP) and lipid uptake transporter CD36 that was increased by tunicamycin treatment. Furthermore, taurine supplementation prevented tunicamycin-induced lipid peroxidation along with decreased glutathione (GSH) level via correction of abnormal cysteine catabolism which is involved in production of both taurine and GSH. These results suggest that taurine supplementation prevents tunicamycin -induced liver injury through manipulating oxidative stress as well as ER stress.

Corresponding author : Young-Suk Jung

Keywords : Non-alcoholic fatty liver disease, Endoplasmic reticulum stress, Oxidative stress, Lipid accumulation, Glutathione

PS-B-039

Dietary restriction alleviates acetaminophen-induced hepatotoxicity

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Dietary restriction (DR) has been revealed to have health benefits as it induces reduction in oxidative stress. Glutathione (GSH), an important cellular antioxidant, is increased in rodent livers owing to DR; however, the exact mechanism and clinical relevance of DR are yet to be fully understood. In this study, male C57BL/6 mice were administered a 50% restricted diet for 7 days, and the hepatic sulfur-containing amino acid (SAA) metabolism was determined to assess the biosynthesis of GSH. The hepatic methionine level was found to decrease, while the homocysteine, cysteine, and GSH levels were increased owing to decreased betaine-homocysteine methyltransferase (BHMT) and increased CβS, CγL, and GCL catalytic subunit (GCLC) proteins in the livers of mice subjected to DR. To determine the effects of DR on drug-induced oxidative liver injury, mice subjected to DR were injected with a toxic dose (300 mg/kg) of acetaminophen (APAP). DR significantly alleviated APAP-induced liver damage and oxidative stress, which might be attributed to the higher levels of GSH and related antioxidant enzyme (GPx, GSTα, and GSTμ) in the livers. The decrease in the levels of hepatic CYP1A, 2E1, and 3A, which imply the inhibition of APAP metabolic activation, could contribute to the lower hepatotoxicity in mice subjected to DR. Overall, our findings revealed that DR stimulated the hepatic transsulfuration pathway and GSH synthesis. The consequent elevation of GSH could thus serve as an important mechanism of DR-mediated liver protection against APAP intoxication.

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Corresponding author : Young-Suk Jung

Keywords : Dietary restriction, Sulfur amino acid metabolism, Glutathione, Acetaminophen, Cytochrome P450

PS-B-040

Synthetic cannabinoids-induced reward behavior is associated with cannabinoid receptor 1 receptor and dopamine transporter function

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New psychoactive substances (NPS) are often developed whilst modifying the basic chemical structures of many abused drugs. But toxicology and pharmacology of NPS is not well-known. Cannabinoids are involved in drug addiction through cannabinoid type 1 receptor (CB1). In this study, we observed that the behavioral effect of synthetic cannabinoids (CBs) among NPS, JWH-018, CB-13 or AKB-48 in C57BL/6N mice. Conditioned place preference (CPP) induced by JWH-018 (0.025, 0.05 mg/kg, i.p.), CB-13 (0.1, 0.3 mg/kg, i.p.) or AKB-48 (0.1, 0.3 mg/kg, i.p.) were evaluated. Furthermore, we measured the intracellular Ca^{2+} signals induced by CBs in PC12 cells to confirm neuronal activity alteration. In addition, we studied the CB1 availability and dopamine transporter (DAT) function in the striatum. JWH-018 (0.05 mg/kg) or CB-13 (0.3 mg/kg) induced CPP development in mice, but not AKB-48. Ca^{2+} signals were decreased by JWH-018 (0.1 μM) or CB-13 (0.1 μM) treatment in PC12 cells, but not AKB-48 (0.1 μM) treated cells. JWH-018 (0.05 mg/kg) or CB-13 (0.3 mg/kg) decreased CB1 availability and DAT function, but not AKB-48. These results suggest that synthetic cannabinoids induced reward behavior through modulation of Ca^{2+} signaling, CB1 availability, and DAT function.

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Keywords : Drug abuse, Conditioned place preference, Cannabinoid receptor 1, Dopamine transporter, Dependency

PS-B-041

Evaluation of the regenerative potential of cell therapeutics using repair-associated cell-containing intestinal organoid

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Mesenchymal stem cell (MSC)-based cell therapeutics possess the abilities of immunomodulation and wound healing. Since it is difficult to specifically evaluate the regenerative potential of a treatment in animal model, apart from its effect on inflammation, we sought to utilize intestinal epithelial organoid (IEO) as a platform for the evaluation of regenerative potential. We focused on the detection of recently reported population of stem cell in IEOs, repair/regeneration-associated cells (RACs). Notably, IEOs cultured with 3D-cultured MSCs (MSC_{3D}) exhibited spheroid-like morphology and enrichment with RACs. We found that the elevated prostaglandin E_2 production from MSC_{3D} accelerated the epithelial regeneration process via EP4 receptor, compared to 2D-cultured MSCs (MSC_{2D}). Moreover, MSC_{3D} exerted more potent therapeutic efficacy against colitic symptoms on murine model of inflammatory bowel disease. To further augment in vivo engraftment efficiency of MSCs, microparticles carrying anti-oxidative agent were incorporated in MSC_{3D} (heterospheroid, MSC_{HS}). In a murine model of colitis, MSC_{3D} and MSCHS exhibited enhanced anti-inflammatory impact than MSC_{2D} via attenuating neutrophil infiltration and regulating helper T cell (Th) polarization into Th1 and Th17 cells. MSC_{HS} exhibited the most prominent therapeutic outcomes owing to their enhanced anti-inflammatory and regenerative effects with prolonged survival capacity. Taken together, we established an organoid-based platform for the evaluation of regenerative potency of cell therapy and suggested a convergent strategy of MSC_{HS} formation to maximize the therapeutic potential of conventional MSC application.

Corresponding author : Jee-Heon Jeong, Hyung-Sik Kim

Keywords : Epithelial regeneration, Intestinal organoids, Reserve stem cells, Cell therapeutics, Inflammatory bowel disease

PS-B-042

A novel strategy to potentiate the therapeutic efficacy of secretome from canine stem cells against atopic dermatitis utilizing microencapsulation technologies

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Atopic dermatitis, one of the most common diseases in companion dogs, is caused by hypersensitive reactions of the innate- and adaptive immune system. Atopic dermatitis causes reccurent, uncurable symptoms such as excessive itching and skin inflammation. Therefore, atopic dermatitis requires continuous treatment, which causes economic burden and mental fatigue on the owner. In this study, we aimed to develop novel technologies to enhance the immunoregulatory efficacy of mesenchymal stem cells (MSCs) derived from canine adipose tissue. Three-dimensional 3D spheroids were generated to enrich the production of immunomodulatory paracrine factors from MSCs. Among previously reported soluble factors, the expressions of LIF, TSG6, and PGE2 were increased in 3D spheroids compared to conventional two-dimensionally cultured cells. Next, chitosan-based microspheres containing 3D spheroid-derived secretome were generated to improve the efficiency of secretome delivery, followed by the injection into murine atopic dermatitis model. We found that the clinical severity was significantly reduced in the microsphere-treated group compared to the vehicle-treated group. In addition, epidermal thickness and immune cell infiltration in the lesion were significantly decreased in the microsphere-injected group. Taken together, in the present study, we suggest novel convergent technologies to enhance the immune regulatory functions of MSCs for the treatment of canine atopic dermatitis.

Corresponding author : Jee-Heon Jeong, Hyung-Sik Kim

Keywords : Atopic dermatitis, Canine adipose stem cell, 3D spheroid, Microsphere

PS-B-043

Atopy-induced stress increases hippocampal neuroinflammatory damage in an atopic dermatitis mouse model

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Atopic dermatitis (AD) is a chronic pruritic skin disease whose severity is associated with increased anxiety, and for which psychological stress is a risk factor. AD can increase psychological stress as well, creating a positive feedback loop that exacerbates the disease, so maintaining psychological equilibrium is important for patients with AD. The hypothalamic-pituitary-adrenal (HPA) axis regulates glucocorticoid hormone release in response to psychological stressors, such that acute, time-limited rises in glucocorticoid levels are adaptive, but chronic elevation is actually maladaptive. Such a chronic inflammatory-like state negatively impacts many organs, including the central nervous system, in which it can induce neurotoxicity, cerebral atrophy, and cognitive impairment. However, it is unknown whether the high levels of glucocorticoids caused by AD-related psychological stress cause neuroinflammatory damage. We therefore assessed in vivo expression of various inflammation biomarkers in a mouse model of AD, the DNCB-induced NC/Nga mouse. Atopy-related stress induced corticotropin-releasing hormone response-related factor in skin, plasma, and brain. It increased glial activity, neuroinflammatory cytokine (iNOS, Cox-2, LIX, MIP-2, and IL-1α) expression, and markers of neuronal and synaptic loss. This is the first study to demonstrate that atopy-related stress can exacerbate neuroinflammation and potentially accelerate neurodegenerative disease states. Our results demonstrate a hypothetical pathway through which HPA hyperactivity related to atopic stress may induce neuroinflammation and its correlated hippocampal damage, or could accelerate the effects of neuroinflammation produced by another disorder, such as Alzheimer's disease.

Corresponding author : Gunhyuk Park

Keywords : Corticotropin-releasing hormone, Neuroinflammation, Neuronal damage, Chronic inflammatory-like state, Atopy

PS-B-044

Pathogenic effect of crystallin alpha B in fibrosis and angiogenesis in mice age related macular degeneration model

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Age-related macular degeneration (AMD) is the leading cause of blindness in Korea in people over 65 years of age. In general, it is classified into two types, dry (dAMD) and wet (nAMD). Macular fibrosis caused by choroidal neovascularization (CNV) in nAMD resulting in blindness with high probability. In our previous study, the concentration of crystallin alpha B (CRYAB), member of the small heat shock proteins, is associated with proliferative vitreoretinopathy (PVR) Grade. PVR is characterized by the growth of dense fibrotic contractile membranes. In this study, we investigated the role of CRYAB on CNV-induced fibrosis. In human aqueous humor, protein level of CRYAB was increased in patients of AMD and fibrosis, moreover, CRYAB was defined as having 0.87 area under the curve regarding fibrosis. Furthermore, proteomics studies identified Prss1, a protease as CRYAB binding protein that may produce cleaved form of CRYAB and augment activity to bind to fibrosis-related molecules. In laser-induced CNV mice model, CNV area was reduced in CRYAB knockout mice than WT mice in mice eyeballs, moreover, deficient of CRYAB decreased the expression of angiogenic factor and myofibroblasts marker. These data suggested that CRYAB may be involved on the development of CNV via proliferation of angiogenesis and fibrosis. We also found that CRYAB was present in a cleaved form with N-terminal cut off in eyeballs of laser-induced CNV mice. A CRYAB inhibitor, 3-methylglutamic acid ameliorates laser-induced pathological process, such as CNV development, expression of fibrosis markers and VEGF *in vivo* and *in vitro*. Interestingly, a protease inhibitor, AEBSF co-treatment with CRYAB inhibitor decreased expression of fibrosis markers and VEGF in *in vivo*. Taken together, our data suggested that CRYAB plays a key role in AMD as pathogenic factors via regulation of angiogenesis and fibrosis, and further research is needed on how the cleaved CRYAB form regulates angiogenesis and fibrosis.

Corresponding author : Jaesuk Yun

Keywords : Age-related macular degeneration, Crystallin alpha B, Angiogenesis, Fibrosis, Choroidal neovascularization

PS-B-045

Effect of inhaled 1,2-Dichlorobenzene on cytochrome P450s and lipid [eroxidation in B6C3F1 mice

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1,2-Dichlorobenzene(o-Dichlorobenzene, 1,2-DCB) is a solvent used in various industrial area. Previous studies on the hazards and risks of 1,2-DCB have shown that this substance causes skin corrosion, skin irritability and specific organ toxicity. Although inhalation and dermal exposure are known as the major exposure routes in industrial workers and to be exposed in the mixed substances with organic solvents such as benzene or other DCB complex rather than a single substance, there is still a lack of information on human hazards. In this study, we investigated the specific organ toxicity and sex differences of 1,2-DCB using whole body inhalation of laboratory mice. The male and female mice were exposed to 0-300 ppm of test substance during 13 weeks. After sacrifice, the organs were collected and histopathological assessments and immunohistochemistry (IHC) were performed and lipid peroxidation were investigated. As a result, the test substance related macro and microscopic lesions were observed in liver of male mice and the microscopic alterations were observed in nasal cavity of male and female mice. In addition, the IHC analysis of liver and nasal cavity confirmed an increase in cytochrome P450s induction in males than in female mice, and MDA and HNE were increased in both male and female mice by inhalation of 1,2-DCB. Based on the relevant literature and experimental results, 1,2-DCB is believed to cause specific organ toxicity in the liver and nasal cavity of mice, which is related with sex differences on cytochrome P450 induction and changes in lipid and oxidative products associated with the early metabolites of test substance.

Corresponding author : Eun-Sang Cho

Keywords : 1, 2-Dichlorobenzene, Inhalation toxicity, B6C3F1, Upper respiratory toxicity, Liver toxicity

PS-B-046

Improvements to improve the survival rate of rats in the carcinogenicity test (inhalation toxicity)

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The carcinogenicity test evaluates carcinogenicity by dosing(exposing) the test substance for a long period to the lifespan of an experimental animal for a period similar to that of a human.

The Inhalation toxicity research center of Occupational Safety & Health Research Institute (OSHRI) initiated the first GLP(Good Laboratory Practice) inhalation toxicity test in the Republic of Korea. So far, it is the only institution in the Republic of Korea that can conduct a carcinogenicity test through the inhalation method.

OECD Test Guideline No. 451 recommends termination of the test when the survival rate of the experimental animal control group falls below 25%. This study was to analyze the factors affecting the survival rate of rats in the carcinogenic inhalation toxicity test and to prepare an improvement plan to improve the survival rate.

Physical factors, biological factors, environmental factors, etc. affecting the experimental animals(rats) were reviewed and measured. Physical factors include noise, illumination, temperature, and relative humidity. Biological factors include feed, drinking water, and litter. Chemical factors include gaseous substances, pesticides, disinfectants, and detergents. Other environmental factors include breeding box, welfare products, and the experimenter's work method.

In conclusion, the improvement plan to increase the survival rate of rats when performing the carcinogenicity test among various factors was to change the breeding box, which is an environmental factor. Additionally, the supply of welfare products and the change of the experimenter's work method had a positive effect on the improvement of survival rate.

Corresponding author : Sung-Bae Lee

Keywords : Carcinogenicity study, Inhalation toxicity, Survival rate, Rats

PS-B-047

28-Days inhalation toxicity of 2-Methoxyethanol in B6C3F1 mice

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This study was evaluated the toxic effect of 2-methoxyethanol during repeated dose on the 28 days using total of 40 male and female mouse. The study consisted of a control, T1(200 ppm), T2(400 ppm), and T3(600 ppm) group. Exposure to the test substance was performed for 6 hours/day, 5 days/week for 4 weeks using whole body inhalation toxicity chamber. The average analysis concentrations of the test materials during the test period were 202.62±6.78 ppm, 412.01±10.93 ppm, and 607.17±8.49 ppm, respectively, in T1, T2, and T3. Abnormal general symptoms or dead animals were not observed during the exposure period. Also, significant weight loss or reduction food consumption or ophthalmological findings judged by the influence of the test substance was not observed. A statistically significant reduction in absolute or relative weight of male testes, epididymides, and male and female thymus was confirmed, which was determined to be the effect of the test substance. As a result of gross findings, a decrease in the size of the testes judged to be affected by the test substance was observed in the male T3. The hematological test, a statistically significant increase in the number of neutrophils in the female T3 and the ratio of neutrophils in the male and female T3 was observed. The histopathological tests, testes tubular degeneration and atrophy of male T2 and T3 judged to be influenced by the test substance and atypical residual bodies of T1 were observed. A deduced of sperm in the lumen of the epididymides in the T2 and T3 was observed, and prostate atrophy in the T3 was observed. In addition, an increase in granulocytes and extramedullary hematopoiesis in the spleen were observed in the female T3. As a result of the above, NOAEC of 2-Methoxyethanol is proposed at less than 202.62 ppm.

Corresponding author : Daesik Rha

Keywords : 2-Methoxyethanol, B6C3F1 mice, Male reproductive toxicity, NOAEC, 28-day inhalation

PS-B-048

Subacute(28-days) inhalation toxicity of 1-Ethoxy-2-propanol in wistar rats

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This study was evalated to the toxic effect of 1-Ethoxy-2-propanol during repeated dose on the 28 days using male and female rats and to use it as basic data for setting the dose level of the subsequent test. Exposure to the test substance was performed for 6 hours a day, 5 days a week, and 4 weeks. The study was conducted within the scope of the environmental conditions suggested by the guidelines. The time to reach 95% of the target concentration in chamber was 17.7 minutes, 16.4 minutes, and 16.7 minutes in the T1, T2 and T3 groups, respectively.

The average of analysis concentrations during the exposure period were 495.41±14.58 ppm, 993.76±10.24 ppm, 1527.85±39.60 ppm, respectively, in T1, T2, and T3 groups, and nominal concentrations were 538.76±10.26 ppm, 1059.05±27.43 ppm, respectively. Abnormal general symptoms or dead animals were not observed during the test period. No significant weight loss or reduction in feed intake judged by the influence of the test substance was observed. Ophthalmological tests did not confirm any specific findings related to exposure to test substances. No abnormalities were confirmed as a result of visual observation at the time of autopsy. In organ weight measurement, statistically significant changes were not observed in the absolute and relative organ weight. Hematological tests, biochemical tests, and histopathological tests showed no change judged to be the effect of the test substance.

As a result of the above, NOAEC (No Observed Adverse Effect Concentration) of 1-Ethoxy-2-propanol is proposed at >1527 ppm.

Corresponding author : Daesik Rha

Keywords : 1-Ethoxy-2-propanol, Wistar rat, 28-day inhalation, NOAEC

PS-B-049

Cigarette smoke-mediated alteration of reactive oxygen species (ROS) regulates immunomodulatory and hematopoietic stem cell supporting properties in human mesenchymal stem cells

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The therapeutic availability of mesenchymal stem cells (MSCs) has been becoming more diverse. At the same time, various intrinsic and extrinsic risk factors lead to less efficient clinical outcomes. Cigarette smoking induces systemic abnormality including cellular senescence and impeded recovery process in damage tissue, as well as directly causes the diseases in respiratory organs. In present study, we investigated whether the exposure to cigarette smoking extract (CSE) disrupt the direct or indirect therapeutic potential of MSCs and its underlying mechanism. Bone marrow (BM)-derived MSCs from the mice repeatedly infused with the 3R4F reference CSE showed defective immunosuppressive properties and hematopoietic stem cells (HSCs) supporting function. Furthermore, transcriptomic profile analysis showed that 3R4F up-regulated the expression of ROS-related genes as well as pro-inflammatory cytokines undermining the immunosuppressive property in human Wharton's jelly (hWJ)-derived MSCs. Exposure of CSE to hWJ-MSCs exerted a deactivated niche state to support the maintenance of HSCs as evidenced by diminished the fraction of long-term repopulating (LT)-HSCs in co-culture system. HSCs co-cultured with CSE exposed hWJ-MSCs showed impaired engraftment and lineage reconstitution in humanized mouse model. Notably, impaired immunoregulation and HSC supportive function were restored by pretreatment of ROS inhibitor, N-acetyl-l-cysteine (NAC). In conclusion, these findings indicate that exposure to CSE reduces the therapeutic potential of MSCs including immunosuppressive effect and HSC supportive function by inducing robust ROS production and subsequent oxidative stress.

Corresponding author : Mi-Kyung Oh, Kyung-Rok Yu

Keywords : Cigarette smoking extract, Mesenchymal stem cells, ROS, Immunomodulation, Humanized mouse

PS-B-050

Gunryeong-tang suppresses cardio-renal syndrome in rats with pulmonary arterial hypertension

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Pulmonary arterial hypertension is caused by elevated blood pressure in the pulmonary arteries that supply blood from the heart to the lungs. Pulmonary arterial hypertension is characterized by elevated pulmonary vascular resistance, resulting in right ventricular (RV) failure and death. Gunryeong-tang (君苓湯, GRT) has been used to treat yin deficient (陰虛) induced edema. However, there are no pharmacological studies of GRT for pulmonary arterial hypertension-inducd cardio-renal syndrome. Therefore, the present study was designed to evaluate whether GRT ameliorates cardio-renal dysfunction and related mechanisms in cardio-renal syndrome-induced by pulmonary arterial hypertension in rats. As a result, GRT alleviated symptoms of RV enlargement and fibrosis, suppressed the increase in lung weight, and alleviated pulmonary fibrosis in the pulmonary arterial hypertension model. In addition, it was found that pulmonary artery fibrosis and increase in vessel wall thickness were suppressed, and RV pressure was reduced. In this study, MCT injection increased cardiac hypertrophy marker mRNA expression in RV. However, it was significantly decreased in the GRT administration group. In addition, the effect of GRT on cardiac fibrosis was confirmed by reducing the expression of cardiac fibrosis factor mRNA. This is thought to appear through inhibition of the TGF-β/p-Smad 2 pathway and the high mobility group box-1 (HMGB-1)/toll-like receptor 4 (TLR4)/myeloid differentiation primary response 88 (MyD88)/nuclear factor-kappa B (NF-κB) pathway. Furthermore, GRT treatment decreased blood urea nitrogen, which is indicators of renal dysfunction. In addition, as a result of measuring the creatinine clearance, which is an index of the glomerular filtration rate, the improvement effect was confirmed in the group administered with GRT. The present results showed that GRT has protective effects via inhibition of TGF-β/p-Smad 2 pathway and the HMGB-1/TLR4/MyD88/NF-κB pathway, which is associated with improvement of cardio-renal syndrome in rats with pulmonary arterial hypertension.

Corresponding author : Ho Sub Lee, Dae Gill Kang

Keywords : Gunryeong-tang, Pulmonary arterial hypertension, Monocrotaline, Cardio-renal syndrome

PS-B-051

The factors determining the biological fate of nanodiamond: sp3/sp2 carbon ratio vs hydrodynamic size

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A rapidly growing interest in the usage of nanodiamond as a bioapplication triggered manipulation of nanodiamond in various ways such as physicochemical modification or conjugating drug formulation. Accordingly, safety and toxicity evaluation of nanodiamond in relation to the physicochemical properties has been performed to be commercially available, but little information is still known about biokinetics of nanodiamond. This study was designed to investigate the biodistribution and clearance kinetics depending on the structural parameters(sp3/sp2 carbon ratio) and physicochemical parameters(hydrodynamic size). Four types of nanodiamond(ND)s (low purity nanodiamond; LPND, high purity nanodiamond; HPND, serum coated low purity nanodiamond; sLPND, serum coated high purity nanodiamond; sHPND) were injected into the ICR mice via intravenous administration. The biodistribution and clearance kinetics were evaluated by quantitative(quantification of nanodiamond from the mice organ using UV-Vis) and qualitative analysis(Visuallization of nanodiamond from the mice organ using dark-field microscopy, clearing tissue technique and Bio-TEM). Four types of NDs(LPND, HPND, sLPND, sHPND) were mostly accumulated in the lung, spleen, and liver. No significant differences were found in organ distribution between group LPND and group HPND when it comes to comparing based on structural parameter, whereas remarkable differences were found in organ distribution between group NDs(LPND and HPND) and group sNDs(sLPND and sHPND) when comparing based on physicochemical parameter, which is the accumulation of ND was rarely shown in the lung after intravenous injection of serum coated ND. Our findings revealed that physicochemical parameters which was hydrodynamic size would influence on biodistribution and clearance kinetics rather than structural parameters which was sp3/sp2 carbon ratio. Hence, this data will provide information about biodistribution and clearance kinetics of nanodiamond in relation to hydrodynamic size which would be helpful for developing nanodiamond for bioapplication and the safe-by-design approach.

Corresponding author : Wan-Seob Cho

Keywords : Nanodiamond, Toxicokinetics, sp3/sp2 carbon ratio, Hydrodynamic size, Accumulation

PS-B-052

The toxicity evaluation of cement and nano-cement after intratracheal instillation to rat

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The usage of nano-cement has been gradually increasing in industrial field, because nano-cement has more advantage of strength and durability properties rather than cement. As the safety evaluation of nanomaterials has been considered as important issue, there have been also some reports on safety of nano-cement. However, little information is known about toxicity of the nano-cement. This study was designed to investigate toxicity of nano-cement by analyzing ROS-generating potential and BALF analysis. In order to perform BALF analysis, we intratracheally instilled cement, nano-cement, and DQ12 as a reference material into the lung of rats and the rats were sacrificed at 24h and 1m post injection. As a result, there was no big difference in ROS generation potential between cement and nano-cement unlike DQ12 which showed higher ROS generation potential than cement and nano-cement. In BALF analysis, unlike DQ12 which induced severe inflammation, both materials produced mild inflammation, which showed also no big difference in pattern of inflammation. In conclusion, this study implies there is no significant difference in toxicity between cement and nano-cement which can provide important information on safety of nano-cement. However, further studies will be required to understand the toxicity of nano-cement comprehensively, since we performed the toxicity test merely up to 1m.

Corresponding author : Wan Seob Cho

Keywords : Cement, Nano-cement, Inflammation

PS-B-053

The toxic effects of fragmented microplastics under ultraviolet oxidation

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With the exponential increasing use of polystyrene (PS) food containers, it has produced the global pollution and side effects on human, which lead to raise concerns on potential hazards of microplastics. Although many researchers has conducted study on toxicity of microplastics, there is little information about the biological effects of realistic materials such as weathered secondary microplastic, because the current toxicity studies were performed using spherical primary microplastics. In this study, we investigated the comparative toxic effects of two different sized (1 and 10 μm) fragmented secondary PS particles, w/ wo ultraviolet (UV) oxidation, and primary and secondary microplastics using relevant cell lines (THP-1 macrophages, HepG2, Caco-2, and A549 cells) for human exposure. In THP-1 macrophages, the cytotoxicity and pro-inflammatory cytokines levels showed size (1 μm PS > 10 μm PS), UV oxidation (UV > pristine), and origin (secondary > primary) dependent patterns. The intrinsic or cellular ROS levels showed good correlations with toxicity endpoints, and these effects were reversed by the treatment of N-acetylcysteine, a ROS scavenger. These toxic effects were not observed in non-phagocytic cells including HepG2, Caco-2, and A549 cells. These results imply that the fragmented secondary PS microplastics are more hazardous than the spherical primary ones, and size and UV oxidation aggravate its toxicity by increasing the ROS and inflammasome activation.

Corresponding author : Wan-Seob Cho

Keywords : Secondary microplastics, Fragmented, Weathering, UV oxidation, Toxicity, Oxidative potential

PS-B-054

Acute lung injury induced by 2D materials(single and multi-layered Ti3C2 MXene

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Two dimensional (2D) materials such as MXenes and graphene oxide have gotten a lot of attention due to its potential in catalysis, sensing, and nanomedicine, although their safety and biocompatibility are yet unclear. In this study, we synthesized the Ti3C2 MXenes with different layers [single-layer (SL) and multi-layer (ML)] and graphene oxide (GO) to analyze the inflammation potential in vivo and in vitro, as well as a key characteristic triggering its toxicity by the read-across approach. The toxicity endpoints of bronchoalveolar lavage fluid (BALF) increased significantly when test materials were inhaled by mice, with a strong dose dependency. The correlation of test material oxidative potential and toxicity endpoints revealed that both the intrinsic and intracellular oxidative potential of Two dimensional materials are the key factors producing inflammation and damaging plasma membrane, as supported by scanning electron microscopy and in vitro study using THP-1 macrophages. Furthermore, administering N-acetyl cysteine (NAC), a scavenger for reactive oxygen species (ROS), reduced the toxicity of test materials, which confirms the oxidative stress paradigm demonstrated in this study. These findings suggest that the oxidative stress paradigm applies to Two dimensional materials, which aids in the understanding of toxicity mechanisms and safer by design.

Corresponding author : Wan-seob Cho

Keywords : Acute inflammation, MXene, Graphene oxide, Oxidative potential, Read-across

PS-B-055

Effects of 2-week repeated intratracheal instillation of vehicles on mice lung

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The present study investigated the potential toxic effects of distilled water (DW), phosphate buffered saline (PBS), saline, 5% or 10% dimethyl sulfoxide (DMSO), 0.1% or 0.5% tween 20, 0.1% or 0.5% tween 80, 1% or 2.5% sodium carboxymethyl cellulose-Na (CMC), and corn oil. The most animals (except corn oil treated mice) received a fixed instillation volume of 50 μL and corn oil treated mice were intratracheally instilled at a dosing volume of 10, 30, or 50 μL. The vehicles were administered once daily by intratracheal instillation for 2 weeks to male C57BL/6 mice. During the test period, mortality, clinical signs, left lung weight, broncho-alveolar lavage fluid (BALF) from the right lung, biochemical analysis of BALF, and histopathology were examined. One animal of 2.5% CMC treated group died within one week after the administration, and all animals of 50 μL of corn oil treated group died within two weeks after administration. The result from histopathological analysis indicated that intratracheal instillation of DW, 0.1% tween 20, and 0.1% tween 80 induced minimal lung damage. Also, notable increases of inflammation cell were observed in the 0.5% tween 20, 0.5% tween 80, 1% or 2.5% CMC, and corn oil treated group. Based on the results, 2-week repeated administration of saline, PBS, and 5% or 10% DMSO did not appear to cause lung damage. Altogether, the results of this study could be helpful for the selection of appropriate vehicles for use in intratracheal instillation studies.

Corresponding author : Sung-Hwan Kim

Keywords : Intratracheal instillation, Lung toxicity, Vehicles, Repeated dose toxicity, Broncho-alveolar lavage fluid

PS-B-056

Effect of neonatal exposure of di-(2-methoxyethyl)phthalate on susceptibility to nicotine-induced locomotor sensitization and nicotine self-administration in rats

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Prenatal or postnatal exposure to environmental endocrine disruptors, such as phthalates or bisphenol A, has been associated with neurobehavioral disorders such as autism and attention-deficit/hyperactivity disorder (ADHD). In addition, recent studies reported that people with ADHD show higher rates of nicotine dependence than that of the general population. Thus, the present study investigated the effect of neonatal exposure to di-(2-methoxyethyl) phthalate (DMEP), one of the phthalates, on susceptibility to nicotine-induced locomotor sensitization and nicotine-taking behavior in adulthood of offspring in rats. We found that neonatal exposure to the DMEP group did not increase general locomotor activity compared to neonatal exposure to the saline group at postnatal (P) 54 in both male and female offspring's adulthood. In addition, repeated administration of nicotine produced development and expression of locomotor sensitization in DMEP pre-exposed rats compared to saline pre-exposed rats in male offspring. However, repeated administration of nicotine had no change in development (P57 - P63) and expression (P67) of behavioral sensitization in DMEP pre-exposed rats compared to saline pre-exposed rats in female offspring. Unexpectedly, there were no significant differences in susceptibility to nicotine self-administration between neonatal exposure to saline and DMEP on male or female offspring. Taken together, neonatal exposure to DMEP produces the susceptibility to repeated nicotine-induced locomotor sensitization in the male offspring in adulthood.

Corresponding author : Eun Young Jang

Keywords : Di-(2-methoxyethyl) phthalate, Repeated nicotine administration, Locomotor sensitization, Nicotine self-administration, Adulthood

PS-B-057

A novel synthetic cathinone, α-pyrrolidinobutiothiophenone, produces psychomotor, rewarding, and reinforcing properties in rodents and increases dopamine level in the striatum of mice

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Synthetic cathinones are chemical derivatives of cathinone, a structural analog to amphetamine, and produce higher abuse potentials by enhancing dopaminergic neurotransmission in the brain. Among the novel synthetic cathinones, α-pyrrolidinopentiothiophenone (α-PVT) has been known to produce psychoactive effects in rodent models. In this study, we demonstrated the effects of α-pyrrolidinobutiothiophenone (α-PBT) on psychomotor, rewarding, and reinforcing behaviors using the locomotor activity test in open-field, conditioned place preference (CPP), and self-administration paradigms. We also investigated the role of the dopamine (DA) D1 or D2 receptor in the psychomotor effect of α-PBT in mice. In addition, using enzyme-linked immunosorbent assay (ELISA) and immunofluorescence, we determined the DA concentration and neuronal activation in the dorsal striatum of mice administered with acute α-PBT. The results showed that acute or repeated α-PBT administration increased locomotor activity and produced behavioral sensitization in mice. In the CPP experiment, α-PBT increased drug-paired place preference in mice. In the self-administration test, α-PBT significantly enhanced self-administration during a 2 h session under fixed ratio 1 (FR1) schedule in rats. Furthermore, acute α-PBT administration increased DA concentration and c-Fos-positive cells in the dorsal striatum of mice associated with these behaviors. Finally, pretreatment with SCH23390 (0.06 mg/kg), a DA D1 receptor antagonist, attenuated acute α-PBT-induced locomotor activity in mice, but not, eticlopride (0.05 mg/kg), a DA D2 receptor antagonist. Taken together, these findings suggest that α-PBT has psychoactive properties by producing psychomotor, rewarding and reinforcing effects via enhanced dopaminergic transmission in the dorsal striatum.

Corresponding author : Eun Young Jang

Keywords : Cathinones, Locomotor activity, Conditioned place preference, Self-administration, Dopamine

PS-B-058

The cytotoxic effects of nanoplastics in mouse preimplantation embryos

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Toxic environmental substances that can cause infertility, such as fine dust, microplatsitcs (MPs) and Nanoplastics (NPs), have become a critical concerning as the number of infertile women increases. Previous studies have shown that nanoplastics(NPs) accumulate in several reproductive tissues, such as the human placenta, altering the integrity of the reproductive organs and causing dysfunction, such as ovarian defects and poor sperm quality. The impairment of MPs/NPs on reproduction can be caused by the redox unbalance since oxidative stress (OS) is fully recognised as a key factor in ovarian dysfunctions and development of infertility. NPs also alter the reproductive systems via mechanisms such as membrane damage, inflammation, genotoxicity and oxidative stress. Despite these toxicities, the effects of NPs exposure on the early embryonic development in terrestrial animals, especially mammals, are not understood. In this study, we investigated the toxic effects of NPs during pre-implantational embryonic development in mouse. Using GFP-conjugated NPs, we confirmed that it was infiltrated into developing embryos and decreased the rate of blastocyst development. We then measured the total cell number and differentiation of blastocysts to investigate the quality of surviving blastocysts. We also found that NPs disrupted the balance of pro- and anti-oxidants and induced reactive oxygen species(ROS) generation. In addition, we found that induced ROS was rescued by antioxidant chemical, melatonin, co-culturing during the pre-implantation embryonic development. Taken together, we confirmed exposure of NPs defects on blastocyst quality, potential capability of implantation, by inducing the ROS generation.

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Keywords : Nanoplastics, Embryonic development, Oxidative stress, Reproduction, Cellular toxicity

PS-B-059

The cytotoxic effects of particulate matter 10 on rhesus monkey in skin fibroblast

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Particulate matter 10 (PM10) is a major component of air pollutants that affects human health all over the world. According to previous research, PM can pass through the skin barrier, thus causing skin diseases such as heat rash, allergic reactions, infections, and inflammation. However, very few studies have been conducted on the cytotoxic effect on PM exposure on large scale animals. Therefore, we investigated if and how PM affects rhesus monkey skin fibroblasts. In an MTT assay, a PM10 concentration above 50ug/ml reduced cell proliferation rate in a dependent manner. PM10 treatment increased TUNEL positive cell numbers while the pro-apoptosis-associated genes, such as caspase3 and BAX, were significantly upregulated. Furthermore, PM10 treatment induced the cellular reactive oxidative stress (ROS). The ROS related genes' (TXNRD1) and antioxidant enzyme related genes' (GPX1, GPX3) mRNA expression was significantly upregulated. We confirmed that PM10 reduced mitochondrial membrane potential. The mitochondrial-apoptosis-related gene, CYCS's, mRNA expression was also significantly upregulated. In conclusion, these results revealed that PM10 triggers apoptosis, mitochondrial damage and ROS accumulation. Therefore, these findings offer more information on PM10 exposure's cytotoxic effects and help to understand the mechanism of skin diseases by air pollution.

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Keywords : Particulate matter(PM), Reactive oxidative stress(ROS), Apoptosis, Non-human primate(NHP), Cytotoxicity

PS-B-060

Dysfunction of female reproductive system is caused by exposure to airborne nanoplatics in mice

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Nanoparticles are small size, which diameter less than 1μm, and known to cause cytotoxic physically because they can penetrate cells freely. Among these small particles, especially nanoplastics(NPs) recently emerged as environmental problem because it cannot decompose naturally, remain or split more smaller in the environment. NPs can exposed to terrestrial and marine species by intake through food, water and/or breathing. Previously research indicate that NPs affect on intestinal barrier dysfunction, cardiovascular damage, neurobehavioral disorder, neuronal damage in marine and mammalian animals. Current studies reveal that NPs cause dysfunction of female reproductive system and embryo development such as fetal growth restriction. Futhermore, exposure of microplastics(MPs; 1μm to 5mm) induce ovarian and testicular inflammation, tissue fibrosis, and disturb reproductive cell formation such as impair the oocyte maturation and spermatogenesis. However it is unclear the effects of NPs, very small size enough to penetrate cells, on the mammalian reproductive system. In this study, we investigated the toxic effect of NPs exposure via intratracheal intubation in female mice. We confirmed the NPs, inhaled through respiratory system, spread to whole body, especially to ovaries. We also checked exposure of NPs affects the concentration of gonadotropin(FSH, LH) and AMH, known as female reproductive hormones. We investigated mRNA and protein levels of ROS, apoptosis, inflammation, and pyroptosis related molecules in lung and ovary. We identified fibrosis in ovary, and lung. Taken together, these results demonstrate that NPs exposure cause ovarian dysfunction, potentially leading to female infertility, as an environmental stressor.

Corresponding author : Ji-Su Kim

Keywords : Nanoplastics, Ovarian toxicity, Reproduction, Intratracheal injection

PS-B-061

Damage to olfactory organs of adult zebrafish induced by diesel particulate matter

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The particulate matter (PM) is one of the air pollutants and its harmfulness has been raised as a social problem. As the human olfactory organ is directly exposed to PM, its damage is occurring quickly and makes our daily life inconvenient. However, studies of harmful effects of PM on the olfactory system are still insufficient. Therefore, we propose a research of the zebrafish olfactory model to identify harmfulness of PM exposure. For this research, we treated Korean diesel particulate matter (KDP20) to the adult zebrafish. KDP20 contains heavy metals and polycyclic aromatic hydrocarbons (PAHs). Under 300ug/mL, the survival rate was 100%, but in 500μg/mL, the rate was decreased by 50%. According to this result, we decided appropriate concentration of 300μg/mL. In addition, we confirmed the damaged olfactory sensory neurons with field-emission scanning electron microscopy (FE-SEM). In the behavior test, the adult zebrafish exposed KDP20 for 3 days had little food reaction than control. In the zebrafish olfactory organ, the number of goblet cells was increased in damage group than control and recovery group in histopathology analysis. While, the cell density was decreased in KDP20 exposed group than the control. Quantitative real-time polymerase chain reaction (RT-PCR) exhibited gene regulation related to KDP20 exposure. We confirmed up-regulation of the cytochrome p450 (*CYP1A* and *AHR2*) which causes cell cytotoxicity, reactive oxygen species (*CAT*) and inflammatory reaction (*IL-1B*). Whereas the olfactory organ (*s100, OMP*) related genes were down-regulated. These results reveal that we acquired meaningful data using zebrafish model. In other words, this model can be used PM-related diseases research in the future. In conclusion, KDP20 exposure causes destruction of olfactory sensory neurons and mucosal layer, this leads the loss of ability of olfactory organ.

Corresponding author : Chan-sik Kim

Keywords : Diesel particulate matter, Toxicity, Olfactory dysfunction, Cilia and epithelial damage, Zebrafish

PS-B-062

Cimicifugae Rhizoma extract attenuates oxidative stress and airway inflammation via the upregulation of Nrf2/HO-1/NQO1 and downregulation of NF-B phosphorylation in ovalbumin-induced asthma

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Cimicifugae Rhizoma is a traditional herbal medicine that has been taken as an analgesic, anti-inflammatory, antipyretic, and antiviral agent in East Asia. Herein, we conducted this study because the effect of Cimicifugae Rhizoma extract (CRE) on allergic asthma has not yet been evaluated. To induce allergic airway inflammation, we intraperitoneally injected ovalbumin (OVA) mixed with aluminum hydroxide into mice twice at intervals of 2 weeks (Days 0 and 14) and then inhaled them thrice with 1% OVA solution using a nebulizer (Days 21 to 23). CRE (30 and 100 mg/kg) was administered orally daily for 6 days (Days 18 to 23). The mice showed remarkable reduction in allergic inflammation at 100 mg/kg of CRE, as evidenced by decreased inflammatory cell counts, pro-inflammatory cytokine levels, OVA-specific immunoglobulin E level, airway hyperresponsiveness (AHR), and production of mucus. Additionally, these effects were involved with the enhancement of heme oxygenase-1 (HO-1), NAD(P)H: quinone oxidoreductase (NQO1), and nuclear factor erythroid 2-related factor 2 (Nrf2) expression and reduction of nuclear factor-B (NF-B) phosphorylation and matrix metalloproteinase-9 (MMP-9) expression. Our findings indicated that CRE effectively protected against OVA-induced inflammation and oxidative stress via upregulation of the Nrf2/HO-1/NQO1 signaling and downregulation of NF-B phosphorylation in asthma caused by OVA.

Corresponding author : In-Sik Shin

Keywords : Cimicifugae Rhizoma, Asthma, Nuclear factor erythroid 2-related factor 2 (Nrf2), Heme oxygenase-1 (HO-1), Nuclear factor-B (NF-B)

PS-B-063

Titanium dioxide nanoparticles exacerbate allergic airway inflammation via TXNIP upregulation in a mouse model of asthma

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Titanium dioxide nanoparticles (TiO_2NPs) are widely used in industrial and medicinal fields and in various consumer products, and their increasing use has led to an increase in the number of toxicity studies; however, studies investigating the underlying toxicity mechanism have been rare. In this study, we evaluated potential toxic effects of TiO_2NPs exposure on lungs as well as the development of asthma through the ovalbumin (OVA)-induced mouse model of asthma. Furthermore, we also investigated the associated toxic mechanism. TiO_2NPs caused pulmonary toxicity by exacerbating the inflammatory response, indicated by an increase in the number and level of inflammatory cells and mediators, respectively. OVA-induced asthma exposed mice to TiO_2NPs led to significant increases in inflammatory mediators, cytokines, and airway hyperresponsiveness compared with those in non-exposed asthmatic mice. This was also accompanied by increased inflammatory cell infiltration and mucus production in the lung tissues. Additionally, TiO_2NPs decreased the expression of B-cell lymphoma 2 (Bcl2) and the expressions of thioredoxin-interacting protein (TXNIP), phospho-apoptosis signal-regulating kinase 1, Bcl2-associated X, and cleaved caspase 3 were escalated in the lungs of asthmatic mice compared with those in non-exposed asthmatic mice. These responses were consistent with in vitro results obtained using human airway epithelial cells. TiO_2NPs treated cells exhibited an increase in the mRNA and protein expression of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α with an elevation of TXNIP signaling compared to non-treated cells. Moreover, pathophysiological changes induced by TiO_2NP treatment were significantly decreased by TXNIP knockdown in airway epithelial cells. Overall, TiO_2NP exposure induced toxicological changes in the respiratory tract and exacerbated the development of asthma via activation of the TXNIP-apoptosis pathway. These results provide insights into the underlying mechanism of TiO_2NP-mediated respiratory toxicity.

Corresponding author : Jong-Choon Kim

Keywords : Titanium dioxide nanoparticle, Asthma, Airway inflammation, Thioredoxin-interacting protein, Apoptosis

PS-B-064

Effect of 28-day repeated oral dose toxicity of aluminum chloride(AlCl3) in rat

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The present study investigated the potential adverse effects of aluminum chloride (AlCl3) following a 4-week repeated oral administration in Sprague-Dawley rats. The test article was administered once daily by gavage to male and female rats at dose levels of 0, 100, 300, and 900 mg/kg/day for 4 weeks. After administration of AlCl3 at 900 mg/kg/day, treatment-related systemic toxicity manifested as significant increases in salivation incidence, neutrophil percentage, reticulocytes, serum triglyceride, adrenal gland and liver weights, and single-hepatocyte necrosis, as well as significant decreases in body weight gain, food intake, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration (MCHC), lymphocyte percentage, serum total protein and albumin, and thymus weight in male rats; and significant increases in salivation incidence, serum triglyceride, and liver weight, as well as a significant decrease in lymphocyte percentage in female rats. At 300 mg/kg/day, a significant decrease in MCHC was found in male rats, but not in female rats. However, this finding was not toxicologically significant because the reduction was minimal and was not accompanied by changes in any other parameters. No treatment-related effects were observed in the 100 mg/kg/day group of both genders. Under the experimental conditions of this study, the target organs of AlCl3 were determined to be the blood, liver, and thymus in rats. The no-observed-adverse-effect level was found to be 300 mg/kg/day in rats of both genders.

Corresponding author : Jong-Choon Kim

Keywords : Aluminum, Systemic toxicity, Target organ, No-observed-adverse-effect level, Rat

PS-B-065

Efficacy evalution of candidate A in DSS-induced inflammatory bowel disease mouse model

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The experiment aimed to investigate the anti-inflammatory effect of the new drug candidate protein (candidate A) by using Dextran Sulfate Sodium (DSS)-induced acute and chronic inflammatory bowel diseases (IBD) mouse model. DSS-induced IBD is caused by loss of epithelial barrier function and entry of luminal organisms or their products into the mucosa or normal lamina propria. Administration of DSS to mice in drinking water is a highly reproducible method of IBD induction, characterized by erosions/ulcers, loss of crypts, and infiltration of granulocytes in the colon.

In the acute IBD model, 2% DSS was supplied as drinking water for 7 days, and candidate A, cyclosporin A, and adalimumab were administered during the 7-day tap water supply period. Different concentrations of candidate A (20, 80, 320 μg/kg) and adalimumab (4 mg/kg) were injected onto the mice via intravenous injection, while cyclosporine A (80 mg/kg) was orally administered to the mice.

In the chronic IBD model, 2% DSS supply as drinking water for 3 days and tap water for 4 days were repeated twice, and 2% DSS was supplied as drinking water for 4 more days. Different concentrations of candidate A (320, 640 μg/kg), infliximab (1.03 mg/kg), ustekinumab (1.03 mg/kg) and vedolizumab (1.03 mg/kg) were administered intravenously on the first day of tap water supply during the induction period.

Mice were sacrificed, and samples were obtained on day 14 for the acute model and on day 18 for the chronic model induction. Drug efficacy was assessed by weight loss, colon length, and histological scores. As a result, in both acute and chronic models, candidate A showed comparable or higher effect versus that of cyclosporin A, adalimumab, infliximab, ustekinumab, and vedolizumab within the evaluation of weight loss, colon length, and histological scores. Therefore, candidate A can be proposed as a potential drug for the treatment of IBD.

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Keywords : Dextran sulfate sodium, Inflammatory bowel disease, Experimental ulcerative colitis, Inflammation

PS-B-066

Comparison of biochemical analysis in rodents using diluted serum

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In toxicity and efficacy studies using laboratory animals, clinical pathology tests are important data for disease diagnosis and monitoring of animal condition. Recently, there has been active research on fetuses and newborns, and which have a small amount of blood. In many studies using mice, hematology tests and biochemical analysis are performed by dividing animals from the same group for limited blood volume. Therefore, a study on sample dilution that can obtain biochemical data using a small amount of serum was attempted.

In order to evaluate the effect of diluted samples in biochemical analysis, the serum was diluted by saline and compared with the results of undiluted serum. Blood was collected from the abdominal vena cava of SD Rat and was centrifuged. Serum samples were diluted 4-fold and 10-fold, and 20 parameters corresponding to the OECD guidelines for repeated toxicity study were analyzed using an automatic biochemical analyzer TBA 120FR (Toshiba, Japan).

In the results of biochemical analysis of diluted serums, 15 parameters including total protein, albumin, albumin globulin ratio, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total cholesterol, triglyceride, phospholipid, creatine phosphatase, blood urea nitrogen, glucose, calcium, inorganic phosphorus and potassium, showed a difference within 2 standard deviation in both 4-fold and 10-fold dilution compared to undiluted serum values. Therefore, above 15 parameters has shown to give comparable results to those undiluted serum, so it can be recommended for obtain biochemical data with diluted serum in case of having difficult to measure due to small blood volume samples.

Corresponding author : Jeongah Song, Mi-Jin Yang

Keywords : Biochemistry analysis, Diluted serum

PS-B-067

Human induced-pluripotent stem cells (iPSc) derived-hepatocyte as an in vitro model for evaluation of cytochrome P450 induction by hepatotoxicant

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Cytochrome P450 enzymes are the major enzymes involved in drug metabolism, accounting for about 75% of the total metabolism. Most drugs undergo deactivation by CYP enzymes either directly or by facilitated excretion from the body. There are various types of subunits in CYP enzyems, and among them, CYP enzymes that are most involved in human drug metabolism are CYP1A2, CYP2B6, CYP2C9, CYP3A4. These enzymes that must be analyzed in the drug development. Although various in vitro assays have been developed to evaluate the cytochrome P450 (CYP) inducing potential of drug candidates, there is a continuing need for development of a reliable model in drug development. For this reason, drug screening models using Hepatocyte-like cells (HLCs) derived from human induced pluripotent stem cells (iPSc) and human primary cells are being actively developed. HLCs has higher liver function activity than HepG2 and HepaRG, which have been used as drug screening models in the past. In addition, compared to primary hepatocytes, HLCs offer relative unlimitied and consistent supply of cell with stable phenotype, and compared to hepatoma cell lines, they are from non-tumor origin and can be obtained from different doners. Therefore, the aim of this study was to compare CYP induction by hepatotoxicants in induced-pluripotent stem cell derived hepatocytes with HepaRG cells. Human iPSc (QIA7) was established from adipose tissue-derived stromal cells from our previous study. As a results, CYP enzyme activity and mRNA expression were higher in QIA7 cells than in hepaRG cells. In addition, the mRNA expression and enzyme activity of the CYP enzyme increased in a concentration-dependent manner with the treated hapatotoxicants. There results suggest that induced-pluripotent stem cell-derived hepatocytes better than HepaRG cells are a more suitable model for screening CYP inducers in drug screening.

Corresponding author : Byung-Suk Jeon

Keywords : Cytochrome P450, Human induced-pluripotent stem cells (iPSc), Hepatocyte-like cells (HLCs), QIA7, Drug screening

PS-B-068

Withdrawal from treatment with 3-Fluoroethamphetamine induces hyperactivity and depression-like behavior in mice

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3-Fluoroethamphetamine (3-FEA) is a stimulant drug of the amphetamine class which acts as a releasing agent of the monoamine neurotransmitters norepinephrine, dopamine and serotonin. 3-FEA acts on the central nervous system and exhibits physical and mental side effects such as euphoria, heart rate increase, and excitement. So, 3-FEA has recently been designated as a banned substance in Japan. Withdrawal symptoms and behavioral changes that may contribute to the adverse effects of 3-FEA administration are not yet known.

This study evaluated the short-term consequences of binge 3-FEA administration (twice a day, 7 days, i.p.; 1, and 10 mg/kg) in adult C57BL/6 (male, 7 weeks) at three behavioral levels following 1~4 days of withdrawal: (1) global withdrawal score (Observation for 30 min at 16 h to 2 days after withdrawal), (2) hyperactivity (Open field test (OF), elevated plus maze test (EPM), and cliff avoidance (CA) test), and (3) negative affect (forced-swim test).

Body weight was significantly increased at 7 days with 3-FEA 10 mg/kg. In the global withdrawal score test, withdrawal behavior increased in all 3-FEA administration groups at 16 hours and 2 days after withdrawal. In the OF, EPM, and CA test, the 3-FEA administered group was hyperactive. In addition, in the forced-swim test, 3-FEA 1, 10 mg/kg increased immobility time.

The data indicating the withdrawal symptoms of 3-FEA presented in the study provide a scientific basis for the control of this new psychoactive substances worldwide.

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Corresponding author : Sunok Choi

Keywords : 3-Fluoroethamphetamine, Amphetamine, Withdrawal symptoms, Hyperactivity

PS-B-069

Hepatoprotective effect of germinated soybean embryo extracts in HFD-fed obese mice

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Soybean is known to have diverse beneficial effects against human diseases, including obesity and its related metabolic disorders like type 2 diabetes, hypertension and dyslipidemia. Furthermore, soybean in diet has been known to show inhibitory effects on hepatic steatosis induced by HFD in animal experiments. Germinated soybean embryos are enriched with bioactive phytochemicals which contained a high concentration of soyasaponin Ab and known to inhibit diet-induced obesity in mice, but their effect on non-alcoholic fatty liver disease (NAFLD) remains unknown. Here, we germinated soybean embryos for 24 h, and their ethanolic extract (GSEE, 15 and 45 mg/kg) was administered daily to mice fed with a high-fat diet (HFD) for 10 weeks. HFD significantly increased the weight of the body, liver and adipose tissue, as well as serum lipid markers, but soyasaponin Ab-rich GSEE alleviated these changes. Hepatic injury and triglyceride accumulation in HFD-fed mice were attenuated by GSEE via decreased lipid synthesis (SREBP1c) and increased fatty acid oxidation (p-AMPKα, PPARα, PGC1α, and ACOX) and lipid export (MTTP and ApoB). HFD-induced inflammation (TNF-α, IL-6, IL-1β, CD14, F4/80, iNOS, and COX2) was normalized by GSEE in mice livers. In adipose tissue, GSEE downregulated white adipose tissue (WAT) differentiation and lipogenesis (PPARα, C/EBP, and FAS) and induced browning genes (PGC1α, PRDM16, CIDEA, and UCP1), which could also beneficially affect the liver via lowering adipose tissue-related circulating lipid levels. Thus, our results suggest that GSEE can prevent HFD-induced NAFLD via inhibition of hepatic inflammation and restoration of lipid metabolisms in both liver and adipose tissue.

Corresponding author : Young-Suk Jung

Keywords : Germinated soybean embryo, Non-alcoholic fatty liver disease, Obesity, Inflammation, Lipid metabolism

PS-B-070

A comparative study of artificial intelligence analysis for diagnosis of liver fibrosis in rats

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Liver fibrosis is one of the hepatic healing responses to chronic liver damage. If the damage continues, hepatocytes are exchanged for extracellular matrix instead of regeneration. Histologically, when hepatic stellate cells are activated, they are converted to a myofibroblast-like phenotype, and if new fibers are formed, excess collagen is accumulated. These collagen fibers are formed around the hepatic lobule and gradually become a portal to portal bridging.

During drug developments to overcome liver fibrosis, pathologists must directly read slides to measure the degree of fibrosis to assess their toxicity or efficacy of them. However, artificial intelligence (AI) analyses are currently being attempted to diagnose liver fibrosis.

This study confirmed the possibility of quantitative analysis of liver fibrosis images by applying several AI models with good performance as accurate classification and detection of digital images became possible with the recent development of AI. Mask R-CNN, DeepLab, and SSD were used for the study. To evaluate the AI algorithm, 2011 cropped images were used. Images were randomly classified as 7:2:1 into three classes: training, validation, and test.

The accuracy of the model was 92.65%, 98.40%, and 94.35% for Mask R-CNN, DeepLab, and SSD, respectively. Each model was compared with the annotation area. The coefficient of determination between the annotation result and the AI algorithm was 0.9007, 0.8785, and 0.5456(Mask R-CNN, DeepLab, SSD). The image was divided into pixel units to measure the size of the results of annotation and each algorithm, and the difference and error rates were shown in graphs. Error rates were 2.31%, 2.54%, and 36.51% for Mask R-CNN, DeepLab, and SSD, respectively.

Overall, all three algorithms showed high results in detecting liver fibrosis. In particular, to quantify liver fibrosis, semantic segmentation model DeepLab or instance segmentation model Mask R-CNN showed better results than object detection model SSD.

Corresponding author : Jae-Woo Cho

Keywords : AI model, DeepLab, Mask R-CNN, SSD, Hepatic fibrosis

PS-B-071

Hepatic steatosis screening analysis study applying artificial intelligence to rats

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Hepatic steatosis is a symptom caused by the storage of extra fat in the liver. Hepatic steatosis can be divided into alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NALD), and ALD is caused by the consumption of heavy drinking. NALD is called hepatic steatosis caused by various factors, not alcohol intake, and there are several factors such as obesity, type 2 diabetes, and pharmaceuticals. If left unmanaged, it leads to liver cirrhosis. In the histopathological examination of nonclinical studies, fatty changes in the normal range are often observed in rat liver.

We confirmed whether steatosis can be detected through artificial intelligence (AI) and proceeded to determine which algorithm is advantageous for analyzing steatosis by comparing the performance of each algorithm. The AI algorithm used YOLO, object detection model, DeepLab, semantic segmentation model, and Mask R-CNN, instance segmentation model.

The accuracy of Mask R-CNN, DeepLab, and YOLO showed 94.21%, 100%, and 63.58% results, respectively. Using these trained models, the remaining 173 images were tested and compared with the annotations of experienced senior pathologists. The coefficient of determination between the results of the AI algorithm and the annotation was 0.8344, 0.8211, and 0.331 (Mask R-CNN, DeepLab, YOLO). And the difference in the area between the result of the algorithm and the annotation was calculated. The measured error rates were 5.80%, 6.22%, and 27.18%.

In the case of YOLO, the object detection model, because the results are derived in rectangle, showed good results in reading observations, but large errors in quantification. On the other hand, the semantic segmentation model and instance segmentation model, DeepLab and Mask R-CNN show high agreement in detection and quantification, and can be used as tools to help pathologists read.

Corresponding author : Jae-Woo Cho

Keywords : AI model, DeepLab, Mask R-CNN, YOLO, Hepatic steatosis

PS-B-072

A study for selecting optimal classification AI model for 1-naphthyl isothiocyanate-induced liver necrosis in the mouse

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Toxicologic pathology assessment usually begins with reading tissue slides and recording their results by pathologists. Recently, numerous studies have been reported showing the possibility that artificial intelligence, not humans, can read slides through the fusion of technology of digital pathology which converts glass slides to digital whole slide image quickly, and artificial intelligence technology.

This study tested some classification algorithms for liver necrosis in the mice induced by ANIT and tried to confirm which algorithm showed the highest accuracy. From the mice given ANIT 75mg/kg, the liver was collected and prepared for tissue slide and whole slide image was obtained with 20x magnification. Data was classified into three categories: necrosis (n=2070), normal liver (n=10687), and blood & background(n=3641). We trained them in ResNet-50, Xception, and EfficientNet-B0. All of these models are known to have good performance. Since the input image size is different for each algorithm, we converted the image size according to the model. The data set was divided into 8:1:1 for learning, validation, and testing, respectively. The batch size was 32 for all models. Adam Optimizer with an initial learning rate of 0.0001 was used and according to the learning rate scheduling, training was conducted by halving the initial learning rate every 10 epoch. There was no data augmentation. If there was no improvement in validation loss during 10 epoch, the training was terminated. Epoch for training was 22, 11, and 7 in ResNet-50, Xception, and EfficientNet-B0, respectively.

The model accuracy for the test set is 99%, 99%, and 99% in ResNet-50, Xception, and EfficientNet-B0, respectively and there were no remarkable differences, so it seems that all of them can be used for hepatic necrosis analysis.

Corresponding author : Jae-Woo Cho

Keywords : Classification algorithms, ResNet-50, Xception, EfficientNet-B0, Liver necrosis

PS-B-073

Using drug discrimination techniques to evaluate the abuse related effects of 3-Fluoroethamphetamine in rats

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3-Fluoroethamphetamine (3-FEA) is amphetamine family that produces a psychedelic and stimulant effects when administered. Recently, an online vendor has made it available for sale in the grey market as a research chemical. The drug has an extremely short history of human recreational use and has not been documented for sale on the streets. So, It is not currently known as subjective effects of 3-FEA on humans have not yet been detailed. Moreover, abuse potential of the drug has not been systemically examined yet.

In this study, we evaluated the behavioral effects for abuse liability of 3-FEA in rat using drug discrimination techniques. First, the experimental protocol was established using both methamphetamine and cocaine as a positive control. As a result, the drug induced the full substitution (94±3%) in methamphetamine-trained rats in dose dependent, whereas partial substitution (approximately 75±15%) in rats trained with cocaine at 2 mg/kg. In addition, the response rate per second of 3-FEA decreased at high dose (2 mg/kg) of both the positive control and decreased rapidly in the cocaine group indeed. Lastly, the median effective dose (ED_{50}) was measured, 3-FEA of the cocaine group had the higher ED50 than cocaine, and 3-FEA of the methamphetamine group showed the opposite result. In conclusion, 3-FEA met the criteria for full substitution for the methamphetamine training dose. These findings suggest the abuse liability for 3-FEA.

Corresponding author : Sun Ok Choi

Keywords : 3-Fluoroethamphetamine (3-FEA), Amphetamine, Abuse liability, Drug discrimination

PS-B-074

The protective effect of *Achyranthes japonica* aqueous extract (USL) in the dry eye model

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Dry eye syndrome (DES) is a disease due to a lack of tear or excessive evaporation of tears. In this study, we examined the effect of USL, the root of *Achyranthes japonica* (Achyranthis Radix, AR), on development of DES in the extraorbital lacrimal gland-excised rats and on inflammation and apoptosis in hyperosmolar stress-stimulated human conjunctiva cell (HCC). In vivo, Tear fluid volume was significantly reduced in the extraorbital lacrimal gland excised rats (dry eyed group). However, oral-administration of USL extracts at 50, 100 and 250 mg/kg remarkably recovered the tear fluid volume compared to dry eyed group. Moreover, the significantly lower corneal smoothness and damage score in dry eyed group was highly improved by oral-administration of USL extracts at 100 and 250 mg/kg. The DES induced by extraorbital lacrimal gland excision induced the loss of goblet cells and mucin4 as well as inflammation in rat's conjunctiva tissue. However, goblet cells and mucin4 expression was increased, and increased MPO and TNF-α protein expression in dry eyed group was remarkably dose-dependently inhibited in USL oral-administrated groups. In addition, mRNA expression of inflammatory genes such as IL-1β, TNF-α, IFN-γ and CXCR1 was decreased by oral administration of USL. In vitro, USL suppressed hyperosmolar stress-induced inflammation through attenuation of the translocation of NF-κB to the nucleus and the mRNA expression of TNF-α, IL-1β, -4, -6, -13, -33, and MMP9. Moreover, the hyperosmolar stress-induced NLRP3 inflammasome pathway and apoptosis were highly decreased by USL treatment. Therefore, the present study demonstrates the protective effect of USL against dry eye syndrome in the extraorbital lacrimal gland excised rat model as well as insight into the mechanism including the anti-inflammation and anti-apoptosis. Our study also provides that USL might be a potential therapeutic agent to preserve eye health.

Corresponding author : Chan-Sik Kim

Keywords : *Achyranthes japonica*, Dry eye syndrome, Extraorbital lacrimal gland excision, Inflammation, Hyperosmolar stress

PS-B-075

Toxicological evaluation of extracellular vesicles derived from canine adipose tissue-derived mesenchymal stem cells (ASC-EVs)

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Mesenchymal stem cells (MSCs), self-renewing cells with the potential to differentiate into various cell types, are being presented as attractive materials to treat various diseases in the medical field. Recent studies have shown that MSCs exert an immunosuppressive effect by producing and releasing extracellular vesicles (EVs) of various sizes consisting of lipid bilayers, rather than through cell-to-cell contact. In a previous study, we found that extracellular vesicles derived from canine adipose tissue-derived mesenchymal stem cells (ASC-EVs) improved AD-like dermatitis in a dose-dependent manner. In the present study, we examined the single and repeated dose toxicity of ASC-EVs in ICR mouse. The cASC-EVs was injected subcutaneously to mouse at dose level of 7.45E+08, 2.98E+09 or 1.19E+10 particles/20g for single-dose toxicity study and repeated-dose toxicity study. In both studies, there were no dose related changes in mortality, clinical sign, body weight change, food and water consumption, opthalmoscopy, organ weights, urine analysis, biochemical examination, and hematological findings of all animals treated with cASC-EVs. Gross and histopathological findings revealed no evidence of specific toxicity related to cASC-EVs. These results suggest that cASC-EVs may show no single and repeated dose toxicity in ICR mouse under the conditions. Therefore, there is potential for the treatment of diseases in animals using cASC-EV.

Corresponding author : Kyung Seuk Song

Keywords : Adipose tissue-derived mesenchymal stem cells (ASCs), Canine, Safety, Skin, Toxicology

PS-B-076

Neurotoxic effects of 3-Fluoroethamphetamine in mice: behavioral pharmacological approach

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3-Fluoroethamphetamine (3-FEA), also called N-ethyl-3-fluoroamphetamine, is a stimulant drug of the amphetamine class which acts as a releasing agent of the monoamine neurotransmitters norepinephrine, dopamine and serotonin. Some cases of using 3-FEA as a recreational drug have been reported, it is classified as a temporary drugs in Korea. In this study, we conducted an evaluation study on the toxicity of the central nervous system, which is directly related to life support, to secure scientific basis in the designation of narcotics. Body weight, body temperature, physiological and neurotoxicity symptoms (Irwin test), locomotion (open field (OF) test), motor function (rota-rod test), and cognitive memory function (Y-maze and novel object recognition (NOR) test) were evaluated in mice. As a result, administration of 3-FEA to mice decreased body temperature and increased body weight. In Irwin test, among 23 evaluation lists, locomotion (increase and decrease), stereotypy, tremor, convulsion, tail elevation, respiration rate, eyelid, salivation, pinna reflex, touch response, pain response, passivity, abdominal tone, traction, catalepsy, righting reflex, and death were observed. Hyperactivity was observed in the OF test. Y-maze and NOR test results showed no effect on cognitive memory function, however 3-FEA 1mg/kg and 10mg/kg treatment groups showed a decrease in sensory-motor function on rota-rod test. Taken together, 3-FEA can adversely affect the central nervous system, and this result provides a scientific basis for temporary drug regulation.

Corresponding author : Sunok Choi

Keywords : 3-Fluoroethamphetamine, Amphetamine, Neurotoxicity

PS-B-077

Dependence potential of 4-Fluoroethylphenidate (4F-EPH); Behavior approaches in mice

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4-Fluoroethylphenidate(4F-EPH) is one of new psychoactive substances (NPSs), acting as a norepinephrine-dopamine reuptake inhibitor (NDRI). NDRIs can be addicted due to the mechanism of action in the central nervous system like methamphetamine. However, there is lack of scientific information on the dependence potential and toxicity of 4-Fluoroethylphenidate. NPSs can be more dangerous because they are likely to be abused/misused to obtain their euphoric effect without recognizing their harmful effects. The purpose of this study was to evaluate the rewarding and reinforcing effects of 4-Fluoroethylphenidate in rodents. The applied dose range of 4-Fluoroethylphenidate was determined based on the results from the locomotor test (0, 0.01, 0.1, 1, 10 and 40 mg/kg, i.p.). In order to evaluate the rewarding effects of 4-Fluoroethylphenidate, conditioned place preference (CPP) test was performed at the selected doses (0, 1, 10 and 40 mg/kg, i.p.) in mice. Then the self-administration (SA) test was undertaken at the doses (1 and 4 mg/kg/infusion, i.v.) which presented the highest effects in the CPP test. As a result of this study, significant response was observed in the locomotion behavior test. In the CPP test, the mice administered with 4-Fluoroethylphenidate showed a significant preference in the drug-paired compartment at 1, 10, and 40 mg/kg. In addition, the mice increased the number of infusions in the SA test. Taken together, 4-Fluoroethylphenidate might be the potential substance to induce rewarding and reinforcing effects suggesting the dependence liability.

Corresponding author : Sun-Ok Choi

Keywords : 4-Fluoroethylphenidate(4F-EPH), Norepinephrine-dopamine reuptake inhibitor (NDRI), Locomotion behavior, Conditioned place preference (CPP), Self-administration (SA)

PS-B-078

Particulate matters-mediated oxidative stress induces airway inflammation and pulmonary dysfunction through TXNIP/NF-κB and SIRT1/p53/caspase-3 pathways in mice

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Growing epidemiological evidence suggests that exposure to particulate matter (PM) is closely associated with decreased pulmonary function and the increasing prevalence of respiratory diseases. In this study, we investigated the potential effects of PM10 on the respiratory system in BALB/c mice and NCI-H292 cells. PM_{10} (0, 1, 2, and 8 mg/kg) was administered to mice by intratracheal instillation for 7 days. PM_{10} treatment markedly increased the inflammatory cell counts, pro-inflammatory cytokines and reactive oxygen species (ROS) in bronchoalveolar lavage fluid in PM_{10}-induced mice and PM_{10}-stimulated NCI-H292 cells. PM_{10}-mediated oxidative stress down-regulated thioredoxin-1 (Trx-1), and up-regulated thioredoxin-interacting protein (TXNIP) concurrent with activation of nuclear factor-kappa B (NF-κB) and matrix metalloproteinase-9 in the lung tissue and in PM_{10}-stimulated NCI-H292 cells. PM_{10} treatment induced suppression of sirtuin 1 (SIRT1) and increased p53 acetylation, which resulted in caspase-3 activation and apoptotic changes in PM_{10}-treated mice and PM_{10}-stimulated NCI-H292 cells. PM_{10} treatment caused inflammatory cell infiltration into airways or interstitial regions, thickening of alveolar walls, and activation of α-smooth muscle actin and collagen type1A2 in the lung tissues. In pulmonary function tests, PM_{10} exposure induced impaired pulmonary function resembling pulmonary fibrosis, characterized by increased resistance and elastance of the respiratory system, and resistance, elastance, and damping of lung tissues, whereas it decreased compliance of the respiratory system and forced expired volume. Taken together, PM_{10}-mediated oxidative stress caused airway inflammation and pulmonary dysfunction with fibrotic changes via activation of the TXNIP/NF-κB and modulation of the SIRT1/p53/caspase-3 pathways.

Corresponding author : In-Chul Lee

Keywords : Particulate matter, Oxidative stress, Pulmonary dysfunction, Thioredoxin-interacting protein, Sirtuin1/acetylated-p53

PS-B-079

Spiraea prunifolia var. simpliciflora downregulates inflammatory responses and oxidative stress in a mouse model of PPE/LPS-induces chronic obstructive pulmonary disease

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Spiraea prunifolia var. simpliciflora (SP) has traditionally been used as a medical food and exhibits diverse beneficial properties, malaria, fever, and emesis. The aim of this study to reveal the protective effects of SP leaves methanolic extract against airway inflammation and oxidative stress in lipopolysaccharide (LPS)-stimulated Raw264.7 cell and in porcine pancreatic elastase (PPE)/LPS-induced chronic obstructive pulmonary disease (COPD) murine model. Male C57BL/6N mice were orally administered SP (50 and 100 mg/kg) and dexamethasone (3 mg/kg) 2 h before intratracheal (i.t.) instillation with 0.5 units of PPE and 5 μg of LPS. SP treatment reduced inflammatory cell counts, particularly macrophages and neutrophils, and pro-inflammatory cytokines in bronchoalveolar lavage fluid in PPE/LPS-induced mice and LPS-stimulated Raw264.7 cells. SP treatment markedly decreased inflammatory cell infiltration and alveolar destruction in PPE/LPS-induced mice with elevation of total elastance, tissue elastance, and forced expiratory volume to forced vital capacity (FEV/FVC) ratio in lung tissues. SP inhibited the NOD-like receptor pyrin domain-containing 3 (NLRP3) inflammasome and downregulated levels of caspase-1 and IL-1β expression in PPE/LPS-induced mice and LPS-stimulated Raw264.7 cells. In addition, SP elevated the expression of nuclear factor-erythroid 2-related factor (Nrf-2), heme oxyngenase-1 (HO-1) and NAD(P)H quinine oxidoreductase 1 (NQO1). SP markedly increased anti-oxidant enzyme activities as well as suppressed the reactive oxygen species and nitric oxide levels in lung tissues of PPE/LPE-induced mice and LPS-stimulated Raw264.7 cells. Taken together, these findings indicate that SP may provide a useful therapeutic strategy for the treatment of COPD.

Corresponding author : In-Chul Lee

Keywords : *Spiraea prunifolia var. simpliciflora*, Oxidative stress, Airway inflammation, Thioredoxin-interacting protein, NOD-like receptor pyrin domain-containing 3 inflammasome

PS-B-080

Effect of mexedrone, a mephedrone analog, on abuse-related behaviors in rodents

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Mexedrone is a novel synthetic cathinone structurally related to mephedrone, which mimics the effect of classic psychostimulants such as amphetamine. However, the abuse potential of mexedrone has not been fully characterized. Thus, in the present study, we evaluated the effect of mexedrone on locomotor activity in open-field as well as its rewarding and reinforcing effect in the conditioned place preference (CPP) and intravenous self-administration procedures in rodents. Acute mexedrone significantly increased locomotor activity in a dose-dependent manner in mice. In the CPP study, mexedrone (50 mg/kg) produced a significant alteration in place preference in mice. In addition, in the self-administration study, mexedrone (0.5 mg/kg/infusion) significantly enhanced mexedrone-taking behavior during a 2 hr session under fixed ratio 1 (FR1) schedule of reinforcement in rats. Furthermore, acute mexedrone at a dose of 50 mg/kg increased c-Fos, a marker of neuronal activation, expression in the nucleus accumbens of mice. Taken together, these findings suggest that mexedroen has a high potential for abuse, given its robust psychomotor, rewarding, and reinforcing properties via neuronal activation in the nucleus accumbens.

Corresponding author : Eun Young Jang

Keywords : Synthetic cathinone, Mexedrone, Locomotor activity, Conditioned place preference, Self-administration

PS-B-081

Anti-atopic effect of Persicaria longiseta (Bruijn) Kitag in atopic dermatitis murine model induced by 2,4-dinitrochlorobenzene

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Atopic dermatitis (AD) is a disorder prevalent during childhood and adulthood, seriously affects the patient's quality of life that characterized by chronic highly pruritic and relapsing inflammatory skin lesions. Despite its growing morbidity, therapeutic treatments remain limited. Natural herbal extracts may be useful for treating AD symptoms. In this study, we investigated the effects of *persicaria longiseta* extract (PLE) on atopic dermatitis murine model. We used 2,4-dinitrochlorobenzene (DNCB) to induced atopic dermatitis-like skin in six-week-old BALB/C mice. Oral administration of PLE 100mg/kg, 200mg/kg, 400mg/kg and dexamethasone 1mg/kg on DNCB-induced BALB/c mice for 6 weeks. After PLE administration to DNCB-induced BALB/c mice there were improvements in SCORing Atopic Dermatitis (SCORAD) scores decrease in trans-epidermal water loss and improved skin hydration. PLE also decreased serum IgE levels, thymic stromal lymphopoietin (TSLP) levels, Macrophage-derived chemokine (MDC, CCL22) levels, melanin index, erythema index and epidermal thickness. These results suggest that PLE has an anti-atopic activity by regulating inflammatory chemokines and may be an effective therapeutic approach for prevention of AD-like disease.

Corresponding author : Young-Rak Cho

Keywords : Atopic dermatitis, BALB/c, Inflammatory, Skin lesion

PS-B-082

Establishment of transfection condition for study to investigate effect of miR-143 on canine mammary gland malignant tumor

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Regulating the expression of microRNAs (miRNAs) has been reported to have an effect on many physiological processes of organisms and miR-143 has been shown to play a role in the inhibition of tumors. In recent studies, the expression of miR-143 was observed to be reduced in malignant canine mammary gland tumors. Based on this, we conducted an experiment to establish the transfection condition for investigating whether miR-143 overexpression and inhibition are involved in tumor cell proliferation. For miRNA overexpression and knockdown, the miR-143 mimic and inhibitor were transfected into the canine mammary gland adenocarcinoma 335 cell line (passaged 32 times) at cell concentrations of 1×10^5 and 5×10^4, respectively, using a Neon electroporation transfection system (Invitrogen) with a 100 μℓ tip and a single pulse of 40 ms. To determine the suitable voltage for further study, we set up four treatment groups: voltages of 1100 (group 1), 1200 (group 2), and 1300 (group 3) and control. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum at 37°C. We repeated the experiment three times and counted the number of cells daily for 5 days. The one-way ANOVA with Tukey's multiple comparisons test was used to determine statistical significance. The number of cells in groups transfected with the miR-143 mimic did not show any significant difference. However, the number of cells in group 2 transfected with the miR-143 inhibitor was higher than that in group 1 on day 2 (P<0.05) and day 3 (P<0.005). Similarly, the number of cells in group 2 transfected with the miR-143 inhibitor was higher than that in group 3 (P<0.05) from day 2 to day 3. As a result, the best cell viability was obtained in group 2 and we concluded that transfection of miR-143 at a voltage of 1200 is the most effective for cell viability. This study was supported by Cooperative Research Program for Agriculture Science and Technology Development (#PJ014990022021), Uijeongbu-si.

Corresponding author : Geon A Kim

Keywords : MiR-143, Tumor cell suppression, Micro RNA overexpression, Micro RNA inhibitor

PS-B-083

Inhibitory effect of gastrodin and 4-hydroxybenzyl alcohol on tumor cell proliferation

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The extracts of Gastrodia elata, gastrodin and 4-hydroxybenzyl alcohol (4HBA), have been used as traditional medicine for hypertension and neuralgia in Korea, China, and Japan. Previous studies have reported that *G. elata* has a potential tumor-suppressing effect. The purpose of this study was to analyze the inhibitory effect of gastrodin and 4HBA on canine mammary gland tumor cell proliferation and establish an appropriate treatment concentration for further study. We divided into 3 groups to treat gastrodin and 4HBA, respectively. Canine mammary gland adenocarcinoma cells, 335 (passaged 32 times) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum at a concentration of 5×10^4 cells per well at 37°C in 12-well plates. After culturing the cells for 0, 24, 48 h, the medium in each well was changed with the addition of either gastrodin at 0, 1 μM, and 10 μM or 4HBA at 0, 0.1 mM, and 1 mM to the DMEM. We repeated the experiment four times after cell seeding, counted the number of cells daily for 3 days. Statistical analysis was performed using a one-way ANOVA with the Tukey's multiple comparisons test. Cell proliferation of the groups treated with 10 μM gastrodin was lower than that of the control (P<0.05) and group treated with 1 μM gastrodin (P<0.5) from day 1 to day 2. However, on day 3, following treatment with 1 μM (P<0.5) and 10 μM (P<0.0005) gastrodin, the number of cells was lower than that of the control. Following exposure to 0.1 mM 4HBA, the number of cells on day 1 was lower than that of the control (P<0.05). On day 2, cell proliferation in the 1 mM 4HBA-treated group was lower than that of the control (P<0.005) and 0.1 mM 4HBA-treated group (P<0.05). In this study, we demonstrated that gastrodin and 4HBA have an inhibitory effect on tumor cell proliferation and concluded that 10 μM gastrodin was the best at suppressing tumor cell proliferation. This study was supported by the Cooperative Research Program for Agriculture Science and Technology Development (#PJ014990022021, #PJ016773), Uijeongbu-si.

Corresponding author : Geon A Kim

Keywords : Gastrodin, 4HBA, Tumor cell line suppression, Proliferation assay

PS-B-084

Setting conditions for performing miRNA-210 transfection experiment

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Micro RNAs (miRNAs), consisting of approximately 23 nucleotides, are small, non-encoding RNA molecules that regulate gene expression. Previous studies have reported that miR-210 expression is upregulated in malignant canine mammary gland tumors. Therefore, the aim of this study was to determine the conditions for miR-210 transfection to analyze whether the regulation of miR-210 expression affects tumor cell proliferation. 335 cell line of the canine mammary gland adenocarcinoma were cultured in advance for this experiment and miR-210 mimic and inhibitor were transfected into cells to regulate the expression level of miRNAs. Cells (passaged 32 times) at a concentration of 5 × 104 were transfected using a Neon transfection system with a 100 μℓ tip and a single pulse of 40 ms. The cells was divided into four treatment groups with voltages of 1100 (group 1), 1200 (group 2), and 1300 (group 3) and the control to investigate the suitable conditions for further experiments. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum at 37°C. We repeated the experiment three times and after cell seeding, the number of cells were counted daily for 5 days. Statistical significance of the results was determined using the one-way ANOVA with the Tukey's multiple comparisons test. On day 3, the number of cells in group 1 transfected with the miR-210 mimic was higher than that in group 3 (P<0.5). Similarly, on day 2, the number of cells in group 1 transfected with the miR-210 inhibitor was significantly higher than that in group 3 (P<0.5). On day 3, the number of cells in group 3 transfected with the miR-210 inhibitor was lower than that in groups 1 and 2 (P<0.005). Therefore, the best cell viability was obtained from group 1 and we concluded that transfection of miR-210 at a voltage of 1100 is the most effective for cell viability. This study was supported by Cooperative Research Program for Agriculture Science and Technology Development (#PJ014990022021), Uijeongbu-si.

Corresponding author : Geon A Kim

Keywords : MiR-210, Tumor cell suppression, Micro RNA overexpression, Micro RNA inhibitor

PS-B-085

Image-based evaluation of adjuvant efficacy in a vaccine candidate against norovirus infection

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An adjuvant is one of the vaccine components that increase the effectiveness of a vaccine by inducing an additional immune response. Existing methods to test the efficacy of a vaccine adjuvant are to evaluate the immune responses through the secretion of cytokines and the formation of antibodies in terms of cellular and humoral immunity. In previous studies, we proposed a method for evaluating the efficacy of vaccine candidates and adjuvants by monitoring immune cell dynamics using multimodality imaging. The purpose of this study is to monitor the biodistribution of Norovirus vaccine components and to test the efficacy of adjuvants using PET, fluorescence (FLI), and bioluminescence imaging (BLI). Norovirus peptide antigen was labeled with [125I] and adjuvants were labeled with a fluorescent dye DiR. Antigen, adjuvants, and a mixture of both were intramuscularly injected into the right leg of B6.albino and BALB/c-nu mice. To measure immune cell dynamics after vaccination, luciferase-expressing immune cells (splenocytes) were transplanted into the tail vein of a B6.albino mouse. SPECT/CT and IVIS imaging were performed every day for 1 to 8 days after injection to monitor the biodistribution of vaccine components and immune cell kinetics. Vaccine candidates showed localization of antigens and adjuvants at the injection site as well as draining lymph nodes. The NoV antigen was degraded 24 hours after injection, but the adjuvant mixture remained at the injection site for up to 144 hours. In addition, we observed the migration of vaccine components from the injection site to the draining lymph nodes where adjuvants-mediated immune response occurs. We also observed that the adjuvant-induced the recruitment of immune cells, and confirmed the recruitment of immune cells at the injection site for 168 hours after vaccination.

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Keywords : Vaccine, Adjuvant, Multimodal imaging, Biodistribution

PS-B-086

Dopamine responsiveness to 3-FEA in rodents: Investigations in the nucleus accumbens

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3-Fluoroethamphetamine (3-FEA) is an amphetamines stimulant that acts as a release agent for monoamine neurotransmitters norepinephrine, dopamine and serotonin. Compared to the unplaced ethyl methamphetamine, 3-FEA has a weak release of noradrenaline, but strong release of dopamine and serotonin. Produced the strongest reinforcing effects in studies out of a range of 3-substituted ethamphetamine derivatives tested, despite not being the most potent dopamine releaser. Methamphetamine produces dependence and addiction through dopaminergic transmission but the abuse potential of 3-FEA has not been characterized. So we assessed abuse liability to identify the insufficient scientific information of 3-FEA. Dopamine level changes were analyzed using in vivo microdialysis to investigate the effect of 3-FEA on the central nervous system. In addition, serotonin level changes were analyzed using microdialysis. Behavioral changes caused by the influence of neurotransmitters were confirmed by testing locomotor activity. A significant response was observed in the microdialysis with 15 mg/kg intraperitoneally administered 3-FEA. In the locomotion test, mice that intraperitoneally received 10 mg/kg showed significant locomotor distance. Synthetically, It increased dopamine levels on in vivo microdialysis tests and change locomotor activity. 3-FEA regulates dopamine and serotonin in the brain and regulates locomotor activity and reward.

Corresponding author : Sun-Ok Choi

Keywords : 3-Fluoroethamphetamine, Microdialysis, Locomotor activity, Dopamine, Serotonin

PS-B-087

Evaluation of the potential immunotoxicity in mice with single exposure to poplypropylene microplastics through intragastric intubation

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The increased use of plastics and its relation to environmental pollution has become a growing concern globally. Many studies have reported the health effects of microplastics (MPs) exposure, especially through oral route. However, the toxicity assessment of polypropylene (PP) MPs is very limited. The present study aimed to evaluate the potential immunotoxicity of PP-MPs exposed to ICR mice via intragastric intubation. PP-MPs (average 5 or 50μm diameter size) were administered once to 6 wk old male and female mice at 500, 1000, or 2000 mg/kg/day. Considering the skewedness of helper T cell immune reactivity, cytokines levels were measured in polyclonally activated splenic T cells culture supernatant. A significant upregulation in the ratio of IFNγ/IL-4 was demonstrated in the male administered with 5μm-500mg PP-MPs compared to the control. Similarly, IL-13 level was significantly downregulated as dose-dependent manner in the male administered with 50μm PE-MPs. No significant alteration in the production of these cytokines was resulted in the female. The ratio of serum IgG2a/IgG1 was higher without statistical significance in the male exposed to PP-MPs of both 5 and 50μm size than the control mice. Meanwhile, the ratio was apparently lowered in the female compared to the control. No significant differences were observed in the thymic T cell subpopuation distribution. While splenic cytotoxic T cell % was significantly downregulated in the male exposed to 5 μm-2000mg compared to the control. Overall, the present study implies that cellular or humoral immunity could be affected under single exposure to PP-MPs and effects of repeated exposure are necessary to further investigate. [Supported by Korea Environmental Industry & Technology Institute (Grant No.2020003120002) and the Ministry of Environment-Educational training program for the hazards and risk of chemical substances]

Corresponding author : Yong Heo

Keywords : Propropylene, Microplastics, Immunotoxicity, Intragastric intubation

PS-B-088

Cyclophilin A-induced M2 macrophage polarization protects inflammation-induced preterm birth in mice

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Preterm birth (PB) is the principal cause of neonatal mortality and contributes to delayed physical and cognitive development. PB is known to be provoked by various causes such as inflammation induced by intrauterine infection. In PB condition, the M1/M2 macrophage polarization plays an important role. Cyclophilin A (CYP-A), a peptidylprolyl isomerase, which is ubiquitously expressed in many cell types, and has multi-facet functions in various inflammatory conditions and diseases. In this study, we examined whether CYP-A can modulate macrophage polarity to protect PB in a mouse model of intrauterine inflammation-induced PB. Lipopolysaccharide (LPS, 25 ug) was injected into the uterine horn between the first and second gestational sacs for uterine inflammation. CYP-A was intravenously administered 12 h and 30 min before LPS injection. Uterine, placental, and decidual tissues were isolated 6 h after LPS injection for experiments. Whereas LPS provoked PB in most mice within 48 h (5/6, 83.3%), administration of CYP-A at 10 ng significantly reduced the rate of LPS-induced PB (1/6, 16.7%) as well as improved the rate of perinatal survival (5/6, 83.3%). Furthermore, CYP-A markedly reduced the expression levels of pro-inflammatory cytokines in tissues of mice with the LPS-induced PB. When RAW 264.7 cells, a mouse macrophage cell line, were polarized toward M1 by LPS *in vitro*, CYP-A pretreatment suppressed expression of *iNOS, Il-6*, and *Socs3* (M1 markers) and increased expression of *Arg1* and *Mrc1* (M2 markers). CYP-A promotes the macrophage polarization toward anti-inflammatory M2 type. To address whether M2 macrophages are the key for preventing PB, we performed adoptive transfer of M2 polarized macrophages *in vitro* could protect inflammation-induced PB. Administration of M2 bone marrow derived macrophages reduced the rate of PB within 48 h (1/4, 25%) after LPS injection. Therefore, CYP-A can be a new therapeutic tool to regulate the polarity of the macrophage toward M2 to prevent preterm labor and increase offspring survival.

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Corresponding author : Haengseok Song

Keywords : Preterm birth, Inflammation, Cyclophilin A

PS-B-089

The deficiency of ABCG1 and ABCG4 transporters by rare earth oxide nanoparticles induces the pulmonary alveolar proteinosis

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Pulmonary alveolar proteinosis (PAP) is a rare disease that accumulates alveolar surfactant. PAP can be induced by nanoparticles (NPs), but there is little information about PAP-producing NPs and their mechanism of action. Here, we evaluated 7 PAP-producing NPs including 6 of rare earth oxide (REO) NPs and NiO NPs, and investigated the magnitude of PAP at 1 and 6 months after a single intratracheal instillation to female Wistar rats. In addition, alveolar macrophages were purely collected from bronchoalveolar lavage fluid (BALF) after each time-point to evaluate the gene expression profile of ATP-binding-cassette (ABC) transporters and related proteins. The 6 REO NPs (Dy_2O_3, Eu_2O_3, In_2O_3, Pr_6O_{11}, Sm_2O_3, and Tb_4O_7) and NiO NPs caused PAP at 1 month, while only In_2O_3 NPs showed a persistent PAP at 6 months. The Pearson correlation test between the levels of phospholipids in BALF and gene expression profile of ABC transporters showed that ABCA1, ABCB4, ABCB8, ABCG1, and ABCG4 showed a significant correlation, which implies that these genes are the main transporters influenced by PAP-producing NPs. Because both ABCG1 and ABCG4 down regulated as exacerbation of PAP, these can be key transporters producing PAP by NPs. While other ABC transporters were up regulated as exacerbation of PAP, which implies compensatory transporters involved in the recovery of PAP. In conclusion, the PAP production by six types of REO NPs and NiO NPs shown in this study suggest that PAP will emerge as an occupational disease in the near future and the ABCG1 and ABCG4 can be a target for the treatment of PAP, which needs further studies.

Corresponding author : Wan-Seob Cho

Keywords : Pulmonary alveolar proteinosis, Rare earth oxide nanoparticles, ABC transporters, Foamy macrophages, Nanoparticles

PS-B-090

Comparison of disease severity by induction period in the Bleomycin-induced mouse model of idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, fibrotic lung disease characterized by expansion of fibroblast/myofibroblast populations and aberrant remodeling, which can lead to respiratory failure and death. More clinically relevant animal models that reproduce key known features of the IPF should be used to investigate pathophysiology and novel treatment approaches.
Historically, among the first to be developed and used widely is the bleomycin model, which is the best-characterized and currently most extensively used animal model due to its ability to reproduce many aspects of IPF, good reproducibility, and ease of induction.
Bleomycin is the primary chemotherapeutic drug for testicular cancer, but lung fibrosis is a side effect in ~10% of patients. Bleomycin-induced lung fibrosis is the most frequently used rodent model of lung fibrosis and produces inflammatory and fibrotic events similar to those seen in human pulmonary fibrosis. Bleomycin administration in rodents leads to the accumulation of leukocytes, especially macrophages, followed by the activation of fibroblasts and fibroblast-like cells and collagen deposition.
Our study's primary purpose is to explore the most effective IPF animal model among the different periods of IPF induction by comparing the phosphorylation of ERK and the expression of alpha-smooth muscle actin (α-SMA), which are mainly identified in IPF. Nine-week-old C57BL/6 (DBL) male mice were used throughout the experiments. Mice were sacrificed on day 10, day 14, or day 21 after 2 mg/kg of bleomycin (intratracheal). As a result, the mortality rate was high in the 21-day model, and disease induction was weak in the 10-day model. Therefore, 14 days after administering bleomycin is considered the most suitable condition.

Corresponding author : Joon-Chul Kim, Geun Ho An

Keywords : Idiopathic pulmonary fibrosis, Lung fibrosis, Bleomycin, Animal model

PS-B-091

Aloe vera prevents lung fibrosis by suppressing TGF-β/Smad pathway

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After the COVID-19 outbreak, growing number of patients are suffering from pulmonary fibrosis. As the final pathway of lung fibrosis appears to be common even in the different type of lung diseases, there is a hope to develop antifibrotic agents that might be effective in fibrotic diseases including post-COVID fibrosis. *Aloe vera* is a popular health supplement with effects such as anti-inflammation, healing of wounds, antioxidant activity, and hepatoprotection. Therefore, we thought that *Aloe vera* could suppress the process of pulmonary fibrosis because it was accompanied by various processes such as damage, recovery, and inflammation

Multiple cell types including lung epithelial cells, mucosal cells, macrophages, and fibroblasts from normal human or IPF patients were used to test the beneficial effect of processed *Aloe vera* gel (PAG). MTT assay was performed to choose treatment dose in each cell lines. The expression of ECM related genes such as collagen, α-SMA, and fibronectin were tested by RT-PCR and Western blot in normal human lung fibroblast WI-38, MRC-5 cells and patient-derived lung fibroblast LL29 cells. In Bleomycin treated lung fibrosis mouse model, PAG was orally treated for 2 weeks. Lung tissues were stained with Masson's trichrome and H&E staining.

Although there was not dramatic beneficial effect PAG on pneumocyte, macrophage, mucous secreting lung cells, in normal and IPF patient-derived pulmonary fibrosis cells PAG suppressed the fibrosis markers such as collagen, fibronectin, and α -SMA in RNA and protein level. Also, production and secretion of TGF- β1 was decreased and pSmad2 was inactivated by PAG in fibroblasts. This study demonstrates potential antifibrotic effects on lung fibrosis through downregulation of TGF β/Smad signaling pathway.

Corresponding author : Seung Hyun Oh

Keywords : *Aloe vera*, IPF, TGF β/Smad signaling, Lung fibrosis

PS-B-092

External electrical stimulation suppresses hepatic lipid accumulation by modulating lipid metabolism in non-alcoholic fatty liver animal models

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Electrical stimulation, one of the non-pharmaceutical methods, can be divided into several categories according to the characteristics of stimulation, especially micro-current stimulation (MCS) is attracting attention in terms of safety and effectiveness. This study aimed to investigate the possibility of MCS as a treatment for non-alcoholic fatty liver (NAFL). The intensity of MCS was selected as 200 μA and 400 μA based on previous studies. MCS with 400 μA increased the expression of proteins related to Sirt1/AMPK signaling in oleic acid-induced lipid accumulation in FL83B cells. Moreover, Factors related to lipolysis increased, while transcription factors related to lipogenesis decreased. In the case of fatty acid β-oxidation, both MCS groups tend to improve, but 400 μA showed more dominant changes. Also, this study indirectly observed lipid accumulation in liver tissue through liver weight and visual observation using two animal models. In the case of the ob/ob mice model, an animal model commonly used as NAFL, liver hypertrophy and color change were severe in ob. Both MCS groups did not show liver hypertrophy, but the weight increased. However, compared to ob, both MCS groups showed a significant reduction in liver weight. The second animal model used a model that intakes an eight-week high-fat diet. Unlike ob, HFD did not show liver hypertrophy, but the color of liver changed to brownish-yellow color. The liver weight was significantly reduced in HFD200, but there was no significant change in the visual observation of liver. On the other hand, in HFD400, not only the liver weight was significantly reduced, but also visual observation showed a similar appearance to CON. Taken together, these results implied that MCS might be a treatment for NAFL by modulating hepatic lipid metabolism.

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Corresponding author : Han Sung Kim

Keywords : Micro-current stimulation, Non-alcoholic fatty liver, FL83B cells, ob/ob mice, High fat diet-induced obese mice model

PS-B-093

The development of alcohol-associated hepatocellular carcinoma mouse model

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Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death and the sixth most prevalent cancer worldwide. Therapeutic approaches for advanced HCC stages have only limited efficacy, and patients whose disease has progressed to hepatocellular carcinoma, 15% die while awaiting liver transplantation due to a shortage of grafts. The heterogenous genetic alterations of HCC, profound alterations in the hepatic microenvironment, and incomplete understanding of HCC biology make treating HCC challenging. Therefore, the development of appropriate HCC animal models is urgently required to improve our understanding of HCC biology (including specific pathways and genetic alterations in the progression of carcinogenesis).

In this regard, animal model development has integrated key features found in HCC development, such as the activation or inactivation of specific pathways by genetic means, as well as the hepatic environment that promotes hepatocarcinogenesis, such as chronic liver injury, inflammation, obesity, and fibrosis. Recently, the prevalence of chronic alcohol drink mediated liver cancer is rapidly increasing, and accelerates various social/economic losses, however, there has been a great difficulty in studying chronic alcohol-induced liver cancer because there is no animal model of alcohol-induced liver cancer so far. Therefore, we would like to suggest a new mouse model of alcohol-associated HCC mouse model through this study.

Corresponding author : Wonhyo Seo

Keywords : Hepatocellular carcinoma, Alcohol

PS-B-094

Assessment of the lung toxic effect of whole cigarette smoke extract on repeated intratracheal instillation

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Existing inhalation exposure methods for tobacco smoking have required expensive equipment and wasted labor to induce potential toxicity. Therefore, we sought to develop a novel method using intratracheal instillation (ITI) of whole tobacco smoke condensate (WCSC). WCSCs were prepared at 20 mg/mL, diluted to 10 and 5 mg/mL, and administered by ITI using an automatic video dropper. ITI, 12 reps, 12 days (daily), group 2 (6, 12 days). After the final ITI, bronchoalveolar lavage fluid (BALF) and lung tissue were collected. BALF was analyzed using a commercially available sandwich ELISA method. Mild histopathological changes were observed in lung tissue treated with WCSC (20 mg/mL) for 12 days. On the other hand, as a result of repeating the ITI of WCSC for 6 days, body weight, neutrophil and lymphocyte count increased compared to the control group. In addition, histopathological markers were observed in lung tissue treated with high-dose WCSC. BALF analysis of the 6- and 12-day groups confirmed that inflammatory changes and changes in secretion of the related cytokine MCP-1 were observed in this study. In addition, activation of tissue remodeling markers MMP-9 and TGF-β1 in lung tissue was regulated by WCSC administration. And as a result of RNA sequencing analysis to confirm the change in gene expression in the lung tissue, the expression of CCl2, a type of chemokine, was induced in the WCSC-administered group compared to the control group. However, there was no change in the secretion of IL-6 and TNF-α in BALF. In summary, ITI exposure may be a simpler and more effective method to evaluate adverse effects of WCSC with short exposure periods of less than 2 weeks.

Corresponding author : Min-Seok Kim

Keywords : Whole cigarette smoke extract, Intratracheal instillation, Lung injury, Inflammatory response

PS-B-095

Processed Aloe vera gel accelerates reversal of liver fibrosis in vitro and in vivo through regulation of TGFβ1 pathway

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Aim of the study: Liver fibrosis is a common stage of multiple different chronic hepatic injuries when damage regions are encapsulated by an excessive amount of extracellular matrix (ECM). When being left untreated, pathologically abundant deposit of fibrous proteins may induce further irreversible distortion of hepatic architecture and debilitate patients' hepatic functions. Unfortunately, there is no FDA-approved pharmacotherapy targeting fibrotic stage. In this research, we discovered the antifibrotic effect in vitro and in vivo of cellulase-processed Aloe vera gel (PAG), with main component is acetylated mannan or Acemannan at various sizes, ranging from 5 to more than 500 kD.
Materials and methods: In vitro and in vivo liver fibrosis models were used to test the effects of PAG. Firstly, we explored the antifibrotic effects of PAG in C57BL/6 mouse hepatic stellate cells and LX-2 cells. Furthermore, six-week-old male C57BL/6 mice were concomitantly intraperitoneally injected with CCl4 twice weekly and force-fed with PAG at 3.14 and 12.5 mg/mouse daily for 6 weeks. Liver tissues were stained with Masson's trichrome and sirius red methods.
Results: PAG treatment reduced accumulation of fibrotic markers like Collagen I A1 (Col1A1), Fibronectin, and α smooth muscle actin (α-SMA) in TGFβ1-treated primary mouse hepatic stellate and LX-2 cells. In concordance with the in vitro experiments, PAG reduced scarring proteins in livers of CCl4-induced liver fibrosis in C57BL/6 mice. We found out that PAG also reduced TGFβ1 expression at both protein and mRNA levels, suggesting its mechanism of action through this signaling pathway.
Conclusions: To recapitulate, this study demonstrates antifibrotic effects of PAG on both in vitro and in vivo models through downregulation of TGFβ1 expression.

Corresponding author : Seung Hyun Oh

Keywords : Liver fibrosis, Aloe vera gel, TGFβ1, Antifibrotic, Polysaccharides

PS-B-096

Methoxychlor and Bisphenol A have more negative effects on male reproductive system when used together

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Endocrine Disrupting Chemicals(EDCs) are found in many commercial products and cause many problems, such as interfering with the normal function of the endocrine system. EDCs are more dangerous because they affect the human body in combination. However, there is a lack of research on combination administration of EDCs. So we studied the effects on male reproductive function, especially when administered together with methoxychlor(MXC) and Bisphenol A(BPA), which are toxic to male reproductive organs.
In this experiment, male mice were divided into four groups of control, 400mg MXC, 1mg BPA and 400mg MXC + 1mg BPA/kg/day. And MXC and BPA were dissolved in sesame oil and acetone and administered oral gavage for three weeks. After administration, weight and histological changes in testicles and epididymis, sperm count calculation, parameters measurement of sperm, in vitro sperm survival test, biochemical tests, and complete blood count (CBC) were performed.
The testicular weight was reduced in the experimental group compared to the control group. In the case of sperm, the number and parameter of sperm in the combined administration group were significantly reduced. And in the in vitro sperm survival test, it was confirmed that the sperm survival ability of the combined administration group was lower than that of other groups. As a result of biochemical tests, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) increased.
This study suggests that MXC and BPA have a more negative effect on male reproductive capacity when acting together, resulting in damage to testicular tissue and increased AST, ALT, and LDH in the experimental group. In particular, it shows that it reduces sperm functions such as sperm concentration, activity, and viability.

Corresponding author : Dae Young Kim

Keywords : Methoxychlor, Bisphenol A, Male reproductive system, Endocrine disrupture, Testis

PS-B-097

Understanding the etiology and establishing the therapeutic strategies for menopausal xerostomia using ovariectomized mice

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Salivary gland (SG) dysfunction leads to xerostomia (dry mouth). Importantly, the senior population is susceptible to xerostomia and the prevalence of the disease is much higher in elderly women than in men, implying the correlation between salivary function and sex hormone, such as estrogen. To understand the etiology of post menopause-associated xerostomia, we investigated the functional alteration of the salivary gland (SG) of ovariectomized (OVX) mouse model. Notably, OVX leads to SG dysfunction with reduced salivary flow. In histological assessment, aquaporin 5 (AQP5)-expressing acinar compartment was significantly reduced in SG of OVX mice (OVX-SG), while ductal cells were profoundly increased instead. In the gene set enrichment analysis (GSEA), we found that GO terms associated with structural alterations such as extracellular matrix (ECM) and cell junctions were significantly enriched in OVX-SG compared with controls. Interestingly, TGF-β2 expression in the SG was significantly upregulated after OVX. Next, we utilized salivary gland organoids (SGOs) as a modeling system for the investigation of salivary epithelial stem/progenitor cell homeostasis and differentiation. Established mouse and human SGOs were able to maintain stably and differentiate into mature cells, including acinar and duct cells, that are capable of recapitulating functions of SG. It was noted that treatment of TGF-β2 significantly reduced the formation of SGO, which was recovered by co-treatment of TGF-β inhibitor SB431542 or Estradiol. We found that TGF-β2 could increase the susceptibility of SG cells against oxidative stress, partially via induction of ferroptosis. To downregulate the oxidative stress in OVX-SG, we administrated extracellular vesicles (EV) isolated from mesenchymal stem cells overexpressing SOD3, one of antioxidant enzymes. Interestingly, treatment of EV-SOD3 could restore the salivary flow rate in OVX mice with reduction in damage-associated markers. These findings suggest a novel insight regarding the pathogenesis and effective treatment strategy of menopausal xerostomia.

Corresponding author : Hyung-Sik Kim

Keywords : Xerostomia, Salivary gland, Organoid, Extracellular vesicle, Ovariectomy

PS-B-098

Adverse affects of chlopyrifos on reproductive and immune system

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Chlorpyrifos(CPF) is easily used to prevent pests as a organophosphate paesticide. CPF has toxicity that inhibits acetylcholineesterase(ACHE), which causes Oxidative stress, affects hormone and meatabolomics and reproductive system. Our study focus on how CPF affect to male mice's reproductive system. The reproductive toxicity of CPF was studied in male mouse (ICR mouse 6 week old). Male mouse were treated by gavage with CPF doses at Control (2ml corn oil), 5mg/kg(1/12 0f the LD50) 10mg/kg(1/6 0f the LD50), 15mg/kg(1/4 0f the LD50), 20mg/kg(1/3 0f the LD50) for 28 days. Totally 25 Mouse was randomly assigned 5 groups. The oral LD50 for CPF to male mice is 60mg/kg. We progress western blot, Histopathological test of testes and epididymis by using H&E staining, measures sperm count and mobility using Computer-assisted sperm analysis (CASA) and progress white blood cell(WBC) test, and complete blood count (CBC). Also, after the gavage during 28 days, we measure mouse's organ weight. In 20mg/kg group and 15mg/kg group there are significant differences in western blot and histopathological test and CASA. Through western blot we found that increase of CPF dose causes denaturalization of reproductive system associated protein. Especially 15mg/kg and 30mg/kg group, denutalized protein has been increase rapidly. In histopathological test empty space and inflammation observes in all grop except control. In casa, abnormal sperm and mortality sperm increased as CPF doses increased. There is no significane difference in whole weight in all group. But, weights of testis was reduced. According to this study we found that CPF exposure may have effects on male mouse reproductive system and immune system.

Corresponding author : Dae Young Kim

Keywords : Chlorpyrifos, Male, Mouse, Sperm, Testicular tissue

PS-B-099

Compound K-enriched Korean red ginseng regulates induces apoptosis of lung cancer cells through STAT3 down-regulation

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Despite the development of anticancer drugs, still recurrence, metastasis, and resistance frequently arise in patients. The increase in extracellular matrix has been known to contribute not only to the fibrosis but also so the resistance to anticancer drugs. The ECM is a non-cellular meshwork of cross-linked macromolecules, which provides a physical barrier to cancer cells from cytotoxic drugs as well as anchorage sites for cancer cells. Therefore, the use of agents which can modulate the ECM will enhance the intra-tumoral diffusion of anticancer drugs and therapeutic effect will be improved.

Korean red ginseng and its various components have been reported have direct anticancer effects. When red ginseng is taken, various components are metabolized to form compound K and its anticancer effects were shown in cancers. Recently we made a ginseng product enriched with compound K (CKP) and tried to demonstrate its therapeutic and/or beneficial effect on lung cancer.

The ingredients of CKP were analyzed by HPLC. Antiproliferative effect of CKP was tested in 8 different lung cancer cells and lung fibroblast (WI-38) with MTT and colony formation assay. Anti-proliferation and apoptosis effects were confirmed by Western blot, RT-PCR and FACS analysis. The expression of ECM related genes such as collagen, α-SMA, and fibronectin were tested by RT-PCR and Western blot in WI-38 cells.

The amount of Compound K increased up to 31.14 % in CKP, while it was not detectable in red ginseng extract. The proliferation of the most lung cancer cells were dramatically decreased by CKP. The apoptotic effect of CKP was confirmed by Western blot and FACs experiments. Interestingly, we found the reduction of collagen, α-SMA, and Fibronectin protein/RNA expression by CKP in WI-38 cells.

In this study, we suggest that CKP can be used for combined therapy with traditional drugs and as an anti-fibrotic agent.

Corresponding author : Seung Hyun Oh

Keywords : Korean red ginseng, Compound K, Lung cancer, Fibrosis

PS-B-100

The impact of vanadium oxide exposure on sperm motility and function in mice

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Air pollution is a serious environmental problem that is caused by the advancement of industrialization and urbanization. PM contains sulfate, nitrate, heavy metal (vanadium pentoxide, lead, iron, cadmium), and polycyclic aromatic hydrocarbons. Vanadium is a chemical element that enters the atmosphere via anthropogenic pollution. Exposure to vanadium affects cancer development and can result in toxic effects. Multiple studies have focused on vanadium's detrimental effect on male reproduction using conventional sperm analysis techniques. This study focused on vanadium's effect on spermatozoa following capacitation at the molecular level, in order to provide a more detailed assessment of vanadium's reproductive toxicity. We observed a decrease in germ cell density and a structural collapse of the testicular organ in seminiferous tubules during vanadium treatment. In addition, various sperm motion parameters were significantly decreased regardless of capacitation status, including sperm motility, rapid sperm motility, and progressive sperm motility. Curvilinear velocity, straight-line velocity, average path velocity, beat cross frequency, and mean amplitude of head lateral displacement were also decreased after capacitation. Capacitation status was altered after capacitation. Vanadium dramatically enhanced protein kinase A (PKA) activity and tyrosine phosphorylation. Taken together, our results suggest that vanadium is detrimental to male fertility by negatively influencing sperm motility, motion kinematics, and capacitation status via abnormal PKA activity and tyrosine phosphorylation before and after capacitation. Therefore, we suggest that exposure to intemperate vanadium may have a negative influence on male reproductive function and fertility.

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Keywords : Capacitation status, Protein kinase A, Sperm motility, Tyrosine phosphorylation, Vanadium oxide

PS-B-101

Chlorpyrifos affects reproductive system in female mice

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Background: Today Endocrine disruptors (EDCs) are used in many industries, but most people are unaware of the dangers of EDCs. Among them, Chlorpyrifos is the most commonly used insecticide causing a lot of damage to the reproductive system when it enters the body. However, the effects of chlorpyrifos (CPF) on reproductive organs are not known exactly, so we investigated through this experiment.
Methods: Female ICR mice were divided into four groups (negative control, 5, 10 and 20 mg/kg/day) of seven, and oral gavage was administered three days a week for four weeks. To determine the effect on reproductive organs, the estrous cycle was checked twice a week. Subsequently, histopathological analysis of ovary and uterus, measurement of steroid (estrogen, progesterone) level, blood biochemical test, IHC and WBC count were performed to study the reproductive toxicity of CPF.
Results: Estrous cycle showed irregular cycle compared to negative control, and steroid (estrogen, progesterone) level decreased. In the case of surface epithelium and luminal epithelium of the uterus and ovary, hypertrophy and abnormalities of tissue were observed. The toxicity was confirmed through a blood test, IHC and WBC count.
Conclusion: Our findings suggest that Chlorpyrifos affects the reproductive system in female mice. However the impact on the reproductive system is related to reproduction, and the effects on the next generation are required to reduce use of CPF in future.

Corresponding author : Dae young Kim

Keywords : Chlorpyrifos, Female mice, Reproductive system, Toxicity

PS-B-102

The effects of micro-current stimulation on alleviating non-alcoholic steatoheptitis in CDAHFD-induced mice model

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Non-alcoholic steatohepatitis (NASH) is the most common cause of chronic liver disease, with the potential to develop cirrhosis and hepatocellular carcinoma. Recently, micro-current stimulation (MCS) is known as one of the non-pharmacological treatments of using for anti-inflammation and anti-obesity purposes. This study aimed to investigate whether MCS can alleviate the progression of NASH. For the animal experiments, we used the choline-deficient L-amino acid-defined high-fat diet (CDAHFD)-induced mouse model. MCS with the intensity of 400 μA was applied to the NASH+MCS group. To evaluate the efficacy of MCS, the experimental animals divided the male 4-week-old C57BL/6J into three groups: control group (CON), CDAHFD group (NASH), and CDAHFD and MCS applied group (NASH+MCS). NASH and NASH+MCS fed CDAHFD for 8 weeks, and MCS was applied 40 minutes daily for 4 weeks only for NASH+MCS. Western blot analysis for liver tissue was conducted to observe changes in protein expression in indicators, such as NLRP3, TLR4, αSMA, and COL1A1, closely related to NASH. NLRP3, which plays a major role in the progress of NASH, was significantly decreased in NASH+MCS compared to NASH. TLR4, αSMA, and COL1A1 were also decreased. In the case of the body weight and liver weight, it also decreased significantly compared to the NASH group. Taken together, these results suggest that MCS can alleviate NASH by modulating various inflammatory factors associated with NASH.
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Corresponding author : Han Sung Kim

Keywords : Non-alcoholic steatohepatitis, Choline-deficient L-amino acid-defined high-fat diet, Micro-current stimulation, Nucleotide-binding domain leucine-rich containing family, Pyrin domain-containing-3

PS-B-103

A comprehensive mechanism in inhibiting gap junction intercellular communication by DMBA

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Gap junctional intercellular communication (GJIC) is composed of the connexin protein and plays an important role in maintaining intracellular homeostasis. 7,12-dimethylbenz(a) anthracene (DMBA) belonging to polycyclic aromatic hydrocarbons (PAH) is widely known as a carcinogen, and has been reported to inhibit GJIC. However, the mechanism of GJIC inhibition by DMBA is not well understood. The purpose of this study is to elucidate the comprehensive regulatory mechanism of GJIC inhibition by DMBA. This study showed that Cx43 promoter activity was increased by DMBA treatment in WB-F344 rat liver epithelial cells. The mitogen-activated protein kinase (MAPK) pathway is known to enhance Cx43 promoter activity through phosphorylation of c-Fos protein. In this study, the activity of the Cx43 promoter by DMBA treatment was increased by upregulation of the MAPK/c-Fos pathway. In a previous study, an increase in HNF3β by a decrease in Wnt/β-catenin signaling upregulated the activation of the Cx43 promoter. In fact, it was confirmed that the Wnt/β-catenin signal was decreased by DMBA along with Cx43 promoter activation. Interestingly, DMBA treatment increased Cx43 promoter activity but decreased the amount of Cx43 mRNA. The RNA binding protein human antigen R (HuR) was found to enhance mRNA stability by preserving the unique adenylate-uridylate-rich (AU-Rich) sequence of Cx43 mRNA. Therefore, it was found that the reduction of HuR by DMBA treatment was associated with the inhibition of Cx43 mRNA stability. In addition to a decrease in Cx43 mRNA stability, we also observed an increase in Cx43 proteolysis by DMBA treatment. The MAPK pathway is known to inhibit GJIC through phosphorylation of Cx43 protein. Indeed, the MAPK pathway was increased by DMBA treatment. This is considered to be related to the loss of GJIC through phosphorylation of Cx43. In conclusion, our study suggested that AP-1, HuR, and MAPK can be important factors for GJIC inhibition by DMBA treatment.

Corresponding author : Jun-Won Yun

Keywords : DMBA, Gap junction, MAPK, C-Fos, HuR

PS-B-104

Identification of potential mechanism through miRNA-target gene profile of cisplatin-induced hepatotoxicity and nephrotoxicity and discovery of circulating miRNA diagnostic biomarker

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miRNA is being studied as a miRNA biomarker candidate for the diagnosis of various diseases due to its physiological roles such as regulation of cell growth, proliferation, and metabolism. Our study aimed to verify liver and kidney miRNA changes after cisplatin administration, predict their target genes, and identify changes in the cellular pathways of difference expressed genes (DEGs) through RNA-sequencing and discover organ-specific serum miRNA biomarkers. At 2 days post-treatment with cisplatin, hepatotoxicity and nephrotoxicity were exhibited through increases in liver and kidney-related serum biochemical markers, the degree of histological alteration, and changes in oxidative stress and apoptosis-related protein. Small RNA sequencing was performed and 32 liver differentially expressed miRNAs (DE-miRNAs) and 51 kidney DE-miRNAs were identified by cisplatin treatment. The target gene of liver and kidney DE-miRNAs was predicted using miRDB. Identifying the intersection of the predicted gene with cisplatin-induced hepatotoxicity and nephrotoxicity-related DEGs, it was classified as a gene regulated by DE-miRNA. KEGG pathway analysis was performed with the above gene using the DAVID database. PI3K/Akt signaling pathway, which is important for cell proliferation, survival, was significantly lower expressed in cell cycle progression genes following cisplatin administration in liver. Peroxisome, which plays a major task in fatty acid oxidation (FAO) and hydrogen peroxide turnover, the FAO-related gene was expressed low-level by cisplatin in kidney. Among many serum miRNAs changed by cisplatin, we identified 7 liver-related serum miRNAs and 14 kidney-related serum miRNAs that are expressed only in liver or kidney. Top 5 miRNA candidates were further selected for each organ according to the expression levels of serum circulating miRNA level after cisplatin administration. Especially, according to the time course and injury level after cisplatin administration, 3 liver-circulating miRNAs (miR-511-5p, miR-146a-5p, miR-187-3p) and 3 kidney-circulating miRNAs (miR-219a-2-3p, miR-409a-3p, miR-379-5p) tended to decrease in tissue and significantly increased in serum. Collectively, serum circulating miRNA can be a novel biomarker for diagnosing cisplatin-induced hepatotoxicity and nephrotoxicity, and the identification of potential mechanisms through miRNA-regulated genes provides valuable insight into cisplatin-induced toxicity.

Corresponding author : Jun-Won Yun

Keywords : Cisplatin, Hepatotoxicitiy, Nephrotoxicity, Biomarker, MicroRNA

PS-B-105

Brain structural alteration by cocaine administration is regulated by dihydropyrimidinase-related protein 2 and crystallin alpha B in the marmoset

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Cocaine is one of most abused drugs. Chronic cocaine abuse is associated with structural modification of brain regions, such as white matter reduction. Moreover, cocaine-induced rapid head movement in common marmoset (Callithrix jacchus) can serve as a model for stereotype. However, molecular mechanism underlying cocaine-induced brain damage. Here, we studied the effects of binge cocaine administration on the common marmoset (Callithrix jacchus) white matter structure. Cocaine (0.1 to 0.5 mg/kg/infusion) was self-administered (SA) intravenously to adult marmosets for 30 days. After the last cocaine administration, we performed diffusion tensor imaging to elucidate white matter structure in the brain. The cocaine induced reduction in fractional anisotropy value of the corpus callosum. In proteomics study, level of dihydropyrimidinase-related protein2 (DPYSL2) that is related with axon development and guidance regulation molecules are most altered in cocaine treated marmoset acute brain slice, and protein expression of DPYSL2 tended to decrease by treatment of cocaine. Interestingly, gene-gene interaction analysis revealed that crystallin alpha B (CRYAB), a chaperone expressed in oligodendrocyte is associated with DPYSL2, and we also confirmed that cocaine significantly induced expression of CRYAB and p-CRYAB in oligodendrocytes-positive cells in the cortex. These results suggest that white matter modification induced by cocaine is associated with axon development regulatory molecules via oligodendrocytes function.

Corresponding author : Jaesuk Yun

Keywords : Cocaine, Common marmoset, Self-administration, Oligodendrocyte, Corpus callosum

PS-B-106

Brain functional alteration by cocaine administration is involved in stereotyped behavior in the marmoset

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Dysfunction and structural changes in the brain associate with repetitive and stereotyped behaviors. Here, we studied the effects of binge cocaine administration on the common marmoset (Callithrix jacchus) behaviors and brain connectivity. Cocaine (0.1 to 0.5 mg/kg/infusion) was self-administered (SA) intravenously to adult marmosets for 30 days. After the last cocaine administration, the behaviors were observed in freely moving animals for 2 h. We performed resting-state functional magnetic resonance imaging (rs-fMRI) to elucidate resting-state functional connectivity (rsFC). Additionally , we evaluated differences in metabolic profiles of the plasma between pre- and post-cocaine conditions. A total of around 30 mg/kg of cocaine injected for one month induced repetitive and stereotyped behaviors, such as rapid head movements in the freely moving marmosets. We confirmed the brain connectivity at 23 areas, including the prefrontal cortex, motor cortex, and nucleus accumbens that were significantly changed by cocaine administration, and glutamine/glutamate metabolism were identified as altered in the cocaine administrated marmosets' plasma. Cocaine induced changes in cannabinoid receptor type 1 availability and glutamate decarboxylase expression in marmosets' cortex. Taken together, The study suggests that cocaine induces disturbance in the cortex connectivity along with stereotype behavior in marmoset via regulation of endogenous cannabinoid system and GABAergic neurotransmission.

Corresponding author : Jaesuk Yun

Keywords : Cocaine, Common marmoset, Self-administration, Cortex, Stereotyped behavior

PS-C-001

Comparative analysis of gut microbiome related to weight gain of the same individual before and after gut microbiome replacement

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The precise action mechanisms of gut microbiome in its host at the level of its constituting bacteria are obscure in most cases despite its definitive role. Here, we developed an *in vivo* divergent method to analyze the gut microbiome by using a mouse model to overcome the ambiguity. The original gut microbiome of conventional C57BL/6 mice was randomly replaced with the subset of human gut microbiome, followed by the comparison of weight gained by the same individual mouse before and 3 months after the replacement to analyze only the effect of gut microbiome by offsetting gene factors. The human gut microbiome replacement affected the body weight gain in three different ways: positive, medium, and negative. The gut microbiome analysis showed that the differences in body weight gain were associated with the replacement of their original gut microbiomes with different kinds of gut microbiomes in each of the three groups of mice. Also, body weight gain is not determined by a group of bacteria such as Firmicutes, Bacteroidetes, etc., which was consistent with recent findings. Uncultured bacteria NR_074436.1, NR_144750.1, and NR_0421101.1 were positively associated with body weight gain, while *Trichinella pseudospiralis*, uncultured bacteria NR_024815.1 and NR_144616.1 were negatively associated. This work shows that the comparative analysis of the original gut microbiome with the newly established gut microbiome together with body weight changes successfully identified the intestinal microbes associated with weight gaining at the species level.

Corresponding author : Hea-Jong Chung

Keywords : Gut microbiome, Comparative analysis, Gut microbiome replacement

PS-C-002

Anti-malarial effect of PJ extract by inhibiting platelet activation in rodent model

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Natural herb extracts have been an important source of antimalarial compounds. This study aimed to evaluate the impact of the PJ extract on malarial infection. Antimalarial activity of a PJ extract was evaluated *in vitro* using chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) *Plasmodium berghei* strains. Also, the *in vivo* activity of the extract was evaluated in *P. berghei*-infected mice via oral administration followed by a four-day suppressive test to measure the hematological parameters. In addition, platelet activation signaling induced by the PJ extract in *P. berghei* infection was evaluated.
Exposure to the PJ extract significantly inhibited both CQ-sensitive (3D7) and resistant (Dd2) strains of *P. falciparum* with IC_{50} values of 8.48 ± 1.70 and 7.83 ± 6.44 μg/ml, respectively. Administration of the PJ extract also resulted in potent antimalarial activities in *P. berghei*-infected mice with no associated toxicity. The treatment also improved the hematologic parameters. In addition, the survived mice from *P. berghei* infection exhibited the inhibition of collagen-induced platelet aggregation by attenuated glycoprotein VI (GPVI) downstream signaling. PJ extracts promote antimalarial effects both *in vitro* and *in vivo*. In addition, the effects appear to be induced by the inhibition of collagen-induced platelet activation related to attenuated GPVI downstream signaling. Further studies to identify and characterize the antimalarial compounds in PJ extract will promote the development of new drugs.

Corresponding author : Youn-Kyoung Goo

Keywords : Herb extract, *Plasmodium falciparum*, *Plasmodium berghei*, Antimalarial effect, Platelet activation

PS-C-003

Regulation of the SARS-CoV-2 pseudovirus infection by using engineered exosomes

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused an epidemic of coronavirus illness 2019 (COVID-19) has been declared a pandemic. Because of the lung infection, the majority of infected patients show symptoms. To enter host cells, SARS-CoV-2 requires the spike, a surface-located trimeric glycoprotein. The receptor-binding domain (RBD) of the S1 site of the spike interacts with the host receptor angiotensin-converting enzyme-2 (ACE2), which is followed by spike cleavage at the S2 region by the transmembrane serine protease 2 (TMPRSS2). Although a clinical trial for COVID-19 using soluble ACE2, TMPRSS2 is underway, our knowledge of how ACE2 is delivered via small extracellular vesicles (EVs) is still limited.
We used exosomes that overexpressed ACE2 from several types of cell lines to determine the degree of infection with SARS-CoV-2 pseudovirus. And then, we generated a SARS-CoV-2 pseudovirus to explore the viral entrance. Moreover, we developed a human induced pluripotent stem cell (iPSC)-derived lung organoids model to identify the possibility of protection by using EVs and determine whether lung organoids were damaged by SARS-CoV-2 infection. We investigated SARS-CoV-2 pseudovirus infected lung organoids to identify viral entrance, infection, and protection. SARS-CoV-2 will continue to multiply in humans, mutations will occur, and potentially dangerous variations will arise. The advent of mutant strains poses a higher danger to the containment of the COVID-19 pandemic. For these reasons, we used the Omicron variant of SARS-CoV-2 pseudovirus and showed the efficiency of viral infections by using overexpressed ACE2 EVs in lung organoids.

Corresponding author : Kyung-Rok Yu, Hee Min Yoo

Keywords : SARS-CoV-2, ACE2, Exosome, Lung Organoid, Pseudovirus

PS-C-004

The pneumococcal pep27 mutant infection enhances intracellular uptake and immune mediation, and provides apoptosis resistance via Mcl-1 upregulation in Raw264.7 cells

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Streptococcus pneumoniae is the major causative agent of pneumonia, which is highly associated with high morbidity and mortality in the young and the elderly. Macrophages, especially alveolar macrophages (AM), are the first-line sentinels that play a key role in initiating inflammatory response against invading pathogens through the release of cytokines and chemokines in the respiratory tract. Previously, immunization with *pep27* mutant (*Δpep27*) provided an enhanced protection against infection by *Klebsiella pneumoniae, Staphylococcus aureus*, and *S. pneumoniae* in mice, however, how *Δpep27* mediates the early innate response remains unknown. In this study, we used murine macrophages (Raw264.7 cells) for *Δpep27* infection and the fate of the Raw264.7 cells and *Δpep27* was characterized. The results showed that adhesion, internalization, and intracellular survival rates of *Δpep27* were significantly higher than those of wild-type (WT). However, annexin V staining and cell viability assay revealed that apoptosis of Raw264.7 cells infected with *Δpep27* was significantly attenuated than that infected with the WT. Also, ROS analysis using a DCFDA assay demonstrated lower intracellular ROS production from *Δpep27* infection than the WT infection. In contrast, *Δpep27* infection elicited higher levels of inflammatory mediators, i.e., *iNOS, COX-2*, and TNF-α, and anti-apoptotic *Mcl*-1 expressions than the WT infection. These results suggest that *Δpep27* up-regulates host immune-related factors, however, attenuates apoptosis of Raw264.7 cells via up-regulation of anti-apoptosis than the lethal WT infection.

Corresponding author : Dong Kwon Rhee

Keywords : *Streptococcus pneumoniae*, *Pep27*, Internalization, Apoptosis, Macrophages

PS-C-005

Comparisons of diagnostic methods for identifying *Pasteurella pneumotropica* in experimental mice in Korea

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Pasteurella pneumotropica is a gram-negative coccobacillus that lives on mucous membranes as commensal parasites. It usually causes subclinical infections in mice, but is occasionally pathogenic in immunodeficient mice with respiratory disease. *P. pneumotropica* infect laboratory animals at a high rate worldwide including Korea. However, many recent reports represented the genetic diversity of *P. pneumotropica*. Consequently, the genus of this bacterial species was reclassified as a new genus, *Rodentibacter* (R), further divided into *R. pneumotropicus* and *R. heylii*.

Furthermore, to investigate the genetic and taxonomical diversity of *P. pneumotropica*, we studied 86 strains of *P. pneumotropica* isolated from Korean laboratory animals. We compared and analyzed the results of the currently used diagnostic methods; biochemical test (API 20NE), 16S *rRNA* sequence, and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). Identification based on the molecular method, *R. heylii* (68.6%%, n=59) and *R. penumotropicus* (31.4%, n=27) were diagnosed in Korean laboratory animals.

While comparing the results obtained using them, the MALDI-TOF method was mostly similar to the molecular method (95.3%), and biochemical method (90.7%) in total. The number of bacteria that could not be identified or were incorrectly identified in the API 20NE tests reached 9.3%. In addition, this method could not differentiate between R. *pneumotropicus* and *R. heylii*, and all of them were identified as *P. pneumotropica*. The number of erroneous or unidentified bacteria in MALDI-TOF MS was 4.7% indicating a lower error rate than that of the biochemical test method.

The common identification methods used currently have some limitations, thus complementing this approach with other methods may provide more accurate results when used for rapid identification testing.

Corresponding author : Young Suk WON

Keywords : Pasteurella, *Rodentibacter*, MALDI-TOF MS, Health monitoring, Laboratory mice

PS-C-006

Galectin-4 enhances the immunostimulatory function of M2 macrophages to upregulate the antiviral CD4$^+$ T cell response and antibody production

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Galectin 4 (Gal-4), a member of the galectin family, is a β galactoside-binding protein involved in modulating cell-cell and cell-matrix interactions. To date, the roles of Gal-4 in physiological processes of the gastrointestinal tract have been well studied, however, its immunological function remain unclear. In this study, we investigated the immunostimulatory role of Gal-4 in M1 and M2 macrophages. Gal-4 treatment drove more robust changes in the gene expression of M2 macrophages compared to M1 macrophages. Of interest, antiviral immune response-related genes were significantly upregulated in M2 macrophages that were treated with Gal-4. Consistently, Gal-4 treatment significantly enhanced the immunostimulatory function of M2 macrophages upon Toll like receptor 7 (TLR7) stimulation or lymphocytic choriomeningitis virus (LCMV) infection. Importantly, the LCMV-specific antibodies and the antiviral CD4$^+$ T cell responses, but not the antiviral CD8$^+$ T cell responses, were significantly increased by M2 macrophages treated with Gal-4 *in vivo*. Furthermore, the administration of Gal-4 into mice that were infected with LCMV significantly upregulated the proportion of germinal center B cells and plasma B cells in the spleen, which subsequently augmented the serum LCMV-specific antibody level. In conclusion, Gal-4 enhances the immunostimulatory function of M2 macrophages, leading to the increased antiviral adaptive immune responses. Therefore, the novel immunomodulatory role of Gal-4 could be applied to a development of therapeutics for diseases caused by the viral infection.

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Keywords : Galectin-4, M2 macrophage, CD4$^+$ T cell, Antibody response, Lymphocitic choriomeningitis virus

PS-C-007

Identification of fecal microbiomes characteristics and transcriptome in blood of growing Jindo dogs

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Microbiome has been associated to various aspects of health, including host immunity. Interest in dogs' health and microbiota has risen recently since more households raise dogs. Jindo dogs, in particular, Korean natives, with an estimated 15,000 are being raised in the country for different purposes. However, the research on microbiome and transcriptome of Jindo dog has not been conducted previously. The goal of this study therefore was to analyze the microbiome of Jindo dogs during their growth and blood transcriptome to understand how to raise healthy Jindo dogs. We focused on the 8-month-old and 9-month-old dogs, whose growth rates increase dramatically. The 16S rRNA sequencing result showed that Actinobacteria, Bacteroidota, Firmicutes, Fusobacteria, and Proteobacteria phylum dominated the microbiome of Jindo dogs. The microbial composition among 8-month-olds and 9-month-olds differed significantly (PCoA, Bray-Curtis dissimilarity), where *Lactobacillus* was more prevalent in 8-month-olds and *Fusobacterium* in 9-month-old (LEfSe analysis, LDA > 4). In addition, we conducted the blood transcriptome analysis of Jindo dog and identified a total of 107 DEGs at FDR < 0.05. At the age of 8 and 9 months, 53 and 54 DEGs were identified, respectively. The functional analysis of these DEGs highlighted immune-related and sexual maturity-related GO terms enriched for 8 and 9-month-old dogs, respectively. For 8-month-old dogs, the most up-regulated gene, *FANCD2* was found to involve in immunological regulation. Then, the top-most up-regulated genes, *ZBTB16* and *RARG* were found to be involved in male maturity, such as spermatogenesis for the 9-month-old dogs. Our future study will be the relationship between Jindo dogs' microbiomes and blood transcriptomes to examine if there are any ways to improve dog health through gut microbial control.

Corresponding author : Woncheoul Park

Keywords : Jindo-dog, Microbiome, Transcriptome

PS-C-008

Identification of age-related compositional changes of intestinal microbiota in piglets

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The composition of intestinal microbiota changes rapidly due to various causes such as stress and dietary changes in the early growth stage of piglets. The correlation between piglet intestinal microbiota and growth performance has been reported in several studies. Therefore, it is important to understand the intestinal microbial changes in the early growth stages of the piglet for enhancing the growth performance of the pigs. This study characterized the fecal microbial shifts of 78 piglets at the early growth stage (7, 14, 21, and 28 days) using the 16s rRNA V3-V4 region. We observed significant drifts in the intestinal microbiota along the growth stages. The alpha diversity showed an overall increasing trend along growth stages. PCoA (Principal Coordinate Analysis) plots showed a clear separation according to the growth stages ($p < 0.05$). The relative abundances of the 8 bacterial families (Lachnospiraceae, Oscillospiraceae, Ruminococcaceae, Christensenellaceae, Succinivibrionaceae, Muribaculaceae, Tannerellaceae, and Campylobacteraceae) significantly increased, whereas the relative abundances of the 7 bacterial families (Bacteroidaceae, Enterobacteriaceae, Streptococcaceae, Enterococcaceae, Lactobacillaceae, Fusobacteriaceae, and Pasteurellaceae) significantly decreased as piglets aged. In addition, we predicted the functions of the intestinal microbiota using the PICRUSt2. The result showed that several KEGG pathways including galactose and methane metabolism were significantly different between the suckling piglets (7 days old) and weaning piglets (28 days old). This study will help to expand the understanding of the microbial ecology in piglets during the early growth stage.

Corresponding author : Jin A Lim

Keywords : Piglets, Microbiome

PS-C-009

Development of mouse lethal models for Japanese encephalitis virus and Dabie bandavirus

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Recently, vector-borne viral infectious diseases are becoming a public health concern worldwide and are monitored as emerging or re-emerging threats. Japanese encephalitis virus (JEV) is an emerging mosquito-borne flavivirus in the Asia-Pacific region that causes Japanese encephalitis with an average mortality rate of 30%. Dabie bandavirus, also known as Severe Fever with Thrombocytopenia Syndrome virus (SFTSV), is a tick-borne phlebovirus that causes severe fever with thrombocytopenia syndrome (SFTS) with an average mortality rate of 12% or up to 30% higher in certain areas or specific genotypes. Developing diverse animal models of JEV or Dabie bandavirus is crucial for better understanding viral pathogenesis and the immune response. In this study, we established a lethal animal model using ICR (CD-1) mice to study pathogenicity of JEV or Dabie bandavirus infection and evaluate the efficacy of therapeutic agents or vaccine candidates *in vivo*. We calculated the 50% of lethal dose (LD50) of JEV or Dabie bandavirus, and the effects of viral infection in vivo were analyzed by histopathological and molecular biological analyses including blood-brain barrier permeability test. We tried to use this mouse models to test therapeutic candidate(s) at concentrations higher than LD50. In future studies, this experimental animal model will be advanced through the comparisions between genetically diverse mouse strains.

Corresponding author : Chi Ho Yu

Keywords : Vector-borne, Viral infectious disease, Lethal mouse model

PS-C-010

Effect of genetic background differences between FVB and C57BL/6 mice in SARS-CoV-2 infection

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In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged, resulting in a worldwide coronavirus disease 2019 (COVID-19) pandemic. This study was conducted to select an animal model for preclinical experiments by comparing the differences between human angiotensin-converting enzyme 2 (hACE2) transgenic mice with different genetic backgrounds. FVB-Tg(K18-hACE2)A [FVB-K18-hACE2] mice and B6.Cg-Tg(K18-hACE2)2 [B6-K18-hACE2] mice were compared susceptibility to SARS-CoV-2 infection of different concentrations ($1x10^2$, $1x10^5$, $5x10^5$ $TCID_{50}$/20ul, intranasal route) at 2 and 7 days post-infection. As infectious dose increases, both mouse groups of different genetic backgrounds exhibited an increase in lethality and loss in body weight. The decrease in activity score occurred in both groups, but mortality tended to be more severe in B6-K18-hACE2 mice. Lung to body weight ratios were higher in B6-K18-hACE2 mice than FVB-K18-hACE2 mice. Viral titer of the lungs elevated over time in both groups, but B6-K18-hACE2 mice exhibited a higher viral titer in the lungs than FVB-K18-hACE2 mice. In histopathology, the lesion scoring of pneumonia, bronchiolitis and perivascular edema in the lungs were higher in B6-K18-hACE2 mice. SARS-CoV nucleocapsid proteins were observed in the lungs, spleens and small intestines of both groups by immunohistochemistry staining. These results indicate that B6-K18-hACE2 mice are relatively more susceptible to SARS-CoV-2 than FVB-K18-hACE2 mice. Based on these results, choosing an appropriate genetic background in mice is essential to the evaluation of vaccines and therapeutics against SARS-CoV-2 infection.

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Keywords : COVID-19, SARS-CoV-2, K18-hACE2, Genetic background

PS-C-011

Comparative analysis of complex IBD model in Il2rg-deficient mouse and C1qa/Rag2 double knockout mouse

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Inflammatory bowel disease(IBD), represented by Crohn's disease and ulcerative colitis, is caused by a combination of environmental factors such as diet and intestinal flora change based on the host's genetic traits. In this study, IBD was induced in two different ways. First, applying a high-fat diet to Il2rg-deficient mouse and C1qa/Rag2 double knockout(KO) mouse, and second, applying Citrobacter rodentium with dextran sodium sulfate to Il2rg-deficient mouse and double-KO mouse. Each mouse was grouped into normal diet group (ND), high-fat diet group (HFD), normal diet with C. rodentium and DSS combination group (NCD), and high-fat diet with C. rodentium and DSS combination group (HCD). The mice of each group fed with high-fat diet or normal diet for 8 weeks ad libitum. After 8 weeks, C. rodentium and DSS were administered via intragastric route to NCD and HCD groups. After each study, histopathological and microbiome analyses were performed to compare the pathological differences between each group. The colon length was significantly shortened in all groups of Il2rg-deficient mouse. However, in double-KO mouse, only HFD group was significantly shortened. Also, severe inflammatory cell infiltration on colonic lamina propria not occurred in double-KO mouse but in Il2rg-deficient mouse. Significant differences were detected in each group of both Il2rg-deficient mouse and doubld-KO mouse in alpha-diversity and beta-diversity analysis. In each group of Il2rg-deficient mouse and double KO mouse, Muribaculaceae, lachnospiraceae, ruminococcaceae, bifidobacteriaceae, lactobacillaceae, clostridiaceae family, and bacteroidiales class were decreased and enterococcaceae, enterobacteriaceae famliy and eryspelatoclostrium and mucispirillum genus were increased in the mucous layer of the colon, compared to each ND group. In conclusion, Il2rg-deficient mouse can be used as a proper animal model for IBD etiology study and therapy than C1qa/Rag2 double-KO mouse.

Corresponding author : Yang-Kyu Choi

Keywords : IBD model, High-fat diet, Citrobacter rodentium, DSS, Il2rg

PS-C-012

Establishment of a Hataan Orthohantavirus infection model in mice

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Hataan orthohantavirus (HTNV) is an enveloped, single stranded negative sense RNA virus species of Old World Orthohantavirus. It causes hemorrhagic fever with renal syndrome (HFRS) with a case fatality rate ranging from < 1 to 15% in human. Hantavax is a vaccine against the Hantavirus, which has been conditionally approved by the Ministry of Food and Drug Safety (MFDS). However, only 50% of volunteers had neutralizing antibodies 1 year following the boost. Effective antiviral treatments against HTNV infection are limited. the development of antiviral strategies for HTNV has been partly hampered by the lack of efficient animal models to evaluate the efficacy of the candidate antiviral drugs. In this study, we presented an asymptomatic animal infection model of HTNV. The animal experiments were performed at the animal bio safety level 3 (ABL3) facility. BALB/c mice were inoculated with HTNV via intramuscular (IM) route. Mice were sacrificed and lung tissues were harvested 1, 3, 5, and 7 days after inoculation. Viral RNAs were analyzed by RT-PCR. Viral RNA began to be detected in lung tissue five days after infection. In this study, we established infection animal models for HTNV. In adult BALB/c mice, intramuscular HTNV inoculation led to asymtomatic infection. These in vivo model is helpful to evaluate the efficacy of therapeutics against HTNV infection.

Corresponding author : Chi Ho Yu

Keywords : Hantaan orthohantavirus, Animal model, Biological agent, Mouse

PS-C-013

Health monitoring system of "K-MEDI hub Preclinical Research Center" for high quality improvement of laboratory animals

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K-MEDI hub (Daegu-Gyeongbuk Medical Innovation Foundation; DGMIF) is established for the purpose of developing cutting-edge drugs and medical technology to affiliated public institution with the ministry of health and welfare. The Preclinical Research Center (PRC) of K-MEDI hub is a specialized research support facility in the field of animal experiments that supports the evaluation to efficacy of new synthetic drugs and performance of medical devices, health monitoring for quality control of laboratory animals etc. PRC of K-MEDI hub joined as a member of the Performance Evaluation Program of the Diagnostic Laboratory (PEP) of the International Council for Laboratory Animal Science (ICLAS) in order to improve the quality of health monitoring technique. The health monitoring report proves that the laboratory animals are of high quality. PRC of K-MEDI hub is conducting serological, molecular biological, bacteriological, parasitological tests for health monitoring and, it supports health monitoring of rodents and non-human primates etc. Services for health monitoring are for various organizations such as industries, academia, research institutes etc., and provide quality management consulting, education for health monitoring. In addition, PRC of K-MEDI hub is research for development of next-generation diagnostic methods and diagnostic kits and working to improve technology for quality control of laboratory animals through alternative health monitoring. PRC of K-MEDI will continue to work to improve the accuracy and reliability of health monitoring results.

Corresponding author : Eui-Suk Jeong

Keywords : Health monitoring, Laboratory animals, Quality control

PS-C-014

Protective effect of *Lactobacillus kunkeei* on Dextran Sodium Sulfate-induced colitis mouse model

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Recently, inflammatory bowel disease has been on the rise cause by western eating habit. However, the exact pathogenesis is not known and there is no suitable treatment. There are many reports that the microbiome in the gut regulates the immune system of our body and is correlated with various diseases. The association between Inflammatory Bowel Disease and the gut microbiome has been continuously reported. Among the microbiota, lactic acid bacteria are well known to have a protective effect on inflammatory bowel disease. In this study, we report that *Lactobacillus kunkeei*, a lactic acid bacteria derived from bees, has a protective role in DSS-induced ulcerative colitis mouse model. *L. kunkeei* was administered per oral every day for 18 days, and on the 7th day of *L. kunkeei* administration, 2% Dextran Sodium Sulfate(DSS) was administered in sterile tap water for 6 days to induce colitis. In the DSS-induced collitis group, *L. kunkeei* administration recovered body weight and clinical disease index compared to the negative control group. According to the *L. kunkeei* administration, colon length was also relieved compared to the negative control group. In histological evaluation, it was observed that the degree of damage to the intestinal epithelium and the degree of infiltration of inflammatory cells were also alleviated compared to the negative control group. In conclusion, *Lactobacillus kunkeei*, derived from bees, has potential as a therapeutic agents for ulcerative colitis.

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Keywords : Inflammatory bowel disease, Dextran sodium sulfate, *Lactobacillus*, Probiotics

PS-C-015

Hydroxy fatty acid produced by gut bacteria protects diet-induced obesity through improving energy expenditure

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Probiotics including *Bifidobacterium* and *Lactobacillus* species are well known for their beneficial roles in metabolic diseases with multiple mechanisms. However, by which specific metabolites from these bacteria play a key role and what the underlying mechanism is not well understood yet. As we found that the bacterial culture supernatant inhibits lipid accumulation in FA-stimulated AML12 hepatocyte but not bacterial lysate, we hypothesized that particular metabolites produced by these bacteria can affect host metabolism. By using mass spectrometry-based metabolic approaching, we identified hydroxy fatty acids as a top-increased metabolites in the bacterial supernatant during the bacterial growth. In high fat-diet (HFD) induced obese mice model, dietary supplementation of Hydroxy palmitic acid (HPA) protected obesity and hepatic steatosis, and accounted by an increased energy expenditure without altering food intake or locomotor activity. Furthermore, HPA supplementation shows beneficial effects in glucose metabolism as it reduced plasma glucose and insulin concentration during intraperitoneal glucose tolerance test in HFD fed mice. By using XF-24 respiration analyzer, we found HPA was not able to undergo to fatty acid oxidation contrary to non-hydroxylated PA, which may likely to trigger adaptive energy expenditure mechanism responded to the disability of mitochondrial beta-oxidation. Taken together, this study implicates that a specific metabolite produced from gut bacteria can protect diet-induced obesity and improves glucose metabolism through improving energy expenditure, and suggests HPA as candidates of therapeutic drugs for improving glucose and lipid metabolism.

Corresponding author : Hui-Young Lee

Keywords : Bacterial metabolites, Hydroxy fatty acid, Obesity, Energy expenditure, Metabolism

PS-C-016

Gut bacteria-derived leucic acid attenuates diet-induced obesity and improves insulin sensitivity

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Leucic acid, which is also referred as 2-hydroxyisocaproic acid, is a branched chain hydroxy acid (BCHA) produced through catabolic pathway of leucine, an essential amino acid, in humans. Also, leucic acid has been reported to be a gut bacterial metabolite and mainly *Lactobacillus* sp. have been reported to synthesis leucic acid. Recently, it has been reported that a fermented food yogurt consumption increased the levels of fermentation-derived BCHAs including leucic acid in plasma and tissues, which were decreased in the diet-induced obesity mice and the metabolic beneficial effects of yogurt could be mediated by BCHAs, especially leucic acid *in vitro* studies. However, it has not been studied whether gut bacteria-derived leucic acid improves diet-induced obesity *in vivo*. Thus, we investigated whether leucic acid improved high-fat diet-induced obesity and glucose intolerance.

Mice fed a HFD supplemented with leucic acid (1g Leucic acid/kg HFD) for 6 weeks showed significantly decreased fat mass gain as well as body weight gain compared with control mice, while there was no difference in food intake. Additionally, we identified increased energy expenditure during calorimetric study and improved insulin sensitivity via glucose tolerance test and glucose-stimulated insulin secretion measurement in leucic acid-fed mice compared to control mice. In conclusion, these results suggest that leucic acid attenuates diet-induced obesity and improves insulin sensitivity and could be considered as a therapeutic target for diet-induced obesity.

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Keywords : Leucic acid, Gut bacterial metabolite, Obesity, Insulin sensitivity

PS-C-017

A bacteria-mediated tryptophan derivative, tryptamine, reduces fat accumulation in diet-induced obese mice

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It has been known that gut microbiome can affect to metabolism of tryptophan, one of essential amino acids, and generate several derivatives in host intestine. However, the effects of tryptophan derivatives produced by gut bacteria on obesity is not well understood. Here, we tested tryptophan and five tryptophan derivatives produced by gut microbiome whether these play a role against obesity by using 2-way routes (short-term intraperitoneal (IP) and long-term oral supplementation (PO)) of administration in diet-induced obesity mouse model. Mice were injected intraperitoneal with tryptophan and tryptophan derivatives daily for 2 weeks after a week of feeding high-fat diet (HFD). Among the five tryptophan derivatives, only tryptamine reduced body weight and fat mass without altering diet intake or energy expenditure during the short-term IP injection. During the long-term PO study for 8 weeks, mice fed a HFD supplemented with tryptamine (1g tryptamine/kg HFD) showed consistent effects in decreased body weight and fat mass similar to those in IP group under pair-feeding condition. Furthermore, *in vitro* study showed that tryptamine treatment in 3T3-L1 decreases lipid accumulation during the differentiation of pre-adipocytes, whereas the other tryptophan or tryptophan derivatives were not. In conclusion, these data demonstrate the anti-obese effects of tryptamine in both *in vitro* and *in vivo*, and suggest a mechanism how gut microbiome can affect host tryptophan metabolism via producing specific derivatives.

Corresponding author : Hui-Young Lee

Keywords : Gut microbiome, Tryptophan, Tryptamine, 3T3-L1, Obesity

PS-C-018

Use of nanobody as a treatment for influenza disease

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Influenza virus, causative pathogen of annual flu disease, contribute to morbidity and mortality of human with respiratory symptoms. The flu disease is caused by an annual mutated influenza virus, and can occasionally be pandemic. In the case of the flu pandemic that occurred in 2009, three types were mixed and it was caused by a recombinant influenza virus, and this virus strain was represented by A/California/07/2009. In the pandemic situation, the number of seriously ill patients increases, and at this time, one of the things that can be considered as a method for rapidly improving the symptoms of patients is the serum of convalescent patients. Because this serum contains a neutralizing antibody, it can play a major role in directly defending against the invasion of virus cells. In the 1990s, it was revealed that camel IgG had a structure different from that of general mammals, and several advantages such as small size and high affinity were also revealed as a result of the study. In addition, it became known through several papers that the nanobody has a greater efficacy than a neutralizing antibody when a virus treatment test through animal testing is carried out. Our research team wanted to make a nanobody treatment for the pandemic 2009 virus, and accordingly, a nanobody for A/California/04/2009(like A/California/07/2009) was produced in alpaca. To confirm the therapeutic efficacy of the produced nanobody, an animal experiment was conducted on mice, and as a result, the perfect protective effect was confirmed against A/California/04/2009 of lethal dose. It is expected that the results of this study will enrich the treatment options for virus diseases.

Corresponding author : Woonsung Na

Keywords : Influenza virus, Nanobody, Therapeutic agent, Mice experiment

PS-C-019

Lactobacillus spp. isolated from Honey bee can ameliorate high fat diet-induced obesity

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In recent years, there have been many reports regarding a correlation between lactic acid bacteria and amelioration of metabolic disease. However, despite the considerable evidence for the beneficial effects on health of probiotics representatively containing *Lactobacillus* spp., their anti-obesity effects have not been well examined. This study is concerned with a pharmaceutical composition capable of preventing or treating obesity, which consists of *Lactobacillus kunkeei* NCHBL-003, *Lactobacillus plantarum* NCHBL-004 isolated from Honey bee. In particular, treatment with *L. kunkeei* NCHBL-003 CFS(cell free supernatant) to 3T3-L1 cells inhibited the differentiation to adipocyte suggesting the effect of suppressing the prevalence of obesity, but not *L. plantarum* NCHBL-004 CFS. We also confirmed anti-obesity effects of *L. kunkeei* NCHBL-003, *L. plantarum* NCHBL-004 in high-fat-diet-induced obesity model for 16 weeks. The oral intake of *L. kunkeei* NCHBL-003, *L. plantarum* NCHBL-004 resulted in decreased weight gain, and ameliorated insulin resistance in both glucose tolerance test and insulin tolerance test. In adipose tissue, gene expression associated with lipid metabolism was improved in *L. kunkeei* NCHBL-003-treated mice. And gene expression concerned with adipogenesis and inflammation was decreased in *L. plantarum* NCHBL-004-treated mice. These findings indicate that *Lactobacillus* isolated from Honey bee mitigates obesity and metabolic disease, including type 2 diabetes. It can be safely used as an anti-obesity agent or as a functional food, without the side effects caused by appetite control.

Corresponding author : Jong-Hwan Park

Keywords : *Lactobacillus*, Honey bee, Obesity, Insulin resistance, Adipogenesis

PS-D-001

The therapeutically useful sleep aid and sedative of Betulinic acid

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A major constituent of Zizyphus seeds that have been long used as thera-peutic agents for sleep-related issues in Asia. Betulinic acid (BA) is a major constituent of Zizyphus seeds that have been long used as thera-peutic agents for sleep-related issues in Asia. BA is a pentacyclic triterpenoid. It also possesses various anti-cancer and anti-inflammatory effects. Current commercially available sleep aids typically use GABAergic regulation, for which many studies are being actively conducted. However, few studies have focused on acetylcholine receptors that regulate wakefulness. In this study, we utilized BA as an antagonist of α3β4 nicotinic acetylcholine receptors (α3β4 nAChRs) known to regulate rapid-eye-movement (REM) sleep and wakefulness. Effects of BA on α3β4 nAChRs were concentration-dependent, reversible, voltage-independent, and non-competitive. Site-directed mutagenesis and molecular-docking studies confirmed the binding of BA at the molecular level and showed that the α3 subunit L257 and the β4 subunit I263 residues affected BA binding. These data demonstrate that BA can bind to a binding site different from the site for the receptor's ligand, acetylcholine (ACh). We utilized BA as an antagonist of α3β4 nicotinic acetylcholine receptors (α3β4 nAChRs) known to regulate rapid-eye-movement (REM) sleep and wakefulness This suggests that BA may be an effective antagonist that is unaffected by large amounts of ACh released during wakefulness and REM sleep. Based on the above experimental results, BA is likely to be a therapeutically useful sleep aid and sedative.

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Keywords : Insomnia, Sleep regulation

PS-D-002

Similarities and differences in immunity between Rag2 knock out mice derived from two different sources

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Rag2 knockout (KO) mice are widely used in various research fields including vaccine development, transplantation studies and hematopoiesis research, but there are little comparison of properties between them. To compare similarities and differences of immunity between Rag2 KO mice derived from different sources, alterations on the weight, histological structure and population of T and B cells in spleen and thymus of C57BL/6-Rag2^{em1hwl}/Korl (Rag2-Kr KO) and B6.Cg-Rag2$^{tm1.1Cgn}$/J (Rag2-J KO) mice. The weight of spleen and thymus was similarly decreased in Rag2-Kr KO and Rag2-J KO mice although other organs were kept at the same weight. A similar alteration was observed in the number of WBC, LYM, NEU and HGB, while a different pattern was detected in HCT and PLT between Rag2-Kr KO and Rag2-J KO mice. The white pulp of spleen was similarly diminished in both mice, and the cortex region of thymus significantly decreased in both mice. The number of CD3$^+$/CD4$^-$ and CD3$^+$/CD4$^+$ cells were remarkably decreased in the spleen of Rag2-Kr KO and Rag2-J KO mice, while the number of CD3$^-$/CD4$^-$, CD3$^-$/CD4$^+$ and CD3$^+$/CD4$^+$ cells were changed in the thymus of the same group. A similar response was observed in CD3$^+$/CD8$^-$ and CD3$^+$/CD8$^+$ cells as well as CD3$^-$/CD4$^-$, CD3$^-$/CD4$^+$ and CD3$^+$/CD4$^+$ cells. But, the increased rate in the number of CD43$^-$/B220$^-$ cells was greater with 3.1 times in the spleen of Rag2-Kr KO than Rag2-J KO mice. Therefore, these results indicate that Rag2-Kr KO than Rag2-J KO mice exhibit similar immunity in the spleen and the thymus except the number of CD43$^-$/B220$^-$ cells.

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Keywords : Rag2, Knockout mice, Immunity, Spleen, Thymus

PS-D-003

Loperamide-induced constipation activates inflammatory signaling pathways in transverse colon of SD rats via Complement C3 and its receptors

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Complement component 3 (C3) receptors play an important role as inflammatory mediator in innate immune system although their mechanism was not well studied during constipation. The aim of this study is to investigate the regulatory role of C3 and its receptors downstream signaling during constipation. Alterations in the C3, C3a receptor (C3aR) and C3b receptor (C3bR) expressions, PI3K/AKT pathway, RhoA/MLC pathway, MAP kinase pathway, and inflammatory cytokine expressions were measured in the transverse colon of loperamide (Lop) treated SD rats. Lop treatment successfully induced constipation phenotypes, including decreased stools parameters and histological structure alterations. Expression levels of C3 were significantly increased, whereas expressions of C3aR and C3bR were observed to decrease during Lop-induced constipation. Moreover, significant upregulation was observed in the phosphorylation levels of PI3K, AKT and GSK3b in transverse colons of Lop treated SD rats. The expression of RhoA and phosphorylation of MLC were also enhanced in the Lop treated group. Furthermore, a similar pattern was detected in the MAP kinase pathway and inflammatory cytokine expressions. Subsequent to Lop treatment, the phosphorylation of ERK and p38, as well as the mRNA levels of NF-kB, TNF-a, IL-6 and IL-1b were remarkably increased in the transverse colon. Therefore, these results indicate that Lop-induced constipation is tightly linked to the downregulation of C3aR and C3bR expressions, and upregulation of the C3, C3Rs downstream signaling pathway including PI3K/AKT, RhoA/MLC, and MAP kinase pathways as well as inflammatory cytokine expressions in the transverse colon of SD rats.

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Keywords : Complement C3, Constipation, C3a/b receptor, PI3K/AKT pathway, Loperamide

PS-D-004

Mig-6 in BAT controls brown adipogenesis and thermogenesis in mice.

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Stimulating the metabolic function of BAT, due to special ability to dissipate energy as heat, represent potential therapeutic strategies for increasing energy expenditure and reducing obesity. Mitogen-inducible gene 6 (Mig-6), a tumor suppressor gene, is a negative regulator of the EGFR signal. Recently, Mig-6 has an important role in the regulation of cholesterol homeostasis and lipid metabolism in the liver. In previous study, we demonstrated the association between EGFR signaling and NAFLD. However, the roles of Mig-6 in BAT remain poorly understood.

We down-regulated the expression of Mig-6, using lentivirus mediated shRNA by transducing immortalized brown adipocytes. We generated BAT specific Mig-6 knock-in (UCP1Cre;ROSA LSL) and knock-out (UCP1Cre;loxP/loxP) models using a genetic strategy. BKI and BKO mice were measure GTT, ITT, biochemical parameters and energy expenditure. Western blot and Q-PCR were performed to analyze related genes.

Here, we showed that the inhibition of Mig-6 declined adipogenesis and thermogenesis in the BAT cell. Mig-6 BKI mice were improved glucose metabolism, lipid levels and fasting glucose. Mig-6 BKO mice were impaired glucose metabolism, increased fasting glucose. We detected a reduction in the size of adipocyte and a relative increase of UCP1 expression by anti-UCP1 IHC in Mig-6 KI BAT. Of note, Mig-6 augmented the expression of thermogenesis relative genes (UCP1, Pgclα, Cieda, PPARα, Elovl3), consistent with the increased UCP1 in the BAT of mice. In addition, the absence of Mig-6 reduced the thermogenesis relative genes.

In conclusion, our findings demonstrate that Mig-6 is as potential factor improving obesity by regulating adipogenesis and thermogenesis in the BAT.

Corresponding author : Bon Jeong Ku

Keywords : Energy homeostasis, Thermogenesis, Mig-6, UCP1, Differentiation

PS-D-005

Ablation of *CrebH* accelerates the progression of inflammatory bowel disease–associated liver injury

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Cyclic adenosine monophosphate (cAMP)-responsive element-binding protein H (CrebH, known as CREB3L3) is a transcription factor expressed mostly in the liver and small intestine. However, CrebH's roles in the pathogenesis of inflammatory bowel disease (IBD) and extraintestinal manifestations remain unknown. We investigated CrebH's roles in these diseases and their underlying mechanisms. IBD and primary sclerosing cholangitis (PSC) disease models were established in wild-type and *CrebH*$^{-/-}$ mice treated with dextran sulfate sodium, dinitrobenzene sulfonic acid, and diethoxycarbonyl dihydrocollidine diet, respectively. *CrebH*$^{-/-}$ mice exhibited accelerated liver injury progression without substantial differences in the gut and higher expression of adhesion molecules, such as MAdCAM-1, in the liver and small intestine than wild-type mice. Exosomes play a pivotal role in IBD-associated liver injury through a different miRNA profile. Furthermore, we identified miR-29a-3p as an effective mediator for MAdCAM-1 regulation. Finally, we found that altered genes in the *CrebH*$^{-/-}$-dextran sodium sulfate vs. wild-type-DSS were highly overlapped with the genes differentially expressed in 3,5-dieythoxycarbonyl-1,4-dihydrocollidine vs control groups. *CrebH* deficiency led to increased susceptibility to IBD-induced liver diseases via enhanced expression of adhesion molecules and concomitant infiltration of T lymphocytes. This study provides novel insights into the role of CrebH in IBD-induced liver injury.

Corresponding author : Jung Hwan Hwang

Keywords : *CrebH*, Exosomes, Inflammatory bowel disease, Liver damage, Primary sclerosing cholangitis

PS-D-006

Novel biomarkers for pre-diabetes and diabetes mouse model

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Diabetes mellitus (DM) is a highly prevalent metabolic disease characterized by uncontrolled elevation of blood glucose levels. Type 2 DM (T2DM) is a major global health issue. The development of T2DM is gradual and is preceded by the pre-DM (pre-DM) stage, which is often undiagnosed. This study aimed to identify novel pre-DM biomarkers in a high-fat diet (HFD)-induced pre-DM mouse model. Male mice were fed with HFD for 12 weeks. Serum and liver were isolated in a time dependent manner. Semi-quantitative assessment of secretory cytokines was performed by cytokine array analysis, and 13 cytokines were selected for further analysis based on changes in expression levels in pre-DM and T2DM stages. HFD-fed mice gained body weight and exhibited higher serum lipids, liver enzymes, glucose, and insulin levels during the progression of pre-DM to T2DM. Gene expression of novel biomarkers was elevated in the pre-DM; these findings were confirmed by measurement of protein levels. Our study identified novel pre-DM biomarkers which may help to delay or prevent the progression of T2DM. This work was supported by an NRF grant funded by the Korea Government (MSIP) [NRF-2021R1A4A1029238] and the Basic Science Research Program through the NRF funded by the Ministry of Education [2020R1I1A1A01069401].

Corresponding author : Seung-Soon Im

Keywords : Diabetes mellitus, Type 2 Diabetes mellitus, Pre-diabetes, Biomarkers, Metabolic disease

PS-D-007

Protective role of *DAX-1* deficiency against acetaminophen-induced liver injury in animal model

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Acetaminophen (APAP) is a commonly used analgesic and antipyretic drug but leads to severe hepatotoxicity in overdose and this is the major cause of acute liver failure globally. The dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1 (DAX-1, NR0B1) is an orphan nuclear receptor acting as a transcriptional co-repressor of various genes. In this study, we identified the role of DAX-1 in APAP-induced liver injury using hepatocyte-specific *DAX-1* knockout (*DAX-1* LKO) mice. In response to APAP overdose, plasma ALT and AST levels were decreased in *DAX-1* LKO mice than in wild-type (WT) controls, accompanied by reduced liver necrosis. Moreover, *in vitro* studies showed that APAP-induced cell death was attenuated in *DAX-1* LKO hepatocytes treated with APAP than in WT hepatocytes. Expression of the genes encoding the enzymes catalyzing GSH synthesis and metabolism (GCLC, GCLM, GSS, GR, and GPx1) was increased in APAP treated *DAX-1* LKO mice. In the mitochondrial fraction of APAP treated *DAX-1* LKO mice liver, the ROS level was decreased with an augmented GSH level. Furthermore, the expression of antioxidant enzymes (NQO1 and GSTA1) was increased in *DAX-1* LKO mice. Most of these increased factors are related to the transcription factor Nrf2, which mediates cellular anti-oxidative response. Intriguingly, while DAX-1 deficiency increased the protein expression of Nrf2 compared to WT controls after APAP administration, there was no significant difference in gene expression. Meanwhile, Keap1, an important factor that can regulate Nrf2, was not affected by DAX-1 deficiency. Taken together, this study demonstrates that DAX-1 deficiency protects against APAP-induced liver injury by affecting the post-translational regulation of Nrf2.

Corresponding author : Jong Soo Lee, Chul Ho Lee

Keywords : Acetaminophen, *DAX-1*, Nrf2

PS-D-008

Anti-inflammatory effect of *DAX1* against ConA-induced acute liver injury in mice

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Fulminant liver failure is defined by the sudden onset of liver dysfunction and immune-mediated inflammation of the hepatic parenchyma resulting in massive hepatocellular damage by recruitment of immune cells into the liver. Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (DAX1, NR0B1) is one of the orphan nuclear receptors and acts as a transcriptional co-repressor of various genes. In this study, we investigated whether DAX1 serves as a critical regulator of inflammatory liver failure induced by concanavalin A (ConA). C57BL/6J (WT) mice, myeloid cell-specific *Dax1* knockout (MKO) mice, and hepatocyte-specific *Dax1* knockout (LKO) mice received single intravenous administration of ConA (15 mg/kg). Histopathological changes of liver and plasma levels of ALT and AST in *Dax1* MKO mice were comparable to WT mice at 9h after ConA injection. Unlike *Dax1* MKO mice, *Dax1* LKO mice were greatly susceptible to ConA induced liver injury which was confirmed by histopathological necrotic changes of hepatic parenchyma, increased plasma ALT and AST levels, and upregulated expression of pro-inflammatory cytokine, TNF-α. Immunohistochemical staining and FACS analysis revealed that the recruitment of $CD4^+$ and $CD8^+$ T cells into the liver was markedly increased in *Dax1* LKO mice. Moreover, augmented gene expression of chemokines including CCL5, CXCL9, CXCL10, and CXCL11 and adhesion molecule, ICAM1, in the liver of *Dax1* LKO mice, indicated a correlation with T-cell chemotaxis. Further studies showed that hepatocyte-specific *Dax1* deficiency dramatically increased the level of NF-κB p65 phosphorylation by enhancing Akt and p38 MAPK activity. Our results demonstrate that deficit of *Dax1* exacerbates ConA-induced acute liver injury by upregulating the NF-κB p65 signaling pathway, which leads to the infiltration of T lymphocytes driven by inflammatory chemokines modulation.

Corresponding author : Chul Ho Lee

Keywords : *DAX1*, Concanavalin A, NF-κB p65

PS-D-009

LRH-1 regulates hepatic triglyceride metabolism via modulation of the expression of Perilipin 5

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Liver receptor homolog-1 (LRH-1) plays a critical role in the regulation of development, cholesterol transport, methyl group homeostasis and steroidogenesis. This study investigated the regulatory function of LRH-1 in lipid metabolism in maintaining a normal liver physiological state during fasting. The Lrh-1f/f and LRH-1 liver-specific knockout (Lrh-1LKO) mice were either fed or fasted for 24 h, and the liver and serum were isolated. During fasting, the Lrh-1LKO mice showed increased accumulation of triglycerides in the liver compared to that in Lrh-1f/f mice. Interestingly, in the Lrh-1LKO liver, decreases in perilipin 5 (PLIN5) expression and genes involved in β-oxidation were observed. In addition, the LRH-1 agonist dilauroylphosphatidylcholine also enhanced PLIN5 expression in human cultured HepG2 cells. These findings directed us to analyze the Plin5 promoter sequence, which revealed -1620/-1614 to be a putative binding site for LRH-1. Additionally, fasted Lrh-1f/f primary hepatocytes showed increased co-localization of PLIN5 in lipid droplets (LDs) compared to that in fasted Lrh-1LKO primary hepatocytes. Overall, these findings suggest that PLIN5 might be a novel target of LRH-1 to mobilize LDs, and manage the cellular needs during fasting. This work was supported by an NRF grant funded by the Korea Government (MSIP) [NRF-2021R1A4A1029238] and the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare, Republic of Korea (HI14C1324).

Corresponding author : Seung-Soon Im

Keywords : Fasting, Liver receptor homolog-1, Perilipin 5

PS-D-010

Insulin resistance improving effects of *Circium japonicum* extract from type 2 diabetic mice model

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Type 2 diabetes (T2D), one of the most common diseases of metabolic syndrome, causes diseases such as obesity, hyperlipidemia, and hyperglycemia. In particular, T2D has a symptom called insulin resistance, which is extended to loss of insulin sensitivity. *Cirsium japonicum* (CJ) is a plant native to Asia and has recently been reported to have various physiological activities against antioxidants, antineoplastic and anti-inflammatory. In this study, we aimed to confirm the effects of CJ extracts on symptoms such as hyperglycemia, hyperinsulinemia, fatty liver, and insulin resistance in T2D mice models. After administering CJ extract (CJE) to db/db mice for six weeks, we proceeded with the evaluation of various biomarkers. In mice administered CJE, the weight of body weight and accessory testicular fat decreased and insulin resistance, lipid profile, and liver and kidney damage improved. CJE also helped normalize HDL-cholesterol reduced by diabetes and confirmed improved insulin resistance by volume. These results were similar to those of mice administered glimepiride, a hypoglycemic agent. These results improved insulin resistance of the T2D-induced mouse model and suggested the possibility of antidiabetic resistance. Therefore, CJ is potentially valuable as functional food material for diabetes.

Corresponding author : Jungkee Kwon

Keywords : Type 2 diabetes, *Cirsium japonicum*, Obesity, Metabolic syndrome

PS-D-011

Thymosin beta 4 regulates NLRP 3 inflammasome through multiple singaling pathways

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Thymosin beta 4 (Tβ4) is a ubiquitous protein that helps heal wounds and plays an essential role in various functions such as tissue regeneration, anti-cellular death, anti-inflammatory, cell proliferation, and differentiation. Risk signals, characteristic of inflammatory diseases, perform functions to activate NLRP3, a congenital immune signal receptor in the cytoplasm. An activated NLRP3 generates an inflammasome assembly and induces secretion of interleukin-1beta (IL-1β) and IL-18, activation of caspase-1, and initiation of an inflammatory process. Therefore, it is essential to inhibit the activation of the NLRP3 inflammasome, which plays an important role in immune response and inflammatory induction. In this study, we investigated the effect of Tβ4 on NLRP3 inflammasome. Tβ4 prevents LPS and ATP-induced NLRP3 priming by suppressing NF-kB, JNK/p38MAPK expression, and LPS- and ATP-induced ROS production. Also, Tβ4 induced autophagy by controlling autophagy markers (LC3A/B and p62) by suppressing the PI3K/AKT/mTOR signaling pathway. In conclusion, Tβ4 attenuated NLRP3 inflammasome by inhibiting NLRP3, ASC, IL-1β, and caspase-1 proteins. Our results show that Tβ4 attenuated the NLRP3 inflammasome through multiple signaling pathways. Based on the above findings, there is a hypothesis that Tβ4 may be a potential inflammatory agent targeting the NLRP3 inflammasome.

Corresponding author : Jungkee Kwon

Keywords : Autophagy, NLRP3 inflammasome, MAPK, Inflammation, Thymosin beta 4

PS-D-012

KLF10 as a tumor suppressor gene and its TGF-B signaling

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Krüppel-like factor 10 (KLF10), originally named TGF-b inducible early gene 1 (TIEG1), is a DNA-binding transcriptional regulator containing a triple C2H2 zinc finger domain. By binding to Sp1 sites on the DNA and interactions with other regulatory transcription factors, KLF10 encourage and suppresses the expression of multiple genes in many cell types. Many studies have investigated its signaling cascade, but those other than the TGF-β/Smad signaling pathway are still not clear. KLF10 plays a role in proliferation, differentiation as well as apoptosis, just like other members of the SP/KLF. Recently, several studies reported that KLF10 KO is associated with defects in cell and organs such as osteopenia, abnormal tendon or cardiac hypertrophy. Since KLF10 was first discovered, several studies have defined its role in cancer as a tumor suppressor. KLF10 demonstrate anti-proliferative effects and induce apoptosis in various carcinoma cells including pancreatic cancer, leukemia, and osteoporosis. Collectively, these data indicate that KLF10 plays a significant role in various biological processes and diseases, but its role in cancer is still unclear. Therefore, this review was conducted to describe and discuss the role and function of KLF10 in diseases, including cancer, with a special accent on its signaling with TGF-b.

Corresponding author : Woon Kyu Lee

Keywords : KLF10, TGF-B, Disease, Cancer, Tumor suppressor

PS-D-013

Establishment of canine cancer organoids using patient-derived cells from hepatocellular carcinomas and mammary gland tumors

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Organoids represent a breakthrough for disease modeling as proven by their ability to recapitulate pathophysiological morphology and functional features of the original tissue. Recently, cancer organoids derived from human cancer have been proposed as a useful model for predicting drug response by reflecting consistent status of actual patients. Cancer organoids will compensate for the limitations of current *in vitro* models to recapitulate key features of Cancer.

We established cancer organoids from primary cells dissociated from dog patients with spontaneously occurred liver tumors and mammary gland tumors. All specimens were collected with the consent of the owners under the approval of the Ethical Committee of Konkuk University. Morphologic heterogeneity and architecture of organoids were assessed with haematoxylin and eosin-stained slides. We also confirmed expression of cellular proteins of hepatocellular carcinoma (HCC), combined hepatocellular-cholangiocarcinoma (CHC) and mammary gland tumor (MGT). Histological characteristics of organoids and primary tumors were consistent.

We evaluated the formation efficiency of organoids and compared the efficiency between benign and malignant tumors. Moreover, the expression of proliferation and epithelial-mesenchymal transition (EMT) markers demonstrated different levels between benign and malignant tumors. Organoids generated from a patient with both liver and kidney cancers showed enhanced EMT characteristics.

In addition, organoids showed varying levels of stem cell markers (CD133, CD44, CD24 and ALDH1A1) associated with cancer progression.

Our results suggest that canine cancer organoids, as in humans, are good materials for understanding characteristics focusing on disease modeling and developing therapeutic approaches.

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Corresponding author : Kyong-Ah Yoon

Keywords : Organoid, Canine cancer, Mammary gland tumor, Hepatocellular carcinoma, Cholangiocarcinoma

PS-D-014

Antioxidation and anti-inflammatory effects of gamma-irradiated silk sericin and fibroin in H2O2-induced HaCaT Cell

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Oxidative stress in skin cells can induce the formation of active oxygen species (ROS), which is important for pathogenic processes such as immunosuppression, inflammation, and skin aging. In this study, we confirmed the improvement of gamma-irradiated silk sericin (I-sericin) and fibroin (I-Fibroin) in the human keratinocyte cell line HaCaT damaged by oxidative stress. First, we confirmed that sericin and fibroin had no toxic effects on cells and could be used safely. Especially in H2O2-induced cell treatments gamma-irradiated, and non-gamma-irradiated are simultaneously performed, I-sericin and I-fibroin had more excellent cytotoxicity protection at the same concentration (100ug/mL). This was a similar result for fibroin, which helped prevent cytotoxicity. Analysis of the indicators on oxidative stress showed that I-sericin and I-fibroin are useful for inhibiting ROS production and SOD activity. Furthermore, when the inflammatory markers induced by oxidative stress were identified, gamma-ray irradiated I-sericin and I-fibroin influenced inflammatory response reduction. The I-sericin and I-fibroin effects were balanced by competition with skin regenerative protein factors. I-Sericin and I-Fibroin were more effective for expressing skin regeneration-related proteins than non-irradiated sericin and fibroin. These results indicated that gamma-irradiation promoted skin regeneration. Overall, compared with uninvestigated sericin and fibroin, I-sericin and I-fibroin have antioxidant and anti-inflammatory activity and protective effects against skin cell damage caused by oxidative stress. Our results demonstrated the potential of gamma-irradiation for development of beneficial cosmetics to enhance skin health to prevent skin aging.

Corresponding author : Jungkee Kwon

Keywords : Gamma-irradiation, Silk sericin, Silk fibroin, Skin regeneration

PS-D-015

Effect of protecting phosphatidylcholine against liver and kidney cell damage by advanced glycation end products (AGEs)

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Activation of Receptorof advanced glycation endproducts (RAGE) induces cell death and various pathological conditions, leading to diabetes complications. At this time, advanced glycation endproducts (AGEs) play a vital role in the pathogenesis of diabetes complications. The accumulation of AGEs in the blood vessel wall causes chronic glucose toxicity, causing various diabetes complications, and contributes to ROS and cytokine production. Recently, AGEs and RAGE reported that interactions have increased in diabetic Mellitus. These interactions increase oxidative stress and activate inflammatory cytokines by activating various intracellular signaling pathways such as nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK). Phosphatidylcholine (PC) is a polyunsaturated fatty acid found in egg yolks, mustard, and beans. The PC is known to mainly affect anti-inflammatory properties, acute renal failure, and attenuation of liver dysfunction. Therefore, this study aims to evaluate the protective effect of PCs against AGE liver and kidney injury by evaluating RAGE expression regulation related to NF-kB/MAPK signal pathway suppression. There is also human kidney (HK2) cells. As a result, the PC consistently reduced RAGE expression with weakened levels of inflammatory cytokines and NF-kB/MAPK signaling. In addition, PC-treated cells showed a significant reduction in cytotoxicity levels and a reduction in oxidative stress and inflammatory factors. These findings suggest that the PC can be an effective functional material for liver and kidney injury related to oxidative stress caused by AGE among diabetic conditions.

Corresponding author : Jungkee Kwon

Keywords : Diabetes, HepG2 cell, HK2 cell, Phosphatidylcholine, Receptor of advanced glycation end products

PS-D-016

Effects of particulate matter (PM) on the Tau-BiFC transgenic mouse

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As a part of air pollution, particulate matter (PM) exposure has been recognized as a critical issue and potential risk factor for health. Several epidemiological studies suggest that PM could lead to a direct penetration to the brain and increase risk for neurodegeneration. In this study, we evaluated the effects of particulate matter on the brain by direct inhalant-based exposure system to our tau transgenic mouse named, TauP301L-BiFC. The TauP301L-BiFC system is a bimolecular fluorescence Turn-ON sensor that indicates neuronal degeneration associated with tauopathies that induce Alzheimer's disease. We exposed the mouse three-week, 8-hour daily and behavioral tests were performed when the exposure is finished. There was significant change in PM exposed group. PM exposed group showed higher anxiety and impairment of recognition function, comparing control group. Moreover, brain tissue in hippocampus and cortex showed that PM exposure lead to tau hyperphosphorylation and inflammation in astrocytes by increased intensity in BiFC, AT8 and GFAP. Our mRNA sequencing results further indicated that the mostly affected cell type within the brain were epithelial cells, which are known to form the basis of blood vessel and secondly affected cells were astrocytes. These results suggest that PM penetrates the brain via blood vessels and subsequently activates astrocytes, which in turn may lead to tau pathology activation.
Our data showed that PM exposure caused anxiety-like behavioral and impaired recognition functions. Increased BiFC, AT8, and GFAP intensity in brain tissue and mRNA sequencing result assure that PM exposure were closely related with tauopathy.

Corresponding author : Yun Kyung Kim

Keywords : Particulate Matter, Tau transgenic mouse, Tauopathy

PS-D-017

Comparison of phenotype expression between Leprdb/Korl and Leprdb/J mice

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Recently, the utilization of disease-model animals escalated due to human-like disease symptoms in pharmacological research. The most of disease-model animals are imported from foreign. However, the commercial animal models imported abroad have difficulty obtaining research results because of the freight forwarding process. The Ministry of Food and Drug Safety (MFDS) developed disease-model animals including Leprdb/Korl mice. The Leprdb/Korl mice induced human-like diabetes using the clustered regularly interspaced short palindromic repeats/cas9 (CRISPER/Cas9) system. In this study, the phenotype of the Leprdb /Korl mice was compared to the Leprdb/J mice used as the commercial animal model. In result, Leprdb/Korl and Leprdb/J mice upregulated the fasted blood glucose (FBG) in the blood for the experimental period. Leprdb/Korl and Leprdb/J mice exhibited glucose intolerance on the glucose tolerance test (GTT). Leprdb/Korl and Leprdb/J mice displayed insulin intolerance on the insulin tolerance test (ITT). In addition, therapeutic effects were investigated by administering metformin on domestic and commercial animal models to confirm the availability of domestic animal models in metabolic diseases research. Metformin was being widely used as a positive control group in the metabolic research. Metformin downregulated FBG in the blood of the Leprdb/Korl and Leprdb/J mice. Metformin reduced hemoglobin A1c (HbA1c) in the blood of the Leprdb/Korl and Leprdb/J mice. Metformin decreased insulin level in the serum of the Leprdb/Korl and Leprdb/J mice. Overall, the present study indicated that the phenotype of Leprdb/Korl mice has similar to Leprdb/J mice.

Corresponding author : Jong Kwon Lee

Keywords : Disease-model animal, Leptin receptor, db/db mice, Diabetes

PS-D-018

Establishment and characterization of six canine hepatocellular carcinoma cell lines

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Hepatocellular carcinoma(HCC) is the most common primary hepatic tumor in dogs, however, the cause of naturally occurring canine hepatic neoplasms is unknown. Canine HCCs are morphologically classified into massive, nodular and diffuse types. The prognosis for dogs with massive HCC is good after surgical resection, whereas nodular and diffuse HCC is poor, and there is no effective therapy.

We established novel canine HCC cell lines from six primary tumor tissue and one metastatic kidney tumor that were resected from canine patients. Two of which were nodular types, and the others were massive types. One patient was histopathologically diagnosed with mixed hepatocellular and cholangiocellular carcinoma(HCC-CC). All specimens were collected with the consent of the owners under the approval of the Ethical Committee of Konkuk University. Protein expression of alpha-fetoprotein(AFP), an onco-fetal protein known to be increased in serum and tumor tissue of dogs with HCC, and EpCAM as a carcinoma marker were evaluated to confirm HCC origin of the cell lines. We also evaluated the general growth characteristics of cell lines by cell doubling time, proliferation rate, and tumorigenicity by colony formation assay, which would be further verified in a mouse xenograft model. The migratory ability of cell lines was evaluated by wound healing assay and compared with their EMT characteristics. Furthermore, we tested drug sensitivity to several anticancer drugs in cell lines to evaluate their applicability to canine HCC. Toceranib, a multitargeting receptor tyrosine kinase inhibitor approved for canine mast cell tumors nowadays showed an excellent effect in inducing apoptosis. Following RTK-ERK pathway inhibition and cell apoptosis were evaluated in protein level.

We established six HCC cell lines from canine patients with liver tumors. These cell lines would be valuable research materials to understand molecular characteristics and develop therapeutic strategies for canine HCC patients.

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Corresponding author : Kyong-Ah Yoon

Keywords : Canine cancer cell line, Canine cancer, Hepatocellular carcinoma, Hepatocholangiocarcinoma

PS-D-019

Cytosolic $Ca2^{+}$ levels signal by Per2 down regulation is related to amphetamine-like drugs induced reward behavior

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Based on the basic chemical structure of psychoactive drugs, new psychoactive substances (NPS) are produced, leading to abuse. Therefore, the possibility of dependence of NPS such as amphetamine is being studied. methamphetamine induced dopamine release into the synaptic cleft. Dopamine release triggered by influx of Ca2+ The relationship between Ca2+ regulation of Period circadian regulator 2 (PER2) gene and reward behavior is not well known. To compare the drug reward behavior, intracranial self-stimulation (ICSS) induced by amphetamine-like substances (methamphetamine, PMMA) were studied in wild type (WT) and PER2 knockout (PER2KO) mice. We also measured Ca2+ fluorescence evoked by treatment of amphetamine-like substances using PC12 cells. Methamphetamine (2 mg/kg, i.p.) and PMMA (3.2 mg/kg, i.p.)-induced ICSS threshold of PER2KO mice is lower than those of WT mice. Ca2+ fluorescence induced by methamphetamine (10 μM) and PMMA(10 μM) in PER2 knockdown (PER2KD) PC12 cells was increased in comparison with naive PC12 cells. These results showed that amphetamine-like drugs induced reward behavior is associated with cytosolic Ca2+ levels regulated by PER2.

Corresponding author : Jaesuk Yun

Keywords : New psychoactive substances, Amphetamine, ICSS, Calcium, Reward behavior

PS-D-020

Establishment and evaluation of a novel mouse model of Fabry disease

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Fabry disease is a rare genetic disease that X-linked lysosomal storage disease caused by deficient activity of α-galactosidase A (α-GLA). α-galactosidase A is a glycoside hydrolase enzyme that hydrolyses the terminal alpha-galactosyl moieties from glycolipids and glycoproteins. α-GAL catalyzes the removal of the terminal α-galactose from oligosaccharides. Defects in human α-GAL result in lysosomal storage disease (LSD), a rare lysosomal storage disorder and sphingolipidosis that results from a failure to catabolize α-D-galactosyl glycolipid moieties. Complications for this disease can be life-threatening and may include progressive kidney damage, heart attack, and stroke. Although the Gla-/- mice used as a lysosomal storage disease model seem to have a normal, complication-free life span. Recently, overexpression of thrombospondin-1 (TSP-1) secondary to Gb3 accumulation is primarily for the observed LSD-vascular endothelial cells (VECs) dysfunction. Therefore, we tried to product the mouse model by producing double transgenic (DB, Gla-/- and human TSP-1 overexpression) mouse and evaluated the Fabry disease. DB mice showed Gla levels and high TSP-1 level. In Gla-/- and DB mice, Left ventricular posterior wall was thicker and cardiac systolic ability was lower than normal aged mice. Interestingly, DB mice increased inflammation and fibrosis markers. These data suggest that DB mice is suitable for studying the pathogenesis of Fabry disease and for preclinical studies of candidate therapies. This research was supported by the Korean Fund for Regenerative Medicine (KFRM) grant funded by the Korea government (the Ministry Science and ICT, the Ministry of Health & Welfare). (KFRM 21C0726L1).

Corresponding author : Yong-Mahn Han

Keywords : Fabry disease, Double transgenic mouse, A-galactosidase, Thrombospondin-1, Glogotrioasylceramide accumulation

PS-D-021

Breeding and characterization of Spinocerebellar Ataxia type 1 model mice

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The Spinocerebellar Ataxia type 1(SCA1) is an autosomal dominant neurodegenerative rare disease with gait impairment, dysarthria, and swallowing disorders. SCA1 is caused by an increase in the number of glutamines in the ataxin1 protein due to the expanded CAG repeats of the *ATXN1* gene, so it is classified as polyglutamine diseases. Despite the fact that glutamine expanded ataxin1 aggregates in purkinje cell of cerebellum, it is unclear how the mutant protein have specific toxicity to cerebellum. SCA1 knock in mice (B6.129S-Atxn1$^{tm1Hzo/J}$, Atxn1 $^{154Q/2Q}$), having 154 CAG repeats in the endogenous mouse locus, were purchased from The Jackson Laboratory, breeded, and characterized for development of therapeutics for SCA1. The mice were monitored th body weight once a week, behavior test with rotarod once every 10 week, and histological analysis of cerebellum. The Atxn1 $^{154Q/2Q}$ mice showed less body weight than wild mice from 4 weeks old, both in male and female. Both males and females mutant mice showed decreased exercise capacity in rotarod test from 10 weeks old. In histological analysis of cerebellum, decreased ratio of molecular layer per granular layer, and loss of purkinje cell appear at 28 weeks old, but not at 10 weeks old. And tibialis anterior muscle weight was significantly reduced from 10 weeks old.

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Keywords : Neurodegerative rare disase, Spinocerebellar ataxia type 1, Model animal

PS-D-022

Auditory or audiovisual stimulation ameliorates cognitive impairment and neuropathology at different stages of ApoE4 knock-in mice

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Background & Objective: Alzheimer's disease (AD) causes progressive loss of cognition, exacerbated by ApoE4, the greatest genetic risk factor for AD. We hypothesized that auditory or audiovisual stimulation could reduce the progression of cognitive decline and ameliorate the pathology of AD. Thus, the objective of this study was to investigate the effects of auditory or audiovisual stimulations on cognition and neuropathology at different stages of in AD mouse model.
Methods: We Apoetm1.1(APOE*4)Adiuj (ApoE4-KI) mice. Animals were divided into 3 groups : N, no stimulation; A, auditory stimulation; AV, audiovisual stimulations. We performed behavioral test, such as the Morris Water Maze (MWM) test and Y-maze test before and after (7 and 14 days) each sensory stimulation. Mice were sacrificed 14 days after each sensory stimulation. Apoptotic cell death was evaluated in hippocampus sections using the terminal dUTP nick-end labeling (TUNEL) of fragmented nuclei assay and the morphometric measurements were done by an image analyzer system. Amyloid-beta plaques (Aβ) expression was assessed in the hippocampus by quantitative immunohistochemistry.
Results: In the MWM test, 5 months ApoE4-KI mice significantly decreased mean escape latency in the AV group, whereas 12 months ApoE4-KI mice showed a decrease mean escape latency in the A group compared with the N group. In the Y-maze test, mean alternation percentage of 12 months ApoE4-KI mice was significantly increased A and AV groups compared with N group. The number of TUNEL positive-cells in the CA3 region of hippocampus showed a significant decrease in 5 months ApoE4-KI mice after AV stimulation, but not 12 months. Also, only in 12 months ApoE4-KI mice, Aβ expression levels in the hippocampal CA3 region showed significant reductions after A and AV stimulation.
Conclusions: Our results revealed that auditory or audiovisual stimulation improved cognitive impairment and showed significant reduction of neuronal cell death or Aβ levels in the hippocampal region at early and late stage of ApoE4-KI mice. Further studies are needed to know the impact of auditory or audiovisual stimulation on cognition depending on the stages of ApoE4-KI mice.

Corresponding author : Jong-Hee Sohn

Keywords : ApoE4, Alzheimer's disease, Cognitive impairment, Auditory stimulation, Audiovisual stimulation

PS-D-023

Anti-adipogenic effects of citrus flavonoid, Nobiletin in 3T3-L1 cells

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Nobiletin is a citrus flavonoid widely known for its antioxidant, anti-inflammatory, anti-tumor, anti-diabetes, hepatoprotective, and neuroprotective effects. The anti-obesity effect of nobiletin has also been reported in the literature, but studies on brown fat cell differentiation have not been sufficiently conducted. The expression of adipogenesis genes and proteins essential for obesity was also investigated to explain the mechanism of action of the anti-obesity effect of nobiletin, which was found to inhibit lipid accumulation during 3T3-L1 cell differentiation. The expression of peroxisome proliferator-activated receptor-gamma (PPARγ), sterol regulatory element-binding transcription factor 1 (SREBP1), CCAAT/enhancer-binding protein alpha (C/EBPα), and fatty acid synthase (FAS) were shown to be downregulated according to the dose of nobiletin. The expression of peroxisome proliferator-activated receptor-gamma adjuvant activator-1 alpha (PGC-1α), which plays a pivotal role in cell energy metabolism in 3T3-L1 cells, was upregulated, which can be considered the subject of pharmacological intervention in obesity treatment. These results highlight the anti-obesity effect of nobiletin, which may be due to the suppressing of adipogenesis due to the down-regulation of PPARγ, SREBP1, C/EBPα, and FAS and upregulation of PGC-1α, a transcriptional coactivator that regulates the genes involved in energy metabolism. Therefore, we intend to analyze molecular mechanisms for the anti-adipogenic effect and propose nobiletin as a candidate for anti-obesity treatment through the additional *in vivo* study. This work was supported by the "Cooperative Research Program of the Center for Companion Animal Research (Project No. PJ01398402)" of the Rural Development Administration, Republic of Korea.

Corresponding author : Hae-Jeung Lee

Keywords : 3T3-L1 cell, Flavonoid, Nobiletin, Adipogenesis

PS-D-024

Production of transgenic piglets for BRAF mutation-based melanoma model using Jeju native pig fetal fibroblast

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Pigs are suited to biomedical research, sharing many similarities with humans, including body size, physiology, and pathophysiology, and there are several species, such as Yucatan, Hanford, and Sinclair. Jeju native pigs are also considered suitable the animal models for the disease models because of their relatively small body size. Meanwhile, melanoma is the malignancy cancer of the pigment-producing melanocytes in the skin. For more advanced therapeutics in melanoma, proper *in vivo* models are required to understand cancer cell-intrinsic factors and complicated intercellular molecular mechanisms. Here, we established the porcine melanoma model using a Jeju native pig fetal fibroblast (JNPFF) inserted with the CreER^{T2} inducible system to control the melanoma-inducing BRAF^{V600E}. First, we produced JNPFF inserted with an induction system for use as donor cells. Then, after conducting somatic cell nuclear transfer (SCNT), we evaluated common embryonic development and established pig embryonic stem cells (pESCs), which were performed the whole seeding using SCNT blastocysts. The generated two pESCs lines were characterized by alkaline phosphatase (AP), immunostaining, and gDNA PCR. Further, transgenic embryos were transplanted into surrogate mothers. As a result, four transgenic piglets were born on Day 115 after embryo transfer. Analysis of umbilical cord gDNA showed that all piglets were transfected. In addition, EGFP expression was confirmed in all cell lines established by the primary culture of umbilical cord tissue and detected in the hoof of piglets. Interestingly, in number two piglet (P#2), unintended tumors were generated on the head and back before induction, and H&E analysis confirmed that they were cutaneous melanocytoma. In conclusion, for the first time, the present study showed that BRAF mutation-based melanoma pig model was developed using JNPFF. Further studies are needed to induce oncogene expression by *in vitro* and *in vivo* editing through the Cre recombination.

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Corresponding author : Sang-Hwan Hyun

Keywords : Somatic cell nuclear transfer, Transgenic cancer model, Melanoma, Jeju native pig fetal fibroblast, Cre-LoxP system

PS-D-025

Assessment of OKT-3 induced cytokine release syndrome in hPBMC-humanized mouse model

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The humanized mouse models with engraftment of human peripheral blood mononuclear cells (hPBMCs) have been considered as a useful tool for human immunological studies. This model with rapid and efficient T cell engraftment enables to investigate the cytokine release syndrome (CRS) induced by some biotherapeutics. It has been reported that immunodeficient mouse models transplanted with hPBMCs can detect CRS stimulated by monoclonal antibodies. In this study, we aimed to evaluate a murine anti-CD3 antibody (OKT3) induced CRS in the hPBMC-engrafted humanized (hPBMC-NSG) mice. The mice were administered with $2x10^6$ viable hPBMCs following 25mg/kg busulfan treatment. To stimulate CRS, 0.5 or 2 mg/kg of OKT3 was injected intravenously into the hPBMC-NSG mice. The mice were euthanized for sample collection at 4 hours post-OKT3 administration. Organ weight and frequency of engrafted rate of immune cells by flow cytometry were determined. The levels of serum cytokines were examined by multiplex ELISA. As a result, the cell population of leukocytes was 24% and the total T-cell from the leukocytes was 84% in the blood of the hPBMC-NSG mice at 3 weeks post-PBMC transplantation. Although there was no significant difference in the organ weights of the brain, heart, kidney and liver, the spleen weight was decreased in the mice administered with OKT3. Of 12 cytokines, levels of GM-CSF, IFN-γ, IL-1b, IL-2, IL-4, IL-8, TNF-α, and VEGF were higher in the mice treated with OKT3 than those in the control mice. In conclusion, these results indicate that hPBMC-engrafted humanized mouse models enable the assessment of CRS induced by OKT3.

Corresponding author : Kyoung-Sik Moon

Keywords : Human peripheral blood cells (hPBMCs), Immunodeficient mouse, OKT3, Cytokine release syndrome (CRS)

PS-D-026

Adipocyte PHLPP2 inhibition prevents obesity-induced fatty liver

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Increased adiposity confers risk for systemic insulin resistance and type 2 diabetes (T2D), but mechanisms underlying this pathogenic inter-organ crosstalk are incompletely understood. We found PHLPP2 (PH domain and leucine rich repeat protein phosphatase 2), recently identified as the Akt Ser473 phosphatase, to be increased in adipocytes from obese mice. To identify the functional consequence of increased adipocyte PHLPP2 in obese mice, we generated adipocyte-specific PHLPP2 knockout (*A-PHLPP2*) mice. *A-PHLPP2* mice show normal adiposity and glucose metabolism when fed a normal chow diet, but reduced adiposity and improved whole-body glucose tolerance as compared to Cre-controls with high-fat diet (HFD) feeding. Notably, HFD-fed *A-PHLPP2* mice show increased HSL phosphorylation, leading to increased lipolysis *in vitro* and *in vivo*. Mobilized adipocyte fatty acids are oxidized, leading to increased peroxisome proliferator-activated receptor alpha (PPARα)-dependent adiponectin secretion, which in turn increases hepatic fatty acid oxidation to ameliorate obesity-induced fatty liver. Consistently, adipose PHLPP2 expression is negatively correlated with serum adiponectin levels in obese humans. Overall, these data implicate an adipocyte PHLPP2-HSL-PPARα signaling axis to regulate systemic glucose and lipid homeostasis, and suggest that excess adipocyte PHLPP2 explains decreased adiponectin secretion and downstream metabolic consequence in obesity.

Corresponding author : KyeongJin Kim

Keywords : Adipocyte, PHLPP2, Obesity, Fatty liver

PS-D-027

A mouse model with genetic defect of mitochondrial complex I to study neurodegeneration

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Mitochondria is essential for the cellular respiration and energy metabolism for human cells. The activity of mitochondria is gradually decreased during aging. Several neurodegenerations including Parkinson's disease and Alzheimer's disease has been is implicated with the deficiency of mitochondrial function. In Parkinson's disease, the second most common neurodegeneration characterized by death of dopaminergic neurons in Substantia nigra (SN) and movement impairments, mitochondrial complex I dysfunction has been a major pathological hypothesis. In Alzheimer's disease, mitochondrial defect may contribute on neuronal dysfunction and degeneration by itself or accelerate neuronal stress initiated by other pathological factors including amyloid beta and/or hyperphosphorylated tau. However, pharmacological inhibitors for mitochondrial activity have various side effects which limits the study for the effect of mitochondrial defect on neurodegenerations. In this study, we used mouse models which have genetically decreased mitochondrial complex I activity. These mice showed key behavioral symptoms which is experienced by neurodegeneration patients. These results would facilitate the understanding for the pathogenesis of neuron death in neurodegenerations.

Corresponding author : Won-Seok Choi

Keywords : Mitochondria, Mouse model, Neurodegeneration, Knock-out mouse

PS-D-028

Effect of gunryeong-tang on heart and kidney damage in diabetic mice model

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Diabetes mellitus (DM) is a metabolic disease featuring chronic hyperglycemia. Despite advances in diagnosis and management, the prevalence rate of DM-complications remains high. GRT (Gunryeong-tang) is a formula of traditional oriental herbal herbs for the treatment of acute and chronic nephritis, which has been used to reduce water retention and swelling. However, no study has shown that GRT improves renal and cardiac function. This study was investigated whether GRT alleviated diabetic nephropathy and cardiovascular damage in *db/db* mice. The experiment was conducted for 8 weeks, distributed into four different groups; control group, *db/db* control group, *db/db* treated with Vildagliptin (50 mg/kg/day), and *db/db* processed with GRT (200 mg/kg/day). The GRT group showed significant improvements in plasma creatinine (CRE) and blood urea nitrogen (BUN) levels. In addition, GRT reduced the intensity of the periodic acid schiff (PAS) staining, glomerular dilation, and tubular fibrosis in *db/db* mice. GRT significantly inhibited the expression of renal fibrosis biomarkers collagen I and transforming growth factor beta (TGF-β). Moreover, administration of GRT significantly improved cardiac dysfunction and myocardial hypertrophy induced in *db/db* mice. GRT also suppressed the cardiac inflammatory protein expression such as the nuclear translocation of nuclear factor-κB (NF-κB), tumour necrosis factor-α (TNF-α), and IL-1β in the diabetic heart. Administration of GRT reduced the expression of TGF-β1 and collagen I, which are the target of the molecules downstream of NF-κB/Smads signaling pathway. Furthermore, GRT remarkably improved the expression of factors involved in cardiac apoptosis in the *db/db* model. Taken together, these results indicated that GRT has a protective role against heart failure and renal injury in the *db/db* mice.

Corresponding author : Ho Sub Lee, Dea Gill Kang

Keywords : Gunryeong-tang, Fibrosis, Inflammation, Diabetes cardio-renal syndrome

PS-D-029

Modulation of PI3K/PTEN-mTOR signaling pathway in the antibody class switching in activated B cells

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B lymphocytes undergo germinal center (GC) reactions and plasma cell differentiation to provide a robust humoral immunity against invading pathogens. Although PI3K signaling is essential for the clonal expansion of antigen-experienced GC B cells, how this pathway regulates antibody isotype switching, affinity maturation and generation of plasma cells remains unclear. In this study, we have dissected the impact of PI3K/PTEN-mTOR signaling pathway on antibody responses of B cells using an *ex vivo* cocultivation system mimicking the GC reactions and plasmablast (PB) differentiation. Inhibition of mTOR complexes by rapamycin and torin-2 upregulated immunoglobulin class switching to IgG1 in the B cell cultures while blocking generation of $CD138^+$ PBs. On the other hands, hyperactivation of PI3K pathway by depleting PTEN impaired IgG1 isotype switching but did not affect generation of $CD138^+$ PBs. Intriguingly, torin-2 but not rapamycin partially restored the IgG class switching in PTEN-deficient GC B cells, implying a requirement of down modulation in mTOR activities during antibody responses of GC B cells. Moreover, genetic inactivation of mTORC2 but not that of mTORC1 was sufficient to rescue the impaired class switching in PTEN-deficient GC B cells. In an infection model, mice depleted PTEN in GC B cells exhibited prominently IgM responses lacking class switched antibodies against influenza virus, whereas inactivation of mTORC2 in the PTEN-deficient GC B cells elicited IgG3 antibody responses comparable to control mice. Taken together, our data highlight a fine tuning in the PI3K/PTEN-mTOR signaling pathway to generate an appropriate antibody response against pathogen infection.

Corresponding author : Keunwook Lee

Keywords : Germinal center B cells, Plasma cells, Antibody, PI3K/PTEN-mTOR signaling pathway, Antibody isotype switching

PS-D-030

Augmented antitumor effect of unripe *Rubus coreanus* Miquel combined with oxaliplatin in a humanized PD-1/PD-L1 knockin colorectal cancer mouse model

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Immune checkpoint inhibitors (ICIs) such as anti-programmed death-1 (PD-1) or anti-programmed death-ligand 1 (PD-L1) antibodies have been shown to be extraordinarily effective, with durable responses in patients with colorectal cancer (CRC). However, the current ICIs still have adverse effects, long half-life, and poor permeability, and due to the limited efficacy of ICI monotherapy, ICIs have been administered in combination with Oxaliplatin (Oxa), which has provided synergistic advantages to patients with CRC. Oxa increases the infiltration of T cells in tumors, thus enhancing the response to ICIs. We used a natural product to overcome the vulnerability of ICIs, which are administered intravenously and pose several problems to patients with CRC, and tried a combination therapy with Oxa to enhance the PD-1/PD-L1 blockade anticancer effect. We used a natural product containing a bioactive ingredient that can easily be ingested orally, has better dose adjustment possibilities, and is absorbed fast in the body for the development of novel alternative anti-PD-1/PD-L1-based immunotherapies. Previously, we explored Unripe Black Raspberry (*Rubus coreanus* Miquel extract, RCE), which exerts anticancer properties via PD-1/PD-L1 blockade. In the present study, we evaluated the T cell-mediated antitumor immunity with RCE alone or in combination with Oxa in a co-culture cell model of tumor-infiltrating hPD-1 T cell in coexistence with hPD-L1 MC38 cells and a responsive allograft tumor humanized PD-1 mice. RCE plus Oxa considerably reduced tumor growth in humanized PD-1 allograft of humanized PD-L1-expressing mouse MC38 CRC. Moreover, RCE plus Oxa remarkably increased the infiltration of $CD8^+$ T cells in tumor tissues more than either RCE or Oxa alone, as well as increasingly produced GrB of tumor infiltrating $CD8^+$ T cells in the tumor microenvironment. Our study delineated combination therapy with RCE as a PD-1/PD-L1 blockade and Oxa to improve the response to immune checkpoint blockade therapy in conjunction with standard chemotherapy regimens in CRC.

Corresponding author : Jang-Gi Choi, Hwan-Suck Chung

Keywords : PD-1/PD-L1 inhibitor, Cancer immunotherapy, Humanized PD-1 mice, Rubus coreanus Miquel, Oxaliplatin

PS-D-031

The anti-tumor effects of sotorasib in a patient-derived organoid and xenograft mouse model of KRASG12C pancreatic cancer

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Pancreatic cancer regarded as a highly refractory cancer has a 5-year survival rate of less than 10% because of the lack of early detection markers and targeted therapy, severe drug resistance. Furthermore, the preclinical model are limited due to quantitative limitation of patient's tissue because the majority of patients are unresectable. Despite the fact that KRAS, the most potent target protein, is mutated at the G12 position in approximately 95 % of pancreatic adenocarcinomas (PDAC), it has been thought be structurally impossible to develop inhibitors due to the structural properties. Fortunately, sotorasib, a $KRAS^{G12C}$ inhibitor recently developed by Amgen, demonstrated activity in solid cancer and its efficacy was confirmed in clinical trials. Here we aimed to demonstrate the applicability of sotorasib in pancreatic cancer using patient-derived preclinical model. Organoids were generated from patient tissues harboring G12C or G12D single mutation identified through ddPCR, and drug response was assessed. Sotorasib showed remarkable cell killing effect for $KRAS^{G12C}$ PDO in comparison to KRASG12D PDO (IC50 = 0.098 μM vs. 11.71 μM). Furthermore, only in $KRAS^{G12C}$ PDO was reduced ERK phosphorylation and increased apoptosis in a dose-dependent manner. These results were replicated in the PDO-implanted xenograft PDOX mouse model. The tumor volume was reduced by 1.9 to 3.2 times in the sotorasib treatment group compared to the vehicle group after about 10 days, accompanied by a decrease in Ki-67 expression and an increase in α-SMA expression. Collectively, we were able to reliably assess sotorasib's $KRAS^{G12C}$-specific efficacy using an organoid model derived from pancreatic cancer tissue that reflects the patient's genetic/clinical characteristics. The stable establishment and utilization of organoids as a preclinical model closely resembling patient characteristics, as well as evaluating drug response and prognostic factors based on this, will greatly increase the possibility of realizing precision medicine for pancreatic cancer.

Corresponding author : Yun-Hee Kim

Keywords : Pancreatic adenocarcinoma (PDAC), Patient-derived organoid (PDO), Patient-derived xenograft mouse model (PDX), KRAS, Sotorasib

PS-D-032

The regulatory role of nuclear factor erythroid-2-related factor in autoimmune disease animal model

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The balance of immunity and tolerance is critical in immune homeostasis. This unbalance leads to immune-mediated diseases, such as autoimmune diseases. Interestingly, autoimmune diseases have a high reactive oxygen species (ROS) environment. Therefore, oxidative stress (OS) inducing factors are expected to play an important role in the regulation of autoimmune diseases. We focus on nuclear factor erythroid-2-related factor 2 (Nrf2) since Nrf2 is key one of the OS-responsible factors and regulates CD4 T cell differentiation. Based on this, we hypothesized that Nrf2 is involved in the differentiation and function of Th17 and Treg cells, which are central regulators of autoimmune diseases, and thereby regulating disease severity. First, we found that CD4 T cells were skewed toward Th17 cells in naïve Nrf2KO mice than WT mice, while naïve Nrf2Tg mice had less Th17 cells than WT mice. Contrary to the in vivo results, regulatory role of Nrf2 could not be confirmed in vitro Th17 differentiation. On the other hand, Treg cell differentiation was regulated by Nrf2 expression levels. In functional assay of Treg cells, suppressive function of Nrf2KO Treg cells was more effective than WT and Nrf2Tg Treg cells. Finally, to determine the in vivo role of Nrf2 in the autoimmune environment, we induced experimental autoimmune encephalomyelitis (EAE) and analyzed disease progression and effector T cells. As a result, significantly the progression and severity of EAE disease was reduced in Nrf2Tg mice compared WT and Nrf2KO mice. Moreover, we found that the number and percentage of pathogenic Th17 cells in spinal cord increased in Nrf2KO EAE mice. Collectively, Nrf2 deficiency skews naïve CD4 T cells into Th17 and induces autoimmune inflammation. Through in vitro differentiation experiments, we suggest that Nrf2 specifically regulates the in vivo differentiation and function of Th17 during autoimmune disease. Because we showed that Nrf2 regulates Treg differentiation and the suppressive function, further studies are needed to elucidate in vivo roles of Tregs in Nrf2KO EAE mice. Our data indicate that Nrf2 regulates the differentiation and function of Th17 and Treg cells and could be a potential target for future treatment of autoimmune diseases.

Corresponding author : Changwan Hong

Keywords : Autoimmune disease, Nrf2, Th17, Treg, Oxidative stress

PS-D-033

Preclinical study of NGUL, a novel matched-pair theranostic agent labeled with Cu-64 and Cu-67 targeting prostate cancer

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Prostate cancer is the second most prevalent form of cancer in males. We developed the simplest structure of Glutamate-Urea-Lysine(GUL) derivative, NOTA-GUL(NGUL) for $^{64}Cu/^{67}Cu$ labeling. In this study, we aimed to confirm the specific binding of ^{64}Cu-NGUL to 22Rv1 (PSMA-positive cells) xenograft model in-vivo. 22Rv1 cells were injected 5*10^6 in balb/c nude mouse 5 weeks of age, it was confirmed that the tumor size grew to 100mm^3 after 21 days. As a result of biodistribution study, when ^{64}Cu-NGUL μCi/100 μL was injected, absorption [%ID/g(20)] was high in the order of kidney, tumor, spleen, and bone. As a result of the Positron emission tomography(PET) image study, it was confirmed that the tumor targeting ability of ^{64}Cu-NGUL was high. Both the lysine-treated group and the non-lysine-treated group confirmed tumor targeting ability. The lysine treatment group was able to confirm the renal protective effect at the initial time point 2 hours. The lysine-treated group was confirmed at time points 2 hours and 4 hours, and the protective effect was high in the order of bladder, kidney, and tumor. In the group not treated with lysine, the order of the most renal protective effect was the same, but only after 2 hours. After 4 hours, the kidney uptake was highest in kidney, followed by bladder and tumor. The ratio of kidney uptake was generally higher in the group not treated with lysine. After 24 hours, bladder uptake was not significantly present in both groups. As a result, the kidney protection effect was confirmed in the lysine-treated group, and it was confirmed that the tumor-targeting ability of ^{64}Cu-NGUL was excellent in both groups and at all time points. We successfully synthesized radioisotope ^{64}Cu and PSMA ligand NGUL by one-pot method for prostate cancer imaging. We demonstrated the specific binding of ^{64}Cu-NGUL to 22Rv1 cells xenograft tumor model in mice.

Corresponding author : Yun-Sang Lee

Keywords : PSMA, PET, Prostate Cancer, Theranostic, GUL

PS-D-034

Study on intact retinal vessels and vasculopathies in adult zebrafish eye

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Trypsin digestion is a useful method for separating and analyzing the retinal vasculature in mouse, rat, and animal models. However, the method is technically difficult to perform and has therefore not been reported in zebrafish till date. In this study, we detail the method that can be easily analyzed. Using the trypsin digestion method, retinal vessels were confirmed to have a tight junction structure with anti-ZO-1, and cells constituting the blood vessel in the arterial and capillary areas were identified. In addition, using the antibodies, we identified smooth muscle cells, blood cells, and endothelial cells that make up the retinal vasculature. Finally, using the hyperglycemic model, we observed injury to retinal vessels, loss of tight-junction proteins, and the disappearance of smooth muscle cells. Based on these results, we expect that eye researchers using zebrafish will present a successful and consistent analysis method for retinal vasculature disease.

Corresponding author : Jin Sook Kim, Seung-Hyun Jung

Keywords : Zebrafish, Trypsin digestion, Retinal vasculature, Blood-retinal barrier, Hyperglycemia

PS-D-035

Effects of Glycine max germinated extract and Angelica gigas extract mixture on osteoblast and osteoclast

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Osteoporosis affects about 200 million people worldwide and is a silent disease until a fracture occurs. Glycine max and Angelica gigas are emerging as a natural product for treating osteoporosis, one of the typical symptoms of menopausal women. This study aims to investigate the effects of osteoporosis mitigation of Glycine max germinated extract and Angelica gigas extract mixture(GAM). RAW 264.7 cells have been demonstrated to play an important role, using in vitro studies, on osteoclast formation and function and MC3T3-E1 cells induce osteoblast differentiation. Adding RANKL(50ng/ml) to RAW 264.7 cells induces osteoclast differentiation. RANKL-induced osteoclast differentiation was demonstrated using a tartrate-resistant acid phosphatase (TRACP) assay. Differentiated osteoblast cells adding sodium with vitamin C were demonstrated using alkaline phosphatase(ALP) ELISA kit, Estrogen-like activity analysis, alizarin red s staining, Verified BMP-2, RUNX-2, estrogen receptor, and osteopontin(OPN) with Western blotting, mineralization. In this experiment, the group was carried out by setting the control with only osteocyte differentiation, three groups with treated GAM by concentration and a PC group with only Glycine max extract treated. The significant decrease of osteoclast and increase of osteoblast due to treatment of GAM. Based on the results, the mixture can help mitigate osteoporosis in vitro cell line. This study showed the anti-osteal porosity effect of Glycine max germinated extract and Angelica gigas extract mixture.

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Keywords : Osteoporosis, Osteoblast, Osteoclast, RAW 264.7 cell, MC3T3-E1 cell

PS-D-036

Therapeutic effects of LED fusion of two wavelength bands on Atopic dermatitis of NC/Nga mice

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Atopic dermatitis is a common inflammatory skin disease characterized by chronic and recurrent processes. Although it affects all age groups, more than 90% of patients are known to have developed the disease in childhood and childhood. In the case of blue light, which corresponds to the 400-450 nm region, it is known that there are no side effects even if it is used for atopic dermatitis treatment for a long period of time, unlike the UV area. In this study, 405nm+850nmLED was investigated into NC/Nga mice that induced atopic dermatitis every day for a week, and evaluated the effect of improving atopic dermatitis . The experiment was carried out by separating mice into the normal control (Vehicle), only atopic dermatitis inducing (CON) and 405nm+850nm LED phototherapy groups with atopic dermatitis inducing (LED) using the egg mass method. LED phototherapy was performed for 10 minutes every day for a week. The modified SCORAD evaluation method is a method that evaluates the severity of the skin's lesions without including the extent of the lesions and the degree of the disease. LED light therapy research confirmed the improvement of severity of the skin's lesions and observed the reduction of epidermal tissue thickness caused by dermatitis. The significant decrease of serum IL-1β and transdermal moisture loss and serum IgE concentration due to LED light therapy, Based on the results, LED light therapy can help restore normal skin conditions in mice that cause atopic dermatitis. This study showed the anti-atopic effect of infrared light and blue light. Light in mice with atopic dermatitis led to the simultaneous use of circular LEDs with two wavelengths.

Corresponding author : Jungkee Kwon

Keywords : Atopic dermatitis, Light therapy, LED, Fusion, NC/Nga mouse

PS-D-037

Deciphering transcriptome profiles of whole blood in administration to heroin of cynomolgus monkey

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Heroin, also known as diacetylmorphine and diamorphine, Repeated administration of heroin results in the induction of physical dependence, which is characterized as a behavioral state of compulsive drug seeking and a high rate of relapse even after periods of abstinence. Herein, we examined the alteration effect on gene expression in whole blood transcriptome of heroin addiction cynomolgus monkey. We designed the 4 groups as age (M1;1 years, M2;3-4 years, M3;7-9 years and M4; 11 years) to evaluated the age-dependent differences in blood transcriptome. We identified accumulated differentially expressed genes (DEGs) in each group, which decreased with age. These results different from the pattern in the DEGs accumulation of other drugs, such as methamphetamine and cocaine. The highest number of DEGs of C1 group was associated with a strong heroin cytotoxicity. Furthermore, we identified DEGs that age-specific across the time series analysis. Results from this study would benefit the study of drug addiction at different ages.

Corresponding author : Jae-Won Huh

Keywords : Heroin, Non-human primates, Transcriptome, Drug addiction

PS-D-038

CXCR4 regulates the maintenance of stemness and radio-resistance in chordoma cells

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Chordoma is a rare malignant tumor that occurs in the primitive notochord. It is treated with resection and radiation therapy, but the recurrence rate is very high and metastasis to lung and lymph nodes occur. Expression of CXCR4 in cancer has been reported to promote tumor growth, invasion, angiogenesis, metastasis, therapeutic resistance, and poor prognosis. In a previous study, we compared dedifferentiated-type Chordoma (DTC) cells derived from a chordoma patient with an existing Chordoma cell line, UCH-1, which expressed more CXCR4 on the surface of DTC and had more malignant properties. We investigated the effect on tumor progression, stemness property and radio-resistance caused by differences in CXCR4 expression in Chordoma and identified the mechanism for this. CXCR4 shRNA transfection and CXCR4 antagonist (AMD3100) treatment in DTC showed reduction of stemness and radio-resistance, tumor progression. Conversely, overexpression of CXCR4 in UCH1 increased these properties. *In vivo*, mouse subcutaneously injection of DTC shCXCR4 confirmed that the tumorigenicity decreased compared to the control, and the group treated with ionizing radiation and AMD3100 showed a synergetic antitumor effect. Finally, the expression of CXCR4 is involved in the maintenance of stemness and radio-resistance, tumor progression in Chordoma cells. Based on these results, Treatment targeting CXCR4 in Chordoma is expected to show effective radiation therapy and good prognosis.

Corresponding author : Myung Jin Park

Keywords : Chordoma, CXCR4, Radio-resistance, Stemness

PS-D-039

Endothelial PTP mitigates vascular inflammation via USF1/A20 axis-mediated NF-κB inactivation

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Rationale: The nuclear factor-κB (NF-κB) signaling pathway is critical for the pathogenesis of several vascular diseases. Understanding the modification of NF-κB signaling cascades by kinases and phosphatases is crucial for gaining insight into medicinal targets for vascular inflammatory diseases.
Objective: To identify a novel negative regulator of NF-κB signaling in vascular inflammation, we investigate whether the endothelial cell protein tyrosine phosphatase (ePTP) mitigates vascular inflammation through the inactivation of NF-κB signaling.
Materials and Methods: We used human tissues, human umbilical artery ECs, and a *ePTP*$^{-/-}$ mice model and performed histological analysis, immuno-staining, laser captured microdissection assay, lentiviral infection, siRNA transfection, quantitative real-time PCR, and reverse transcription-PCR, as well as luciferase reporter gene assay and chromatin immunoprecipitation assay.
Results: The shRNA-mediated knockdown of ePTP in ECs up-regulated the expression of cell adhesion molecules (CAMs) and induced NF-κB-mediated gene transcription. Studies using *ePTP* knockout mice demonstrated the protective role of ePTP in inflammatory cytokine-induced vascular inflammation and high-fat high-cholesterol diet-mediated atherogenesis. ePTP increased the transcriptional activity of upstream stimulatory factor 1 (USF1) by dephosphorylation and subsequently inducing the transcription of *A20* and inhibiting NF-κB activity.
Conclusions: These results demonstrate that ePTP inhibits the NF-κB-mediated transcription of inflammatory genes through the USF1-mediated A20 signaling axis; this opens new avenues for the treatment of vascular inflammatory diseases including atherosclerosis and sepsis.

Corresponding author : Jong-Gil Park

Keywords : Vascular inflammation, Endothelial cells, Phosphorylation, Atherosclerosis, Mice

PS-D-040

20 (S)-ginsenoside Rh2 exerts anti-cancer activity through the Axl signaling pathway in colorectal cancer cells

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Colorectal cancer (CRC) is a leading cause of international morbidity and mortality and is the third most deadly and fourth most commonly diagnosed cancer in the world. Thus, effective therapeutic targets and strategies are required. 20 (S)-ginsenoside Rh2 (G-Rh2) is one of the major bioactive ginsenosides from ginseng, which exhibits anticancer effects in cancer. We investigated the antitumor effect and underlying molecular mechanism in CRC cells in vivo. Axl is a receptor tyrosine kinase that plays an important role in the metastatic potential and overall prognosis of many solid cancers. To investigate the functions of Axl in CRC cells, we knocked down Axl in HCT116 cells using lentiviral shRNA. Then, we explored the effect of Axl on CRC cell growth by various experiments. And to verify the role of Axl in CRC cells, we established two stable Axl-overexpressing CRC cell lines. The results revealed that Axl promotes the proliferation, migration, and invasion of CRC cells and that it is the main target of G-Rh2 to inhibit the growth of CRC cells. The antitumor effect of G-Rh2 were examined in tumor-bearing mice. The xenograft model was established by subcutaneously injecting of HCT116 cells into the flanks of nude mice. After 12 days, the mice were divided into three groups, and treated with different doses of G-Rh2 for 3 weeks. The results revealed that G-Rh2 suppresses HCT116 xenograft tumor growth in mice and the G-Rh2 apparently inhibits xenograft tumor growth in vivo by suppressing the Axl signaling pathway with no significant toxicity to mice. Our results indicate that G-Rh2 is a potential therapeutic candidate that should be further tested for use against CRC and other solid tumors.

Corresponding author : Myoung Ok Kim

Keywords : 20(S)-ginsenoside Rh2, Axl, Colorectal cancer, Xenograft

PS-D-041

Monitoring induction of 'cold' to 'hot' tumors by irradiation

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Immunotherapy, represented by immune checkpoint inhibitors (ICIs) therapy, has allowed us to have another solution for cancer treatment. However, ICIs are not effective in 'cold' tumors, characterized by a lack of immune cell infiltration. To increase the effectiveness of immunotherapy, it is necessary to convert immunologically 'cold' tumors into 'hot' tumors. In this study, we monitored immune cell recruitment after irradiation to visualize the radiation-induced conversion of 'cold' tumors into 'hot' tumors using luciferase-expressing splenocytes transplantation. B16F10 and LLC cancer cells were subcutaneously grafted into B6 mice, and harvested to determine the degree of immune cell infiltration through flow cytometry and immunohistochemical staining. Immune cell dynamics were monitored by syngeneic transplantation of splenocytes from transgenic mice expressing luciferase to tumor-bearing mice. B16F10 tumor exhibited fewer CD45+ immune cells and less luminescence signals, characteristics of 'cold' tumors. LLC tumor exhibited more CD45+ immune cells and more luminescence signals, characteristics of 'Hot' tumors. Then, B16F10 tumors were treated with 10 Gy of 6 MeV radiation by LINIAC. Two days after irradiation, splenocytes from luciferase-expressing mouse were transferred to irradiated/or non-irradiated B16F10 tumor bearing mice to track immune cells. After irradiation, tumor volume was decreased in irradiated mice. In addition, 48.2% increase of luminescence signals was observed in the tumor site of an irradiated mouse at 48 hours after splenocytes transplantation. Our immune cell tracking system using splenocytes transplantation using reporter mice successfully demonstrated the induction of immune cell infiltration by irradiation in 'cold' tumors, indicating that this system can be applied to monitoring various immune responses in mouse models.

Corresponding author : Hyewon Youn

Keywords : Tumor, Immune cell infiltration, 'Hot' and 'Cold' tumor, Bioluminescence, Radiation therapy

PS-D-042

Hepatic PTPA ameliorates high-fat diet-induced hepatosteatosis and disruption of glucose homeostasis by the activation of the FGF21

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A precise regulation of kinases and phosphatases is crucial for human metabolic homeostasis. The hepatic protein tyrosine phosphatase A (hPTPA) is localized in nucleus, plasma membrane and involved in various intracellular signaling. However, it remains unknown whether hPTPA directly participates in the regulation of hepatic metabolic diseases. Here, we found that deficiency of hPTPA aggravated glucose homeostasis and hepatosteatosis on mice fed a high-fat (HF) diet. Increased lipid accumulation in hepatocytes of *hPTPA*$^{-/-}$ mice reduced the level of glucose transporter 2 on the plasma membrane of hepatocytes leading to a diminution of glucose uptake. hPTPA prevented hepatosteatosis through the activation of the fibroblast growth factor 21 (FGF21) which is a hormone expressed primarily by the live. Liver-specific hPTPA or systemic FGF21 overexpression in *hPTPA*$^{-/-}$ mice fed an HF diet fully restored the disorder of hepatosteatosis and glucose homeostasis. Finally, liver-specific hPTPA expression ameliorated an HF diet-induced hepatosteatosis and disruption of glucose homeostasis in wild-type mice. Taken together, hPTPA is critical for the regulation of hepatosteatosis and glucose homeostasis through the activation of the FGF21. Our current study provides a novel function of hPTPA in the metabolic disorder, hence, modulating hPTPA may be a potential therapeutic strategy against hepatosteatosis-related diseases.

Corresponding author : Jong-Gil Park

Keywords : Hepatosteatosis, Glucose homeostasis, Protein tyrosine phosphatase, FGF21

PS-D-043

Ovarian cycle control, collection, in vitro maturation and in vitro fertilization for oocytes of common marmoset

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The common marmoset (*Callithrix jacchus*) is most suitable for transgenic research in terms of small body size and reproductive characteristics such as number of litter size, early sexual maturation and short interval between each pregnancy. The development of assisted reproduction technologies such as ovum pick-up (OPU), *in vitro* maturation (IVM), *in vitro* fertilization (IVF), *in vitro* culture and embryo transfer is essential for the transgenic research. In this study, ovarian cycle was monitored by plasma progesterone concentration (P4) and the time of OPU was adjusted using prostaglandin F2 alpha, human follicle stimulating hormone (hFSH) and human chorionic gonadotropin (hCG). Collected oocytes were maturated using modified porcine ovum medium (POM) supplemented with 5% fetal bovine serum, 0.15 IU/mL hFSH, and 10 IU/mL hCG, and were classified according to oocyte stage during maturation. Two healthy nulliparous female marmosets were included in this study. The OPU were performed by laparotomy and direct aspiration from ovaries at 19-20 days after peak day of P4 concentration (19.11 – 41.02 μg/mL). On OPU day, the total number of collected oocytes were 24 (12/animal), and germinal vesicle (GV) metaphase I (MI) and metaphase II (MII) stage were 13 (6-7), 7 (3-4) and 4 (2/animal) oocytes, respectively. The maturation from GV to MI or MII were 9 of 18 (50%) and completed within 24-27 hours. MI or MII oocytes were fertilized with sperm using TYH medium on day of OPU or 1 day after disappear of GV or identifying polar body, and 3 oocytes progressed to pronuclear stage (2PN) within 1 day after IVF. However, all 2PN oocytes didn't progress to 2-cell stage. In conclusion, the adjustment of OPU time based on peak P4 concentration of ovarian cycle was very useful, and modified POM may be suitable for IVM from GV to MI or MII stage in marmoset oocytes.

Corresponding author : Dohyun Lee

Keywords : Common marmoset, Progesterone, In vitro maturation, Ovum pick-up, In vitro fertilization

PS-D-044

MTX loaded nanoparticles (MTX-NPs) ameliorate rheumatoid arthritis by simultaneously upregulation of Treg and Breg

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Background: Rheumatoid arthritis (RA) is a progressive systemic autoimmune disease that is characterized by infiltration of inflammatory cells into the hyperplastic synovial tissue, resulting in subsequent destruction of adjacent articular cartilage and bone. Methotrexate (MTX), the first conventional disease-modifying antirheumatic drug (DMARD), could alleviate articular damage in RA and is implicated in humoral and cellular immune responses. However, MTX has several side effects, so efficient delivery of low-dose MTX is important.
Methods: To investigate the efficacy of MTX-loaded nanoparticles (MTX-NPs) against experimental model of RA, free MTX or MTX-NPs were administered as subcutaneous route to mice with collagen-induced arthritis (CIA) at 3 weeks after CII immunization. The levels of inflammatory factors in tissues were determined by immunohistochemistry, confocal microscopy, real-time PCR, and flow cytometry.
Results: MTX-NPs ameliorated arthritic severity and joint destruction in collagen-induced arthritis (CIA) mice compared to free MTX-treated CIA mice. The levels of inflammatory cytokines, including interleukin (IL)-1β, tumor necrosis factor-α, and vascular endothelial growth factor, were reduced in MTX-NPs-treated mice. Number of CD4 + IL-17 + cells decreased whereas the number of CD4 + CD25 + Foxp3 + cells increased in spleens from MTX- NPs-treated CIA mice compared to MTX-treated CIA mice. The frequency of CD19 + CD25 + Foxp3 + regulatory B cells increased in ex vivo splenocytes from MTX-loaded NPs-treated CIA mice compared to MTX-treated CIA mice.
Conclusion: The results suggest that MTX-loaded NPs have therapeutic potential for RA.

Corresponding author : Heebeom Koo, Mi-La Cho, Sung-Hwan Park

Keywords : Interleukin-17-producing T cells, Methotrexate, Nanoparticles, Regulatory B cells, Rheumatoid arthritis

PS-D-045

Modification of atopic dermatitis animal model using MC903: focusing on shortening of induction period and reduction of adverse effects

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Currently, many researchers have emphasized Thymic stromal lymphopoietin (TSLP) as a key factor in the treatment of atopic dermatitis (AD). Accordingly, the need to confirm the TSLP inhibitory effect of the therapeutic candidate material has emerged. Recently, AD animal models using Vitamin D3 and its analog MC903 were known, but laboratories using BALB/c or C57BL/6 were limited in using the protocol they presented. The most typical problems are hair regrowth due to long induction periods, and significant weight loss by MC903. For these reasons, this study was designed to develop a protocol using MC903 with a shorter time and a lower dose. In this experiment, a totally 15 mice were randomly divided four groups: naïve (n=3) and 2, 3 or 4 nM MC903 treated groups (n=4). Sensitization was performed three times at a concentration of 2 nM from day 1-3. All mice were shaved on day 4. After 2 days of stabilization, challenge was performed at a concentration of 2, 3 or 4 nM. MC903 was topically treated six times on day 7-9 and day 11-13. In our results, AD skin lesions such as erythema, scab and roughness were observed in all MC903 treated groups, among them, the symptoms in the 2 nM treated group did not reach the desired level. The erythema index was significantly increased in all MC903 treatment groups, on the other hand, the melanin index was not changed by topical application of MC903. In addition, water contents in all MC903 groups were decreased significantly compared to that in naïve group. However, mice in 4 nM group showed significant weight loss. In conclusion, these results indicate that 3 nM is as an appropriate concentration which showed a significant change in surface symptoms without showing adverse effects such as weight loss.

Corresponding author : Hyungwoo Kim

Keywords : Atopic dermatitis, MC903, TSLP, Animal model

PS-D-046

MTOR/STAT3 targeting suppresses scleroderma via reciprocal regualtion of TH17 and fibroblast with biguanide compounds

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Scleroderma is an autoimmune disease that causes dermal fibrosis. It occurs when collagen accumulates in tissue as a result of persistent inflammation. Th17 cells and pro-inflammatory cytokines such as IL-1β, IL-6, IL-17, and TNF-α play important roles in the pathogenesis of scleroderma. Metformin is widely used biguanide drug to treat type 2 diabetes. Because metformin has effective immunoregulatory functions, we investigated its therapeutic function in scleroderma. Mice in a model of bleomycin-induced scleroderma were treated with metformin for 2 weeks. Histological assessment demonstrated protective effects of metformin against scleroderma. Metformin decreased the expression of pro-inflammatory factors in dermal tissue and lymphocytes. It also decreased mRNA expression of pro-inflammatory cytokines (IL-1β, IL-6, IL-17, and TNF-α) and fibrosis-inducing molecules both in vivo and in vitro. These results suggest that metformin treatment has anti-inflammatory effects on lymphocytes via the inhibition of IL-17 and cytokines related to Th17 differentiation, such as IL-1β, IL-6, and TNF-α. To investigate how metformin modulates the inflammatory process in skin fibroblasts, we measured mTOR-STAT3 signaling in skin fibroblasts and found that phosphorylated mTOR and phosphorylated STAT3 protein expression were decreased by metformin treatment. These results suggest that metformin has potential to treat scleroderma by inhibiting pro-inflammatory cytokines and anti-inflammatory activity mediated by mTOR-STAT3 signaling.

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Keywords : Metformin, Scleroderma, Inflammation, STAT3, MTOR

PS-D-047

Efficient and specific genome editing of CRISPR/AsCpf1 ribonucleoprotein electroporation with adeno-associated virus infection to produce conditional knockout mice

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Elk-3, a member of Ets family, is sensitive to inflammatory mediators and is down-regulated by bacterial endotoxin in mouse macrophages. Previously, we established CRISPR/AsCpf1 ribonucleoprotein (RNP) electroporation with adeno-associated virus (AAV) infection protocol for genome editing in the mouse embryo. Using this protocol, we successfully introduced loxP sequences into mouse genome to generate *Elk-3* conditional mutant mice in four months with 40% efficiency. In this study, we verified specific targeting of *Elk-3* without off-targets by whole genome sequencing (WGS) and myeloid lineage-specific deletion of *Elk-3* when crossed with *LysM*-Cre mice.

RNP was electroporated into zygotes that were infected with AAV containing a conditional allele of *Elk-3*. Electroporated zygotes were transferred at the two-cell stage to pseudo-pregnant mice to produce live pups. Genomic DNA was isolated from pups whose genotype was confirmed as *Elk-3*$^{f/+}$ and WGS was performed. By crossing over several generations, *Elk-3*$^{f/f}$ was obtained and crossed with *LysM*-Cre mice, resulting in *Elk-3*$^{f/+}$*;LysM*$^{cre/+}$ (F1).

When introns 1 and 2 of the *Elk-3* were targeted by RNP in zygotes that were infected with the AAV, most zygotes (39/40, 97.5%) developed to two-cell embryos, 74.3% (29/39) of which developed into the blastocysts. PCR genotyping showed that 37.9% (11/29) of the blastocysts had loxP sequences in introns 1 and 2. A Cre recombination assay validated that 4 of 11 blastocysts (36.3%) and 2 of 5 live pups (40%) contained two loxP sequences in the same allele. We performed WGS with genomic DNA of one live pup. WGS data validated that there was no off-target indel(s) in the entire genome, and the loxP sequences were integrated into the exact locations. We examined the genotype in various organs of F1 mice. Since macrophages present in all tissues, the activity of *LysM-cre* recombinase was observed in all tissues examined in F1 mice. Collectively, this study provides the evidence that CRISPR;AAV genome-editing system is a safe, efficient, and fast method to generate mutant mice.

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Keywords : AsCpf1/gRNA, Adeno-associated virus, LysM-Cre, Myeloid cell, *Elk-3*

PS-D-048

Plasmid DNA nanoparticles for nonviral oral gene therapy

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Herein, a bile acid-inspired triple padlock oral gene delivery platform is developed, facilitating the protection of the therapeutic gene from gastrointestinal degradation, selective intestinal accumulation through a bile acid-specific transporter, and transportation of pDNA NPs through the enterohepatic recycling system. This nonviral oral gene delivery nanoparticle exhibits excellent gene expression kinetics in *in vitro*, *in vivo*, and *ex vivo* studies. A single oral dose leads to maintaining normoglycemia for up to 7 days in three different diabetes mouse models and 14 days in diabetic monkeys. Also, the optimized dosage form can reduce nonfast blood glucose levels and hemoglobin A1C within a normal range from the last stage diabetes conditions with a reduction of weight gain from changes of food uptake behavior after treatment once weekly for 20 weeks. Taken together, the current findings could improve the current painful treatment experience of diabetics and thus improve their quality of life.

Corresponding author : Yong Kyu Lee

Keywords : Oral delivery, Gene therapy, Diabetes, Glucagon-like peptide 1, Bile acids

PS-D-049

IL-17A promotes *Helicobacter pylori*-induced gastric carcinogenesis via interactions with IL-17RC

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Gastric cancer (GC) is the fifth leading cause of malignancy worldwide, with a major attribution to *Helicobacter pylori*. Interleukin (IL)-17A has been reported to be up-regulated in serum and tumor of GC patients compared to healthy controls. However, the precise mechanisms underlying its involvement in gastric tumorigenesis are yet to be established. Here, we investigated the roles and underlying mechanism of IL-17A in gastric tumorigenesis using *Helicobacter pylori*-induced gastric cancer model and human GC cell lines. pathogenesis of *H. pylori*-induced GC. Deletion of IL-17A in mice suppressed *N*-methyl-*N*-nitrosourea (MNU) and *H. pylori*-induced gastric tumor development accompanied by a decrease in gastric epithelial cell growth, oxidative stress, and expression of gastric epithelial stem cells markers. In human GC cells, recombinant human IL-17A (rhIL-17A) inhibited apoptosis and G1/S phase transition arrest while promoting reactive oxygen species production, sphere formation ability of cancer stem cells (CSC), and expression of stemness-related genes. In addition, rhIL-17A induced expression of IL-17RC, leading to NF-κB activation and increased NADPH oxidase 1 (NOX1) levels. Inhibition of NOX1 with GKT136901 attenuated rhIL-17A-mediated elevation of GC cell growth, ROS generation, and CSC stemness. Clinically, IL-17RC expressions was significantly upregulated in human GC compared with normal gastric tissues. Taken together, these results suggest that IL-17A promotes *H. pylori*-associated gastric carcinogenesis, in part, by stimulating cell growth, oxidative stress, and stemness through regulatory effects on the IL-17RC/NF-κB/NOX1 pathway, supporting its potential as a novel target in human GC therapy.

Corresponding author : Young-Suk Won, Hyo-Jung Kwon

Keywords : Gastric cancer, *Helicobacter pylori*, IL-17A, IL-17RC, NF-κB, NOX1

PS-D-050

Loss of Rab25 reduces tumorigenesis in MMTV-PyMT breast cancer model.

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RAB25, a small GTPase belongs to Rab11 family, regulates vesicle trafficking and membrane recycling of membrane proteins and receptors especially EGFR and β1 integrin. RAB25 has been reported to have dual character in cancer as both tumor suppressor and oncogene depending on tissue type. Loss of Rab25 promotes tumor initiation in colorectal adenocarcinoma and migration in esophageal squamous cell carcinoma. By contrast, RAB25 exhibit oncogenic expression in gastric, bladder and ovarian cancers. The dual character of RAB25 has also reported in breast cancer as luminal type and basal-like. In previous study, over-expression of RAB25 in MDA-MB-231, basal-like human breast cancer cell line, reduced proliferation and metastasis. Conversely, knock-down of RAB25 in MCF-7, luminal type human breast cancer cell line, reduced proliferation and migration. The limitation of RAB25 research in breast cancer is that there were no research using transgenic animal model. Here, we used genetically engineered Rab25 knock-out (KO) mice, crossed with MMTV-PyMT mice. MMTV-PyMT mice spontaneously develop luminal type breast cancer. Rab25 KO mice did not induce developmental impair in mammary gland, but it significantly reduced tumor initiation and metastasis in MMTV-PyMT. At the end point, 24 weeks, number of tumor were observed 2-fold decrease in Rab25 KO with MMTV-PyMT compared to MMTV-PyMT only. In addition, incidence of lung metastasis clearly decreased by almost 9% from 67%. Taken together, we postulated that Rab25 has oncogenic role in breast cancer.

Corresponding author : Ki Taek Nam

Keywords : Rab25, MMTV-PyMT, Breast Cancer, Oncogene

PS-D-051

Discovery of early diagnostic markers of hepatocellular carcinoma based on serum exosomal microRNAs

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Hepatocellular carcinoma (HCC) is one of the most malignant tumors and is the third most common cause of cancer death in the world. Accumulating reports provided various diagnostic markers for detecting the early HCC, but they are still insufficient in clinical fields due to the low sensitivity and specificity. In this study, to overcome variation of human HCC, we introduced the non-alcoholic fatty liver diseases (NAFLD) and HCC mouse models, and then we analyzed the exosomal microRNAs (exo-miRNAs) from mouse and human serum samples. Firstly, the Nanostoring data using mouse and human HCC serum exosomes showed that eight exo-miRNAs were commonly increased in HCC compared with normal mouse or normal healthy (NH) group. Using three different cohorts including human NH (n=30), liver steatosis (n=4), liver cirrhosis (n=13), and HCC (n=98), the eight exo-miRNAs were validated. All exo-miRNAs were significantly up-regulated in HCC compared to NH group. To measure the diagnostic accuracy of identified exo-miRNAs, the Receiver Operating Characteristic (ROC) curve was analyzed for each exo-miRNA, indicating that the six each exo-miRNA had high sensitivity and specificity for discriminating HCC from non-HCC individuals. Notably, two exo-miRNAs can distinguish HCC from other liver diseases such as steatohepatitis and cirrhosis, suggesting that these two exo-miRNAs can be as early HCC diagnostic markers. In conclusion, serum exo-miRNAs can serve as non-invasive biomarkers for detecting early HCC with high accuracy.

Corresponding author : Tae-Su Han

Keywords : HCC, HCC mouse model, Exosomal microRNA, Biomarker

PS-D-052

The inhibited invasion of human glioblastoma cells by copper sulfate with chelators in zebrafish model

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Glioblastoma multiforme (GBM) is one of the most common and aggressive types of brain cancer. Unlike normal brain cells, GBM cells exhibit properties of epithelial-mesenchymal transition (EMT) which is a crucial biological process in embryonic development and cell metastasis with highly invasive behavior. Accumulating scientific evidence shows that copper is involved in a critical role in the progression of a variety of cancer, including brain, breast, and lung cancers. However, excessively increased copper has been shown to be toxic and even deadly to cells in high amounts. D-penicillamine(DPA) and Triethylenetetramine(TETA) are well-known copper chelators of first or second choice in the mainstay of treatment in copper-associated diseases. Following treatment of copper sulfate with DPA, GBM cells showed inhibition of proliferation and suppression of EMT properties including reduced expression level of EMT cell markers (N-cadherin, E-cadherin, and Zeb). In contrast, treating copper sulfate with TETA yielded the opposite effects in glioblastoma. We showed that important genes including TGFbeta, in disorders characterized by an increase in copper levels, suggesting a change of expression level involved in EMT. To analyze the invasion and spread of GBM, we used zebrafish embryo xenografted with glioblastoma cell line U87. The invasion of glioblastoma cells in zebrafish embryos was markedly inhibited by copper with DPA. Our findings suggest that copper with DPA mediated proliferation and EMT by a mechanism involving TGFbeta/Smad signaling in glioblastoma. Therefore, DPA could be used as an adjuvant in glioblastoma with high copper concentration, but not TETA.

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Corresponding author : Jei Ha Lee, Seung-Hyun Jung

Keywords : Glioblastoma, Zebrafish, Copper

PS-D-053

Overexpression of cathepsin S is associated with toll-like receptor 7 in systemin lupus erythematosus

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Systemic lupus erythematosus is a chronic inflammatory disease characterized by the accumulation of autoantibodies in various organs such as the heart, lung, musculoskeletal system, kidney, and skin. Previous studies have already confirmed that cytokines such as TNF-α, IL-17, interferon-α (IFN-α), CCL2, CCL7 and CCL12 are associated with the pathogenesis of lupus and that toll like receptor (TLR7) is overexpressed in systemic lupus erythematosus patients. Cysteine protease cathepsin S is involved in the expression of major histocompatibility complex and antigen presentation and can the immune response. However, the effect of cysteine protease cathepsin S in systemic lupus erythematosus has not been confirmed. Therefore, we induced systemic lupus erythematosus in mice expression cysteine protease cathepsin S and confirmed the effect of cysteine protease cathepsin S in systemic lupus erythematosus. To compare the healthy control group (WT), inflammation was increased in the overexpression of cysteine protease cathepsin S group (TG). To compare the TG group and the lupus induced TG group, it was checked that the expression of cysteine protease cathepsin S was increased by the lupus induction. As a results of staining, the thickness of the skin tissue was ticker. Inflammatory infiltration occurred in skin and kidney. And immune cells and TLR7 expression increased in lupus induced TG group. Therefore, this study demonstrated that cysteine protease cathepsin S exacerbated the pathogenesis of systemic lupus erythematosus.

Corresponding author : Myoung Ok Kim

Keywords : Cysteine protease cathepsin S, Systemic lupus erythematosus, Autoimmune disease, Toll like receptor 7, Transgenic mouse

PS-D-054

Generation of FIX-/- rat as a novel pre-clinical model for severe hemophilia B with anti-FIX inhibitors

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Hemophilia B (HB) is characterized by coagulation factor IX (FIX) deficiency due to genetic mutation of F9. Patients with severe HB, which accounts for 60% of all HB cases, exhibit <1% of FIX in the blood and have frequent spontaneous bleeding episodes especially in joints and muscles which can affect quality of life and lifespan of affected patients. Although recombinant FIX replacement therapy is available, patients require chronic intravenous dosing and repeated administration of recombinant FIX can result in development of anti-FIX inhibitors. Therefore, a novel therapeutic approach is required. To test various therapeutic strategies, FIX-/- mouse was generated, however, these mice failed to exhibit spontaneous bleeding phenotype and there is difficulty in repeated blood collection for chronic analysis due to limited blood volume. Therefore, larger animal model that can reflect phenotypes of severe HB is required. For this, we generated FIX-/- rat using CRISPR/Cas9. Unlike FIX-/- mice, our FIX-/- rats fully mimic severe human HB phenotypes by not only prolongs clot formation time upon injury but also displays spontaneous hemarthrosis, the most common symptom of severe human HB patients. Serum analysis showed complete absence of FIX level along with prolonged activated partial thromboplastin time (APTT). Furthermore, to induce anti-FIX inhibitors in these rats, we repeatedly administered recombinant FIX and identified clinical level of anti-FIX developments in the sera of these rats. Together, our results indicate that novel FIX-/- rat can be a valuable pre-clinical model for severe human HB and warrants testing of various therapeutic candidates.

Corresponding author : Jae Young Lee

Keywords : Transgenic rat, CRISPR/Cas9, Genome editing, Hemophilia B

PS-D-055

In vivo genome editing at the APOC3 locus for long-term correction of hemophilia B

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Hemophilia B (HB) is X-chromosomal recessive inherited disease, caused by clotting factor IX (F9) gene mutations. Due to deficiency in F9, blood does not clot properly in HB patients, and suffer from spontaneous bleeding. Although hepatocyte-directed adeno-associated virus (AAV) mediated F9 gene replacement therapeutic approach has been effective at correcting the disease phenotype in both mouse models and limited clinical experiences, this may not be a long-term cure as loss of episomal transgene expression due to hepatocyte turnover has been reported in both pre-clinical and clinical studies. To overcome this, we designed a dual AAV-mediated genome editing strategy; one AAV carrying donor cassette for F9 transgene integration to human APOC3 (a hepatocyte-specific strong endogenous gene comparable to Albumin) locus by homologous recombination and U6 promoter driven sgRNA targeting human APOC3 intron 1 (AAV-donor-sgRNA), while the other AAV carry hepatocyte-specific promoter driven CjCas9 (AAV-CjCas9). To test this in vivo, we generated hemophilia B rat model with humanized APOC3 intron 1 rat using CRISPR/Cas9. Using this model, we injected AAV-donor-sgRNA and AAV-CjCas9 at postnatal day 10 where high level of hepatocyte proliferation is occurring. When compared to AAV-donor-sgRNA alone control, AAV-donor-sgRNA and AAV-CjCas9 administered animals show stable F9 expression in serum over 16 weeks. Furthermore, dual AAV treated animals show correction of bleeding phenotypes. Altogether, our results indicate that our dual AAV-mediated integration of transgene into human APOC3 locus can lead to efficient and potentially permanent transgene expression in hepatocytes and subsequently therapeutic protein secretion to the bloodstream. This in vivo genome editing platform can be applied to various diseases with lack of systemic protein secretion such as hemophilia and lysosomal storage disorders.

Corresponding author : Dong Woo Song, Jae Young Lee

Keywords : Transgenic rat, Hemophilia B, CRISPR/Cas9, Genome editing, AAV

PS-D-056

DDX53 confers the self-renewal and drug-resistance of ovarian cancer stem-like cells

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Cancer stem-like cells (CSCLs, also called cancer stem cells, CSCs) are a subpopulation of cancer cells whose features are characterized by self-renewal ability, tumorigenesis and drug resistance. In this study, the functional role of DDX53 in the regulation of cancer stemness and drug resistance was investigated in ovarian cancer cells. Spheroid-forming cancer stem cells (SP) were isolated from SKOV3, OVACR3 and SUN840 ovarian cancer cells. Enhanced DDX53 expression was observed in spheroid cells (SKOV3-SP, OVCAR3-SP, SNU840-SP) along with enriched CSCLs properties, showed by the self-renewal ability, increased expression of cancer stemness-related genes (SOX2, Oct4, CD133) compared to adherent cells (SKOV3-AD, OVCAR3-AD, SNU840-AD). Furthermore, SP cells showed higher drug resistance to anti-cancer drugs such as taxol and cisplatin as well as greater cell proliferation, invasion and migration ability. DDX53 overexpression in adherent ovarian cancer cell lines conferred the CSCLs characters including spheroid formation, aldehyde dehydrogenase (ALDH) activity ,anti-cancer drug resistance and tumorigenic potentials.. On the contrary, silencing of DDX53 showed opposite effects in SP-ovarian cancer stem cells in vitro and in vivo.. Furthermore, miR-200b and miR-217 known as DDX53-regulating miRNAs were decreased in spheroid cells (SKOV3-SP, OVCAR3-SP, SNU840-SP) whereas upregulated in adherent cells. Alteration of gene expression using miR-inhibitor or miR-mimics of miR-200b and miR-217 was associated with cancer stemness through the regulation of DDX53 expression level in ovarian cancer cells. These results indicated that the DDX53-miR-200b/miR-271 network has a critical role in the regulation of cancer stemness properties in ovarian cancer cells, thus suggesting that DDX53 may be an important therapeutic target in the anti-cancer drug development for ovarian cancer patients.

Corresponding author : Youngmi Kim

Keywords : Cancer testis antigen, Drug resistance, Ovarian cancer, Cancer stem cell like cell, MicroRNA

PS-D-057

A report on non-clinical study of kidney xenotransplantation from genetically-engineered pigs to monkeys

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Kidney xenotransplantation, the form of transplantation of kidneys from genetically-engineered (GE) pig, has been raised as alternative in response to chronic lack of kidney organ supply. Over recent years, the research of xenotransplantation has advanced from producing multiple GE pigs to overcoming hyperacute xenograft rejection. However, several immunological hurdles are still remained to apply living human patients. Therefore, non-clinical trials using GE pig to monkey model was encouraged before transplanting porcine kidney into human body. Here, we performed kidney xenotransplantation using kidney of *Yucatan* minipig that was knocked out 1,3-Gal epitope, xeno-reactive antigens (GTKO) and knocked in human CD55 and CD39 (described as GTKO/hCD55/hCD39) in *cynomolgus* monkeys. A total of 10 monkeys were replaced their kidneys with the GE porcine kidneys and the health condition was monitored by performing daily clinical observation and sampling blood every week for hematology and serum chemistry. Furthermore, histological analysis, NGS study, and cytokine profiling were performed to compare normal and transplanted kidney and observe their immunological changes. As a result, average time of survival was about 40 days and the longest one was 86 days (Korean record). In addition, C-reactive protein (CRP) was changed at specific time points such as 2nd look operation and necropsy day. IL-6, CCL2, CCL4 and CXCL10 were also changed in different pattern at the time points. In conclusion, our studies were expected to suggest proper pretreatment and surgery process for kidney xenotransplantation to improve the time of survival and offer the possibility of overcoming renal xenograft rejection.

Corresponding author : Jeong Ho Hwang

Keywords : Kidney, Xenotransplantation, Monkey, Genetically-engineered pig

PS-D-058

Prolyl hydroxylation primes CMGC kinases for activation through Tyrosine autophosphorylation

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Activation of DYRK1A and DYRK1B (dual-specificity tyrosine-phosphorylation-regulated kinases 1A and 1B) requires prolyl hydroxylation by prolyl hydroxylase, PHD1. Prolyl hydroxylation of DYRK1 initiates a cascade of signaling events leading to the release of molecular constraints on von Hippel-Lindau (VHL) ubiquitin ligase tumor suppressor function. However, the modification of proline residue of DYRK1 by hydroxylation and the role of prolyl hydroxylation in tyrosine autophosphorylation of DYRK1 are unknown. We found that a highly conserved proline in the CMGC insert of the DYRK1 kinase domain is prolyl hydroxylated by PHD1, and this event precedes tyrosine autophosphorylation. Mutation of the hydroxylation acceptor proline precludes tyrosine autophosphorylation and folding of DYRK1, resulting in a kinase unable to preserve VHL function and lacking glioma suppression activity. The consensus proline sequence is shared by most CMGC kinases, and prolyl hydroxylation is essential for catalytic activation. Thus, formation of prolyl-hydroxylated intermediates is a novel mechanism of kinase maturation and likely a general mechanism of regulation of CMGC kinases in eukaryotes.

Corresponding author : Sang Bae Lee

Keywords : DYRK1, Prolyl hydroxylation, Hypoxia, CMGC kinases, Phosphorylation

PS-D-059

Effect of obesity on anaplastic thyroid cancer progression in immunocompetent orthotopic model

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Westernized dietary habits and sedentary lifestyle are increasing the incidence of obesity. Many studies show that obesity is evidence of several metabolic and cardiovascular disease as well as cacner. Obesity is known to be a risk factor for the onset and progression of thyroid cancer, but the molecular mechanism and changes in the tumor immune microenviroment(TiME) changes are not well known. In this study, we investigated the response to tumor immunotherapy in the growth and progression of thyroid anaplastic cancer in an immunocompetent diet induced obesity mouse model.
5 weeks old female C57BL/6 mice were fed a chow diet or high fat diet for 14 weeks. Then anaplstic thyroid cancer cell line was orthotopic injection into thyroid gland . 3 days after the injection of cancer cell, each dietary group was treated with anti-PD-L1 . Each group maintained their intial diet for 2 weeks.
Threre was no significant difference in tumor growth rate, tumor weight and volume in mice fed an high fat diet compared chow diet. After transplanation of anaplastic thyorid cancer, weight loss was significantly less in the anti-PD-L1 treatment group. In immunocompetent mouse model of thyroid anaplastic cancer, we investigated that high fat diet induced obestiy mice respond better to tumor immunotherapy than chow diet mice.

Corresponding author : Sun Wook Cho, Kyu Eun Lee

Keywords : High fat diet, Obeisty, Anaplastic thyroid cancer

PS-D-060

Creation of mouse models and analysis methods for the investigation of brain disorders

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Brain disorders including brain cancers, Alzheimer's disease, Parkinson's disease and epilepsy are prevalent but it's therapeutics are still undiscovered yet. The cause of brain tumors is not fully understood and the increasing number of patients with neurodegenerative diseases due to an aging population, brain research and figuring out the treatment of brain disorders are considered essential. Here we aimed to build a brain research platform to find out the causes of brain diseases in a variety of ways. We developed a mouse brain tumor model using surgical method and evaluated the effect of new drug candidates using a biological imaging system. In addition, we have established a mouse model for Alzheimer's and Parkinson's disease to study neurodegenerative diseases and confirmed their phenotype by various behavioral tests. At present, we are conducting many research and providing services related to brain diseases with various industries and institutes. K-MEDI hub Preclinical Research Center will continuously develop a new assessment method and more accurate analysis techniques to play a central role in preclinical studies, which are essential for the study of brain diseases.

Corresponding author : Tae-Jun Kwon

Keywords : Brain cancer, Neurodegenerative disease, Alzheimer's disease, Parkinson's disease, Biological imaging system

PS-D-061

Behavioral and neuropathological analysis for Alzheimer's disease preclinical research by the 5xFAD mouse model

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Alzheimer's disease(AD) is the most prevalent neurodegenerative disease that induces cognitive impairment and memory loss. Animal models of AD have been a crucial role in understanding the underlying pathological mechanism. Importantly, mouse models of human disease aid the investigation of therapeutic interventions in the preclinical research. Here we have conducted a characterization of the 5xFAD mice through the comparison of behavioral and neuropathological phenotypes with Wild-type(WT) mice. As a behavioral tool for evaluating cognitive impairment in the old 5xFAD mice, we used the Barnes maze test which assesses spatial memory learning. The old WT and 5xFAD mice impaired spatial memory rather than the young WT mice. And, the old 5xFAD mice showed relatively the delay of spatial memory acquisition compared to the old WT mice. Subsequently, we have analyzed distribution of Aβ plaque formation and astrocytes in the cortical area and hippocampus to confirm the neuropathological phenotype. This characterization of the 5xFAD mouse model indicates that this methodological approach is available as a preclinical research tool of Alzheimer's disease.

Corresponding author : Tae-Jun Kwon

Keywords : Alzheimer's disease, 5XFAD , Barnes maze test, Amyloid beta, Astrocyte

PS-D-062

Metformin ameliorates monosodium-iodoacetate-induced Osteoarthritis via regulation of mitochondria and the autophagy-inflammation- cell death axis

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Osteoarthritis (OA) is the most common degenerative arthritis associated with pain and cartilage destruction in the elderly; it is known to be involved in inflammation as well. A drug called celecoxib is commonly used in patients with osteoarthritis to control pain. Metformin is used to treat type 2 diabetes but also exhibits regulation of the autophagy pathway. The purpose of this study is to investigate whether metformin can treat monosodium iodoacetate (MIA)-induced OA in rats. Metformin was administered orally every day to rats with OA. Paw-withdrawal latency and threshold were used to assess pain severity. Cartilage damage and pain mediators in dorsal root ganglia were evaluated by histological analysis and a scoring system. Relative mRNA expression was measured by real-time PCR. Metformin reduced the progression of experimental OA and showed both antinociceptive properties and cartilage protection. The combined administration of metformin and celecoxib controlled cartilage damage more effectively than metformin alone. In chondrocytes from OA patients, metformin reduced catabolic factor gene expression and inflammatory cell death factor expression, increased LC3IIb, p62, and LAMP1 expression, and induced an autophagy-lysosome fusion phenotype. We investigated if metformin treatment reduces cartilage damage and inflammatory cell death of chondrocytes. The results suggest the potential for the therapeutic use of metformin in OA patients based on its ability to suppress pain and protect cartilage.

Corresponding author : Sung-Hwan Park, Seok Jung Kim, Mi-La Cho

Keywords : Autophagy, Combination therapy, Metformin, Osteoarthritis, Pain

PS-E-001

Balloon angioplasty with elastase for creating wide-necked aneurysm model in rabbit

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Purpose: The reliable and safe models of the elastase-induced aneurysm in rabbits have been proposed for preclinical research; however, the neck size control of aneurysms is still challenging. The purpose was to investigate the technical feasibility and safety of creating a wide-necked aneurysm model using elastase-induced with balloon angioplasty in the rabbit right common carotid artery (RCCA).
Methods and Materials: Fifteen male New Zealand White rabbits were divided into 3 groups as follow: group A received only elastase stimulus, group B received elastase stimulus with balloon angioplasty, group C received balloon angioplasty at 4 weeks after creation of aneurysm by elastase stimulus. All rabbits underwent surgical procedure to create the elastase-induced aneurysm at the RCCA. Balloon angioplasty in groups B and C was performed to induce wide-neck aneurysm. All rabbits were sacrificed 4 weeks after the aneurysmal creation procedure. Size and shape of the created aneurysm and histological changes were analyzed and compared.
Results: The aneurysmal creation procedures were technically successful in 14 (93.3%) of 15 rabbits. One rabbit in group A died during the surgery due to adverse anesthetic response. Saccular aneurysm was created in group A and wide-necked aneurysm was successfully created in groups B and C (all 100%). The neck size, and dome-to-neck ratio were significantly higher in groups B and C than group A (all $p < 0.05$). The thickness of tunica media, vessel area, and luminal area were significantly higher in groups B and A than group C (all $p < 0.001$). The thickness of neointima, and thickness of tunica adventitia were significantly higher in only group B than group A (all $p < 0.05$). However, there was no significant difference among three groups in the inflammatory cell infiltration (all $p > 0.05$).
Conclusion: Creation of the wide-necked aneurysm model using elastase-induced with balloon angioplasty was technically feasible and safe in the rabbit RCCA. Balloon angioplasty after elastase stimulus effectively established a wide-necked aneurysm as a potential model for reproducing the mechanisms of the aneurysm.

Corresponding author : Deok Hee Lee

Keywords : Aneurysm, Elastase, Wide-neck aneurysm, Balloon angioplasty, Aneurysm model

PS-E-002

Novel stent-based electrode for radiofrequency ablation in the rat esophagus: a preliminary study

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Purpose: Endoluminal radiofrequency (RF) electrodes have been developed for the management of inoperable biliopancreatic ductal cancers and Barrett's esophagus but, the formation of a uniform ablation zone is still lacking. The purpose was to investigate technical feasibility and efficacy of RF ablation with use of a novel monopolar stent-based electrode (SE) in the rat esophagus.
Methods and Materials: RF protocol was determined to the exposed rat esophagus reached at 70 °C at 30, 40, and 50 W, respectively. Eighteen of 21 male Sprague-Dawley rats were divided into three groups and received stent-based RF ablation at 40 W, and the remaining three rats received a sham procedure. Histological changes including the thickness of submucosal fibrosis and epithelial layer, degree of inflammatory cell infiltration, and collagen deposition were analyzed and compared with sham control at immediately (n = 6), 1 week (n = 6), and 2 weeks (n = 6). Additionally, TUNEL and HSP70-positive deposition were investigated.
Results: The optimal RF protocol was at 40 W and 480 kHz for 60 sec. The stent-based RF ablation was successful in 16 (88.8%) of the 18 rats. Two rats died of dyspnea due to thicker delivery system and were excluded in this study. The degrees of RF-induced fibrotic changes and inflammatory cell infiltration in the RF-ablated rat esophagus were significantly and gradually increased compared with the sham control at 1 and 2 weeks (all $p < 0.05$). The thickness of epithelial layer was significantly lower at immediately ($p < 0.05$) but, gradually increased at 1 and 2 weeks (all $p < 0.001$) compared with the sham control. TUNEL-positive deposition was significantly increased immediately after RF ablation ($p < 0.001$) and gradually decreased. The HSP70-positive deposition was significantly increased compared with sham control at immediately, 1 and 2 weeks (all $p < 0.001$)
Conclusion: The SE can maximize the RF ablation-induced therapeutic effects by fully contacting the inner wall of the rat esophagus. The stent-based RF ablation is technically feasible and effective to evenly induce thermal damages in the rat esophagus.

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Keywords : Radiofrequency ablation, Self-expandable metallic stent, Radiofrequency electrode, Esophagus

PS-E-003

Current operational issues of the Institutional Animal Care and Use Committee in Korea and suggestions for improvement

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The Animal Protection Act (APA) and the Laboratory Animal Act (LAA) are two important laws in Korea that govern animal experiments and laboratory animal treatment. In 2007, the Korean government mandated that all institutions with animal facilities should review each animal experiment protocol through their respective Institutional Animal Care and Use Committee (IACUC) by the APA. In 2009, the government also enacted, in conjunction with the LAA, the establishment of the Laboratory Animal Management Committee (LAMC). Although the purpose and composition of each committee differ, it is difficult to manage both committees simultaneously. Therefore, the IACUC is considered an LAMC if it meets the requirements of both the APA and the LAA. This was accomplished by recognizing the authority of a committee established by an institution, which is referred to as an "Integrated IACUC." Although certain sections in the integrated IACUC are contradictory, most institutions that operate animal facilities have an integrated IACUC, and the government generally approves these committees. However, Korean IACUC is currently facing some operational pressing issues. 1) Review of the animal protocol with controversial technology. 2) Review of the multi-institution animal protocol. 3) Review of veterinary clinical trials for client-owned animals. 4) Delay the review process in large institutions with a single IACUC. Here, the following three solutions are proposed to address the above issues. 1) Establishment of public IACUC. 2) Establishment of the Veterinary Clinical Study Committee as an advisory body to the IACUC. 3) Operating multiple committees rather than increasing the number of committee members on a single committee. Although there may be other issues that need to be solved, the three suggestions above will help resolve the major issues in the current IACUC operations in Korea.

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Keywords : Animal ethics, Animal protocol review, Animal welfare, Clinical trial, Institutional Animal Care and Use Committee

PS-E-004

Transcriptomic insight into the ion channel genes across tissue types and developmental stages

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Ion channels regulate a large number of cellular functions and their functional role in many diseases makes them potential therapeutic targets. Given their diverse distribution across multiple organs, the roles of ion channels, particularly in age-associated genome-wide expression changes in specific organs, is yet to be fully revealed. Using RNA-seq data, we investigated the rat transcriptomic profiles of ion channel genes across 11 organs and 4 developmental stages in both sexes of *Fischer* 344 rats and identify tissue-specific and age-dependent changes in ion channel gene expression. Organ-enriched ion channel genes were identified, in particular, the brain showed higher tissue-specificity of ion channel genes, including *Gabrd, Gabra6, Gabrg2, Grin2a,* and *Grin2b*. Notably, age-dependent changes in ion channel gene expression were prominently observed in the thymus, including in *Aqp1, Clcn4, Hvcn1, Itpr1, Kcng2, Kcnj11, Kcnn3,* and *Trpm2*. Our comprehensive study of ion channel gene expression will serve as a primary resource for biological studies of age-related diseases caused by abnormal ion channel functions.

Corresponding author : Eun A Ko

Keywords : RNA-seq, Ion channel gene, Rat transcriptome, Age-dependent expression

PS-E-005

Vitamin D suppresses pain and cartilage destruction in OA animals model via regulation of autophagic flux and cell death

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Background: Osteoarthritis (OA) is the most common form of arthritis associated with ageing. Vitamin D has diverse biological effect on bone and cartilage, and observational studies have suggested it potential benefit in OA progression and inflammation process. However, the effect of vitamin D on OA is still contradictory. Here, we investigated the therapeutic potential of vitamin D in OA.
Methods: Six-week-old male Wistar rats were injected with monosodium iodoacetate (MIA) to induce OA. Pain severity, cartilage destruction, and inflammation were measured in MIA-induced OA rats. Autophagy activity and mitochondrial function were also measured. 1,25$(OH)_2$D3 (vitamin-D) and celecoxib were used to treat MIA-induced OA rats and OA chondrocytes.
Results: Oral supplementation of vitamin D resulted in significant attenuations in OA pain, inflammation, and cartilage destruction. Interestingly, the expressions of MMP-13, IL-1β, and MCP-1 in synovial tissues were remarkably attenuated by vitamin D treatment, suggesting its potential to attenuate synovitis in OA. Vitamin D treatment in OA chondrocytes resulted in autophagy induction in human OA chondrocytes and increased expression of TFEB, but not LC3B, Caspase-1 and Caspase-3, in inflamed synovium. Vitamin D and celecoxib showed a synergistic effect on antinociceptive and chondroprotective properties *in vivo*.
Conclusion: Vitamin D showed the chondroprotective and antinociceptive property in OA rats. Autophagy induction by vitamin D treatment may be a promising treatment strategy in OA patients especially presenting vitamin D deficiency. Autophagy promoting strategy may attenuate OA progression through protecting cells from damage and inflammatory cell death.

Corresponding author : Mi-La Cho

Keywords : Osteoarthritis, Autophagy, Inflammatory cell death, Vitamin D, Mitochondria

PS-E-006

Extracts of *Ficus erecta* Thunb. leaves ameliorate cognitive impairment and neuronal loss in an amyloid-β-Induced Alzheimer's disease-like animal model

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Ficus erecta Thunb. is a traditional medicinal plant used to treat inflammatory diseases. In the present study, we investigated the effects of *F. erecta* Thunb. leaves against cognitive deficit and neuronal damage in an amyloid-β (Aβ)-induced Alzheimer's disease-like mouse model. In behavioral tests (passive avoidance task and Morris water maze test), extracts of F. erecta (EEFE) leaves markedly improved cognitive impairment in Aβ-injected mice. EEFE reduced neuronal loss and the expression of neuronal nuclei (NeuN), a neuronal marker, in brain tissues of Aβ-injected mice. EEFE significantly reversed Aβ-induced suppression of cAMP response element-binding protein (CREB) phosphorylation and brain derived neurotrophic factor (BDNF) expression, indicating neuroprotection was mediated by the CREB/BDNF signaling. Moreover, EEFE significantly suppressed the inflammatory cytokines interleukin 1beta (IL-1β) and tumor necrosis factor alpha (TNF-α), and expression of ionized calcium-binding adaptor molecule 1 (Iba-1), a marker of microglial activation, in brain tissues of Aβ-injected mice, suggesting anti-neuroinflammatory effects. Finally, the inhibitory effects of EEFE were observed on Aβ aggregation in brain tissues of the animal model. Overall, EEFE has the protective effects against cognitive impairment and neuronal damage in AD-like mice via activation of the CREB/BDNF signaling and upregulation of the inflammatory cytokines. Our findings support that EEFE is a promising drug candidate for the treatment or prevention of AD or AD-related neurodegenerative conditions.

Corresponding author : Soo-Jin Jeong

Keywords : Amyloid-β, Alzheimer's disease, *Ficus. erecta* Thunb. Leaves, Cognitive impairment

PS-E-007

The protective effect of *Tilia amurensis* honey protective effect on infection of influenza A virus infection through activation of interferon signaling

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Recently, attention has been focused on the prevention and treatment of respiratory viruses including influenza viruses. Here, we evaluated the antiviral effect of *Tilia amurensis* honey (TH) against influenza A virus in murine macrophages. First, the infection of influenza A virus was reduced when pre-treated with TH. In addition, the data shown that pre-treatment of TH in murine macrophages increased the production and secretion of type-1 IFN and pro-inflammatory, and increased phosphorylation of type-1 IFN-related proteins, TBK and STAT. Moreover, it was found that the TH increased the expression of interferon-stimulating genes (ISGs) and also increased the expression of IFN-inducible transmembrane (IFITM3) a protein that interferes with virus replication and entry. Taken together, this funding suggested that TH suppresses influenza A virus infection by regulating the innate immune response in macrophages. This finding can be used as potentially useful information for the development of preventive and therapeutic agents for influenza A virus, and can enhance the economic value of TH.

Corresponding author : Jang-Gi Choi

Keywords : *Tilia amurensis* honey Influenza A virus, Interferon, ISGs, IFITM3

PS-E-008

National primate infrastructure for biomedical and basic science

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Primates are valuable resources for different research fields including genetics, evolutionary biology, biomedical research, neuroscience, regenerative research, microbiology, vaccine development and pharmacology. Because primates have more biological and behavioral similarities and closer genetic relationship to humans than other animal models. However, primate resources are limited to access for individual researchers. In 2005, Republic of Korea government established National Primate Research Center (NPRC). First purpose of NPRC is production and supply of specific pathogen free (SPF) primate in Republic of Korea. Second purpose is supporting the regenerative medicine (bio organ transplantation, stem cell and gene therapy). The last one is supporting the basic biomedical research and basic science. Recently, NPRC established primate resource bank with various primate samples (Tissue deoxyribose nucleic acid (DNA), Blood DNA, ribose nucleic acid (RNA), cDNA, paraffin blocks (brain), etc) from crab-eating monkey, marmoset monkey, rhesus monkey, African green monkey, and squirrel monkey. And also, we established cutting edge medical imaging technique using 3 Tesla magnetic resonance imaging (3T-MRI), positron emission tomographic-computed tomographic (PET-CT), micro PET-CT, and angiography imaging system. Therefore, researchers who want to access the primate resources and use the imaging analysis with primate for research purpose could get various national primate infra service, easily (http://portal.kribb.re.kr/primate).

Corresponding author : Jae-Won Huh

Keywords : Basic science, Biomedical, Infrastructure, Monkey, Primate

PS-E-009

Support center for vaccine commercialization and development

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Gyeongbuk Institute for Bio indurstry is a non-profit research institute that promotes key challenges for the development process in the vaccine industry, industrial hemp, and materials and healthy food industry. The Support Center for Vaccine Commercialization and Development (SCVCD) will become the center of the vaccine hub from southern provinces to nationwide through the establishment of infrastructure dedicated to vaccines, corporate support, and vaccine technology development. As the importance of vaccine development increases worldwide due to the COVID-19 pandemic, the SCVCD plans to build a dedicated infrastructure specialized in the vaccine field, supporting as a one-stop service to improve the product yield and evaluating its/their efficacy by using high-risk pathogen handling facilities and animal testing facilities that are composed of standable Biosafety Levels. The SCVCD is located at 1021, Goejeong-ri, Pungsan-eup, Andong-si, Gyeongsangbuk-do, with a site area of 9,981 m2 and a building area of 4,623 m2. It is scheduled to be completed in December 2022 with the goal of establishing a (A)BL2/3 research facility and GLP as designating a non-clinical laboratory to lay a foundation to promote the industrialization of future vaccines.

Corresponding author : Tae Gyun Kim

Keywords : Gyeongbuk institute for bio industry Vaccine, Biosafety level, Good laboratory practice, Preclinical study

PS-E-010

Assessment tool based non-invasive molecular imaging for pharmacokinetic evaluation of oligonucleotides

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Aptamers are molecules of single-stranded DNA or RNA, ranging from 20 to 100 base pairs in length, derived from a pool of random libraries. These achieve in high affinity through the systematic evolution of ligands by exponential enrichment (SELEX) and can be in picomolar range of binding affinity, comparable to small molecules or antibodies, against target. Over the two decades, numerous aptamers have been proposed with promising result in preclinical and clinical studies for therapeutics either on themselves or aptamer-drug conjuagtes. There is thus a growing demand for viable methods to evaluate and verify aptamers that have already been or are being developed. We aims herein to introduce the utility of positron emission tomography (PET) to evaluate the in vivo tissue pharmacokinetics (PKs), target specificity and applicability of oligonucleotides. PET is a representative imaging modality that enables noninvasive, real-time visualization of biochemical events at the cellular and molecular level in living subjects. For in vivo imaging of aptamers with PET, human epidermal growth factor receptor 2 (HER2)-specific and selective binding aptamer labeled with the radioisotope, fluorine-18, was synthesized by base-pair hybridization using complementary oligonucleotide platform. Then, PET images were acquired up to 6 h using a dedicated small animal PET/CT scanner after intravenous administration to the laboratory animals. The acquired PET images were reconstructed into a three-dimensional images by combining CT and PET images. The PKs and biodistributions of ^{18}F-labeled-aptamer on each organ over time were analyzed including excretion routes and PK parameter such as $T_{1/2}$, T_{max}, AUC and C_{max}. In order to investigate the target specific accumulation, ^{18}F-labeled-aptamer by PET was also measured in HER2-positive tumor (KPL4) bearing mice. In addition, as essential data for clinical trials, the internal absorbed dose for humans was estimated as that of mice. Intravenously administered ^{18}F-labeled-aptamer showed significant and rapid uptake in most tissues except for the initial brain and muscle, and was highest in the order of heart, kidney, liver, lung, gall bladder, spleen, and stomach. The main route of excretion was approximately 77.8% through the renal and about 8.3% through the biliary tract of the total dose. The internal absorbed dose to standard organs of an adult female was evaluated using the residence time in the body and S-value on humans. The effective dose for an adult female was estimated to be 0.00189 mGy/MBq which might be safe. Consequently, the distribution in each organ including HER2 expression could be well visualized and quantified by PET with ^{18}F-labeled-aptamer. The excretion routes and PK parameters in vivo were also successfully determined. These results suggest that PET with radioisotope labeled aptamers could be potentially useful for the development of targeted therapeutics against various diseases of target specific and selective binding aptamers and evaluating their PKs.

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Keywords : Pharmacokinetic, Positron emission tomography, Aptamer, Radioisotope

PS-E-011

Comparative study of domestic and foreign national residue programs

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In the case of Korea, a system for testing harmful residues in domestic meat was introduced in 1991, and since 1996, in accordance with Articles 11 and 12 of the Livestock Products Sanitation Control Act and Article 9 (3) of the Enforcement Regulations of the same Act, Regulations"(Ministry of Food and Drug Safety Notification, and Ministry of Agriculture, Food and Rural Affairs after 2019) is operating the National Residual Program (NRP). The NRP plan is established with monitoring and regulatory inspections, and monitoring inspection includes bioresidue inspection before shipment and carcass inspection after slaughter, and includes inspection plans for cattle, pigs, chickens, ducks, sheep (goats), and horses. For monitoring and regulatory tests, simple microbiological tests such as EEC-4 plate are performed on randomly collected samples. If positive is confirmed in the simple qualitative test result, a precise quantitative test is performed using precision analysis equipment such as LC-MS/MS, GC-MS/MS, and the suitability for the residual substance test is performed depending on whether the test item exceeds the residual tolerance standard. In this study, the management system for residues in foreign countries was analyzed and based on this, it was conducted to find out the level of the management system for residues in Korea compared to the management system with foreign countries. In order to analyze each country's national residual system, related data and literature were searched through the Internet. We collected and analyzed the inspection plans and inspection results of the European Union, the United States, and Japan, including Korea, for the past five years. In addition, related literature and report data were also collected. Also the types of residual substances inspection, inspection methods, number of inspections, and violation rates in each country were compared and analyzed. In korea, inspection items are classified into 74 groups, including antibiotics, synthetic antibacterial drugs, and hormones, and a total of 219 substances are tested. The NRP inspection performance for the last 4 years has some differences by year, but approximately 150% or more were inspected, and the violation rate was 0.23~0.35%. In EU, twenty-eight member countries test for residues of veterinary drugs and specific substances in livestock and animal-derived products, and test targeted and suspected samples in accordance with Council Directive 96/23/EC. Each member country operates a residual substance inspection program by establishing a system to collect and inspect samples according to its own inspection system and some samples from imported goods. Most Member States are testing to meet the minimum requirements for sample frequencies set out in Council Directive 96/23/EC and Commission Decision 97/747/EC, and have been designated non-compliant in the last 10 years. Non-compliant targeted samples averaged 0.25-0.37%. The United States National Residue Program (NRP) begins with the interagency blue book of sampling programs and consists of the collection and analysis of reported samples (red book). As a result of the NRP inspection for the last 4 years, the residual material violation rate was 0.35% to 0.40%, and the violation rate was slightly higher in samples from the inspector generated sampling program than in the US targeted sampling program. In Japan, the inspection results reported by local public organizations and quarantine services, after each food subject to be inspected is divided into domestic and imported products, and analysis is conducted by the Food Ministry of the National Institute of Health Sciences. As for inspection samples, about 260,000 to 270,000 cases of domestic and imported livestock and fishery products have been inspected annually. The violation rate was in the range of 0.01 to 0.014. The NRPs of the countries studied were basically operated in a similar way. Although the inspection items were similar, there was a difference in the substances detected because there was a difference in the chemical substances mainly used in each country. Violation rates were similar in each country, but Japan showed a relatively low violation rate. In the future plan, an optimal sampling plan should be established and the test results should be returned to the farmer quickly. Finally, guidance should be given to minimize the violation rate of residual substances.

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Keywords : National residue programs, Korea, Foreign countries, Comparative study

PS-E-012

Domestic and abroad investigations on bioresidue testing before shipment

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The pre-shipment bioresidue test, one of the domestic monitoring tests, is requested by a livestock product testing and inspection institution established by a local government for sanitary inspection of livestock products by collecting urine or serum samples from livestock to be shipped. Antibacterial in urine or serum such as thin layer chromatography (TLC), BmDA method (B. megaterium disc assay), EEC 4-plate method, enzyme immunoassay (ELISA), microbial receptor assay or fluorescence immunoassay Inspection is carried out using a method suitable for simple substance inspection or a method that can detect less than the tolerance level for residual meat. There is a need for an inspection method that can determine whether it is possible to ship by examining the residual substances remaining in the livestock at the farm before slaughter. This test method is attracting a lot of attention as a method of increasing the income of livestock farmers and helping the public health by supplying safe livestock products as well as reducing the disposal of carcasses due to the residue of veterinary drugs. Therefore, it is required to introduce an on-site inspection method that can prevent unnecessary carcass disposal and supply safe livestock products by inspecting whether residual substances exceed the standard using samples such as urine and blood before slaughtering and shipping livestock from livestock farms. Relevant data and data were collected through the Internet. Domestic and foreign laws, regulations, and pre-slaughter inspection methods by country were investigated. In addition, related literature and report data from each country were analyzed. As for the inspection method, the inspection methods applied in each country and the types of inspection kits on the market were also investigated. As a result, In Korea, pre-shipment bioresidue testing is performed on cattle, chickens and ducks, but there are few cases of violations in cattle and ducks. The existing antibiotic test methods (EEC-4 plate method, TTC method) using microorganisms take a lot of time until the test results, so there are problems such as storage for a certain period after slaughter. Kits are introduced and used. A rapid test kit using immunoassay that detects antibiotics through a competitive reaction between antibiotics and antibiotic conjugates in samples such as milk, meat, eggs, beta-lactam, quinolone, tetracycline, sulfanamide, streptomycin, gentamycin, tylosin/timicosin, spiramycin can be identified. There is no country that applies an inspection program that can be applied at the farm level to the national residue program. However, in Korea, autonomous pre-inspection is conducted by farms or associations, so it is desirable to operate as a pilot system through preliminary preparation before introducing such a system in its entirety. The distribution pattern in tissues and biological samples is different for each drug, and the ratio of distribution in tissues and biological samples may be different depending on the absorption and dissipation time of the drug. In addition, since most of the research is done through device analysis, additional test data using a test kit that can be easily tested on biological samples is required. Therefore it is difficult to apply it uniformly to the drugs and livestock breeds.

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Keywords : Bioresidue test, Before shipment, Antibiotics, Rapid test kit, Livestock

PS-E-013

Research trends of animal-assisted interventions in agro-healing

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Some approaches to regaining control that a growing number of patients' use are complementary and alternative medicine(CAM) techniques. CAM has been defined by the National Cancer Institute as 'a broad range of healing philosophies, approaches and therapies'. CAM includes retaining the need to be needed and having positive affiliation and unconditional acceptance; enter human-animal interaction.

Studies of the effects of animal-assisted interventions (AAI) in agro-healing face a number of theoretical and practical challenges. Proposed theoretical processes for the effects of AAI include those that address primarily the animal's ability to facilitate human–human social engagement, those that emphasize animals' apparent capacity to trigger social attachments and provide nonhuman social support, those that categorize certain animals as supernormal stimuli, those that advance a biophilia hypothesis that living organisms have an innate ability to attract and hold human attention, and those that promote an integrative biopsychosocial model.

The effects of AAI in agro-healing may be influenced by the positive physiological effects of human animal interaction on dopamine, cortisol, oxytocin, prolactin, endorphin, and phenylethylamine levels in both humans and the animals with which they interact. The physiological effects by AAI in agro-healing may lead to a greater sense of control for patients, helping them find meaning, improve their quality of life, and potentially decrease stress, anxiety, and depression in ways similar to other CAM techniques such as hypnosis, guided imagery, or aromatherapy massage.

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Keywords : Agro-healing, Animal-Assisted Intervention, Complementary and alternative medicine(CAM), Hormone

PS-E-014

68Ga-Lactosylated albumin agent for the evaluation of liver function using quantitative imaging

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To treat liver diseases completely such as liver cirrhosis and liver cancer, it is necessary hepatectomy. To avoid the risk of liver failure by hepatectomy, the accurate evaluation of liver functional reserve is important before surgery. However, currently used evaluation methods of liver function test are indirect or not quantitative. In this study, we performed positron emission tomography (PET) imaging for quantifying the normal liver function using hepatocyte targeting agent labeled with a positron emitting radioisotope, ^{68}Ga.

Lactose, which is a targeting moiety of asialoglycoprotein receptors on hepatocytes, was combined with human serum albumin (HSA) to give lactosylated serum albumin (LSA). And 2-S-(4-Isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (p-SCN-Bn-NOTA) was conjugated on LSA or HSA (for control) for ^{68}Ga labeling to give NOTA-LSA or NOTA-HSA.

The liver fibrosis models were induced in the rats by intraperitoneal injection of thioacetamide 3 times per week for 4 weeks. ^{68}Ga-NOTA-LSA or ^{68}Ga-NOTA-HSA was injected into normal and model rats to compare the liver function and evaluate the feasibility of ^{68}Ga-NOTA-LSA as a quantitative imaging agent for liver function test using PET.

In PET images, ^{68}Ga-NOTA-LSA was showed higher standardized uptake value (SUV) in normal rats than in liver fibrosis model rats, and higher than ^{68}Ga-NOTA-HSA in normal rats.

In this study, we confirmed the feasibility of ^{68}Ga-NOTA-LSA as a quantitative imaging agent for liver function test using PET. It can give useful information for the evaluation of liver functional reserve.

Corresponding author : Yun-Sang Lee

Keywords : Albumin, Lactose, Liver, Molecular imaging, Nuclear medicine

PS-E-015

Comparison of biodistribution according to liposome size for the development of nano-based therapeutics

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Macrophages are an important key that plays a role in defending or attacking the immune system in vivo according to the polarization pattern. In this study, molecular imaging was used to compare differences in the distribution of clodronate-liposomes labeled with an isotope capable of targeting immune cells in vivo for the development of therapeutic agents for immune diseases.

Liposomes were prepared with diameters of 50 and 100 nm by encapsulating 20 mg/ml of clodronate (Liposome,Clodronate-Liposome(CL),Mannose-Liposome(ML),Clodronate-Mannose-Liposome(CML)). Mannose targeting macrophages was bound to the surface of the prepared liposome to confirm the target macrophages in vitro. The macrophage target of these liposomes and the removal of macrophages by clodronate were compared in vivo according to liposome size by in vitro cell viability test and PET imaging using normal mice.

As a result of the in vitro study, it was possible to confirm the difference in cell viability according to the concentration of clodronate, but there was no difference in the cell viability according to the size of the liposome. In molecular image, it was confirmed that the liver uptake rate was high when clodronate-liposome was administered, and the liver uptake rate of mannose-labeled clodronate-liposome appeared the fastest among all groups. Comparison according to the size of liposomes, 50nm showed faster uptake in liver than 100nm in all groups.

In this study, the role of clodronate on macrophages was confirmed through in vivo imaging in mice. Looking at the mechanism of bisphosphonate drugs such as clodronate in previous studies, this is a result reflected in the theory that phagocytosis of liposomes introduced into the body occurs by macrophages. Confirmation of biodistribution according to the size of liposomes is expected to play an important role in the development of macrophage-based disease therapeutics.

Corresponding author : Yun-Sang Lee

Keywords : Macrophage, Liposome, Clodronate, Molecular imaging, Click chemistry

PS-E-016

The application of alternatives to animal testing for skin sensitization evaluation on pesticides

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To evaluate skin sensitization of pesticides, the test methods using experimental animal model such as guinea pigs has been commonly used. However, research on alternatives to animal testing to replace laboratory animal is being actively conducted in order to strengthen regulation on animal experiments and to protect ethical protection of experimental animals. In Korea, three institution are promoting the development of alternative animal testing methods focus on Korean Center for the Validation of Alternative Methods (KoCVAM). Alternatives to animal testing for the toxicity test have been developed and applied in National Academy of Agricultural Science (pesticides), National Institution of Food and Drug Safety Evaluation (cosmetics) and National Institute of Environmental Research (chemicals). Because of safety evaluation of pesticides, National Academy of Agricultural Science established eight test guidelines to replace laboratory animal use for skin sensitization evaluation including Local Lymph Node Assay (LLNA; OECD TG 442B), Direct Peptide Reactivity Assay (DPRA; OECD TG 442C), KeratinoSens ARE-Nrf2 luciferase test (OECD TG 442D), and human Cell Line Activation Test (h-CLAT; OECD TG 442E). In our study, DPRA test was predicted to have sensitivity, specificity, and accuracy of 100%. However, KeratinoSens luciferase test was predicted to have sensitivity of 100%, specificity of 83.3%, and accuracy of 90.9%, h-CLAT was also predicted to have sensitivity of 100%, specificity of 66.6%, and accuracy of 85.7%. Skin sensitization evaluation is difficult to completely replace animal testing as a single animal alternative test method. Therefore, it is necessary to apply an integrated evaluation strategy using multiple test methods. National Academy of Agricultural Science is expanding the test guideline for skin sensitization evaluation by conducting research subject for the application of alternatives to animal testing on pesticides. Furthermore, it is expected that the test guideline and test methods for pesticide registration can be improved and applied to pesticide evaluation through research on the application of alternative animal test methods for skin sensitization evaluation of pesticides.

Corresponding author : So Hye Hong

Keywords : Alternatives to animal testing, Skin sensitization, Pesticides

PS-E-017

Study on a new toxicological assessment of endocrine disruptors (EDs) pesticides by in vitro OECD guideline

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Endocrine disruptors (EDs) are altered chemicals of the normal function on the endocrine systems of both environment and humans. A huge number of chemicals have been identified as EDs among the pesticides such as iprodione and simazine. The OECD guideline for the screening test method of EDs has been published. For example, there is an uterotrophic bioassay OECD TG 440 (2007) in rodent for estrogenic properties as in vivo screening test guideline. However, in vitro screening test guideline is still under discussion before application. Thus, the international standardization of in vitro OECD guideline is required. A hormone receptor-binding assay is suggested to identify chemicals that may affect the endocrine system. This study applied the Stably Transfected Transcriptional Activation (STTA) assay using hERα-HeLa-9903 cell line formed by human estrogen receptor α gene and a firefly luciferase gene. The in vitro estrogen receptor-binding assay for the chemicals having the endocrine disrupting potential is placed in level 2 in the OECD conceptual framework (2018) for testing and assessment of EDs. Therefore, this study can be contributed to the global standardization of testing and screening method of EDs.

Corresponding author : Jeong Hyun Lim

Keywords : Endocrine disruptors, Pesticides, Estrogen receptor-binding assay, HERα-HeLa-9903 cell line

PS-E-018

Effect of lactic acid bacteria on the pharmacokinetics and metabolism of ginsenosides in mice

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Ginseng glycoside, called ginsenoside, is a major active pharmacological component, which causes the efficacy of Red ginseng extract (RGE). Ginsenoside has a sugar moiety structure bound to an oleanane and dammarane structure. Further subdivisions occur depending on the length of the sugar chain. The metabolism of ginsenosides is mediated by gut the microbiota, in which the involvement of Lactic acid bacteria (LAB) was reported. This study aims to investigate the effect of lactic acid bacteria (LAB) on in vivo metabolism and the pharmacokinetics of ginsenosides in mice. The amoxicillin pretreatment (20 mg/kg/day, twice a day for 3 days) resulted in a significant decrease in the fecal recovery of CK, PPD, and PPT through the blockade of deglycosylation of ginsenosides after single oral administrations of RGE (2 g/kg) in mice. The plasma concentrations of CK, PPD, and PPT were not detectable without change in GRb1, GRb2, and GRc in this group. LAB supplementation (1 billion CFU/2 g/kg/day for 1 week) after the amoxicillin treatment in mice restored the ginsenoside metabolism and the plasma concentrations of ginsenosides to the control level. In conclusion, the alterations in the gut microbiota environment could change the ginsenoside metabolism and plasma concentrations of ginsenosides. Therefore, the supplementation of LAB with oral administrations of RGE in mice would help increase plasma concentrations of deglycosylated ginsenosides such as CK, PPD, and PPT.

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Keywords : Metabolism, Pharmacokinetics, Lactic acid bacteria, Ginsenoside

PS-E-019

Pharmacokinetics of polynemoraline C in mice

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In this study, We aimed to investigate the pharmacokinetics of polynemoraline C in Mice. We administrated polynemoraline solution (5mg/kg dissolved in 1mL mixture of DMSO : saline = 20:80 (v/v)) via tail vein injection and oral administration also performed (30mg/kg suspended in 2mL of 0.5% carboxymethyl cellulose suspension) using oral gavage. After administration, plasma samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8 and 24 h. Also, we tested protein binding and solubility for more accurate interpretation of pharmacokinetics data. Plasma protein bindng was measured using rapid equilibrium dialysis kit according to the manufacturer's protocol. Solubility was measured by analyzing the filterate after incubating 3mg of polynemoraline C with 1mL water for 24 h.

The plasma concentrations of polynemoraline C after IV??injection showed multi-exponential decay. It declined sharply for 1 h but slowly declined for 4-24 h. The initial concentration (Co) of polynemoraline C was 41.8±7.3 ng/mL but elimination half-life (T1/2) was calculated as 13.84 ± 7.46 h when mice received 5 mg/kg of polynemoraline C intravenously. The results suggested that polynemoraline C may have highly distributed characteristics. Consistently, volume of distribtion (Vd,ss) was calculated as 797.1 ± 211.3 L/kg. Orally administered polynemoraline C showed multiple peaks; first peak appeared in 0.25 – 0.5 h and second peak appeared in 4 – 8 h, which resulted in variable Tmax value (CV of 118%). The lipophilic nature of polynemoraline C and small molecular weight could contribute to the rapid gastrointestinal absorption of polynemoraline C upon administration. But low aqueous solubility (5.46 ± 1.92 μg/mL) of this compound after oral administration of high dose (30 mg/kg/2 mL) may contribute to the delayed second absorption peak. The enterohepatic circulation may also contribute to the multiple peak phenomenone. The AUC of intravenous and oral administration were calculated as 48.69 ± 12.35 and 49.71 ± 24.01 ng·h/mL, respectively, yielding a 17.0% of absolute bioavailability.

Corresponding author : Im-Sook Song

Keywords : Pharmacokinetics, Protein binding, Solubility, Bioavilability, Plasma concentration

PS-E-020

Development of diabetes pig model; pathological study on alloxan or streptozotocin-induced diabetic pig

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Pig is anatomically and physiologically similar to humans and is widely used as experimental animals on the general medical side, so the development of pig disease models for disease research is necessary. Diabetes has an increasing prevalence worldwide, and prevention and treatment of diabetes are important research areas.

In this study, induction of diabetes using the alloxan(ALX) or streptozotocin(STZ), which was commonly used for an experimental diabetic animal model, and a pathological comparison of the two drugs was performed in pigs.

Blood collection was performed before induction and, after induction 0days, 2days, and 7days. Serum biochemical analysis measured AST, ALT, and glucose levels, and histological analysis evaluated insulin and glucagon production by IHC.

As a result, in the serum biochemical analysis, Induction groups were significantly increased AST and glucose levels compared to NC group on 7days, and ALT was increased in induction groups but was not significant. In particular, glucose levels showed that STZ group and ALX group were increased 5.38 times and 1.67 times each compared to NC group, and STZ group showed more increase compared to ALX group. In histological analysis, insulin production was decreased in induction groups, STZ group was dramatically reduced compared to ALX group. The glucagon production was Induction groups were increased compared to NC, but not significant.

In conclusion, STZ induction showed the value diabetic pig model compared to ALX induction, but not enough for research. STZ induction showed higher hyperglycemia compared to research levels, and ALX induction was increased glucose levels but showed no experimental levels. Therefore, the development of an experimental diabetic pig model needs further studies that, in the STZ induction model, injection of insulin to maintain experimental glucose levels, and in the ALX group, controls ALX concentration and experimental schedule.

Corresponding author : Hyunil Kim

Keywords : Pig, Diabetes model, Alloxan, Streptozotocin, Diabetes pathology

PS-E-021

Analysis of CCR2 splice variant expression patterns and functional properties

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C–C motif chemokine receptor 2 (CCR2), the main receptor for monocyte chemoattractant protein-1 (MCP-1), is expressed on immune cells and mediates cell migration toward MCP-1 in inflammation-related diseases. The CCR2 gene encodes two isoforms: CCR2A and CCR2B. The CCR2B open reading frame is localized in a single exon, similar to other chemokine receptors, and CCR2A and CCR2B feature different amino acid sequences in their C-terminal intracellular loops due to alternative splicing. Most biochemical studies on CCR2-related cellular responses in the immune system have focused on CCR2B, with few reports focused on CCR2A. Understanding the functional properties of CCR2A in cellular responses may elucidate the roles played by MCP-1 and CCR2 in pathophysiological responses.

CCR2 gene expression analysis in several cell types revealed that most adherent cells only expressed CCR2A, whereas CCR2B expression was dominant in monocytic cells. The C-terminal Helix 8 region of CCR2A contains few basic amino acids, which may be unfavorable for cell surface localization. CCR2B contains many C-terminal Ser/Thr residues, similar to other chemokine receptors, which may be phosphorylated by G protein–coupled receptor kinases (GRKs) to promote β-arrestin recruitment and subsequent endocytosis. By contrast, CCR2A contains few C-terminal Ser/Thr residues, which are unlikely to be phosphorylated by GRKs. CCR2A localized on the cell surface is resistant to internalization, despite the interaction between Gβ and GRKs induced by ligand binding with CCR2A. CCR2A induced cellular responses at a relatively higher degree than CCR2B, although both receptors mediated signaling events through Gαq and Gαi. HeLa cells lacking CCR2A showed slowed growth compared with parent cells, regardless of MCP-1 stimulation, and their chemotactic activity toward MCP-1, in addition to basal motility, was significantly impaired.

Corresponding author : Jong-Ik Hwang

Keywords : GPCR, CCR2, Gene expression, Protein-protein interaction

PS-E-022

Polymeric micelles including osthol-based drug combination for lung cancer therapy

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Osthol (OTH) is a coumarin derivative that inhibits cancer cell growth, induces apoptosis, and exhibits anticancer effects. Docetaxel (DTX) is a taxane-based anticancer agent that inhibits microtubule degradation to prevent microtubule aggregation and induce cell death. DTX and OTH were solubilized and encapsulated in mPEG-b-PCL polymer micelles, and the synergistic effects of the two drugs were also investigated. In vitro drug release profiles showed that mPEG-b-PCL micelles loaded with DTX/OTH released DTX and OTH more slowly. Cloning analysis using A549 lung cancer cells demonstrated that mPEG-b-PCL micelles loaded with DTX/OTH showed 3.7 times higher inhibitory effects than DTX/OTH solutions. In vivo pharmacokinetic studies have shown that micelles combined with DTX and OTH have increased AUC and decreased clearance values when compared to single micelles, improving biological availability.

Corresponding author : Dae Hwan Shin

Keywords : Osthol, Micelle, Pharmacokinetics

PS-E-023

Increased bioavailability of chrysin by preparing spray dried solid dispersion of chrysin

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The objective of this study was to prepare the solid dispersion formulation of chrysin with surfactant and hydrophilic carriers using spray drying and freeze drying method and to compare the oral bioavailability of chrysin solid dispersion formulatin with chrysin alone. To choose the solubility enhancing surfactant and hydrophilic carriers for chrysin, we measured the solubility of chrysin in the presence of various surfactants (i.e., Tween80 (TW80), Sodium Dodecyl Sulfate(SDS)) as well as various hydrophilic carriers (i.e., Lactose, NaCMC, Pluronic F127(F127), PVP) with varying concentrations. The composition of solid dispersion was selected as a ratio of chrysin: SDS: PVP = 1:5:3 (w/w/w) and the solid dispersion formulation of these combination was prepared using spray drying and freeze drying method and characterized in regards with its pharmacokinetic properties in rats. We investigated the pharmacokinetics of chrysin in rats for 24 h after oral administrations of chrysin alone as well as the mixture of chrysin: SDS: PVP = 1:5:3 (w/w/w), and spray dried formulation, freeze dried formulation with same rate of mixture at dose of 30 mg/kg as chrysin. As results, the AUC24h of chrysin was increased by each 7.0 and 4.8-fold in the spray dried formulation and freeze dried formulation compared with chrysin alone, suggesting increased bioavailability of chrysin using solid dispersion formulation. Also, Tmax value was decreased each 94%, 97% in spray dried group and freeze dried group, while Cmax value was increased 24.5, 20.4-fold. The results indicated that solid dispersion formulaiton not only increased the absorption amount of chrysin but also facilitate the absorption rate of chrysin by formulating solid dispersion. In conclusion, preparation of solid dispersion formulation of chrysin: SDS: PVP = 1:5:3 (w/w/w) showed increased bioavailability by enhancing its solubility as well as its dissolution rate.

Corresponding author : Im-Sook Song

Keywords : Solid dispersion formulation, Increased bioavailability

PS-E-024

Cynomolgus macaque model for COVID-19 Delta variant

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With the spread of SARS-CoV-2 variants, which are randomly mutated, the dominant strains that are problematic in locals and countries are changing. The development of preclinical animal models is imperative to validate vaccines and therapeutics against SARS-CoV-2 variants. The objective of this study was to develop a non-human primate (NHP) model for SARS-CoV-2 Delta variant infection. Cynomolgus macaques infected with Delta variants showed infectious viruses and viral RNA in the upper (nasal and throat) and lower respiratory (lung) tracts during the acute phase of infection. After 3 days of infection, lesions consistent with diffuse alveolar damage were observed in the lungs. For cellular immune responses, all macaques displayed transient lymphopenia and neutrophilia in the early stages of infection. SARS-CoV-2 Delta variant spike protein-specific IgM, IgG, and IgA levels were significantly increased in the plasma of these animals 14 days after infection. This new NHP Delta variant infection model can be used for comparative analysis of the difference in severity between SARS-CoV-2 variants of concern and may be useful in the efficacy evaluation of vaccines and universal therapeutic drugs for mutations.

Corresponding author : Jae Hak Park, Jung Joo Hong

Keywords : SARS-CoV-2Delta variant, Interstitial pneumonia, Immunoglobulin, Non-human primate

PS-E-025

Role of the modified Saengmaeksan(mSMS) in L-NAME-induced hypertension model

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Hypertension is known to be a very important risk factor in cardiovascular disease. Recently, studies on developing various Korean medicines that effectively lower blood pressure(BP) have been conducted. Saengmaeksan was originally used as a prescription to treat dyspnea, thirst, phlegm, etc., which are caused by damage to pep and essence due to heat caused by external heat, and it is a prescription that has been applied to heart diseases such as coronary artery disease, arrhythmia, and heart failure. In this study, we investigated the effect and underlying mechanism of a modified Saengmaeksan(mSMS) in L-NAME-induced hypertension in mice. To improve hypertension, Other herbal medicines other than Schisandra Chinensis and Liriope Muscari were added Saengmaeksan was modified. In addition, were extracted in two extraction methods water and ethanol. ICR mice were fed either normal or L-NAME. L-NAME accelerated the increase in systolic BP and diastolic BP. When an L-NAME group became hypertensive, mSMS was administered for 3 weeks to the L-NAME+mSMS group. mSMS administration lowered BP, and especially, ethanol extract lowered it to a level similar to normotension. As a mechanism study, vasoconstriction factors related to hypertension were investigated in rat primary vascular smooth muscle cells. Taken together, mSMS ameliorated L-NAME-induced hypertension. Therefore, mSMS acts as a novel therapeutic option for hypertension. (NIKOM, Korean medicine industry advancement support project, SJ-2022-03)

Corresponding author : Hyo Jung Kim

Keywords : Hypertension, Saengmaeksan, L-NAME, Vasoconstriction

PS-E-026

Analysis of the animal experiment protocols review by IACUC in 2021 in Korea

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Since 2008, IACUC system has been enforced, all animal research institution in Korea must establish IACUC and report its annual activities to APQA in accordance with the Animal Protection Act. This study was performed to analyze the review of animal experiment protocols (AEP) by IACUC in 2021 in Korea.
Total 481 IACUCs were run and 48,535 protocols were reviewed and voted in Korea in 2021; approval (76.1%), approval after modification (21%), modification request (2.5%) and disapproval (0.4%). Total AEP review request in 2021 increased by 18.1% compared to last year (41,074 protocols).
1,406 protocols were reviewed as either disapproval or modification request and those were 2,677 reasons when counting by reasons; animal experiment design (819), refinement (764), reduction (555), replacement (65), personnel training (58) and others (416). Among 3Rs, especially the refinement related issue was the most pointed out and differentiated as followed; ⅰ) The evaluation of animal pain and distress (33.1%, 253), ⅱ) Humane endpoints and euthanasia (30.6%, 234), ⅲ) Appropriate use of sedatives, analgesics or anesthetics on pain grade D experiments (18%, 138), ⅳ) Animal care and enrichment (9.4%, 72) and ⅴ) Scientific justification performing pain grade E experiments (8.7%, 67)
Even though ⅱ) and ⅳ) have decreased (by 20.8%, 19.5% respectively), ⅰ) and ⅴ) have significantly increased (by 41.3%, 148% respectively) compared to last year.
To improve AEP review approval, practical refinement guidances especially on the evaluation of humane endpoints, animal pain and distress should be developed.
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The IACUC handbook

Corresponding author : Heui-Jin Kim

Keywords : IACUC, 3Rs, Laboratory animal, AEP review

PS-E-027

Establishment of a NanoBiT-Based Cytosolic Ca^{2+} sensor by optimizing Calmodulin-binding motif and protein expression levels

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Cytosolic Ca^{2+} ( ) levels change dynamically in response to inducers, repressors, and physiological conditions, and aberrant concentration ($[Ca^{2+}]_c$) regulation is associated with cancer, heart failure, and diabetes. Therefore, $[Ca^{2+}]_c$ is considered as a good indicator of physiological and pathological cellular responses, and is a crucial biomarker for drug discovery. A genetically encoded calcium indicator (GECI) was recently developed to measure $[Ca^{2+}]_c$ in single cells and animal models. GECI have some advantages over chemically synthesized indicators, although they also have some drawbacks such as poor signal-to-noise ratio (SNR), low positive signal, delayed response, artifactual responses due to protein overexpression, and expensive detection equipment. Here, we developed an indicator based on interactions between Ca^{2+}-loaded calmodulin and target proteins, and generated an innovative GECI sensor using split nano-luciferase (Nluc) fragments to detect changes in $[Ca^{2+}]_c$. Stimulation-dependent luciferase activities were optimized by combining large and small subunits of Nluc binary technology (NanoBiT, LgBiT:SmBiT) fusion proteins and regulating the receptor expression levels. We constructed the binary $[Ca^{2+}]_c$ sensors using a multicistronic expression system in a single vector linked via the internal ribosome entry site (IRES), and examined the detection efficiencies. As a single vector system, the calmodulin-SmBiT-IRES-LgBiT-MYLK2S construct optimized the $[Ca^{2+}]_c$ analysis. Promoter optimization studies indicated that promoter-dependent protein expression levels were crucial to optimize SNR and sensitivity. This novel $[Ca^{2+}]_c$ assay has high SNR and sensitivity, is easy to use, suitable for high-throughput assays, and may be useful to detect $[Ca^{2+}]_c$ in single cells and animal models.

Corresponding author : Jong-Ik Hwang

Keywords : Cytosolic Ca^{2+} sensor, NanoBiT assay, Calmodulin, Myosin light chain kinase 1/2, Internal ribosome entry site (IRES), Promoter

PS-E-028

Nicotinamide Mononucleotide (NMN) enhances cloned mice embryo quality and improves the number of inner cell mass (ICM) cells

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Nicotinamide mononucleotide (NMN), a key NAD+ intermediate, can reverse mitochondrial dysfunction and oxidative stress in cell caused due to insufficient NAD+. Studies have shown that NMN supplementation is effective in improving the fertility of aged mice and to protecting porcine oocyte from pollutant-induced deterioration. However, few studies are available about the effects of NMN on the development and reprogramming of cloned embryos. Therefore, this study aimed to determine the effects of NMN on the developmental potential of cloned mouse embryos. Mice were superovulated by injecting them with 10 IU of PMSG, followed by 10 IU of hCG after 48 h. Oocytes were collected at 14 h after hCG injection. The matured oocytes were enucleated and cumulus cells were injected into enucleated oocytes using a piezo-driven micromanipulator. The reconstructed oocytes were activated for 6 h in an activating medium and supplemented with different concentrations of NMN (500nM, 1 and 2mM). The supplement was continued for the next 22 h till the 2-cell embryo stage was reached. The results showed no significant differences in 2-cell block rates and blastocyst rates. Ratio of ICM number, which is maker of the blastocysts quality, was compared by performing immunofluorescence staining of OCT4. Results showed that 1mM NMN treated had significantly higher ICM cell numbers as compared to the other groups. NMN plays a positive role in the development of SCNT-derived embryos up to the blastocyst stage; therefore, its effect on post-implantation development was analyzed. The efficiency of SCNT-derived embryonic stem cells (SCNT-ESCs) from cloned embryos was observed. There was a numerical increase in the SCNT-ESCs rate in the NMN treated group; however, there was no significant difference. Treatment with 1mM NMN improved the quality of SCNT mice embryos, and significantly increased the number of ICM cells. Although, a positive effect on establishing the SCNT-ESCs was expected, there was no significant difference. These findings will help in determining the quality and establishment of human SCNT-ESC lines by increasing the production efficiency of cloned embryos for human cell therapies. *This study is supported by the 2021R1C1C100954611 of NRF of Republic of Korea.*

Corresponding author : Ah Reum Lee

Keywords : Mouse somatic cell nuclear transfer, Nicotinamide mononucleotide, Somatic cell nuclear transfer-derived embryonic stem cells

PS-E-029

Functional characterization of CXCR3 splicing variants and their ligands

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CXCR3, a receptor of four chemokines, I-TAC, IP-10, MIG, and PF-4, regulates leukocyte trafficking and maturation. It is also implicated in various pathological diseases such as immune system-related diseases as well as cancer. There are three isoforms of CXCR3 driven by alternative splicing in humans: CXCR3A, CXCR3B, and CXCR3Alt. Regarding their functional roles in pathophysiological conditions, many elaborate studies have been conducted on chemokines. However, some biochemical data seem not to be correlated with the biological relevance of the receptor variants and chemokines. According to RT-PCR, most cells may express all splicing variants, suggesting that they may affect each other possibly through receptor complex formation, different expression levels, and chemokine dependency. Here we tried integrative analysis on the functional relation of CXCR3 splicing variants and the chemokines using NanoLuc Binary Technology (NanoBiT) combined with other biochemical methods. Expression patterns and chemokine-dependent signaling of the variants revealed that the N-terminal region of CXCR3 seems to affect expression, especially on cell surface as well as ligand-dependent activation. CXCR3A was efficiently expressed in the membrane and responded to I-TAC, IP-10, and MIG with different potency (I-TAC>>IP-10>MIG), whereas CXCR3B was expressed in the membrane with relatively low efficiency and mediated I-TAC-stimulated signals. CXCR3Alt consisting of four transmembrane domains was rarely expressed on cell surfaces and could not mediate any responses to all chemokines. However, CXCR3Alt negatively affected the membrane expression of the other variants and chemokine-stimulated cellular responses mediated by them. Our results confirmed previous segmented knowledge of CXCR3-mediated signals using NanoBiT technology and further presented an additional layer of cellular responses due to the molecular complexity of the splicing variants, including cell surface expression, ligand-dependent receptor activation, and chemotaxis.

Corresponding author : Jong-Ik Hwang

Keywords : Splicing variants, Chemokine receptors, Cell migration

PS-E-030

Establishment of the Mouse Pathogen Standard Cooperation Center (MPSC) in Korea

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Health monitoring of laboratory animals is essential to correct the accuracy and reproducibility of experimental results and prevent infectious diseases. In order to perform health monitoring quickly and accurately, it is essential to collect type strain of mouse pathogens, and it is also necessary to collect pathogen of mouse infected in Korea. For these reasons, the Mouse Pathogen Standard Cooperation Center (MPSC) was started in September 2021. Eleven mouse pathogens, including 7 viruses, 2 bacteria, and 2 fungi were collected before the start of the Mouse Pathogen Standard Cooperation Center (MPSC). In the year 2021, Mouse Pathogen Standard Cooperation Center (MPSC) collected 13 mouse pathogens including 1 virus and 12 bacteria, additionally. Also, Mouse Pathogen Standard Cooperation Center (MPSC) obtained mouse tissues, serums, and feces infected with mouse hepatitis virus (MHV), murine norovirus (MNV), Sendai virus, Citrobacter rodentium (C. rodentium), and Mycoplasma pulmonis (M. pulmonis). In addition, genetic analysis was performed on two types of bacteria isolated in Korea. Mouse Pathogen Standard Cooperation Center (MPSC) collects and obtains microbial resources of mouse to prepare standard materials for mouse health monitoring depending on researchers' demands. Also, Mouse Pathogen Standard Cooperation Center (MPSC) manages and provides mouse pathogens both domestically and internationally.

Corresponding author : Yang-Kyu Choi

Keywords : Health monitoring, Mouse pathogen, Resources, Genetic analysis

PS-E-031

New types of guided bone regeneration membrane in alveolar bone defect of beagle dog

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Various biomaterials have been developed for the regeneration of periodontal tissues, and in particular, long-term maintenance of materials such as collagen guided bone regeneration (GBR) membranes is required to maintain space. Collagen is a bioabsorbable biomaterial widely used in the production of GBR membranes, and the regenerative effect varies depending on the type, structure, cross-linking degree, and chemical treatment of collagen. For this study, BarriGen, a test material, was applied to beagle dogs and the alveolar bone regeneration effects of 4, 8, and 12 weeks were conducted in beagle dogs to evaluate the critical sized bone (CSD) healing to the beagle dog's mandibular alveolar bone defect for GBR membrane. After 12 weeks healing from extraction of the 2 - 4 premolar, 5 x 5 x 5 mm cubic shape CSD were formed and test materials were applied. After operation, clinical signs were monitored, and radiographic examinations were performed. After the animal sacrificed, micro-CT image analysis, histomorphometrical and histological examinations were performed. BarriGen reliably supports bone regeneration of bone graft materials from both anterior and posterior surfaces compared to commercial positive control.

Corresponding author : Joong-Hyun Kim

Keywords : Alveolar bone, Guided bone regeneration, Collagen, Membrane, Beagle

PS-E-032

Chronic brain–machine interface research in monkey using customized *in vivo* system

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Many studies have focused on brain-machine-interfaces (BMIs) chronically implanted in monkey cortex to allow control of prosthetic limbs. Brain signals or stimulated nerve cells were measured for brain disease treatment and brain research. We developed a custom in vivo system and surgical procedure suitable for chronically implanted minimally invasive hybrid electrocorticography (ECoG) electrode arrays in the brain. Cynomolgus macaque was implanted with a flexible, high-resolution neural interface system with biocompatibility and mechanical properties. This Neural Interface system was used to measure local field potential (LFP) without damaging the brain. Arrays were implanted in the arm representation areas and recorded in 25 channels. In this study, we present the results of a customized in vivo system and NHP brain recording using a developed electrode array.

Corresponding author : Joong-Hyun Kim

Keywords : Brain, Non-human primate, Electrode, Neural interface, Electrocorticography

PS-E-033

Standardization of Cynomolgus monkey (*Macaca fascicularis*) sperm analysis based on computer-assisted sperm analysis (CASA)

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Non-human primates (NHPs) are phylogenetically close to humans (Homo sapiens) and are appropriate animal models because of their physiological similarities to humans. Therefore, NHPs are studied in physiology, neuroscience, and reproductive biology. Cynomolgus monkeys (*Macaca fascicularis*) have menstrual cycles that are similar to those of humans. Consequently, we standardized the sperm of male cynomolgus monkeys, which can be used for developmental research. The purpose of sperm standardization is to assess the critical sperm quality of successful fertilization and proper embryonic development and to accurately assess reproductive ability. Semen from three cynomolgus monkeys was recovered using an automatic electric ejaculation system. The results of the representative sperm analysis indicators, such as motility, viability, morphology, and acrosome reaction, were used to standardize the sperm samples. Average motility (%) of sperm (n=3): rapid progressive, 38.8 ± 15.4; medium progressive, 49.9 ± 17.2; non-progressive, 11.3 ± 2.3; and immobile, 0.0 ± 0.0. Average vitality (%) of sperm (n = 3): live, 57.6 ± 13.3; dead, 42.4 ± 13.3 Average morphology (%) of sperm (n=3): normal, 53.5 ± 7.6; head defects, 9.3 ± 13.7; midpiece defects, 9.3 ± 0.7; tail defects, 21.2 ± 17.8; and cytoplasmic droplets, 0.3 ± 0.6. Average acrosome reaction non-treated group (%) of sperm (n=3): normal, 84.5 ± 5.6; and abnormal 15.5 ± 5.6. Average acrosome reaction treated group (%) of sperm (n=3): normal, 56.4 ± 1.5; and abnormal 43.6 ± 1.5. In conclusion, we confirmed our sperm analyzed data from cynomolgus monkeys, and the results presented are presumed to indicate reproductive ability.

Corresponding author : Ji-Su Kim

Keywords : Non-human Primates, Cynomolgus monkey, Reproduction, Sperm, CASA

PS-E-034

Luteolin supplementation during oocyte maturation improves developmental competence of cloned embryos by reducing oxidative stress in pigs

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Luteolin (Lut), a polyphenolic compound that belongs to the flavones subclass of flavonoids, possesses anti-inflammatory, cytoprotective, and antioxidant activities. However, little information is available on the role of Lut on mammalian oocytes. Therefore, the objective of this study was to examine the effect of Lut supplementation during *in vitro* maturation (IVM) on oocyte maturation and subsequent developmental competence after somatic cell nuclear transfer (SCNT) in pigs. Lut supplementation significantly increased proportion of complete cumulus cell expansion and metaphase II oocytes compared to the control. After SCNT, Lut-supplemented MII oocytes were significantly improved developmental competence in terms of the cleavage rate, blastocyst formation rate, hatching blastocyst rate, cell number, and cellular survival rate compared to the control. Lut-supplemented MII oocytes showed markedly lower reactive oxygen species levels than the control. Moreover, Lut supplementation activated the lipid metabolism including lipid droplet, fatty acid, and ATP levels compared to the control. Interestingly, Lut supplementation significantly increased active mitochondrial content and membrane potential and significantly decreased cytochrome c and cleaved caspase-3 levels compared to the control. These results suggest that Lut supplementation during IVM improves porcine oocyte maturation and subsequent embryonic development after SCNT by reducing oxidative stress and mitochondrial-mediated apoptosis and so could be used to enhance the production of transgenic pig for biomedical research.

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Corresponding author : Bo-Woong Sim

Keywords : Luteolin, Porcine oocyte maturation, Somatic cell nuclear transfer, Oxidative stress, Mitochondrial function

PS-E-035

Bisphenol A –induced developmental impairment can be alleviated with IGF-1 treatment in porcine embryos

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Bisphenol A (BPA) is a most well-known endocrine disruptor, easily accumulating in the body and causing cellular damage in various cell types. Many studies have shown that BPA has toxicity for embryonic development and implantation in mammals. However, it is unclear the mechanism of BPA toxicity in embryonic development. Therefore, we used a porcine embryo to investigate the effect of BPA during *in vitro* culture (IVC) stage. In our study, we used 25, 50, and 75 μM of BPA to assess the effective concentration of BPA during IVC stage. The result showed that 50 and 75 μM of BPA supplement significantly impaired the development rate and cellular survival rate compared to the control during IVC. Especially, 50 μM BPA significantly impaired the trophectoderm (TE) cell rate in blastocysts an important indicator for blastocyst quality. To examine the related mechanism of developmental impairment by BPA, we analyzed the growth factor signals of embryos according to BPA treatment. First, IGF-1, an important factor for TE formation and transplantation, was added to the IVC medium at various concentrations (50, 100, 200 μM) to confirm the optimum condition. 100 μM IGF-1 supplement significantly increased the developmental rate, cellular survival, and TE cell rate compared to the control. In addition, we confirmed whether IGF-1 restored the BPA-induced developmental impairment. The result showed that the decreased embryonic development caused by BPA was relived with IGF-1 supplement in IVC medium. The IGF-1 supplement also significantly restored the apoptosis rate and TE cell rate decreased by BPA. Taken together, our result shows that exposure to BPA impaired early embryonic development by inhibiting the activity of growth factor signaling, including IGF-1. These results are expected to be used as basic data for the inhibition of embryonic development by BPA.

This study was supported by the ministry of Education, Science and Technology (MEST) (No. 2018M3A9H1023142 and 2021M3H9A1096895)

Corresponding author : Bo-Woong Sim

Keywords : Bisphenol A, Porcine embryo, Development, In vitro culture, IGF-1

PS-E-036

Cadmium exposure causes defect in porcine oocyte maturation and subsequent embryonic development by inducing ER stress

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Cadmium (Cd) is one of the toxic metal that can lead to various diseases such as skeletal, cardiovascular, nervous, and reproductive system. However, the mechanism of Cd toxicity on the meiotic maturation of oocytes remains to be clarified. In this study, we investigated the effects of Cd exposure on the meiotic maturation of porcine oocytes and subsequent embryonic development and the underlying mechanism. Porcine cumulus-oocyte complexes (COCs) were cultured *in vitro* maturation (IVM) medium supplemented with Cd at concentrations of 0, 2.5, 5, and 10 μM and Tauroursodeoxycholic acid (TUDCA), an inhibitor of endoplasmic reticulum (ER) stress during IVM. After IVM, COCs were evaluated meiotic maturation, embryo developmental competence, and levels of ER stress by Cd treatment. Here, results showed that Cd exposure inhibited cumulus cell expansion, and meiotic maturation and increased oocyte lysis. After parthenogenetic activation (PA), the addition of Cd in IVM reduced cleavage and blastocyst formation rate in a dose-dependent manner. In addition, Cd exposure decreased the total number of blastomeres, while increasing the apoptosis rate. Moreover, the transcripts of the spliced XBP1, a representative marker of ER stress, were shown to be increased in COCs matured by Cd treatment during IVM. Interestingly, Cd-mediated reductions of cumulus cells expansion, meiotic maturation, and survival rates were ablated by TUDCA supplementation, and the negative effects of Cd on developmental competence of PA embryos were ameliorated by treatment with TUDCA in terms of the blastocyst formation rate, the total number of blastomeres, and rate of apoptosis in blastocysts. Furthermore, transcripts of sXBP1 were significantly reduced in TUDCA-treated COCs compared with Cd treated group. In conclusion, these results suggested that Cd exposure during IVM impaired meiotic progression of oocytes and subsequent embryonic development following parthenogenetic activation through ER stress induction.

This study was supported by the ministry of Education, Science and Technology (MEST) (No. 2018M3A9H1023142 and 2021M3H9A1096895)

Corresponding author : Bong-Seok Song

Keywords : Cadmium, Endoplasmic reticulum (ER) stress, Porcine oocyte maturation, Developmental competence

PS-E-037

Proposal of *in vitro* and *in vivo* studies design to investigate the effect of FLASH radiotherapy

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Radiation therapy is one of the best ways to treat cancer, but inevitably occurs damage of normal tissues. The key to success of radiation therapy is to kill only tumor without causing damage to the surrounding normal tissues. FLASH radiotherapy is the delivery of ultra-high dose rate radiation with several thousand times higher than that used in conventional radiotherapy. FLASH radiotherapy induces a phenomenon known as the FLASH effect, which reduced normal tissue toxicities commonly associated with conventional radiotherapy, while maintaining local tumor control. Considering this advantage, FLASH radiotherapy has emerged new technique in the radiation therapy clinics. However, the mechanisms of FLASH effect are not fully understood. To investigate the effect of FLASH radiotherapy, we aimed to set suitable *in vitro* and *in vivo* studies. When we proceed with FLASH radiotherapy in the future, we first conducted experiments through establishment of *in vitro* and *in vivo* models to determine which parts to consider. *In vitro* study used NCTC 1469 cell, which is normal mouse liver cell and BNL 1ME A.7R.1 cell, which is mouse liver cancer cell. To assess the difference in radiation response between NCTC 1469 and BNL 1ME A.7R.1 cells, we confirmed cell cycle analysis using flow cytometry and proteins regulated cell cycle progression by western blotting. As the result of *in vitro* test, we found that radiation response in liver cancer cell was more sensitive than that in normal liver cell. Next, *in vivo* test used a syngeneic mouse model of BNL 1ME A.7R.1 cell line. We intended to use BNL 1ME A.7R.1 syngeneic mouse model for three efficacy evaluation: liver toxicity, anti-cancer effect, and abscopal effect. In the future study, we planned to study the effect of FLASH radiotherapy using the established *in vitro* and *in vivo* models.

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Keywords : FLASH radiotherapy, Radiation response, Normal cell, Cancer cell

PS-E-038

Suggestions for writing SOPs for maintenance and calibration of GLP equipment

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Good Laboratory Practice (GLP) is the regulation that governs pharmaceutical research, requiring that pre-clinical studies in support of marketing applications to be conducted in accordance with its provisions. As a Standard Operating Procedure (SOP) is a document that describes tests and procedures that are not specified in detail in the protocol or test guidelines, it is required to be maintained to ensure the quality and integrity of the non-clinical study data. The U.S. FDA 21 Code of Federal Regulations, Part 58, Section 58.63 states, "The written standard operating procedures required under Section 58.81(b)(11) shall set forth in sufficient detail the methods, materials, and schedules to be used in the routine inspection, cleaning, maintenance, testing, calibration, and/or standardization of equipment.". In 1986, in order to ensure the reliability of toxicity study data for the approval of new drugs, South Korea first established the 'Regulations for Safety study for Pharmaceuticals, etc. (Health and Welfare Ministry Notification No. 1986-41)', and the GLP system is being operated since the first GLP test facility was designated in 1988. At that time, GLP test facilities have implemented the technical documentation of SOP for the optimal operation and management of equipment by applying the latest practices abroad, but it has to be admitted that the SOP for equipment has not reflected the subsequent revisions of GLP regulations, academic advancements, etc. since then. In particular, it did not fully include the maintenance and calibration of the equipment for data measurement or evaluation. For the reasons, internationality, practicality, and usefulness are to be applied in writing of SOPs for the operation and management of laboratory equipment of testing facilities, and also, user-friendly SOP writing skills reflecting careful planning and regulatory trends are to be suggested. In this review, it will be mainly introduced that considerations when writing SOPs for equipment maintenance and calibration, management of the procedural documents, process-oriented SOP design, and templates for the SOPs.

Corresponding author : Ju-Hyun Bae

Keywords : Equipment, Calibration, Maintenance, SOP

PS-E-039

Suggestions for efficient life-cycle management of GLP standard operating procedures

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A 'Standard Operating Procedure'(SOP) is defined as a document that describes the study processes and activities that are not generally specified in detail in a study plan or test guidelines. In particular, it is required that test facilities have a documented SOP approved by test facility management in order to secure the quality and integrity of raw data. SOP categories that are necessary include organization and personnel, Quality Assurance Unit(QAU), Receipt, Identification, Handling and Storage of Specimens, Maintenance and Management of Equipment, Facilities, Computerized System, etc. When writing SOPs, SOP codes are assigned to each category classified according to the characteristics of the Test Facility and study types to be performed in the Test Facility, such as Management of Studies and Test Facilities, Organization and Personnel, etc. After a list of SOPs for each code is established, the details of each SOP is to be written. According to Good Laboratory Practice regulations, once a SOP is written and reviewed by its drafter, it is reviewed by QAU and then finally reviewed and approved by the Test Facility Management. In general, the table of contents of the SOPs consists of a purpose, scope, responsibilities and authorities, procedures, annexes, etc. and it should be consistent among different codes of SOPs for maintaining uniformity. A valid SOP must be readily available to study personnel performing activities in laboratories. Procedures for distributing copies of the SOPs and retrieving expired versions of the previous SOPs should be clarified so that there should be no confusion in the use of the latest SOPs. SOPs can be revised at whenever according to academic progress, scientific basis, revision of test methods, etc. Careful consideration is required when revising SOPs, and the revision history must be documented so that it can be tracked. The previous version of the SOP will cease to be effective when the SOP revision is distributed. In this review, an efficient management of the SOP life-cycle, establishment-revision-disposal of SOPs, will be introduced

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Keywords : GLP, SOP, Life-cycle, SOP category, SOP code

PS-E-040

Predicting the abuse liability of new psychoactive compounds

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Drug abuse is a major public health problem as a chronic relapsing disorder characterized by compulsion to seek and take the drug despite severe harms. It would be very interesting to assess the abuse liability of novel compounds using behavioral paradigms in animals. We tested new psychoactive substances, desmethyl-8-bromo dragonfly (2C-B-Fly) and 4'-fluoro-4-methylaminorex (4-FPO), which are classified as phenethylamine class with very potent reward and sensitizing properties, in the conditioned place preference (CPP) and intravenous drug self-administration under FR1 schedule. Results showed that 2C-B-Fly produced a significant CPP in dose dependent manner in mice and self-administration at 0.05 mg/kg/infusion dose in rats, while 4-FPO induced CPP at 1 mg/kg (i.p.) in mice. These results suggest the possibility that these new psychostimulants have the rewarding and reinforcing effects.

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Corresponding author : Chae Ha Yang

Keywords : Abuse liability, CPP, Self-administration, 2C-B-Fly, 4-FPO

PS-E-041

Highly efficient human hematopoietic stem and progenitor cell generation from induced pluripotent stem cells using a simple scalable monolayer culture system

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Hematopoietic stem and progenitor cells (HSPCs) are responsible for the lifetime dynamics of hematopoiesis, as they are well known for their self-renewing ability and multipotency to differentiate into all types of blood cells including both myeloid and lymphoid lineages. They are a renewable source that can be used in various applications including cell therapy, disease modeling, and artificial blood production. However, due to their limited amount and accessibility, there is a strong need to search out for alternative methods to produce HSPCs. Recently, induced pluripotent stem cells (iPSCs) have been suggested as a new viable source for HSPCs production. Therefore, in this study, we developed a novel method for the efficient production of HSPCs using a modified simple and scalable monolayer culture protocol. Undifferentiated iPSCs and differentiated HSPCs were characterized through morphological, FACS, qRT-PCR and functional (CFU assay) analysis in a time dependent manner. Our data suggests that HSPCs were successfully generated with significantly enhanced production efficiency through the induction of hematopoietic endothelium (HE) and subsequent endothelial-to-hematopoietic transition (EHT). This process well resembles the definitive hematopoiesis, which allows the generation of true HSPCs with multilineage potential, including lymphocytes and mature erythrocytes. Further studies are in need to confirm the maturity and functionality of iPSC-derived HSPCs, yet in overall, this study was able to show the potential of large scale production of functional HSPCs from iPSCs for future clinical applications.

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Keywords : Induced pluripotent stem cell (iPSC), Hematopoietic stem and progenitor cell (HSPC), Hematopoietic Endothelium (HE), Endothelial-to-Hematopoietic Transition (EHT), Definitive Hematopoiesis

PS-E-042

Therapeutic efficacy of graphene quantum dots for alleviation of acute graft-versus-host disease in a xenogeneic mouse model

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Graphene derivates, especially graphene quantum dots (GQD) have excellent biocompatibility and antioxidant, anti-inflammatory effect, making it a potential candidate in biomedical research for diverse immune-related diseases, including graft versus host disease (GvHD). To investigate the effect of GQD for GvHD in the environment of functional cellular components of the human immune system, we studied the toxicity and immunomodulatory properties of GQD using human peripheral blood-derived mononuclear cells (hPBMCs) and xenogeneic GvHD mouse model. Graphene oxide with a size larger than 100nm showed a significant toxicity to hPBMCs but no detectable apoptosis occurred when less than 80ug/ml GQD treated. Furthermore, GQD suppressed proliferation of activated human T cells and the gene expression of proinflammatory cytokines including IL-1β, TNFα was down-regulated whereas the expression of anti-inflammation related genes including COX2, IDO, IL-10 was up-regulated. In particular, GQD inhibited differentiation to helper T cell 1 (Th1), and induced immune suppressive $CD4^{+}CD25^{high}Foxp3^{+}$ regulatory T cells. We developed a xenogeneic model of GvHD in immunodeficient mice by injecting hPBMCs, and GQD were subsequently administrated to study therapeutic effect of GQD. GvHD mouse models with GQD showed higher survival rate, less weight loss, as well as a significantly lower human blood cell proliferation rate in PB compared to a positive control group. These findings indicate that GQD attenuate immoderate immune reaction by regulating immune cells, implying GQD's potential therapeutic effect to reduce severity of GvHD.

Corresponding author : Kyung-Rok Yu

Keywords : Graft versus host disease, GvHD, Humanized mouse model, Graphene quantum dot, Immunosuppressive drugs

PS-E-043

Quantitative pharmacokinetic analysis using liquid chromatography–mass spectrometry (LC-MS/MS) and desorption electrospray ionization (DESI)-mass spectrometry imaging (MSI) in a rat transient MCAO model

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Ischemic stroke is one of the major causes of death and permanent disability in the world. Transient middle cerebral artery occlusion (MCAO) animal model in rats has been widely used for the efficacy studies for stroke. The extent of neurological injury in the MCAO model was measured by infarct size and blood-brain barrier (BBB) permeability. The most popular techniques for measuring infarct size are a 2,3,5-triphenyltetrazolium chloride (TTC) staining method. The extent of BBB breakdown is mostly determined by a spectrometry technique using Evans-blue staining. However, there are limitations to quantifying the extent of BBB disruption. The concentrations of the drugs that penetrate brain is still unknown and cannot be quantified. Therefore, as an alternative method, mass spectrometry was used to measure the extent of BBB permeability in a rat MCAO model. In this study, liquid chromatography–mass spectrometry (LC-MS/MS) and desorption electrospray ionization (DESI)-mass spectrometry imaging (MSI) were used for evaluating the distribution of drug in the brain of rat transient MCAO model using intraluminal suture MCAO. The drug concentration in brain tissue was quantified by LC-MS/MS and total brain-to-plasma concentration ratio was calculated. DESI-MSI were able to visualize drug distribution in the ischemic hemisphere of the brain. These quantifying methods were able to obtain accurate and statistically reliable results for the pharmacokinetic studies.

Corresponding author : Woori Jo

Keywords : MCAO, LC-MS/MS, DESI-MSI

PS-E-044

Biodistribution study of microplastics in mouse following intratracheal instillation by PET/CT

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Microplastics have been found to be persistent and ubiquitous pollutants in a variety of environments, including sea water, fresh water, soil, and air and consequently ingested and inhaled. There is also an increasing awareness that plastic fragments are dispersed in the air and can be inhaled by humans, which may cause adverse effects on the respiratory system and on other systems. An essential component of the study of the health effects of these pollutants is the accurate determination of their pharmacokinetic behavior in vivo.
In this study, we report the use of nuclear imaging to track inhaled microplastic particles in vivo. We have modified PVC particles with the chelator NODA-GA and radiolabeled NODA conjugated particles with the positron emitting radiometal copper 64 ($t_{1/2}$=12.7hr). The radiolabeled microplastic ([^{64}Cu]Cu-PVC, 18.5 MBq) was instilled intratracheally after the mice were anesthetized with isoflurane. Subsequently, positron emission tomography (PET) was used to visualize the biodistribution of radiolabeled plastics at 0, 3, 6, 24, 48, and 72 h after inhalation.
The imaging data reveal that radiolabeled plastics is only slowly cleared, with approximately 50% of inhaled particles persisting in the lungs after 72 h. These results suggest that large absorption and slow clearance may underlie toxicity from chronic and sustained exposure of microplastics in vivo. In addtion, this study suggests that PET imaging can be a sensitive and effective tool for studying in vivo behavior.

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Keywords : Radioisotope, Microplastics, PET/CT, Intratracheally instillation

PS-E-045

Glutathione (GSH) – sensitive magnetic resonance imaging (MRI) agent, a new indicator of Alzheimer's disease (AD) diagnosis

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Oxidative stress (OS) is implicated in Alzheimer's disease (AD) and mild cognitive impairment (MCI). The oxidative capacity of cell is dependent on its ability to produce glutathione (GSH) [1]. Especially, increase of oxidative stress related to AD has been attributed to decrease of the brain antioxidant, GSH [2]. Consequently, to detect GSH concentration in 5xFAD mouse brain reflects the degree of AD implying oxidative stress level.

To feasibility study of imaging for this event, the use of a contrast agent to detect molecular level is necessary. Chemical exchange saturation transfer (CEST) is one of MRI techniques, CEST MRI contrast is generated based on the decrease of the water signal intensity in target site in the body [3]. To overcome some limitations of general diaCEST agent, the paraCEST agents based on paramagnetic magnetic lanthanide ions, such as Europium (III) is used to increase the observable CEST effect.

In this study, the GSH expression levels of 5xFAD-AD mouse brain in 3-month, 6-month, and 9-month after birth were presented by immunohistochemistry (IHC) study. Also, we report a new type of GSH sensitive MR paraCEST agent, EuL by structure change of functional group in Eu (III) ligand. The fluorescence response of MRI agent to GSH was linearly dependent on the concentrations of GSH. And paraCEST signal intensity of EuL was decreased according to the concentrations of GSH.

Therefore, Eu-probe give promise of future MR paraCEST agents for GSH sensitive brain imaging, and the detection of GSH concentration may shed light on the degree of AD.

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Corresponding author : Yongmin Chang, Hee-Kyung Kim

Keywords : Alzheimer's disease (AD), Glutathione (GSH), ParaCEST agent, Magnetic Resonance Imaging (MRI)

PS-E-046

Performance study of anticoagulant (ASTM F1830) for establishment of in vitro blood circulation loop system

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In vitro blood circulation loop system is used to evaluate haemocompatibility and performance of medical devices in contact with blood. The origin of blood, storage conditions and anticoagulants to prevent clotting of blood are important factors to consider first to establish the system.
Common anticoagulants used in loop systems are listed in ASTM F1830. The anticoagulants listed in ASTM F1830 are the anticoagulants citrate dextrose solution A (ACD-A), citrate phosphate dextrose adenine anticoagulant solution (CPDA-1), and heparin. heparin is the most common anticoagulant used in ECMO, VAD, or dialysis therapies. Blood was collected from healthy rabbits, pigs, Rhesus monkeys, and Cynomolgus monkeys for evaluation of whole blood preserved in anticoagulants (ACD-A, CPDA-1 and heparin). After blood collection, the blood was stored in a blood refrigerator at 1~6℃. Samples were taken at 24, 48 and 72 hours. For each sample Red blood cells were observed over time and their morphological grade was recorded.
The morphological grade of erythrocytes tended to decrease gradually over time. Not significant difference was observed depending on the type of anticoagulant applied in the experiment but there were differences by species. The choice of anticoagulant may depend on the requirements for post-blood analysis or comparison with clinically relevant conditions.

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Keywords : Haemocompatibility, Anticoagulant, ISO 10993-4, ASTM F1830, In vitro blood circulation system

PS-E-047

Non-human primate diagnostic testing services of K-MEDI hub preclinical research center

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Non-human primate (NHP) is the most similar laboratory animals to human, and is generally used for preclinical research for neuroscience, ophthalmic and toxicity research. Recently, the use of NHP as animal models is increasing due to the development of modern medicine and the growth of research in the field of life science such as the development of new drugs and medical devices, and the value is constantly improving. In order to secure reliable and quality animal experiment results, it is important to use high-quality NHP in research, and it is essential to use healthy managed animals. Also, these are directly linked to the ethical handling of laboratory animal. Preclinical Research Center (PRC) of KMEDI-hub is a specialized research support facility in the field of animal experiments and providing health monitoring services of laboratory animal. In addition, PRC joined as a member of the "Performance Evaluation Program for Diagnostic Laboratories (PEP)" one of the Laboratory Animal Quality Network (LAQN) of International Council for Laboratory Animal Science (ICLAS) in order to provide better health monitoring. Health monitoring of NHP is using serological, molecular biological, and biochemical analysis method for diagnostic of microorganism such as virus, pathogenic bacteria, fungi and parasite. Furthermore, according to type pathogen, additional confirmatory test are performed to provide more reproducible and accurate results. We will continue to develop various diagnostic manuals for the maintenance and improvement of quality of NHP health monitoring to provide more accurate and reliable diagnosis services.

Corresponding author : Eui-Suk Jeong

Keywords : Non-human primate, Simian, Health monitoring, Diagnostic testing

PS-E-048

Characterization of a rat plantar heel pain model using fascial distortion and efficacy test

Na-Hye Park, Da-Sol Lee, Yoon Beom Lee, Hye Yoon Choi, Gwang-Hoon Lee, Tae-Gu Kang, Seonghwa Lee, Woori Jo*, Kil-Soo Kim*

Preclinical research center, Daegu-Gyeongbuk Medical Innovation Foundation (K-MEDI hub), Dae-gu, South Korea

Pain control is important to facilitate overall recovery, improve patient satisfaction, decrease morbidity, and reduce health care cost. Plantar Fasciitis is one of the most common causes of heel pain. It involves pain and inflammation of a thick band of tissue, called the plantar fascia, which runs across the bottom of foot which connecting heel bone to toes. It is necessary to set up the preclinical model reflecting plantar heel pain and establish an animal model that have close similarities to postoperative pain in patients and evaluate analgesic efficacy. Briefly, the rat is anesthetized and 1 cm longitudinal incision was made through plantar fasciitis skin, fascia and muscle of the plantar aspect of the hindpaw. The flexor digitorum brevis muscle was elevated and incised longitudinally, with the muscle origin and insertion remained intact. After hemostasis, the skin was closed with 4-0 black silk. Paw withdrawal threshold (PWT) was measured using von Frey filaments at same areas around the wound before surgery and for the next 3 days. The results of this study for PWT suggest that a surgical incision of the rat plantar heel pain causes a reliable and quantifiable mechanical hyperalgesia lasting for several days after surgery. After administration of positive drug, PWT value was increased which means the pain was relieved in the rat plantar heel pain model.

In conclusion, we characterized the rat plantar heel pain model, and established the platform for the analgesic efficacy test using von frey test.

Corresponding author : Woori Jo, Kil-Soo Kim

Keywords : Plantar heel pain model, Fascial distortion, Paw withdrawal threshold, Von frey test

PS-E-049

Clinical evaluation of rapid diagnostic test kit for canine parvovirus and coronavirus

Chaeyeong Min, Yong Lim*

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Canine parvovirus (CPV-2) and canine coronavirus (CCoV) are major pathogens that can induce gastroenteritis in dogs. Co-infection with CPV-2 and CCoV can affect the turnover of small intestine cells. Such co-infection is more fatal than a single infection by CPV-2 or CCoV. Prompt and accurate diagnosis is important because there is no specific treatment for such viral infections. Rapid diagnostic test (RDT) is prompt and easy to use for diagnosing an infectious disease early and preventing the spread of an infectious disease in the field. Nevertheless, it has a lower sensitivity than polymerase chain reaction, thus limiting the use of RDT. Additionally, RDT for animals has a low reliability for test results due to the lack of information about *in vitro* diagnostics, making it necessary to confirm the clinical usefulness of an RDT for animals. Thus, the aim of this study was to confirm the clinical utility of an RDT for diagnosing both CPV-2 and CCoV simultaneously through clinical evaluations. Items evaluated were: limit of detection (LoD), cross-reactivity, interference, sensitivity, specificity, and agreement. Results revealed that LoD values for CPV-2 and CCoV were 9.7 × 10 $TCID_{50}$/mL and 2.5 × 10^2 $TCID_{50}$/mL, respectively. Cross-reactivity by nine pathogens and interference by interfere materials were not observed. Compared with PCR or RT-PCR used as a reference method, RDT for the diagnosis CPV-2 and CCoV showed a sensitivity of 90.0 % and a specificity of 100.0 %. Agreement between the two methods showed a kappa value of 0.900, indicating a high agreement. Since the RDT developed in this study could concurrently detect both CPV-2 and CCoV, it will be useful as a screening test to prevent the spread of CPV-2 and CCoV infection through prompt and accurate diagnosis in the field.

Corresponding author : Yong Lim

Keywords : Rapid diagnostic test, Sensitivity, Specificity, Canine parvovirus, Canine coronavirus

PS-E-050

Evaluation of biocompatibility for electrospinning PLLA-based absorbable dural substitute in rat brain dura mater defect model

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Purpose: In intracranial surgeries, the dura mater must be repaired by bio-scaffold, to prevent the outflow of cerebrospinal fluid, brain edema, or infection. Among the bio-scaffolds, natural-derived materials may not be easy to operate. And they may have antigenicity in the living body, so it is necessary to develop a new bio-scaffold using the advantages of synthetic materials. This study evaluated the biocompatibility by applying a newly developed synthetic electrospinning PLLA-based absorbable dural substitute (PADS), comparing it with a predicate device (Redura®, Medprin biotech, Germany) to a rat brain dural defect model.
Results: All rats showed no behavioral abnormalities and were euthanized at Week 4. As a result of histological analysis (H&E stain), the dural substitution was well maintained in the defect and showed connectivity with the surrounding dura mater. The degree of cell infiltration inside the graft was high in both the T group (PADS) and the R group (Redura®), and malformed cell morphology was rarely observed. Comparing the density of the infiltrated cell nucleus, the degree of infiltration was lower in the T group to an insignificant degree.
Conclusion: This study evaluated the biocompatibility of the newly developed electrospinning PLLA absorbable dural substitute. As a result, the biocompatibility was similar as there was no significant difference in gross findings and histology compared to the Redura®. For further study, a long-term study on graft absorption will be needed.
References: Lima, Frederico de Melo Tavares de, et al. "Biocompatible bacterial cellulose membrane in dural defect repair of rat." Journal of Materials Science: Materials in Medicine 28.3 (2017): 1-7.
Acknowledgment: This study was conducted with research funds from the Advanced Technology Center+ (Grant no. 20014237) dedicated to the Korea Evaluation Institute of Industrial Technology (KEIT), an affiliate of the Ministry of Trade, Industry, and Energy.

Corresponding author : Se Eun Kim

Keywords : PLLA, Electrospinning, Absorbable dural substitute, Dura mater defect model, Rat

PS-E-051

Advanced quarantine program of non-human primate in K-MEDI hub

Gwang-Hoon Lee[1], Yoon Beom Lee[1], Tae-Ku Kang[1], Da-Sol Lee[1], Na-Hye Park[1], Hye-Yoon Choi[1], Woori Jo[1*], KilSoo Kim[1,2*]

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[2]Department of Veterinary Toxicology, College of Veterinary Medicine, Kyungpook National University, Daegu, South Korea

Non-human primates (NHPs) are important species for the development of new drugs and medical devices in the field of efficacy or performance studies due to the similarities to human. The quarantine program is the first step before starting research using NHPs and the procedures contain the steps for eliminating zoonotic diseases. In this study, three cynomolgus monkeys were introduced to preclinical research center in K-MEDI hub. Monkeys are anesthetized after visual inspection. Under anesthesia, body weights were measured and took a medicated bath at 37°C. Collections for blood and feces, cystocentesis were performed to test complete blood count, biochemistry, urinalysis, and microbiology. After then, purified protein derivative skin (PPD) skin test was performed to diagnose silent tuberculosis. Auscultation, measuring body temperature, physical examination, and dental examination were conducted and ivermectin was injected subcutaneously. Food intake and clinical symptoms were checked daily and body weights are measured on the 15th day. On the 29th day, monkeys were anesthetized again and conducted tests for complete blood count, biochemistry, urinalysis, auscultation, body temperature, and physical examination. On the 30th day, all the results were analyzed to determine the end of quarantine. The negative results for PPD skin test, B virus, Mycobacterium tuberculosis, Varicella virus, Salmonella SPP, Shigella spp., Yersinia spp., ectoparasites and endoparasite. Food intake was low at the beginning of quarantine, but gradually increased, and became normal at the end day of quarantine. Soft stools were found in monkeys up to the 12th day of the quarantine. The results of complete blood count test, biochemistry test, urinalysis, and dental examinations were normal, however, abrasions, microbleeds in the ear canal, and mild corneal damages were found under physical examinations. The clinical symptoms were treated with povidone disinfection and eye protection drops. After quarantine, these three monkeys will be used for the pharmacokinetic studies for exosomes, and the following other 5 monkeys will be additionally introduced at the end of this year for drug delivery studies in eyes and ears in the K-MEDI hub.

Corresponding author : Woori Jo, KilSoo Kim

Keywords : Non-human primates, Monkeys, Quarantine, K-MEDI hub

PS-E-052

HepaRG cell line as an in vitro model for measurement of CYP450 enzyme activity by metabolic induction

Byung-suk Jeon, Nam-Ju Kim, Ji-Hyun Bang, Hee Yi, Moon Her, Hyun-Ok Ku*

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Drug-induced liver injury (DILI) and drug-drug interactions (DDIs) are concerns when developing safe and efficacious compounds. Most of drug metabolism happens in the liver by Cytochrome P450 (CYP450) isozymes and the metabolism by CYP450 enzymes. The prediction of DDIs mediated by the induction or inhibition of CYP450 enzymes is of great relevance in the development of new drugs. Since HepaRG, a new human cell line derived from a hepatocellular carcinoma, is being considered a promising model to evaluate the in vitro metabolism of drugs, it was herein used for investigating metabolic-based drug interactions mediated by metabolic induction. In this study, omeprazole and rifampicin were used as probe inducers of acetaminophen (AAP) and amiodarone (ADR) as a model drug. HepaRG cells were firstly seeded in the supplemented Williams' E, and then differentiated in the same culture medium, supplemented with 2% dimethyl sulfoxide for 2 weeks. For metabolic induction studies AAP and ADR were incubated during 24 h in HepaRG cells which were pre-incubated with omeprazole or rifampicin for 24 h. This study evidenced that omeprazole and rifampicin are powerful inducers of the metabolism of AAP and ADR, including at therapeutic drug concentrations. These experimental findings demonstrated, the applicability of HepaRG cells as a useful in vitro model for the prediction of metabolic-based DDIs, namely those mediated by metabolic induction. Thus, this model could potentially be a worthy alternative to the primary human hepatocytes.

Corresponding author : Hyun-Ok Ku

Keywords : HepaRG, Drug interaction, Metabolites, Alternative methods

PS-E-053

Combined regenerative effect of human umbilical cord blood-derived mesenchymal stem cells, polydeoxyribonucleotides, and microcurrent therapy on chronic rotator cuff tear in a rabbit model

Dong Rak Kwon[1*], Yong Suk Moon[2]

[1]*Department of Rehabilitation Medicine, Muscle Research Center, Catholic University of Daegu School of Medicine, Daegu, South Korea.*
[2]*Department of Anatomy, Catholic University of Daegu School of Medicine, Daegu, South Korea*

Objective: To investigate synergic therapeutic effects of combined injection of intralesional mesenchymal stem cells derived from human umbilical cord blood (UCB-MSCs) and polydeoxyribonucleotide (PDRN) combined with microcurrent therapy (MIC) on full thickness rotator cuff tendon tear (FTRCTT) in rabbit models.
Methods: Thirty-two rabbit models were assigned to 4 different groups. FTRCTT in the supraspinatus tendon was created. After 6 weeks, 4 types of procedures (0.2 mL normal saline injection, group 1 (G1-NS); 0.2mL SC injection, group 2 (G2-MSC); 0.2 mL SC and weekly four injections of 0.2 mL PDRN with sham MIC, group 3 (G3-MSC+PDRN+sham MIC); and 0.2 mL SC and weekly four injections of 0.2 mL PDRN with MIC for four weeks, group 4 (G4-MSC+PDRN+MIC) were performed in FTRCTT. All injections were administered under ultrasound guidance. Euthanasia was performed on all the rabbits 4 weeks after first injection. Gross morphologic changes were evaluated before and 4 weeks after the treatment in all of the rabbits. The tendon tears were identified as either partial-thickness tendon tear or full-thickness. Each supraspinatus tendon tear healing was classified as nearly complete healing (regeneration of tendon > 80%), partial healing (5% < regeneration of tendon < 80%), and no healing (regeneration < 5%). Histological changes of proliferating cell nuclear antigen (PCNA), vascular endothelial growth factor (VEGF) and platelet endothelial cell adhesion molecule (PECAM-1), and motion analysis were performed.
Results: There was a significant difference in gross morphologic changes between baseline and week 4 post-treatment in group 4 compared to the other three groups (p=0.01)
In group 3 and 4, all parameters of histochemical and motion analysis have been found to be significantly greater than ones in group 1 and 2 ($p < 0.05$). In group 4, PCNA, VEGF, and PECAM-1 stained cells, as well as walking distance were significantly greater than ones in group 3 ($p < 0.05$).
Conclusion: The treatment with UCB-MSCs and PDRN combined with MIC might be the most effective in rabbit models' traumatic FTRCTT.

Corresponding author : Dong Rak Kwon

Keywords : Human umbilical cord blood-derived mesenchymal stem cells, Polydeoxyribonucleotides, Microcurrent therapy, Rotator cuff

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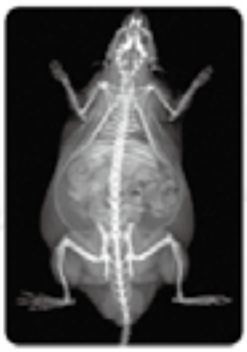
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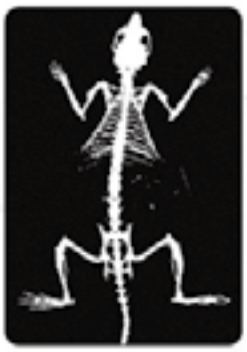
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SCID	Prkdc^{scid} (Chromosome16)	Yes (Albino)	No	No	Functional	Functional
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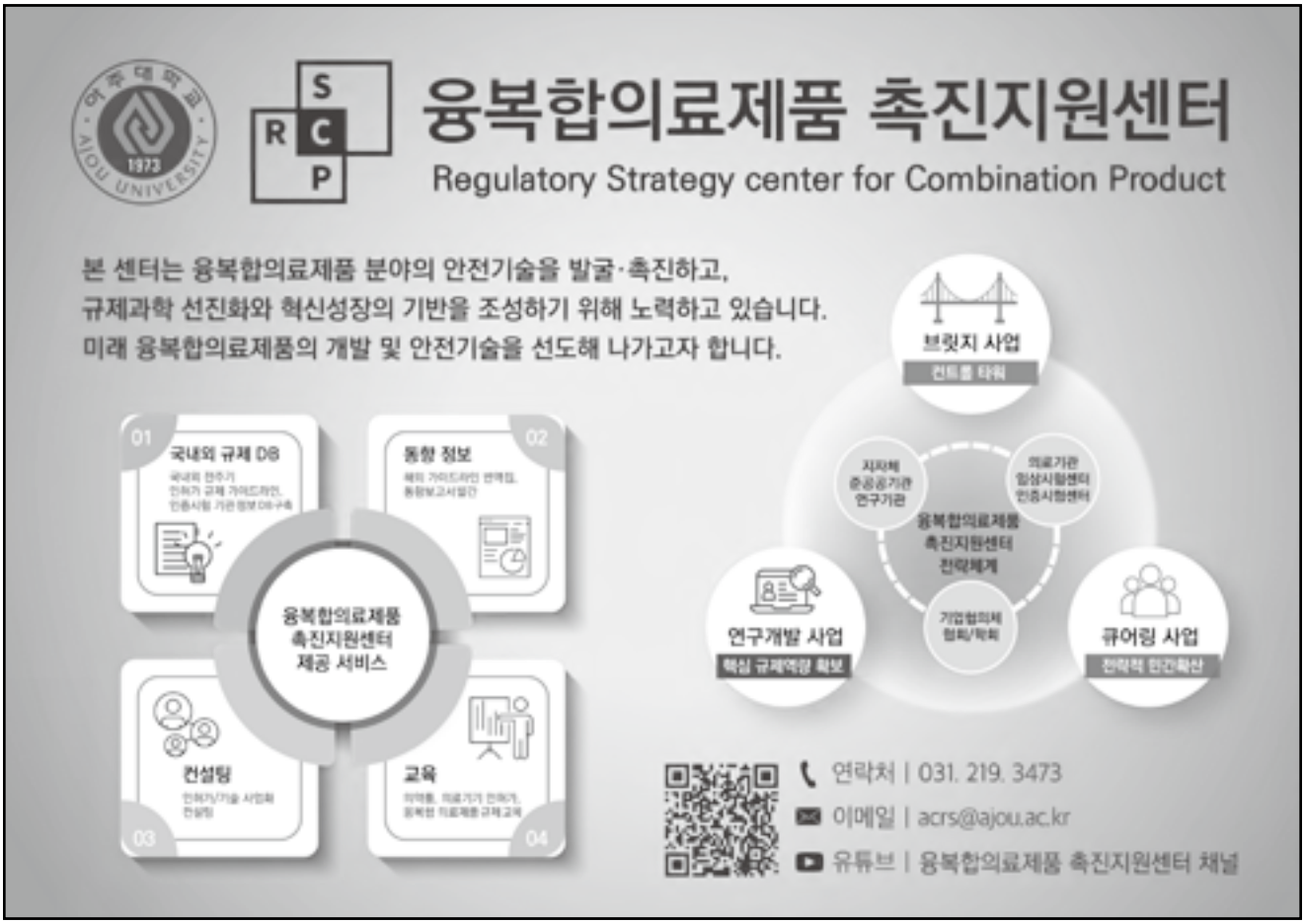
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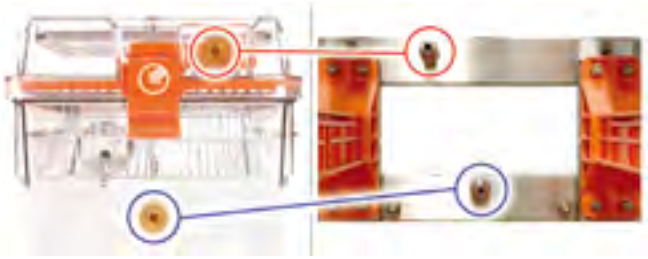

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