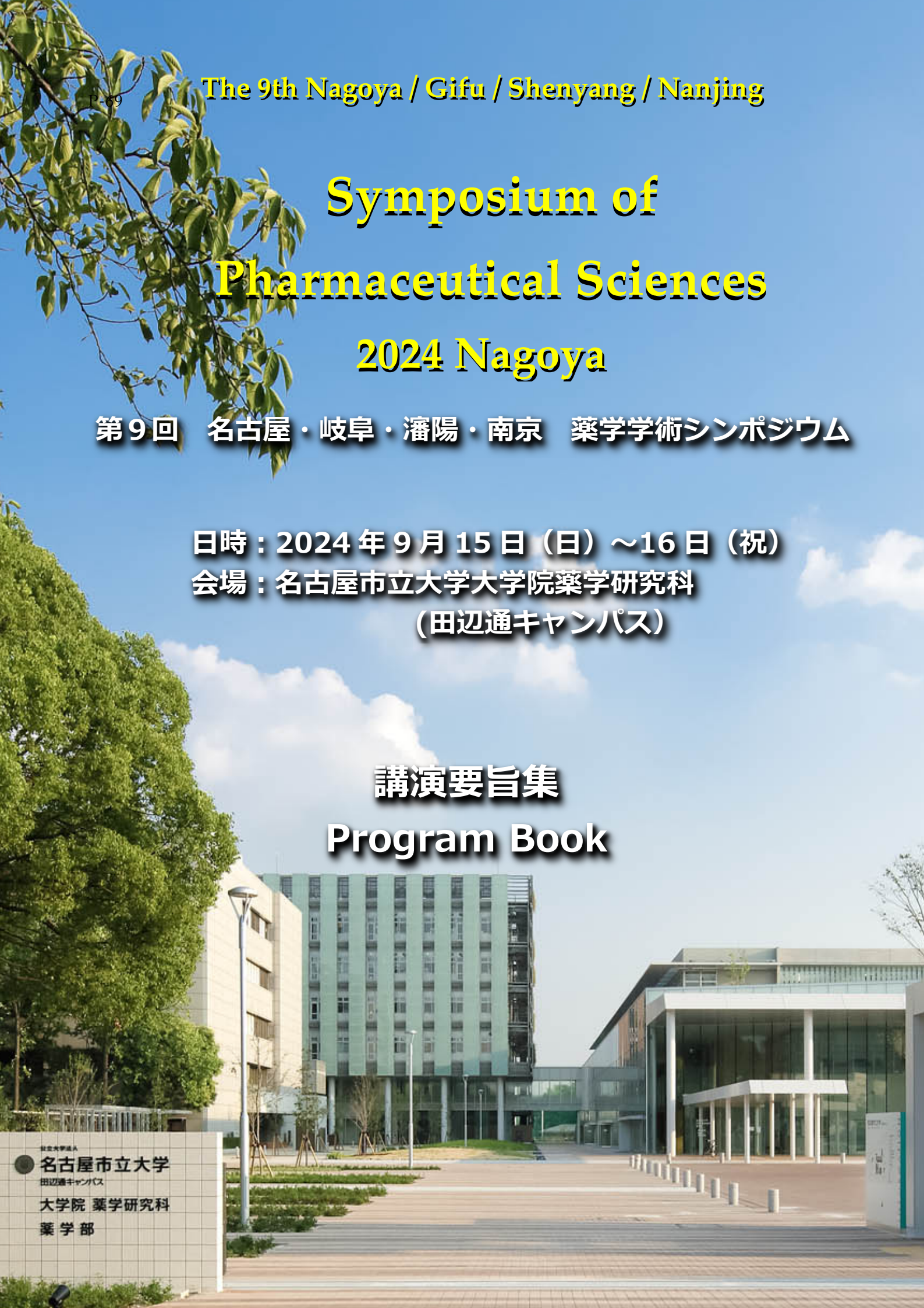
The 9th Nagoya / Gifu / Shenyang / Nanjing

Symposium of Pharmaceutical Sciences 2024 Nagoya

第９回　名古屋・岐阜・瀋陽・南京　薬学学術シンポジウム

日時：2024 年 9 月 15 日（日）～16 日（祝）
会場：名古屋市立大学大学院薬学研究科
（田辺通キャンパス）



講演要旨集
Program Book

FREUND 60 years ANNIVERSARY

原薬開発の約80%が 難溶解性原薬…
ナノ化が製剤開発の未来を照らす

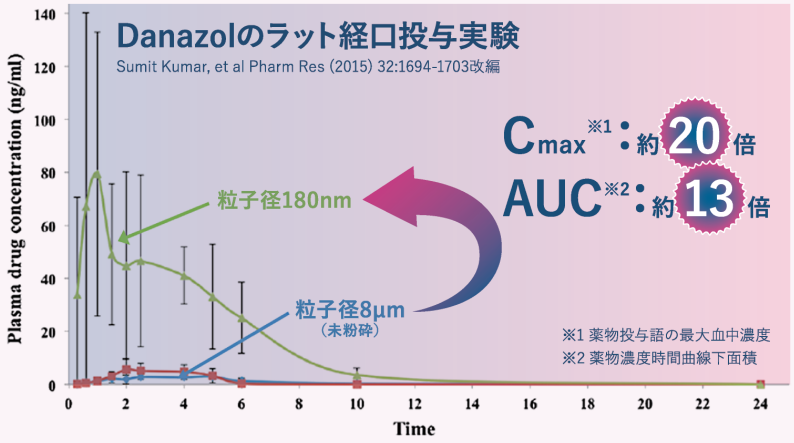
湿式シールレスビーズミル

Apex Mill F&M

金属系コンタミ 1/30 に抑制※　処理速度 1.6 倍※

※共に従来製法比

Value1 難溶解性原薬をナノサイズ化して薬剤吸収性を改善



難溶解性薬物の課題

- ☑ バイオアベイラビリティ小
- ☑ 食事の影響大
- ☑ 吸収のバラツキ大
- ☑ 粘膜作用がある添加剤

〈出典〉Title:Formulation and Performance of Danazol Nano-crystalline Suspensions and Spray Dried Powders
Journal:Pharm Res (2015) 32:1694–1703,
Author:Sumit Kumar & Rajan Jog & Jie Shen & Banu Zolnik & Nakissa Sadrieh & Diane J. Burges
Formulation and Performance of Danazol Nano-crystalline Suspensions and Spray Dried Powders | Pharmaceutical Research (springer.com)

Value2 低融点化合物でも 200nm以下の粉砕が可能

モデル薬物	融点(℃)	溶解度(μg/mL)
スルファメトキサゾール	168	610
イトラコナゾール	168	0.01
メフェナム酸	230	20
フェニトイン	295	32
フェノフィブラート	83	0.25

Value3 錠剤の崩壊性が良く 薬物溶出時間が短い

FL法

FL法は、ビーズミルによる粉砕スラリーを流動層造粒工程でスプレーし、適当な賦形剤に粉砕薬物を担持させ、打錠用顆粒を調製する手法です。
乾燥工程を軽減させ、打錠性の良好な造粒物を得られ、流動層造粒をしているため崩壊性に優れ、溶出時間も短い錠剤を作成できます。

FREUND フロイント産業株式会社 https://www.freund.co.jp

本社
〒160-0023
東京都新宿区西新宿6丁目
25-13 フロイントビル
(03)6890-0769

大阪事業所
〒564-0051
大阪府吹田市豊津町9番1号
EDGE江坂 13階
(06)6338-6745

浜松事業所・
技術開発研究所
〒431-2103
静岡県浜松市浜名区新都田1-2-2

製品についてのご質問や
お問い合わせはこちら▶
Email:cssp@freund.co.jp

The 9th Nagoya / Gifu / Shenyang / Nanjing

Symposium of Pharmaceutical Sciences

2024 Nagoya

第９回　名古屋・岐阜・瀋陽・南京　薬学学術シンポジウム

日時：2024 年 9 月 15 日（日）～16 日（祝）

会場：名古屋市立大学大学院薬学研究科

(田辺通キャンパス）

講演要旨集

Program Book

The 9th Nagoya / Gifu / Nanjing / Shenyang
Symposium of Pharmaceutical Sciences, 2024 Nagoya

Organizer: Symposium Organizing Committee in Nagoya City University.
Co-organizers: Faculty of Pharmacy, Meijo University.
Gifu Pharmaceutical University.
China Pharmaceutical University.
Shenyang Pharmaceutical University.
Lyceums: Consulate General of the People's Republic of China in Nagoya.
The Pharmaceutical Society of Japan and their Tokai Branch Office.
The Society of Powder Technology, Japan. Division of Particulate Design and Preparations.
Aichi Pharmaceutical Association.

Organizing Committee:
Dean of Graduate School of Pharmaceutical Science, Nagoya City University
Prof. Hidehiko NAKAGAWA
Secretariat General
Prof. Toshiaki MAKINO (Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University)

Committee Members:
Graduate School of Pharmaceutical Sciences, Nagoya City University
Prof. Kazuhiko KUME (Department of Neuropharmacology)
Prof. Tetsuya OZEKI (Department of Drug Delivery and Nano Pharmaceutics)
Prof. Hiroaki YUASA (Department of Biopharmaceutics)
Assoc. Prof. Hirokazu YAGI (Department of Multilevel Biofunctional Analytics)
Assoc. Prof. Tsuyoshi UDAGAWA (Department of Biological Chemistry)
Assoc. Prof. Jun TOMITA (Department of Neuropharmacology)
Faculty of Pharmacy, Meijo University
Prof. Yukihiro NODA (Division of Clinical Sciences and Neuropsychopharmacology)
Prof. Hiroyuki KAMEI (Office of Clinical Pharmacy Practice and Health Care Management)
Assoc. Prof. Susumu IMANISHI (Analytical Chemistry)
Assoc. Prof. Yoshiaki TAKAYA (Department of Natural Product Chemistry)
Gifu Pharmaceutical University
Prof. Yukihiro ESAKA (Department of Pharmaceutical Analytical Chemistry)
Assoc. Prof. Naohito ABE (Department of Pharmacognosy)
Assoc. Prof. Ayumi MATSUKA (Department of English Studies)

Welcome Message

It is my great pleasure to welcome all the participants of the 9th Nagoya-Nanjing-Shenyang Symposium on Pharmaceutical Sciences convened on September 15th and 16th at the Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya Japan.

The Japan-China Symposium was first held in 1998, and it was followed by seven more until 2018. Then, now in 2024, we will have 9th symposium with all the alliance member universities in-person after the long break from the 8th symposium in 2018 due to pandemic. Beyond such patient days, we are now anticipating warming up again our interaction and communication for the development of pharmaceutical sciences in both countries.

For this opportunity, we are now planning to make the symposium attractive and interesting, and so that this coming symposium will be a nice platform for scientific and innovative exchange to all the participants from the member universities. Furthermore, I hope this symposium an important opportunity for fermenting warm relationship and mutual understanding of all of us. To accelerate scientific communications, we anticipate coming to our campus in Japan and to have a plenty of oral and poster presentations and discussions.

Again, we hope to see many professors, scientists, and students from all alliance universities in our campus and join fruitful scientific communication and discussion, to encourage further development of sciences and technologies in pharmaceutical sciences for all the participant of this symposium. We are looking forward to seeing all of you in Japan soon.

Prof. Hidehiko Nakagawa, Ph.D.
Dean of Graduate School of Pharmaceutical Science
Nagoya City University

Symposium Information

1. Venue

Graduate School of Pharmaceutical Sciences, Nagoya City University
(Tanabe-Dori Campus)
3-1 Tanabe-Dori, Mizuho-ku, Nagoya 4678603, Japan
TEL: +81-52-836-3402 / FAX: +81-52-834-9309
https://www.nagoya-cu.ac.jp/phar/english/

2. Office

Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University
TEL & FAX: +81-52-836-3416
E-mail: makino@phar.nagoya-cu.ac.jp

3. Registration and Banquet fee

Free for the participants from Shenyang Pharmaceutical University and China Pharmaceutical University.
For faculties from Nagoya City, Meijo, and Gifu Pharmaceutical Universities, the registration and banquet fee are 5,000 JPY and 3,000 JPY, respectively.
For students (undergraduate and graduate) from Nagoya City, Meijo, and Gifu Pharmaceutical Universities, the registration fee is free, and the banquet fee is 500 JPY.

For the participants already registered via Organizing Committee
Please show your name and your university at the registration desk.

Registration on site:
Please write your personal information on the registration sheet, and pay the registration and banquet fee at the registration desk.
Registration desk is open 8:30 – 17:30 on September 15,
and 8:30 – 12:00 on September 16.

4. Tanabe-Dori Campus, Nagoya City University

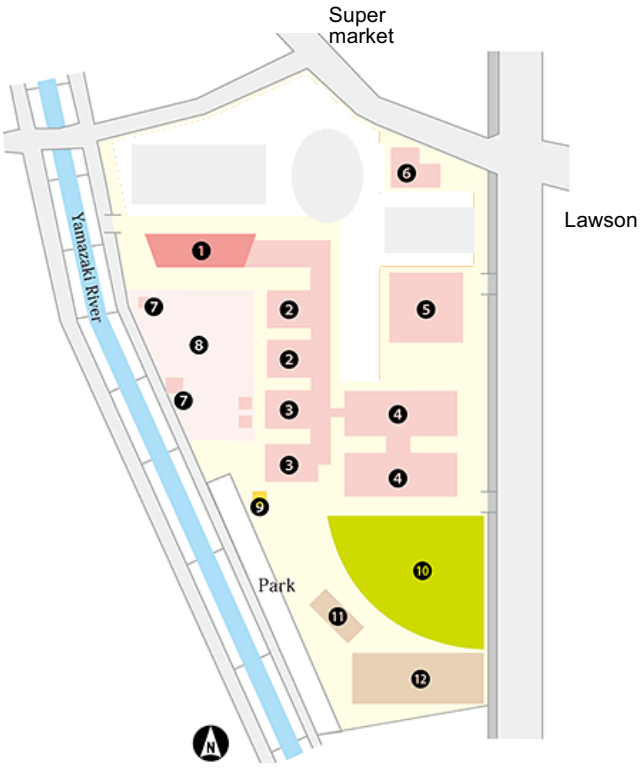
From Chubu Centrea International Airport to Nagoya City: About 30 min by Meitetsu train to Kanayama Station and 35 min to Nagoya Station.

From Nagoya Station to the campus: 22 minutes by Subway Sakuradori Line to Mizuhokuyakusyo (瑞穂区役所) station. Then 15 min walk from the Station. Subway runs almost every 10 minutes, and 270 JPY.

From Kanayama Station to the campus: 25 minutes by Nagoya City Bus #14 or #16 to Shidaiyakugakubu (市大薬学部) bus station.
Each bus runs almost every 30 minutes, and 210 JPY.

From Sakae Station in the downtown of Nagoya City to the campus: 30 minutes by Nagoya City Bus #20 to Shidaiyakugakubu (市大薬学部) bus station.
Each bus runs almost every 1 hour, and 210 JPY.

Please check https://www.kotsu.city.nagoya.jp/en/pc, and you can choose languages.

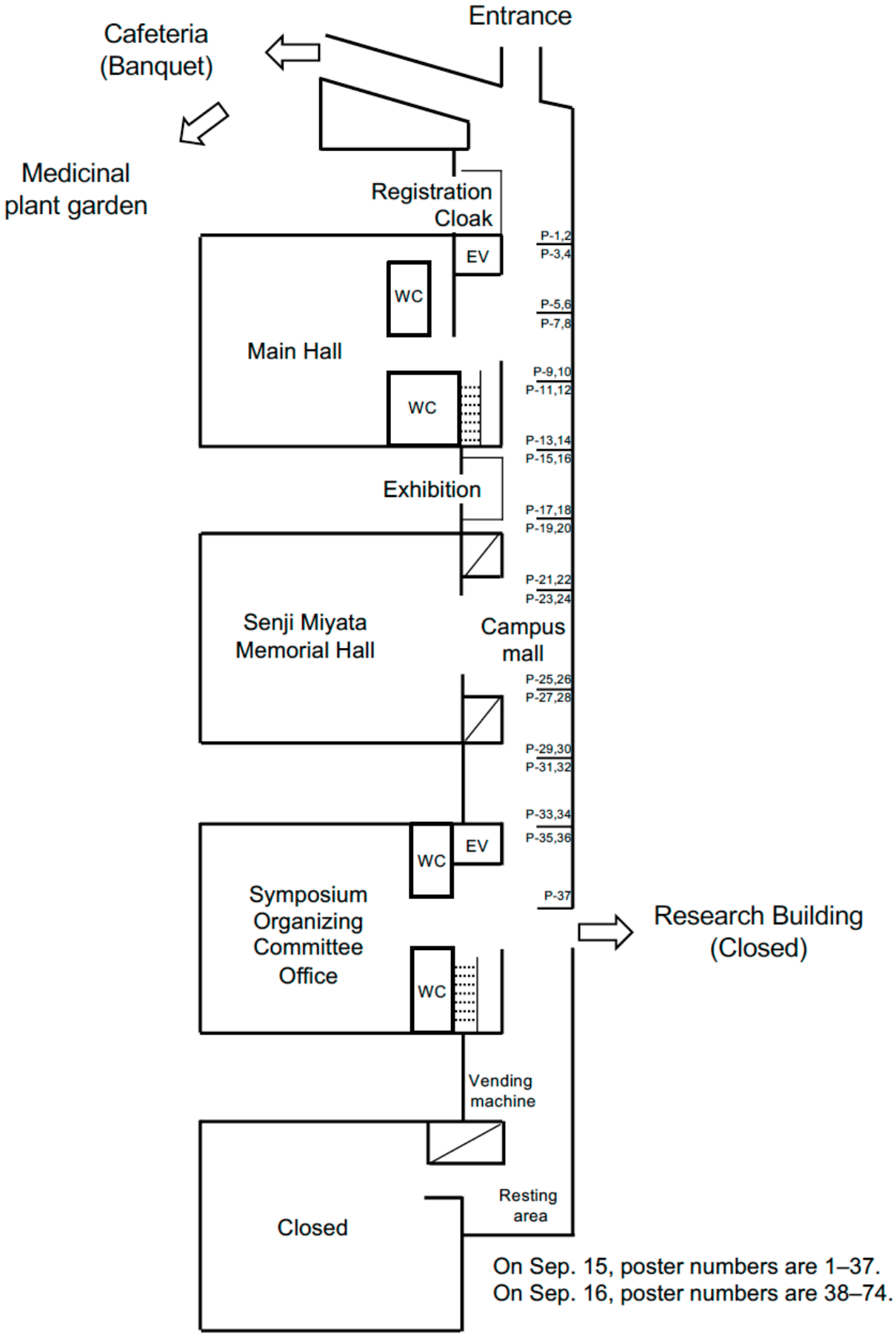


1. Cafeteria (Banquet)
2. Main Hall and Senji Miyata Memorial Hall
3. Pharmacy and practical room for training

The open space (lobby, corridor etc.) in front of #2 and #3, it calls "Campus Mall", and poster sessions will be held there.

8. Medicinal plant garden

Main Hall and Senji Miyata Memorial Hall



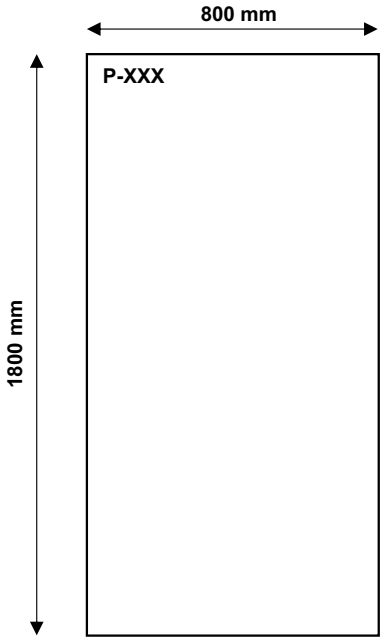
6. Cloak

Cloak is open 8:30 – 17:30 on September 15, and 8:30 – 12:30 on September 16 at the registration desk.

7. General Information for Poster Presenters

Poster Specifications:

Each poster presenter will be provided with a space in which a poster can be mounted. Each poster must be within 1800 mm height and 800 mm width. Please drop your poster number at the upper left area (about 15 cm × 15 cm). Supplies to hang posters will be available to you onsite (push pins).



Poster Session Schedule:

All posters will be set up at Campus mall.

Presenters MUST stand in front of their posters during scheduled poster sessions to discuss with participants.

There are no chairpersons in scheduled poster sessions, and you can talk with participants freely.

There will be four separate Poster Session as follows:

- ODD poster numbers in P-1 –P-37: 14:15 – 14:55 on September 15.
- EVEN poster numbers in P-1 –P-37: 16:48 – 17:28 on September 15.
- ODD poster numbers in P-38 –P-74: 10:06 – 10:46 on September 16.
- EVEN poster numbers in P-38 –P-74: 11:04 – 11:44 on September 16.

Set-Up:

Each poster can be set up from 8:30 to 9:30, and kept it by 17:28 on September 15 or by 12:10 on September 16.

If you do not remove your poster after 19:30 on September 15 (P-1 – 37) or 13:00 on September 16 (P-38 – 74), your poster will be discarded.

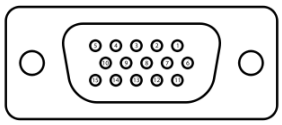
8. General Information for Oral Presenters

Digital projector:

Organizing Committee will prepare note-type computers installed with Windows

10 and PowerPoint 2020 that is connected with digital projectors to conduct oral presentation.
If you would like to use our computers, please bring your PowerPoint data in USB memory at the symposium.

You can also use your computers to connect our digital projectors.
Since our digital projector has only three-row 15-pin DE-15 VGA connector like this figure, not HDMI,
we will prepare the adapter from HDMI to VGA.



In Japan the standard voltage and frequency is 100 V and 60 Hz.
The power sockets that are used are of type A.
Please prepare the power sockets and corresponding plugs.

Plenary lectures are for 45 min, and general presentations are for 10 min plus discussion 2 min.

9. Language

English is official language of this symposium.

10. Lunch

On Sep. 15 and 16, School Cafeteria in Tanabe-Dori campus is closed.
You can buy foods at a supermarket in front of Tanabe-Dori campus or a convenience store Lawson at the east side of the campus.

11. Banquet

Place: Cafeteria in Tanabe-Dori Campus, Nagoya City University.
Date and Time: 17:40 on September 15.

12. Time table

Date	Time	Senji Miyata Memorial Hall	Main Hall
2024/9/15		Opening Address	
	9:30 – 9:35	Greeting from Prof. Hidehiko NAKAGAWA, Dean of Graduate School of Pharmaceutical Sciences, Nagoya City Univ.	
	9:35 – 9:40	Greeting from Mr. JI Wenbin, Vice Consul General, Consul in Consulate General of the People's Republic of China in Nagoya	
	9:40 – 9:45	Greeting from Prof. Hiroyuki KAMEI, Vice Dean of Faculty of Pharmacy, Meijo Univ.	
	9:45 – 9:50	Greeting from Prof. Yukihiro ESAKA, Chair of Internatonal Communication Committee of Gifu Pharmaceutical Univ.	
	9:50 – 9:55	Greeting from Prof. GAO Mingyu, Secretary General of Shenyang Pharmaceutical Univ.	
	9:55 – 10:00	Greeting from Prof. YUAN Hao, Vice Chancellor of China Pharmaceutical Univ.	
	10:00 – 10:10	Group Photo	
		Oral session 1 (10:15 – 10:51)	Oral session 7 (10:15 – 10:51)
	10:15 – 10:27	O-1	O-18
	10:27 – 10:39	O-2	O-19
	10:39 – 10:51	O-3	O-20
	10:55 – 11:40	PL-1 (Prof. Matsunaga, Nagoya City Univ.)	
	11:40 – 12:45	Lunch	
	12:45 – 13:30	PL-2 (Prof. Fu, Shenyang Pharmaceutical Univ.)	
	13:30 – 14:15	PL-3 (Prof. Jiang, China Pharmaceutucal Univ.)	
	14:15 – 14:55	Poster session P1 – P37 (odd number)	
		Oral session 2 (14:55 – 15:31)	Oral session 8 (14:55 – 15:43)
	14:55 – 15:07	O-4	O-21
	15:07 – 15:19	O-5	O-22
	15:19 – 15:31	O-6	O-23
	15:31 – 15:43		O-24
		Oral session 3 (15:33 - 16:09)	
	15:33 – 15:45	O-7	
	15:45 – 15:57	O-8	
	15:57 – 16:09	O-9	
			Oral session 9 (16:00 – 16:48)
	16:00 – 16:12	Oral session 4 (16:12 – 16:48)	O-25
	16:12 – 16:24	O-10	O-26
	16:24 – 16:36	O-11	O-27
	16:36 – 16:48	O-12	O-28
	16:48 – 17:28	Poster session P1 – P37 (even number)	
	17:40 – 19:30	Banquet	

2024/9/16		Oral session 5 (9:30 – 10:06)	Oral session 10 (9:30 – 10:06)
	9:30 – 9:42	O-13	O-29
	9:42 – 9:54	O-14	O-30
	9:54 – 10:06	O-15	O-31
	10:06-– 10:46	Poster session P38 – P74 (odd number)	
		Oral session 6 (10:40 – 11:04)	Oral session 11 (10:40 – 11:04)
	10:40-– 10:52	O-16	O-32
	10:52-– 11:04	O-17	O-33
	11:04 – 11:44	Poster session P38 – P74 (even number)	
	11:50 – 12:00	Closing Address, Dean Prof. Nakagawa, Nagoya City Univ.	

13. Program

Plenary lectures

10:55 – 11:40

Chair: Prof. Xiaohong Hou (Shenyang Pharmaceutical University)

PL-1 Development of human iPS cell-derived cells useful for drug metabolism and pharmacokinetic studies
Tamihide MATSUNAGA (松永民秀)
Graduate School of Pharmaceutical Sciences, Nagoya City University

12:45 – 13:30

Chair: Prof. Professor JIANG Hulin (China Pharmaceutical University)

PL-2 Artificial mucus layer formed in response to ROS for oral treatment of inflammatory bowel disease
Qiang FU (付强)
Wuya College of Innovation, Shenyang Pharmaceutical University

13:30 – 14:15

Chair: Prof. Tetsuya OZEKI (Nagoya City University)

PL-3 Multi-organic fibrosis therapy
Hu-Lin JIANG (姜虎林)
State Key Laboratory of Natural Medicines, China Pharmaceutical University

Oral Presentation

On September 15

Senji Miyata Memorial Hall

Oral session 1 (10:15 – 10:51)

Chair: Prof. Weizhuo Xu (Shenyang Pharmaceutical University)

O-1 Structures of new polyketides isolated from *Hypoxylon* sp. LY94, a Lycopodiaceae-derived endophytic fungus
Mayu OTAKI, Yusuke YAMADA, Wen-Ping JIANG, Dai HIROSE, Kan'ichiro ISHIUCHI
Graduate School of Pharmaceutical Sciences, Nagoya City University; School of Pharmacy, China Medical University; School of Pharmacy, Nihon University

O-2 Structures of ingenan-type diterpenes isolated from the roots of *Euphorbia kansui*
Misato OGAWA, Kan'ichiro ISHIUCHI, Shohei MIYATA, Susumu KITANAKA
Graduate School of Pharmaceutical Sciences, Nagoya City University; College of Humanities and Sciences, Dios Medical Science Institute

O-3 Synthesis of fused cyclic amines via the alkylation–cyclization–isomerization–3-aza-Cope cascades
Takeo SAKAI, Tomoki FURUHATA, Kota HOSOE, Kaho UMEMURA, Fumiho SAITO, Yuji MORI
Faculty of Pharmacy, Meijo University

Oral session 2 (14:55 – 15:31)

Chair: Prof. ZHANG Huaqing (China Pharmaceutical University)

O-4 Quality control and evaluation of Black chokeberry (*Aronia melanocarpa*) by three-wavelength fusion fingerprinting and electrochemical fingerprinting combined with antioxidant activity analysis
Ming CHEN[1], Peifei GU[2], Guoxiang SUN (孙国祥)[3]
[1]School of Pharmacy, Shenyang Pharmaceutical University; [2]Department of Sport Medical, Institution of Sport and Health, Shenyang Sport College

O-5 Discovery of novel small-molecules targeting transcriptional factor GLI
Jiachen WEN (闻家辰), Wangzhi QIN, Linxiang ZHAO
Department of Medicinal Chemistry, Shenyang Pharmaceutical University

O-6 Biosynthesis of vanillin from natural substrates
Weizhuo XU (徐慰倬), Qi YE, Yongbo SONG, Jinghai ZHANG
Shenyang Pharmaceutical University

Oral session 3 (15:33 – 16:09)

Chair: Prof. Takuhei Yamamoto (Gifu Pharmaceutical University)

O-7 Nasal delivery of polymeric nanoDisc mobilizes a synergy of central and peripheral amyloid-β clearance to treat Alzheimer's disease
Huaqing ZHANG, Yun CHEN (陈赟), Yang DING
Department of Pharmaceutics, China Pharmaceutical University

O-8 Biomimetic Elasticity Compressed Assembly Controls Rapidly Intracerebral Drug Release to Reverse Microglia Dysfunction
Guochen Han (韩国臣)[1], Xiaochen Gu[2], Jianping Zhou[1], Huaqing Zhang[1], Yang Ding[1,3,4]
[1]State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University; [2]Faculty of Pharmacy, University of Manitoba

O-9 Postoperative dynamic pathology-directed sequential drug release by an implantable nanofiber in glioma chemoimmunotherap
Mingjie SONG (宋明杰), Jianping ZHOU, Huaqing ZHANG, Yang DING
Department of Pharmaceutics, China Pharmaceutical University

Oral session 4 (16:12 – 16:48)

Chair: Assoc. Prof. Takayoshi MAMIYA (Meijo University)

O-10 Lipoprotein-mimicking Nanoparticles Mobilize Directional Drainage of Amyloid-β Efflux in Alzheimer's Disease Treatment
XI Yilong (习艺龙), ZHANG Huaqing, DING Yang
State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University

O-11 Preparation and characterization of liposome containing tosufloxacin-cyclodextrin inclusion complex
Hemat MOSTAFA[1], Hamid ALGHURABIi[2], TATSUAKI Tagami[1], KOKI Ogawa[1], TETSUYA Ozeki[1]
[1]Drug Delivery and Nano Pharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Pharmaceutics, College of Pharmacy, University of Kerbala

O-12 Preparation of gold nanostars/extracellular vesicles nanocomplex and their photothermal therapeutic effect on cancer cells

Kazuki SATO, Koki OGAWA, Tatsuaki TAGAMI, Tetsuya OZEKI
Drug Delivery and Nano Pharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University

On September 16

Oral session 5 (9:30 – 10:06)

Chair: Prof. Shuhei YAMADA (Meijo University)

O-13 Mitochondria-targeted Gene Delivery System
Yi WANG (王译)
State Key Laboratory of Natural Medicines, China Pharmaceutical University

O-14 iNKT17 cells play a pathogenic role in cholestasis through CXCR3 mediated recruitment and activation
WANG Xinzhi (王欣之)
New Drug Screening and Pharmacodynamics Evaluation Center, State Key Laboratory of Natural Medicines, China Pharmaceutical University

O-15 In Vivo Cell Engineering for Tumor Tracking Therapy
Hao CHENG (程皓), Jianping ZHOU, Yang DING
Department of Pharmaceutics, China Pharmaceutical University

Oral session 6 (10:40 – 11:04)

Chair: Prof. ZHANG Hao (China Pharmaceutical University)

O-16 Metabolomic analysis for unveiling a novel mechanism of breast cancer formation induced by long-term estrogen exposure
Yoshinori Okamoto (岡本誉士典), Akira Aoki, Hideto Jinno
Faculty of Pharmacy, Meijo University

O-17 Unraveling the Mechanism of Cotinine-Induced Stabilization of Androgen Receptor Protein in Prostate Cancer Cells
Riri HAYASHI (林莉々)[1], Yuta YOSHINO[1], Masaki SHIOTA[2], Naohiro FUJIMOTO[3], Akira IKARI[1], Satoshi ENDO[4,5]
[1]Laboratory of Biochemistry, Gifu Pharmaceutical University; [2]Department of Urology, Graduate School of Medical Sciences, Kyushu University; [3]Department of Urology, University of Occupational and Environmental Health; [4]United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University; [5]Center for One Medicine Innovative Translational Research (COMIT), Gifu University

On September 15

Main Hall

Oral session 7 (10:15 – 10:51)

Chair: Assoc. Prof. Takao KOHNO (Nagoya City University)

O-18 Overcoming prostate cancer drug resistance by targeting lipid peroxidation-derived reactive aldehyde metabolism by AKR1C3

Shinya KAWANO (河野真也)[1], Jin SEGAWA[1], Mina KAWAI[1], Atsumi OTA[1], Yuta YOSHINO[1], Masaki SHIOTA[2], Naohiro FUJIMOTO[3], Akira IKARI[1], Satoshi ENDO[4,5]

[1]Laboratory of Biochemistry, Gifu Pharmaceutical University; [2]Department of Urology, Graduate School of Medical Sciences, Kyushu University; [3]Department of Urology, University of Occupational and Environmental Health; [4]United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University; [5]Center for One Medicine Innovative Translational Research (COMIT), Gifu University

O-19 Disruption of intracellular iron homeostasis and mitochondrial damage by lysosomal disfunction in PARK9 model cells

Takanori MURAKAMI (村上貴規), Kazuki OHUCHI, Hisaka KURITA, Masatoshi INDEN

Laboratory of Medical Therapeutics and Molecular Therapeutics, Gifu Pharmaceutical University

O-20 Functional analysis of type III sodium-dependent phosphate transporter in neurons

Junya MURATA (村田潤哉), Yuto MURAYAMA, Ayane MISIMA, Kazuki OHUCHI, Hisaka KURITA, Isao HOZUMI, Masatoshi INDEN

Laboratory of Medical Therapeutics and Molecular Therapeutics, Gifu Pharmaceutical University

Oral session 8 (14:55 – 15:43)

Chair: Prof. Yukihiro NODA (Meijo University)

O-21 Mitochondrial damage activates the cGAS/STING/type I IFN pathway in neurons

Ayaka FUJIMAKI (藤牧綾香)[1], Asuka NAKAJIMA[1], Takanori MURAKAMI[1], Kazuki OHUCHI[1], Hisaka KURITA[1], Yoki NAKAMURA[2], Norimitsu MORIOKA[2], Masatoshi INDEN[1]

[1]Laboratory of Medical Therapeutics and Molecular Therapeutics, Gifu Pharmaceutical University; [2]Department of Pharmacology, Hiroshima University

O-22 Effects of activated α7 nicotinic acetylcholine receptor against α-synuclein-induced neurotoxicity

Shinnosuke TAKIZAWA (滝沢進之佑), Kazuki OHUCHI, Taisei ITO, Takanori MURAKAMI, Hisaka KURITA and Masatoshi INDEN
Laboratory of Medical Therapeutics and Molecular Therapeutics, Gifu Pharmaceutical University

O-23 Development of a Novel Strategy to Suppress Hepatic Stellate Cell Senescence by NMN Supplementation
Tomofumi SAKA (坂智文)[1], Riri HAYASHI[1], Rina KODERA[2], Taichi MITSUI[2], Mitsuyasu KAWAGUCHI[3], Hidehiko NAKAGAWA[3], Yuta YOSHINO[1], Akira IKARI[1], Satoshi ENDO[4,5]
[1]Laboratory of Biochemistry, Gifu Pharmaceutical University; [2]Nagaragawa Research Center, API Co., Ltd.; [3]Graduate School of Pharmaceutical Sciences, Nagoya City University; [4]The United Graduate School of Drug Discovery and Medical Information Science, Gifu University; [5]Center of One Medicine Innovative Translational Research (COMIT), Gifu University

O-24 Effect of external environment pH on the characteristics of mRNA-encapsulated lipid nanoparticles
Toma SHINKAI, Koki OGAWA, Maiko TSUDA, Tetsuya OZEKI
Drug Delivery and Nano Pharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University

Oral session 9 (16:00 – 16:48)

Chair: Prof. Naoyoshi Kurita (Gifu Pharmaceutical University)

O-25 Removal of microplastics and analysis of additives in plastics based on new adsorbent materials
Xiaohong Hou (侯晓虹), Yingying Li, Mengdan Zhang, Sijia Zhang
Department of Environmental Science, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University

O-26 A Simple and Wallet-friendly Method of α-Glucosidase Inhibitory Activity Assay
Natsuki Ichihara[1,2], Nagisa Yamamoto[1], Rina Shibata[1], Chihiro Ito[1], Yoshiaki Takaya[1]
[1]Faculty of Pharmacy, Meijo University; [2]Faculty of Pharmacy, Kinjo Gakuin University

O-27 Total glucosides of paeony alleviates Sjogren's syndrome by inhibiting Th1 and Th17 responses
Weirong FANG (方伟蓉)

Department of Physiology, China Pharmaceutical University

O-28 Paeonol and its metabolites improve LPS/D-GaLN-induced acute liver injury mice by regulating Ndufs7 in Mitochondria of RAW264.7

Xinru LYU (吕心如)[1], Ziya ZHAO[1], Na SU[1], Guoxia LU[1], Qingqing WANG[1], Qiang FU[2], Ying PENG[1], Natalie HUGHES-MEDLICOTT[3], Guangji WANG[1], Jianguo SUN[1]
[1]Key Lab of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing, Jiangsu, China ;[2]Department of Pharmacology of Chinese Materia Medica, China Pharmaceutical University, Nanjing, Jiangsu, China; [3]School of Pharmacy, University of Otago, Dunedin, New Zealand

On September 16

Oral session 10 (9:30 – 10:06)

Chair: Assoc. Prof. Saotomo ITOH (Nagoya City University)

O-29 Combination therapy of silybin-ursodeoxycholic acid improved nonalcoholic steatohepatitis in mice via selectively activating hepatic farnesoid X receptor (FXR) as well as inhibiting intestinal FXR

Dongchun LIU (劉东春), Yu FU, Xinyu LI, Linghe ZANG, Nan LIU
School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University

O-30 Exploring the substance Basis, metabolic toxicity mechanism, and mechanism based prevention and intervention of hepatotoxicity of *Dioscorea bulbifera* L.

Zixia HU, Jiang ZHENG, Ying PENG (彭缨)
[1]Department of Regulatory Science, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University

O-31 Preparation and Application of a PEC immunosensor for pancreatic cancer Marker-CA19-9

Chunling MAO (毛春玲), Longshan ZHAO
Department of Pharmacy, Shenyang Pharmaceutical University

Oral session 11 (10:40 – 11:04)

Chair: Prof. Satoshi Endo (Gifu Pharmaceutical University)

O-32 Oridonin is a covalent p52 inhibitor with anti-proliferative activity of bladder cancer

Lu ZHAO[1], Xialu WANG[2], Rong ZHANG (张嵘)[1]

[1]Department of Biopharmaceutics, School of Life Science and Bio-Pharmaceutics, Shenyang Pharmaceutical University, Shenyang, Liaoning, China; [2]Department of Biomedical Engineering, School of Medical Devices, Shenyang Pharmaceutical University

O-33 Comparison of Multiple Disease-Modifying Antirheumatic Drugs Combination Therapies with Methotrexate in Rheumatoid Arthritis: A Systematic Review and Bayesian Network Meta-Analysis of Efficacy and Safety

Linfeng LIU[1,2], Kaori AMBE[1], Mayu ONISHI[1], Yuka YOSHII[1], Toshiaki MAKINO[2], Masahiro TOHKIN[1]

[1]Department of Regulatory Science, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University

Poster Presentation

On September 15

P-1 Studies toward the total synthesis of serratinine

Yuto FUMIMOTO, Yuji MORI, Takeo SAKAI

Faculty of Pharmacy, Meijo University

P-2 Development of FHIT Imaging Fluorescence Probes with Well-tuned Hydrophobicity for Intracellular Uptake

Mitsuyasu Kawaguchi, Yuri Furuse, Naoya Ieda, Yuhei Ohta, Hidehiko Nakagawa

Graduate School of Pharmaceutical Sciences, Nagoya City University; Graduate School of Pharmaceutical Sciences, Hokkaido University

P-3 Effect of MPBD and DQ Alkyl Side Chain Length in Dictyostelium discoideum Cell Aggregation and Its Antibacterial and Antiproliferative Activity

Titah Aldila Budiastanti[1], Salma Zulqaida[1,2], Tamao Saito[3], Yumiko Komori[1], Chihiro Ito[1], Yoshiaki Takaya[2]

[1]Faculty of Pharmacy, Meijo University; [2]Postgraduate School, Airlangga University; [3]Faculty of Science and Technology, Sophia University

P-4 Comparison of the pharmacological activities of glycyrrhizin and its human metabolites

XIN Jingxiao (辛竟潇), Ryota Sakoda, Kan'ichiro Ishiuchi, Toshiaki Makino

Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-5 One-pot Biosynthesis of (S)-Equol Using Enzymes Cascade
Jiaqi WANG (王珈琪)
Shenyang Pharmaceutical University

P-6 Substrate specificity of antharaquinone glucosyltransferase from *Rheum palmatum*
Toranosuke Narita, Aoi Yoshino, Toshiaki Makino, Kazuyoshi Terasaka
Garduate School of Pharmaceutical Sciences, Department of Pharmacognosy, Nagoya City University

P-7 Tissue and Cell Dual-Penetrating Dendritic Lipopeptide Liposomes for Hypertrophic Scar Treatment
CONG Rui (丛瑞), LEI Lei, WANG Xulei, ZHOU Yinhui, TU Jiasheng, JIANG Lei
Department of Pharmaceutics. School of Pharmacy, China Pharmaceutical University

P-8 A Nanodisc-Paved Biobridge Facilitates Stem Cell Membrane Fusogenicity for Intracerebral Shuttling and Bystander Effects
DING Yang (丁杨), JIN Yi, ZHOU Jianping, ZHANG Huaqing
Department of Pharmaceutics, China Pharmaceutical University

P-9 Construction of cell membrane biomimetic nanoplatform for effective screening of active ingredients from Sanwei Tanxiang capsule
Yi Qina[1], Jiahe Ren[2], Longshan Zhao (赵龙山) [1], Xuefeng Guan[2]
[1]Department of Pharmacy; [2]Department of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University

P-10 Toward hydrogel-based photothermal therapy against skin cancer: fabrication of Ag-nanoparticles and 3D-printed hydrogel scaffold
Jayita Das, Koki Ogawa, Tetsuya Ozeki
Drug Delivery and Nano Pharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-11 Development of Gold Nanostars Coated with Mesoporous Silica for Laser-Triggered Chemo-Photothermal Therapy
Fadilah ASRIL, Koki OGAWA, Tatsuaki TAGAMI, Tetsuya OZEKI
Drug Delivery and Nano Pharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-12 Establishment and application of deep eutectic solvent-assisted reverse thermal proteome profiling (DATPP)

Liu YANG (杨柳), Chen-wan GUO, Wen GAO
State Key Laboratory of Natural Medicines, School of Traditional Chinese Pharmacy, China Pharmaceutical University

P-13 Establishment of Cancer Therapy Strategies Targeting a Core Autophagy Protein ATG4B

Yudai KUDO (工藤優大)[1], Yuta YOSHINO[1], Akira IKARI[1], Satoshi ENDO[2,3]
[1]Laboratory of Biochemistry, Gifu Pharmaceutical University; [2]United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University; [3]Center for One Medicine Innovative Translational Research (COMIT), Gifu University

P-14 Relationship between Anaplastic Lymphoma Kinase (ALK) Inhibitors and Epileptic Seizure Disorder: A Post-Marketing Surveillance Study.

Yoshihiro Noguchi[1,2], Hiroki Asano[3], Rikuto Masuda[1], Yuta Teshigawara[1], Makiko Go[3], Michio Kimura[1,3], Eiseki Usami[2,3], Tomoaki Yoshimura[1,2]
[1]Laboratory of Clinical Pharmacy; [2]Laboratory of Medical Collaborative Pharmacy, Gifu Pharmaceutical University; [3]Department of Pharmacy, Ogaki Municipal Hospital

P-15 Development of Novel Therapeutic Strategies for Refractory Cancer Targeting DDI[2]

Atsumi OTA (太田篤実)[1], Souta MARUYAMA[1], Yuta YOSHINO[1], Akira IKARI[1], Satoshi ENDO[2,3]
[1]Laboratory of Biochemistry, Gifu Pharmaceutical University; [2]The United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University; [3]Center for One Medicine Innovative Translational Research (COMIT), Gifu University

P-16 Involvement of adrenaline beta2 receptors in clozapine-induced lipid droplet accumulation in 3T3-L1 cells

Ayano FUKAMI (深見彩乃)[1], Tomomi UMEMURA[1,2], Akira YOSHIMI[1,3], Yukihiro NODA[1,3]
[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University; [2]Department of Patient Safety, Nagoya University Hospital; [3]Clinical OMICs and Translation Research Center, Meijo University

P-17 Involvement of TNF-α/TNFR1 signaling in microglia and glutamatergic neurotransmission of mice exposed to social defeat stress as juveniles

Yuya ISOZUMI (五十住優弥)[1], Mikio YOSHIDA[1], Hikari KATADA[1], Akira YOSHIMI[1], Norio OZAKI[2], Yukihiro NODA[1]

[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University; [2]Pathophysiology of Mental Disorders, Nagoya University Graduate School of Medicine

P-18 Effect of lithium on hematopoietic toxicity induced by clozapine in HL-60 cells
Akari KATO (加藤朱莉)[1], Aya TORII[1,2], Akira YOSHIMI[1], Yukihiro NODA[1]
[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University; [2]Kinjo Gakuin University

P-19 Mice with deficiency in Pcdh15, a gene associated with bipolar disorders (BD), exhibit BD-like behaviors and monoaminergic properties
Takuma KATO (加藤拓真)[1], Ayaki TAKAHASHI[1], Takahiro ITO[1], Masaki KANO[1], Miyu KUSUMOTO[1], Mikio YOSHIDA[1], Daisuke MORI[2], Norio OZAKI[3], Akira YOSHIMI[1], Yukihiro NODA[1]
[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University; [2]Brain and Mind Research Center, Nagoya University; [3]Pathophysiology of Mental Disorders, Nagoya University Graduate School of Medicine

P-20 Effect of clozapine on cognitive behaviors function and neurotransmitters in a schizophrenia-like mouse model
Shunya KAWAI (川合竣也)[1], Akira YOSHIMI[1], Ayaka KATO[1], Mikio YOSHIDA[1], Mizuki UCHIDA[1], Shinji KITAGAKI[2], Norio OZAKI[3], Yukihiro NODA[1]
[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy; [2]Department of Medical Chemistry, Graduate School of Pharmacy, Meijo University; [3]Pathophysiology of Mental Disorders, Nagoya University Graduate School of Medicine, Nagoya, Japan

P-21 Influence of environment adversity during neurodevelopment on future behavioral responses and neuromorphogenesis in astrotactin[2] (ASTN2) heterozygous mice
Amane KIMURA (木村天音)[1], Mikio YOSHIDA[1], Atsuki KURODA[1], Akira YOSHIMI[1], Tomomi AIDA[2], Koichi TANAKA[2], Norio OZAKI[3], Yukihiro NODA[1]
[1]Division of Clinical Sciences and Neuropsychopharmacology, Meijo University, Faculty and Graduate School of Pharmacy; [2]Laboratory of Molecular Neuroscience, Medical Research Institute, Tokyo Medical and Dental University; [3]Pathophisiology of Mental Disorders, Nagoya University, Graduate School of Medicine

P-22 Analysis of heparan sulfate proteoglycans in mice lacking EXTL3 specifically in glomerular podocytes

Naho Matsubara (松原奈穂), Rikuma Ishigaki, Shuji Mizumoto, Taji Matsuzaka, Akira Sugawara, Shuhei Yamada
Department of Pathobiochemistry, Faculty of Pharmacy, Meijo University

P-23 Involvement of nicotinic acetylcholine receptor α7 subunit in the impairment of social behaviors in mice exposed to social defeat stress as juveniles.
Kazuna MORIKAWA (森川和那)[1], Mizuki UCHIDA[1], Honami TANAKA[1], Mayu OZAKI[1], Norio OZAKI[2], Akira YOSHIMI[1], Yukihiro NODA[1]
[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University; [2]Pathophysiology of Mental Disorders, Nagoya University Graduate School of Medicine

P-24 Involvement of serotonin transporter in chronic orofacial pain with depressive symptoms before and after duloxetine treatment
Mariko NAKAMURA(中村真理子)[1], Akira YOSHIMI[1,2], Tatsuya TOKURA[2], Hiroyuki KIMURA[2], Shinichi KISHI[2], Tomoya MIYAUCHI[3],Kunihiro IWAMOTO[2], Mikiko ITO[4], Aiji SATO-BOKU[4], Akihiro MOURI[5], Toshitaka NABESHIMA[5], Norio OZAKI[2], Yukihiro NODA[1,2]
[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University; [2]Department of Psychiatry, Nagoya University Graduate School of Medicine; [3]Department of Psychiatry, KACHI Memorial Hospital; [4]School of Dentistry, Aichi Gakuin University; [5]Graduate School of Health Science, Fujita Health University

P-25 Measurement of the xylosyltransferase activity of mutants of XYLT1 and XYLT2 involved in glycosaminoglycan biosynthesis, that cause inherited diseases
Wakana NOHARA (野原若菜), Shinya KIYOMASU, Shuhei YAMADA, and Shuji MIZUMOTO
Department of Pathobiochemistry, Faculty of Pharmacy, Meijo University

P-26 Effects of hyaluronidase 4 deficiency on a mouse model of diabetes mellitus
Seriha OGAWA (小川芹葉), Riko SUZUKI, Shuji MIZUMOTO, Shuhei YAMADA
Department of Pathobiochemistry, Faculty of Pharmacy, Meijo University

P-27 Comprehensive gene expression analysis of lymphoblastoid cell lines from schizophrenia patients and blood and brain samples from schizophrenia-like mouse models.
Takumi OYA (雄谷拓海)[1], Akira YOSHIMI[1,2], Hirotake HIDA[1], Miku ASAI[1], Hiromasa ANDO[1], Souta MIZUNO[1], Shinji KITAGAKI[3], Norio OZAKI[4], Yukihiro NODA[1,2]

[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University; [2]Clinical OMICs and Translation Research Center, Meijo University; [3]Laboratory of Medicinal Chemistry, Faculty of Pharmacy, Meijo University; [4]Pathophysiology of Mental Disorders, Nagoya University Graduate School of Medicine

P-28 Spatial brain proteomic analysis using a schizophrenia-like model mouse treated with clozapine

Akira YOSHIMI (吉見陽)[1,2], Susumu Y. IMANISHI[3], Tomoya OGUCHI[1], Shinji KITAGAKI[4], Norio OZAKI[5], Yukihiro NODA[1,2]
[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University; [2]Clinical OMICs and Translation Research Center, Meijo University; [3]Laboratory of Analytical Chemistry, Faculty of Pharmacy, Meijo University, Nagoya, Japan; [4]Laboratory of Medicinal Chemistry, Faculty of Pharmacy, Meijo University, Nagoya, Japan; [5]Pathophysiology of Mental Disorders, Nagoya University Graduate School of Medicine, Nagoya, Japan

P-29 High-sensitive measurement of abasic sites in genomic DNA

Sakura Hida (樋田桜花)[1], Yoshinori Okamoto[1], Akira Aoki[1], Hideto Jinno[1]
Faculty of Pharmacy, Meijo University, Nagoya, Japan

P-30 Inhalation therapy for patients with bronchial asthma during the COVID-19 pandemic: appropriate instructions amidst infectious disease spread

Masaki KANO (加納正暉)[1], Mariko NAKAMURA[1], Noriaki MATUMOTO[2], Mikio SAKAKIBARA[2], Toshiki KANAI[3], Akira KURACHI[4], Masayuki HIRAMATU[5], Yukihiro NODA[1]
[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University; [2]Sugi Pharmacy; [3]Cocokarafine Healthcare Pharmacy; [4]Walnut Pharmacy; [5]Department of Chemical Pharmacology, Meijo University

P-31 Kinetic Characterization of FLVCR2 as an Intestinal Choline Transporter in the Rat Model

Mione YAMASAKI (山﨑美音), Chitaka NAMBA, Takahiro YAMASHIRO, Tomoya YASUJIMA, Hiroaki YUASA
Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-32 Functional Characteristics of SLC19A3 for the Transport of Amiloride as a Newly Found Fluorescent Substrate

Riku YAMAUCHI (山内利玖), Takahiro YAMASHIRO, Michihiro YAMAMOTO, Kaito MATSUI, Tomoya YASUJIMA, Hiroaki YUASA
Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-33 Staphylococcal superantigen-like protein 3 triggers murine mast cell adhesion and enhancement of mast cell activation by binding to CD43

Sae Kawano[1], Chisaki Noda[1], Saotomo Itoh[1], Ayaka Urabe[1], Chifumi Fujii[2,3,4], Isamu Ogawa[5], Ryo Suzuki[1], Shigeaki Hida[1]
[1]Department of Molecular and Cellular Health Sciences, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Biotechnology, Institute for Biomedical Sciences, Shinshu University; [3]Department of Molecular Pathology, Shinshu University School of Medicine; [4]Center for Medical Education and Clinical Training, Shinshu University School of Medicine; [5]Laboratory of Hygienic Chemistry, Faculty of Pharmaceutical Science, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University

P-34 Mechanisms of allergic disease suppression by molecules derived from indigenous skin bacteria

Reina MUKAINAKA (向井中玲菜), Yuma ITOH, Isamu OGAWA, Saotomo ITOH, Shigeaki HIDA
Department of Molecular and Cellular Health Sciences, Graduate School of Nagoya City University

P-35 HMG-CoA reductase inhibitors regulate helper T cell differentiation

Ishikawa Satoshi
Department of Molecular and Cellular Health Science, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-36 Development of a platelet generation evaluation system using human iPS cell-derived megakaryocytes

Tomoe Kajita, Tadahiro Hashita, Eisei Hori, Takahiro Iwao
Educational Research Center for Clinical Pharmacy, Nagoya City University

P-37 Phospholipid flippases ATP8A1 and ATP8A2 modulate inhibitory neurotransmission in hippocampal neurons

Muneyuki KAWASE (川瀬宗之)[1], Takuto MATSUDA[1], Yuta UMEMURA[1], Hisashi OISHI[2], Takashi SAKURAI[3], Mitsuharu HATTORI[1]
[1]Department of Biomedical Science, Nagoya City University; [2]Department of Comparative and Experimental Medicine, Nagoya City University; [3]Department of Cellular and Molecular Pharmacology, Juntendo University

On September 16

P-38 Myosin Va regulates the terminal translocation of migrating neurons in the neocortex
Takao KOHNO (河野孝夫), Mone SATO, LI Minqian, Motsuharu HATTORI
Department of Biomedical Science, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-39 Elucidation of the pathogenesis of neurological diseases caused by phospholipid flippase deficiency
Noritaka SASSA (佐々徳啓)[1], Yuta UMEMURA[1], Muneyuki KAWASE[1], Hisashi OISHI[2], , Mitsuharu HATTORI[1]
[1]Department of Biomedical Science; [2]Department of Comparative and Experimental Medicine, Nagoya City University

P-40 TREK1 channels are involved in fibrogenic matrix expression and cell proliferation in human hepatic stellate LX-2 cells
Naoki KAWATA (川田成紀), Rubii KONDO, Akari DEGUCHI, Yoshiaki SUZUKI, Hisao YAMAMURA
Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-41 Upregulated expression of two-pore domain potassium channels, KCNK1 and KCNK2, in pulmonary arterial hypertension
Taiki AMANO (天野泰樹)[1], Natsumi Shima[1], Aya Yamamura[2], Moe Fujiwara[1], Kazuyuki Matsumoto[1], Taiga Sekine[1], Haruka Okano[1], Rubii Kondo[1], Yoshiaki Suzuki[1], Hisao Yamamura[1]
[1]Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Physiology, Aichi Medical University

P-42 Optogenetic investigation of the role of excitation-transcription coupling in vascular smooth muscle cells
Tsukasa Koide (小井手司)[1], Yoshiaki Suzuki[1], Kondou Rubii[1], Gerald Zamponi[2], Hisao Yamamura[1]

[1]Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Clinical Neurosciences, Hotchkiss Brain Institute, Alberta Children's Hospital Research Institute, University of Calgary

P-43 Downregulation of Kv1.6 channel expression in chondrocytes leads to osteoarthritis via enhanced Ca^{2+} signaling

Tomo KURATA (倉田朋)[1], Yoshiaki SUZUKI[1], Shinya TATENO[1], Shigeru MIYAKI[2], Eiva BERNOTIENE[3], Wayne GILES[4], Hisao YAMAMURA[1]
[1]Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Orthopaedic Surgery, School of Medicine, Hiroshima University; [3]Department of Regenerative Medicine, Innovative Medicine Center; [4]Department of Physiology & Pharmacology, University of Calgary

P-44 Inhibitory effects of melatonin on voltage-gated K^+ ($K_V4.2$) channels in rat pinealocytes

Hibiki Kuzuhara (葛原響), Hiroki Mishima, Shunsuke Ando, Rubii Kondo, Yoshiaki Suzuki, Hisao Yamamura
Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-45 Pharmacologic inhibition of BMI1 activates the p53 pathway to suppress MYCN-amplified neuroblastoma

Masahiro Hirayama[1], Eri Yamada[1], Hiromasa Aoki[1], Kazuya Izumi[1], Ayumi Amano[1], Kohki Toriuchi[1], Koichi Ogami[2], Mai Nagasaka[3], Yasumichi Inoue[3], Hidetoshi Hayashi[3], Satoru Takeshita[1.4], Hiroki Kakita[1.4], Yasumasa Yamada[4], Mineyoshi Aoyama1
[1]Department of Pathobiology, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Division of Molecular Oncology, Center for Neurological Diseases and Cancer, Graduate School of Medicine, Nagoya University; [3]Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University; [4]Department of Perinatal and Neonatal Medicine, Aichi Medical University

P-46 Nanaomycin A exerts antitumor effect on neuroblastoma cells with DNA demethylation.

Kazuya IZUMI[1], Hiromasa AOKI[1], Kohki Toriuchi[1], Hiroki KAKITA[1,2], Satoru TAKESHITA[1,2], Hiroko UEDA[2], Yasumichi INOUE[3,4], Hidetoshi HAYASHI[3,4], Yasumasa YAMADA[2], Mineyoshi AOYAMA[1]

[1]Department of Pathobiology, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Perinatal and Neonatal Medicine, Aichi Medical University; [3]Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University; [4]Department of Innovative Therapeutic Sciences, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-47 Thrombopoietin Enhances Neuronal Cell Proliferation and Axonal Elongation in Intrauterine Growth Restricted Rats

Yuka SUZUKI[1], Satoru TAKESHITA[1,2], Hiroki KAKITA[1,2], Nami NAKAMURA[2,3], Mari MORI[2], Kohki TORIUCHI[1], Hiromasa AOKI[1], Yasumichi INOUE[4,5], Hidetoshi HAYASHI[4,5], Yasumasa YAMADA[2], Mineyoshi AOYAMA[1]

[1]Department of Pathobiology, Nagoya City University Graduate School of Pharmaceutical Science; [2]Department of Perinatal and Neonatal Medicine, Aichi Medical University; [3]Department of Pediatrics, Aichi Medical University; [4]Department of Cell Signaling, Nagoya City University Graduate School of Pharmaceutical Sciences; [5]Department of Innovative Therapeutic Sciences, Cooperative Major in Nanopharmaceutical Sciences, Nagoya City University Graduate School of Pharmaceutical Sciences

P-48 Hypothermic culture attenuates neurotoxic activation of microglia via TRPV4 channel

Rina MIMOTO[1], Naoya FUKUDA[1], Kohki TORIUCHI[1], Hiromasa AOKI[1], Hiroki KAKITA[1,2], Yoshiaki SUZUKI3, Satoru TAKESHITA[1,2], Tetsuya TAMURA[4], Hisao YAMAMURA3, Yasumichi INOUE[5,6], Hidetoshi HAYASHI[5,6], Yasumasa YAMADA[2], Mineyoshi AOYAMA[1]

[1]Department of Pathobiology, Nagoya City University Graduate School of Pharmaceutical Science; [2] Department of Perinatal and Neonatal Medicine, Aichi Medical University; 3Department of Molecular and Cellular Pharmacology, Nagoya City University Graduate School of Pharmaceutical Sciences; [4]Department of Anesthesiology and Intensive Care Medicine, Nagoya City University Graduate School of Medical Sciences; [5]Department of Cell Signaling, Nagoya City University Graduate School of Pharmaceutical Sciences; 6Department of Innovative Therapeutic Sciences, Cooperative Major in Nanopharmaceutical Sciences, Nagoya City University Graduate School of Pharmaceutical Sciences

P-49 The activity of Pinellia tuber raphides onto TRPA1 and the effect of ginger, organic acids

Itsuki NOSE (能瀬逸紀)[1], Taiki AMANO[2], Tsukasa FUEKI[1,3,4], Hisao YAMAMURA[2], Toshiaki MAKINO[1]

[1]Dept. of Pharmacognosy; [2]Dept. of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University; [3]Department of Traditional Medicine, Toho University School of Medicine; [4]Matsuya Pharmacy

P-50 TMEM55B promotes calcium induced calcium release between the endoplasmic reticulum and lysosomes

Nagi MUKAE (向江凪), Keitaro YAMAMOTO, Wakana OKUDA, and Michiko SHIRANE
Department of Pharmacy, Nagoya City University

P-51 The mechanism of inflammation related to Alzheimer's disease in the brain of PDZD8-deficient mice

Honoka MAKI (眞木穂香), Mariko WADA, Hikari KITANO, Yumi MORISUGI, Michiko SHIRANE
Department of Pharmacy, Nagoya City University

P-52 Elucidation of molecular mechanisms involved in proteasome inhibitor resistance in multiple myeloma

Airi NAKAGAWA (中川愛理), Shogo YAMANAKA, Chiharu MIYAJIMA, Hodetoshi HAYASHI, Yasumichi INOUE
Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-53 Mechanism of induction of stress-responsive transcription factor ATF4 through HRI activation by compounds from Myanmar plant

Shogo YAMANAKA (山中翔悟)[1], Kotaro HIRAMARU[1], Yuya MIZUNO[1], Chiharu MIYAJIMA[1], Kan'ichiro ISHIUCHI[2], Toshiaki MAKINO[2], Michiyo MATSUNO[3],Hajime MIZUKAMI[3], Yasumichi INOUE[1], Hidetoshi HAYASHI[1]
[1]Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University; [3]The Kochi Prefectural Makino Botanical Garden

P-54 The deubiquitinating enzyme USP2 stabilizes TAZ to mediate cancer cell proliferation

Takuto FUJIWARA (藤原巧斗), Chiharu MIYAJIMA, Yasumichi INOUE, Hidetoshi HAYASHI
Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-55 ID3 is a target gene of p53 and modulates lung cancer cell metastasi

Sakura HASHIGUCHI (橋口咲良)[1], Mai NAGASAKA[1], Chiharu MIYAJIMA[1], Hiromasa AOKI[2], Kohki TORIUCHI[2], Mineyoshi AOYAMA[2], Hidetoshi HAYASHI[1], Yasumichi INOUE[1]
1Department of Cell Signaling; [2]Department of Pathobiology, Graduate School of Pharmaceutical Sciences, Nagoya City University

P-56 Physiological roles of nicotinic acetylcholine receptors in human pulmonary arterial smooth muscle cells.
Koya NAKAHAMA (中浜光哉)[1], Aya YAMAMURA[2], Rubii KONDO[1], Yoshiaki SUZUKI[1], Hisao YAMAMURA[1]
[1]Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University
[2]Department of Physiology, Aichi Medical University

P-57 Exploration of Novel CaMKK[2] Inhibitors Using a FRET-based Assay System
Haruya TANABE (田邊晴也)[1], Yoshiaki SUZUKI[1], Itsuki OKADA[1], Nanaho MICHIGAMI[1], Takashi MURAYAMA[2], Rubii KONDO[1], Hisao YAMAMURA[1]
[1]Department of Pharmaceutical Sciences, Nagoya City University, [2]Department of Medicine, Juntendo University

P-58 Paxilline blocks calcium-activated chloride TMEM16A channels
Taiga Sekine (関根大雅)[1], Rubii Kondo[1], Akane Suzukawa[1], Yoshiaki Suzuki[1], Aya Yamamura[2], Hisao Yamamura[1]
[1]Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Physiology, Aichi Medical University

P-59 Ameliorative effects of sweetening agents on jet lag
Jiayi LI (李佳憶), Tianxiang GAO, Kazuhiko KUME
Department of Neurophamacology, Nagoya City University

P-60 Enhanced Sleep in Drosophila melanogaster under the Presence of Predators
Haruki Kato, Namiki Shima, Yoshinori Suzuki, Jun Tomita, Kazuhiko Kume
Department of Neuropharmacology, Nagoya City University

P-61 Regulation of sleep by dAWP1, a homolog of the anesthetic sensitivity gene AWP1
Ryo FURUKAWA (古川稜)[1], Taro UENO[2], Yusaku MORI[1], Jun TOMITA[1], Shoen KUME[3], Kazuhiko KUME[1]

[1]Department of Neuropharmacology, Nagoya City University; [2]Toho University; [3]Tokyo Institute of Technology

P-62 Identification of Novel HDAC6-selective Inhibitor as Potential Treatment of Triple-negative Breast Cancer through Induction of PANoptosis
Siyuan WANG (王思远), Meidi LUO, Dan LIU
Department of Medicinal Chemistry, Shenyang Pharmaceutical University

P-63 LPS-inspired engineering of phage-separating Pt(IV)-graft-glycopeptides sequentially sensing pH and redox for deep penetration and targeting chemotherapy of breast cancer
Dali CHEN (陈大力), Yunai DU, Jiasheng TU, Chunmeng SUN
NMPA Key Laboratory for Research and Evaluation of Pharmaceutical Preparations and Excipients, and Department of Pharmaceutics, School of Pharmacy, China Pharmaceutical University

P-64 Does long-term care insurance increase social participation among the younger elderly? Quasi-experimental evidence from China
Yifan Chen (陈一凡), Xia Hu
School of International Pharmaceutical Business, China Pharmaceutical University

P-65 Scutellarein protects angaint cardiac hypertrophy though peroxiredoxin 3 mediated mitochondrial quality control in heart failure
Xiaobing LIN (蔺小兵), Wen GAO
State Key Laboratory of Natural Medicines, School of Traditional Chinese Pharmacy, China Pharmaceutical University

P-66 Effect of *Ginkgo biloba* extract on pharmacology and pharmacokinetics of atorvastatin in rats with hyperlipidaemia
Qingqing Wang (王庆庆)[1], Zihou Liu[1], Rui Wang[1], Run Li[1], Xiaoru Lian[1], Yanquan Yang[1], Jiao Yan[1], Zhiqi Yin[2], Guangji Wang[1], Jianguo Sun[1], and Ying Peng[1]
[1]Jiangsu Provincial Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University; [2]Department of TCMs Pharmaceuticals, School of Traditional Chinese Pharmacy, China Pharmaceutical University

P-67 A novel anti-inflammatory oxaliplatin (IV) prodrug nanomedicine to enhance colorectal cancer therapy
Lei Xing (邢磊), Wen-Jia Wang, Hu-Lin Jiang
State Key Laboratory of Natural Medicines, China Pharmaceutical University

P-68 Tumor microenvironment-initiated lipid redox cycling for efficient triple-negative breast cancer therapy
Tian-Jiao Zhou (周天娇), Hu-Lin Jiang
State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University

P-69 Improving effects of additional administration of brexpiprazole to antipsychotics on cognitive function in schizophrenia patients - A pilot study -
Yuma SHIMIZU (清水侑真)[1], Ippei TAKEUCHI[2,3], Manako HANYA[1,2], Kiyoshi FUJITA[3], Hiroyuki KAMEI[1,2]
[1]Office of Clinical Pharmacy Practice and Health Care Management, Graduate School of Pharmacy, Meijo University; [2]Office of Clinical Pharmacy Practice and Health Care Management, Faculty of Pharmacy, Meijo University; [3]Department of Psychiatry, Okehazama Hospital

P-70 *In vivo* monitoring of pancreatic acetylcholine using microdialysis in rats
Arisa Hayashi (林亜里紗), Tokina Imura, Yoshinori Okamoto, Akira Aoki, Hideto Jinno
Faculty of Pharmacy, Meijo University

P-71 Evaluation of hot flashes using non-invasive continuous measurement of tail skin temperature in ovariectomized rats
Shin-nosuke Hayashi (林信之介), Yoshinori Okamoto, Akira Aoki, Hideto Jinno
Faculty of Pharmacy, Meijo University

P-72 *In silico* analysis for prediction of metabolites from halogenated estrogens
Kohki Kurihara (栗原皓紀), Yoshinori Okamoto, Akira Aoki, Hideto Jinno
Faculty of Pharmacy, Meijo University

P-73 Association between the daily intake of herbal drugs and adverse events: a retrospective study using the Japanese Adverse Drug Event Report database
Koumi MIYASAKA[1], Keita OURA[1], Yamato KATO[1], Mika MAEZAWA[1], Sakiko HIROFUJI[1], Moe YAMASHITA[1], Nanaka ICHIHARA[1], Yuka NOKURA[1], Kana SUGISHITA[1], Tomofumi YAMAZAKI[1], Satoshi NAKAO[1,2], Hirofumi TAMAKI[3], Kazuhiro IGUCHI[3], Jun LIAO[4], Mitsuhiro NAKAMURA[1]

[1]Laboratory of Drug Informatics, Gifu Pharmaceutical University; [2]Department of Pharmacy, Kyushu University Hospital; [3]Laboratory of Community Pharmacy, Gifu Pharmaceutical University; [4]Department of Information Science and Information System, China Pharmaceutical University

P-74 The incentive mechanism for fostering industry-university-research collaboration in pharmaceutical innovation

Xing Wang, Xiaoliang Yuan (袁小量)
Shenyang Pharmaceutical University

第51期 東海漢方入門講座ご案内

目　的：　正しい漢方知識とその運用の習得並びに医薬業職能の向上・確立

期　間：　2024年4月1日から1年間（10回）　原則第2日曜日 午前10時30分～午後4時30分

会　場：　名城大学　八事キャンパス

内　容：　座学による集合研修

感染状況等により、座学による集合研修を中止しWeb配信講座に変更する場合があります。

講座カリキュラム(以下に記載)

主　催：　東海漢方協議会

共　催：　一般社団法人日本生薬学会

～詳しい講師紹介は東海漢方協議会のホームページより確認出来ます～

ここがポイント！
「実際に東海地区で漢方系の職種に携わっている先生方ばかりなので現場のリアルな話が聞けたり、質問等出来ます。」

＜第51期10月以降の講座カリキュラム＞

11/10	10:30～12:30	12:30～13:30	13:30～15:00	15:00～15:15	15:15～16:30
	誰にも分かる漢方基礎 「五臓　脾」 講師 林誠一先生	休憩	冷え性と漢方 講師 所崇先生	休憩	講師 生田悠起先生 生薬解説(附子) 講師 蓑輪暁美先生
12/8	10:30～12:30	12:30～13:30	13:30～15:00	15:00～15:15	15:15～16:30
	誰にも分かる漢方基礎 「五臓　肝」 講師 林誠一先生	休憩	婦人病の漢方 講師 小塚陽介先生	休憩	方剤解説(臓腑) 講師 生田悠起先生 傷寒論解説 講師 三品尚弘先生
1/12	10:30～12:30	12:30～13:30	13:30～15:00	15:00～15:15	15:15～16:30
	誰にも分かる漢方基礎 「五臓　心」 講師 林誠一先生	休憩	「現代病に用いる漢方薬」 講師 榎本楠紀先生	休憩	一貫堂処方解説 臓毒証体質 防風通聖散の考察 講師 伊藤晴夫先生 傷寒論解説 講師 三品尚弘先生
2/9	10:30～12:30	12:30～13:30	13:30～15:00	15:00～15:15	15:15～16:30
	誰にも分かる漢方基礎 「五臓　腎」 講師 林誠一先生	休憩	特別講演 「花粉症の東洋医学的理解と治療」 講師 仙頭正四郎先生 （仙頭クリニック院長）		
3/9	10:30～12:30	12:30～13:30	13:30～15:00	15:00～15:15	15:15～16:30
	誰にも分かる漢方基礎 「四診と経絡」 講師 林誠一先生	休憩	瘀血 (桂枝茯苓丸の 適応症および疾患) 講師 浮亀浩先生	休憩	一貫堂処方解説 その他の汎用処方 五積散　疎経活血湯 講師 伊藤晴夫先生 傷寒論解説 講師 三品尚弘先生

＜申込方法＞

以下URLもしくはQRにて募集内容を確認し、

東海漢方協議会ホームページ内の51期入会申込フォームより申込みをしてください。

東海漢方協議会HP https://tokaikanpo-kyogikai.net/

学生の方は、入会費2,000円（テキスト2冊付）で、講座を年間無料で受講できます！

＜事務局＞　〒464-0084
名古屋市千種区松軒1丁目5番12号（大晃生薬有限会社内）　東海漢方協議会事務局
mail: 東海漢方協議会協議会HP「お問い合わせフォーム」よりお願いします。

Plenary Lectures

PL-1

Development of Human iPS Cell-Derived Cells Useful for Drug Metabolism and Pharmacokinetic Studies

Tamihide Matsunaga (松永民秀)[1]

Department of Clinical Pharmacy, Graduate School of Pharmaceutical Sciences, Nagoya City University

In drug disposition, the small intestine is involved in the absorption and metabolism of orally administered drugs. Using primary human small intestinal epithelial cells for the evaluation of drug membrane permeability would be desirable, but they have poor viability and other characteristics such as short life span that limit their application. In addition, it is very difficult to obtain primary human small intestinal epithelial cells for drug discovery research such as pharmacokinetic studies and drug safety assessment testing of drug candidates. Therefore, caucasian colon adenocarcinoma (Caco−2) cells have been used to evaluate oral drug absorption in the small intestine, although expression patterns of drug transporters in Caco−2 cells differ from those of the small intestine in humans. For predicting drug metabolism in the small intestine, small intestinal microsomes are widely used; however, microsomes can only evaluate drug metabolism via enzymes present in the fraction. Because human induced pluripotent stem (iPS) cells have abilities of pluripotency and almost infinite proliferation, it is expected to stably provide the high-quality cells that have similar characteristics to human normal tissue cells by using human iPS cells.

At a lecture, I will introduce the development research of small intestinal epithelial cells differentiated from human iPS cells as a new research tool for drug metabolism and pharmacokinetic studies.

PL-2

Artificial mucus layer formed in response to ROS for oral treatment of inflammatory bowel disease

Guangshuai Zhang[1], Qiang Fu (付强)[1]

[1]Wuya College of Innovation, Shenyang Pharmaceutical University, No. 103, Wenhua Road, Shenyang 110016, China.

Artificial mucus layer, such as hydrogels, used to repair the damaged intestinal barrier, are a promising treatment for inflammatory bowel disease (IBD). However, the currently reported hydrogel-based artificial barriers are administered via rectal injection, causing unnecessary discomfort to patients. Herein, we report an oral hydrogel precursor solution based on thiol-modified hyaluronic acid (HASH). Owing to the reactive oxygen species (ROS)-responsive gelling behavior, our precursor solution formed an artificial mucus coating over the inflamed regions of the intestines, blocking microbial invasion and reducing abnormally activated immune responses. Notably, HASH also modulated the gut microbiota, including increasing the diversity and enhancing the abundance of short-chain fatty acid-associated bacteria, which play a key role in gut homeostasis. We believe that the ROS-responsive artificial mucus layer is a promising strategy for the oral treatment of IBD.

PL-3

Multi-organic Fibrosis Therapy

Hu-Lin Jiang (姜虎林)[1]

[1]State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009

[1]Hu-Lin Jiang: Tel (+86-25-83271027), e-mail (jianghulin3@163.com)

Background and Purpose. Fibrosis is an outcome of the tissue repair response that becomes dysregulated following many types of tissue injury. It is a pathological process of abnormal increase and excessive deposition of extracellular matrix (ECM) in tissues. Pulmonary fibrosis (PF) is an age-related interstitial lung disease. FDA-approved drugs can decelerate the progression of PF, however, curing aged patients with severe fibrosis is ineffective because of insufficient accumulation of these drugs and wide necrocytosis of type II alveolar epithelial cells (AEC IIs).

Method.

The mesenchymal stem cell (MSC)-based nanoengineered platform via the bioconjugation of MSCs and type I collagenase-modified liposomes loaded with nintedanib (MSCs-Lip@NCAF) were prepared for treating severe fibrosis.

Results.

MSCs-Lip@NCAF were guided to injured lungs and Lip@NCAF was sensitively released. Lip@NCAF degraded collagen fibers, efficiently deliver nintedanib to inhibit fibroblast overactivation. Moreover, MSCs differentiated into AEC IIs to reestablish alveolar structure, which was crucial for treating aged mice with PF.

Conclusion.

MSCs-Lip@NCAF could be used as a promising therapeutic candidate for PF therapy, especially in aged patients.

Keywords: Fibrosis therapy, extracellular matrix, mesenchymal stem cell, nanoengineering, collagen

Oral Presentations

O-1

Structures of new polyketides isolated from *Hypoxylon* sp. LY94, a Lycopodiaceae-derived endophytic fungus

Mayu OTAKI (大瀧真由)[1], Yusuke Yamada[1], Wen-Ping JIANG[2], Dai HIROSE[3], Kan'ichiro ISHIUCHI[1]

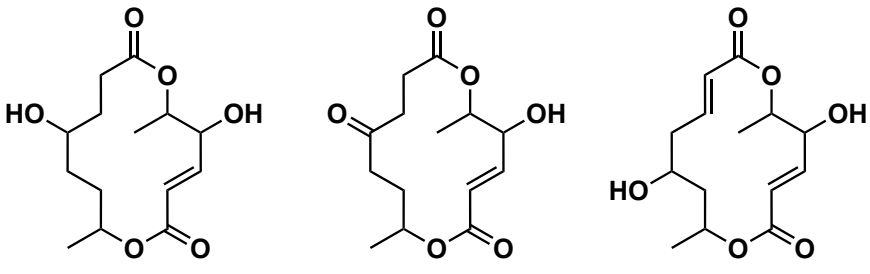
[1]Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]School of Pharmacy, China Medical University; [3]School of Pharmacy, Nihon University

【Objective】

Club moss belongs to Lycopodiaceae family, and is well-known to contain *Lycopodium* alkaloids. While these plants have been surveyed for new alkaloids discovery by many research groups, recently, new secondary metabolites isolated from the Lycopodiaceae plants-drived endophytic fungi have been also reported. For example, Li *et al.* found a new metabolite possessing a unique tricyclic skeleton from *Paraphaeosphaeria neglecta* FT462 isolated from *Lycopodiella cernua*. In our continuing efforts to find structurally and biologically attractive natural products from plant-inhabiting fungi, we focused the Lycopodiaceae plant-derived endophytic fungi as sources of new natural products. In this study, we investigated chemical constituents of *Hypoxylon* sp. LY94 isolated from a root of *Lycopodium serratum* var. *longipetiolatum*.

【Methods and Results】

Hypoxylon sp. LY94 was cultured on potato dextrose agar plates at 28 °C for 14 days. The grown mycelia and media were extracted with methanol, and the extracts were partitioned between ethyl acetate and water. The ethyl acetate-soluble materials were separated by silica gel column chromatography, C_{18} column chromatography, and C_{18} HPLC to obtain three new polyketides (**1**−**3**). On the basis of spectroscopic data, the palaner structures of **1**−**3** was revealed to be new clonostachydiol analogs. The analyses of stereochemistries of these compounds are currently in progress.



1 **2** **3**

O-2

Structures of ingenan-type diterpenes isolated from the roots of *Euphorbia kansui*

Misato OGAWA (小川美怜)[1], Kan'ichiro ISHIUCHI[1], Shohei MIYATA[2], Susumu KITANAKA[3]

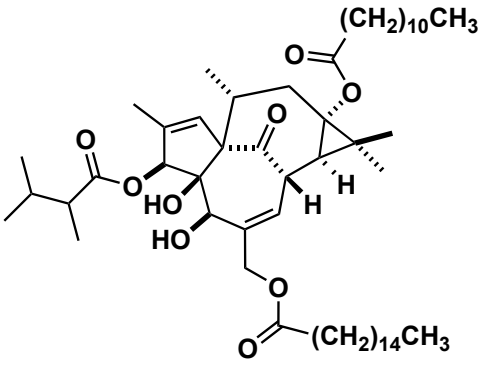
[1]Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]College of Humanities and Sciences, Nihon University; [3]Dios Medical Science Institute

【Objective】

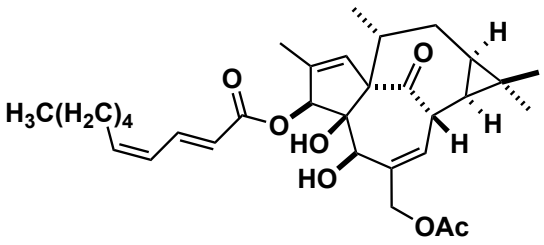
The dried roots of *Euphorbia kansui* (Euhorbiaceae) are known as "Kan Sui" in Chinese medicine. "Kan Sui" was recorded in *Sheng Nung's Herbal* as a low-grade drug and has been used as an herbal remedy for edema, ascites, and cancer in mainland China. *E. kansui* is well-known to contain triterpenes such as tirucallol, *α*-euphol, and *α*-euphorbol, and diterpenes such as kansuinines A and B, kansuiphorins A and B, and 13-hydroxyingenol. Previously, we isolated several diterpenes possessing an ingenan skeleton from this crude drug. Among them, 3-*O*-(2'*E*,4'*E* -decadienoyl)-20-*O*-acetylingenol (**4**) was reported to induce apoptosis in chemoresistant cancers with Cyclin D1 accumulation. In this study, we further explored the chemical constituents of the dried roots of *E. kansui*.

【Methods and Results】

The dried roots of *E. kansui* were extracted with chloroform/methanol, and the extracts were partitioned between ethyl acetate and water. The ethyl acetate-soluble materials were separated by silica gel column chromatography, C_{18} column chromatography, and C_{18} HPLC to obtain two diterpenes (**1**−**2**) along with kansuiphorin A (**3**). The analyses of chemical strucrtures of isolated compounds are currently in progress.



3



4

O-3

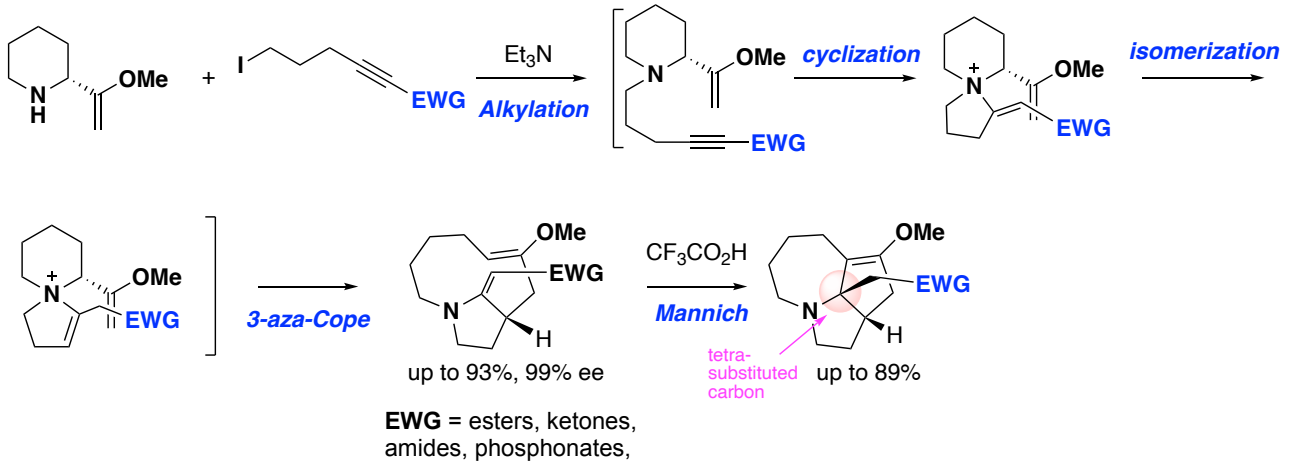
Synthesis of fused cyclic amines via the alkylation–cyclization–isomerization–3-aza-Cope cascades

Takeo SAKAI,* Tomoki FURUHATA, Kota HOSOE, Kaho UMEMURA, Fumiho SAITO, Yuji MORI

Faculty of Pharmacy, Meijo University, Nagoya, Japan

Cascade reactions are useful for constructing complex molecules. Herein, we report our recent research on the construction of fused-cyclic amines via cascade reactions involving the 3-aza-Cope rearrangement and subsequent Mannich cyclization.

We disclose a new cascade reaction using piperidines and alkyl iodides bearing electron-withdrawing alkynes to give bicyclic enamines. The reaction conditions are very simple: heat a mixture of piperidines and alkyl iodides bearing electron-withdrawing group in the presence of triethylamine. A range of electron-withdrawing groups, from ketones to amides, were tolerated on the alkyne moieties under the reaction conditions. The bicyclic enamines underwent trifluoroacetic acid-mediated cyclization to form tricyclic amines bearing tetrasubstituted carbons that could be useful for constructing a library of pseudo-natural alkaloids.



References

1) Sakai, T.; Furuhata, T.; Hosoe, K.; Umemura, K.; Mori, Y. *Org. Lett.* **2023,** *25,* 2986–2990. (Highlighted in Synfacts **2023,** *19,* 651)

2) Sakai, T.; Saito, F.; Hosoe, K.; Mori, Y. *Tetrahedron* **2024,** *153,* 133851.

O-4

Quality control and evaluation of Black chokeberry (Aronia melanocarpa) by three-wavelength fusion fingerprinting and electrochemical fingerprinting combined with antioxidant activity analysis

Ming Chen[1], Peifei Gu[2], Guoxiang Sun (孙国祥)[1]

[1]School of Pharm1acy, Shenyang Pharmaceutical University, [2]Department of Sport Medical, Institution of Sport and Health, Shenyang Sport College.

In this study, Black chokeberry (Aronia melanocarpa) was used as an example to provide reference for improving the safety, efficacy and quality consistency of homologous foods. In this study, two quality markers (Q-markers) of 27 batches of Black chokeberry were determined using high performance liquid chromatography (HPLC), and there were some differences among the 27 samples. Origin B samples had the highest levels of Q-markers for S15, and origin C had lower than average levels overall. Samples were analyzed qualitatively and quantitatively by Systematic Quantitative Fingerprinting (SQFM). Subsequently, a three-wavelength fusion analysis (TWFP) was established on the chromatographic data to compensate for the lack of a single wavelength. Fourteen batches of TWFP samples were rated at Level 5 or above in the SQFM assessment, indicating that there is some variation in the content of samples from different origins. Principal component analysis (PCA) was used to observe the differences in chemical composition and content of TWFP samples. Subsequently, electrochemical fingerprinting (ECFP) was established and nine characteristic parameters were recorded, showing that the samples were suppressed for all electrochemical Belousov-Zhabotinsky oscillation systems (B-Z oscillation systems). Finally, antioxidant tests were performed using DPPH. The antioxidant capacity was predicted using Partial Least Squares (PLS) analysis with $R^2Y=0.84$, $Q^2=0.77$, a good model fit and accurate prediction. The fingerprint-potency relationship between $\boldsymbol{IC}_{50}$-peak area showed that 17 of the 19 shared peaks were negatively correlated, indicating that 17 peaks contributed significantly to the antioxidant. The methods established in this study for the determination of TWFP and ECFP, as well as the spectral relationships with peak area and $\boldsymbol{IC}_{50}$, can be used for the quality inspection and antioxidant capacity test of Black chokeberry, which provides a new research direction for improving the quality standard of medicinal and foodstuffs.

[1] E. Mastrangelo and M. Milani, Role and inhibition of GLI1 protein in cancer, Lung Cancer: Targets Ther. 9 (2018) 35–43.

[2] S. Pietrobono, S. Gagliardi and B. Stecca, Non-canonical hedgehog signaling pathway in cancer: activation of GLI transcription factors beyond smoothened, Front. Genet. 10 (2019) 556.

[3] M. Axelson, M.K. Liu and X. Jiang, et al, U.S. food and drug administration approval: Vismodegib for recurrent, locally advanced, or metastatic basal cell carcinoma, Clin. Cancer Res. 19 (2013) 2289–2293.

[4] D. Casey, S. Demko and S. Shord, et al, FDA approval summary: Sonidegib for locally advanced basal cell carcinoma, Clin. Cancer Res. 23 (2017) 2377–2381.

O-5

Discovery of Novel Small-molecules Targeting Transcriptional Factor GLI

Jiachen WEN (闻家辰), Wangzhi QIN, Linxiang ZHAO

Department of Medicinal Chemistry, Shenyang Pharmaceutical University

The Glioma-associated oncogene (GLI) protein family consists of three transcription factors (GLI1, GLI2, and GLI3) that can serve as either activators or repressors of gene expression depending on the particular homolog and cellular context [1,2]. Historically, GLI-mediated transcriptional regulation has been associated with the roles played by the GLI proteins as downstream effectors of the hedgehog (Hh) signaling pathway. This type of Hh/GLI1 signaling is typically termed canonical and is essential for proper cellular proliferation and differentiation during embryonic development and is critical for the maintenance of some stem cell populations. Constitutive activation of canonical Hh/GLI1 signaling has been observed in various cancers, including basal cell carcinoma and medulloblastoma and several SMO antagonists have been approved for the treatment of Hh-dependent BCC [3-4].

Non-canonical GLI1 activation through Hh-independent mechanisms has been recently implicated as a driver for multiple forms of cancer not traditionally associated with the Hh pathway [1,2]. In these cancers, GLI1 activation is a downstream result of a variety of well-characterized oncogenic signaling pathways, including pro-inflammatory cytokines, the KRAS oncogene, and the PI3K/AKT/mTOR pathway [1,2]. Recent studies also suggest that GLI1 plays a role in DNA repair and may induce replication stress and enhance cytotoxicity in combination with small molecules targeting DNA repair pathways.

We utilized a virtual screening approach to identify an 8-hydroxyquinoline (compound **1**) as a promising hit with the potential to form strong binding interactions with GLI1. We demonstrated that compound **1** binds to GLI1 with high affinity, does not disrupt the GLI1/DNA complex, and inhibits Hh/GLI1 signaling in multiple cell lines. To further develop this new class of GLI1 inhibitors, we performed a systematic structure–activity relationship (SAR) study to determine structural requirements for potent anti-GLI1 activity.

[1] E. Mastrangelo and M. Milani, Role and inhibition of GLI1 protein in cancer, Lung Cancer: Targets Ther. 9 (2018) 35–43.

[2] S. Pietrobono, S. Gagliardi and B. Stecca, Non-canonical hedgehog signaling pathway in cancer: activation of GLI transcription factors beyond smoothened, Front. Genet. 10 (2019) 556.

[3] M. Axelson, M.K. Liu and X. Jiang, et al, U.S. food and drug administration approval: Vismodegib for recurrent, locally advanced, or metastatic basal cell carcinoma, Clin. Cancer Res. 19 (2013) 2289–2293.

[4] D. Casey, S. Demko and S. Shord, et al, FDA approval summary: Sonidegib for locally advanced basal cell carcinoma, Clin. Cancer Res. 23 (2017) 2377–2381.

O-6

Biosynthesis of vanillin from natural substrates

Weizhuo Xu (徐慰倬)[1], Qi Ye[2], Yongbo Song[2], Jinghai Zhang[2]

[1]Department of Functional Foods and Wine, [2]Department of Life Science and Biopharmaceuticals, Shenyang Pharmaceutical University

Vanillin is the most important flavoring ingredients in food industries. Traditional chemical synthesis had been used for years and renders environmental issues.

In this research, natural reproducible material ferulic acid had been used as substrate, by engineered *E. coli* with feruloyl-CoA synthase (Fcs) and enoyl-CoA hydratase/lyase (Ech) vanillin had been generated by biosynthesis.

Enoyl-CoA hydratase/lyase (Ech) enzyme sequence had been analyzed according to their conservation and structural features using computational techniques including sequence comparison and molecular dynamics simulation, detailed the major binding modes and key amino acid residues between Ech and substrates. Then a series of mutations (F74W, A130G, A130G/T132S, R147Q, Q255R, ΔT90, ΔTGPEIL, ΔN1-11, ΔC260-287) by rational design had been obtained. Finally, the yield of vanillin produced by the mutants was verified by whole-cell catalysis. Results showed that mutants F74W, Q147R and truncated ΔN1-11 showed higher yields than wild-type Ech, with mutation efficiency above 30%. Molecular dynamics simulations and residue energy decomposition identified the basic amino acid residues K37, R38, K561, and R564 as the key amino acid residues affecting the free energy of binding between Ech and Feruloyl-Coenzyme A (FCA). The large changes in electrostatic interacting and polar solvating energies caused by the mutations may lead to decreased enzyme activity.

This study provides important theoretical guidance as well as experimental data for the biosynthetic pathway of vanillin.

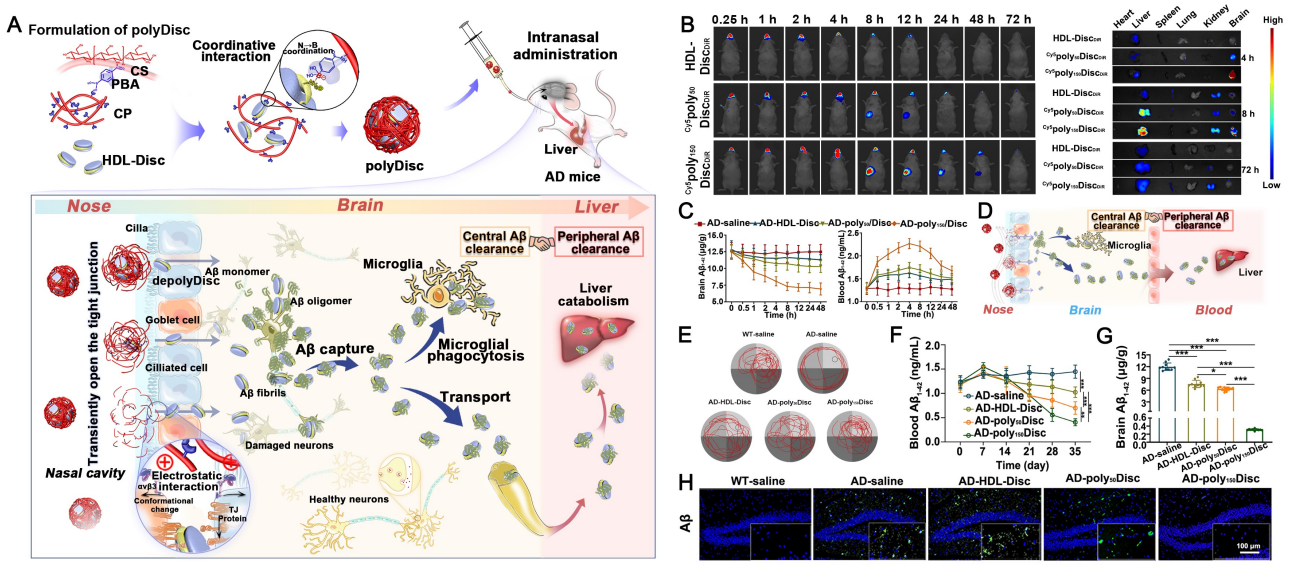
O-7

Nasal delivery of polymeric nanoDisc mobilizes a synergy of central and peripheral amyloid-β clearance to treat Alzheimer's disease

Huaqing ZHANG[#], Yun CHEN（陈赟）[#], Yang DING*

Department of Pharmaceutics, China Pharmaceutical University.

The disequilibrium of amyloid β-peptide (Aβ) between the central and peripheral pools has been claimed as an initiating event in Alzheimer's disease (AD). In this study, we employ discoidal high-density lipoproteins (HDL-Disc) mimicking Aβ antibody for directional flux of Aβ from central to peripheral catabolism, with desirable safety and translation potential. Structurally, HDL-Disc assembly (polyDisc) is prepared with aid of chitosan derivative polymerization. After intranasal administration and response to slightly acidic nasal microenvironment, polyDisc depolymerizes into carrier-free HDL-Disc with chitosan derivatives that adhere to the mucosal layer to reversibly open tight junctions, helping HDL-Disc penetrate the olfactory pathway into brain. Thereafter, HDL-Disc captures Aβ into microglia for central clearance or ferries Aβ out of the brain for liver-mediated compensatory catabolism. For synergy therapy, intranasal administration of polyDisc can effectively reduce intracerebral Aβ burden by 97.3% and vascular Aβ burden by 73.5%, ameliorate neurologic damage, and rescue memory deficits in APPswe/PS1dE9 transgenic AD mice with improved safety, especially vascular safety. Collectively, this design provides a proof of concept for developing Aβ antibody mimics to mobilize a synergy of central and peripheral Aβ clearance for AD treatment.



Scheme. 1 (A) Schematic illustration of polyDisc preparation and transportation for Aβ systemic clearance in AD therapy; (B-H) The polyDisc was polymerized after intranasal administration, following with the site-specific transport pathway that could be described as "nose→brain→liver". Consequently, the polyDisc effectively reduce intracerebral Aβ burden via central degradation and peripheral clearance, rescuing memory deficits in APPswe/PS1dE9 transgenic AD mice.

O-8

Biomimetic Elasticity Compressed Assembly Controls Rapidly Intracerebral Drug Release to Reverse Microglia Dysfunction

Guochen Han (韩国臣)[1], Xiaochen Gu[2], Jianping Zhou[1]*, Huaqing Zhang[1]*, and Yang Ding[1,3]*

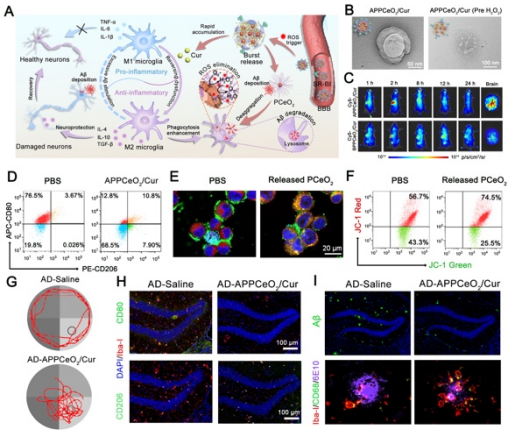
[1]State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University

[2]Faculty of Pharmacy, University of Manitoba

Background: In patients with Alzheimer's disease (AD), the accumulation of amyloid-β (Aβ) and pathophysiological stresses persistently activate microglia differentiation into M1 phenotype, which will stimulate inflammatory responses in the brain by releasing degenerate pro-inflammatory factors and reactive oxygen species (ROS). M2 microglia on the contrary are capable of secreting various protective mediators to mitigate inflammation, subsequently facilitating neuron repair and mobilizing phagocytosis to eliminate pathogenic proteins. Therefore, selective tuning of microglia polarization from M1 to M2 phenotypes will ameliorate inflammatory response and accelerate pathogenic Aβ clearance in AD physiological conditions.

Objective: Develop a combinative assembly of Curcumin (Cur) and ceria nanoenzyme (CeO_2), which is compressed by elastic polymer and packaged with ROS-responsive polydopamine (PDA) shell. The combinative drug assembly can regulate microglia polarization (M1→M2) in a concentration-dependent manner and remove pathogenic Aβ and cascade product ROS.

Results: In lesions of AD, PDA shell was stripped by ROS, and the inner polymeric assembly spread



compressive structure and restored hydrophilicity immediately, leading to burst exposure of Cur and polymer-linked CeO_2 ($PCeO_2$). The concentrated Cur switched microglial phenotype from M1 toward M2, which decreased the level of inflammatory factors by more than 60% and mobilized phagocytosis. $PCeO_2$ possessed high Aβ affinity ($K_D = 10^{-7}$ M) to accelerate Aβ plaques decomposition, and further facilitate microglia-mediated uptake (increasing ~1.8 times) and degradation of Aβ deposition. Subsequently, CeO_2 performed sustainable antioxidant capacity to prevent mitochondria damage of microglia. After administration, we observed that the assembly of Cur and CeO_2 synergistically reversed neurologic damage, leading to ameliorate memory and cognition impairments in AD mice.

Conclusion: Collectively, a biomimetic assembly with intracerebral elastic spreading for concentration-dependent drug therapy could open a new avenue for reversing microglia dysfunction, and suppressing AD progression and related central nervous degenerative diseases.

O-9

Postoperative Dynamic Pathology-Directed Sequential Drug Release by an Implantable Nanofiber in Glioma Chemoimmunotherapy

Mingjie SONG (宋明杰), Jianping ZHOU*, Huaqing ZHANG*, Yang DING*

Department of Pharmaceutics, China Pharmaceutical University

The keys to inhibit glioblastoma multiforme (GBM) postoperative relapse are the elimination of residual tumor satellites and the stimulation of tumoricidal immunity that induces immunologic memory. After surgical resection of orthotopic GBM, we found that residual tumor cells rapidly proliferate (Ki67 > 38.5%) followed by macrophages recruited and activated into immunosuppressive M2-type in a time-dependent manner, which constitutes the postoperative dynamic pathology. To match this process, we design a cavity-implantable nanofiber that comprises a core-shell structure for doxorubicin (DOX) and BLZ945 loading and sequential release to induce “in-situ vaccine” immunogenic death of tumor cells and sequentially repolarize M2-macrophages into immune-promoting M1-type, respectively. Based on the evaluation results in the orthotopic tumor models, the dual drug release from the nanofiber was programmed to match the disease process. *In vivo* results revealed that the designed nanofiber led to the increased lymphocyte levels by 3.76 times and a long-term survival rate of 60%. This study explores the concept to clarify the postoperative dynamic pathology and further directs programmed drug release by an implantable nanofiber to inhibit GBM recurrence, which can be extended to other postoperative tumor treatment regimens by simply adjust drug release profile.

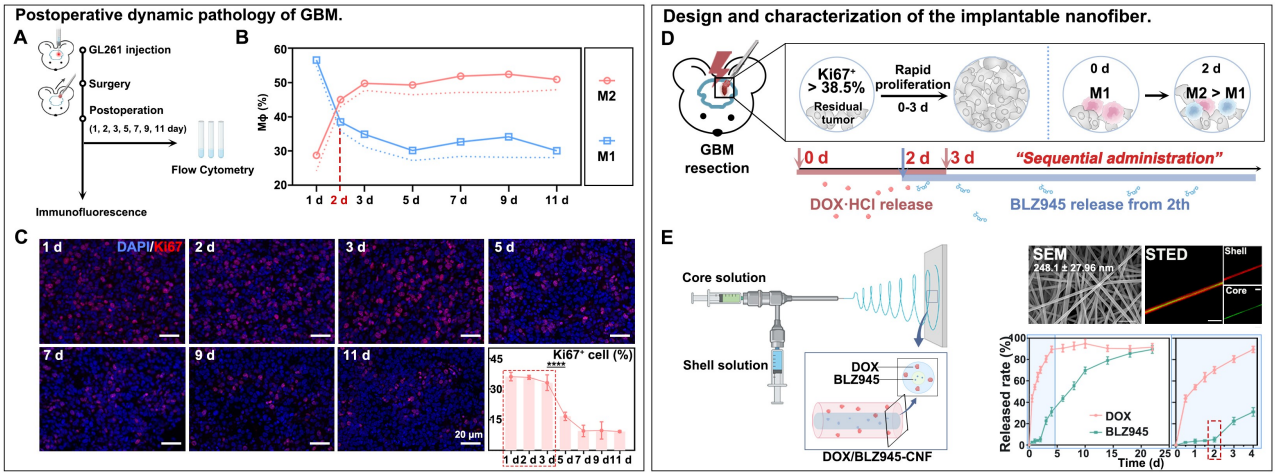


Fig 1. The implantable nanofiber was designed based on the postoperative dynamic pathology of GBM. A. Schematic illustration of the proliferation rate of tumor cells and macrophage phenotype analysis after surgery of glioma-bearing mice. **B**. Variation curves of macrophages on different postoperative days showing percentages of M1 and M2 types (n = 5). **C**. The $Ki67^{+}$ immunofluorescence staining of tumors slices and quantification of the relative percentage on different postoperative days (n = 5). **D**. Schematic illustration of sequential administration. **E**. The preparation process and release profiles of the nanofiber at different times and the SEM micrographs and super-resolution images of coaxial electrospinning nanofibers (n = 5).

O-10

Lipoprotein-mimicking Nanoparticles Mobilize Directional Drainage of Amyloid-β Efflux in Alzheimer's Disease Treatment

XI Yilong (习艺龙), ZHANG Huaqing, DING Yang.

State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University

Recent approvals of anti-amyloid-β (Aβ) antibodies have almost clarified the clinical remission for Alzheimer's disease (AD) by mobilizing Aβ clearance. However, the emergence of adverse events, attributed to excessive intracerebral clearance burden, has sparked safety concerns regarding central Aβ clearance therapies. Herein, we have demonstrated a sustained and secure peripheral Aβ clearance mediated by hepatocyte compared to the central immune elimination. Therefore, we introduced an initiative Aβ efflux pathway drainage by mimetic natural high-density lipoprotein (HDL) particles, the demonstrated Aβ chaperone enabling specific capture and systemic transport. Firstly, we coupled the apolipoprotein-derived α-helical fragments with angiopep-2 (αAng) to direct endothelial low-density lipoprotein receptor-related protein-1 (LRP1) recognization and enable blood-brain barrier (BBB)-mediated efflux. Subsequently to ensure the introduction of particles into the central, we exploited the transdocytosis pathway mediated by endothelial receptor for advanced glycation end products (RAGE), engineering a gallic acid terminated natural substrate fragment (KGA) (Fig. 1A). The reassembled lipoprotein-mimicking nanoparticles (KGA-αLNP) performed ascending brain permeability with the pathological progression in APP/PS1 mice due to the sustained upregulation of RAGE. After KGA liberation in response to the reactive oxygen species in AD lesions to combat oxidative stress, the remaining carrier captured Aβ aggregates with a high affinity of 25.8 nM to promote rapid depolymerization, and drainage Aβ for outflowing across BBB in a LRP1-depended manner (Fig. 1B-D). The brain-derived Aβ was enriched in liver through peripheral circulation and eliminated cerebral Aβ by 63%. This strategy was demonstrated to effectively improve the cognitive decline in APP/PS1 model mice without causing central overload or cerebrovascular amyloidosis. Collectively, we provided an efficient Aβ clearance strategy for relieving Aβ clearance burden to achieve sustainable and secure AD therapy.

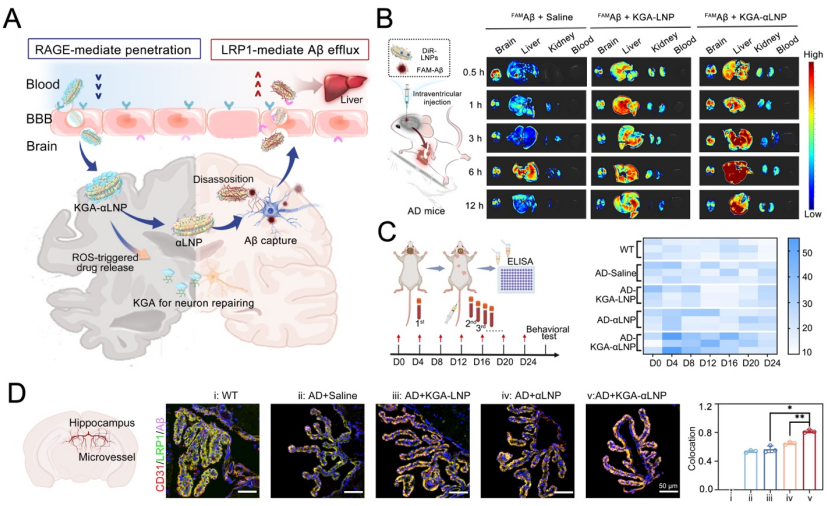


Fig. 1 (A) Schematic diagram of KGA-αLNP transportation. (B) Ex vitro monitored of Aβ efflux drained by KGA-αLNP. (C) Continuous monitoring of plasma Aβ in treated APP/PS1 mice. (D) Co-localization of efflux Aβ with cerebrovascular LRP1.

O-11

Preparation and characterization of liposome containing tosufloxacin-cyclodextrin inclusion complex

Hemat MOSTAFA [1], Hamid ALGHURABIi [2], TATSUAKI Tagami [1], KOKI Ogawa [1] and TETSUYA Ozeki [1]

[1] Drug Delivery and Nano Pharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University
[2] Department of Pharmaceutics, College of Pharmacy, University of Kerbala,

Background and objective: Delivering liposomal antibiotics directly to the respiratory tract is an effective strategy to achieve drug concentrations above the minimum inhibitory concentration at the infection site in the airways, owing to sustainable release and retention. Tosufloxacin tosylate (TFLX) has a low water solubility. This study aimed to enhance the antibiotic effect of TFLX by loading it into the liposomes. Initial attempts showed an encapsulation efficiency of only 17%, as liposomes only provided the lipophilic domain to entrap the lipophilic drugs, which decreased the room to encapsulate more drug.

To address this, we employed hydroxypropyl-beta-cyclodextrin (HP-β-CD) to increase TFLX solubility of TFLX. The TFLX/HP-β-CD complex, which enhances the solubility of TFLX, was loaded into liposomes, resulting in a drug-in-cyclodextrin (CDs)-in-liposome delivery system. This approach aimed to improve the encapsulation efficiency and optimize the delivery of TFLX. This study evaluated the physicochemical and biological properties of a TFLX/CD liposome formulation.

Methodology: Initially, we demonstrated that TFLX forms an inclusion complex with HP-β-CD at a molar ratio of 1:3. Subsequently, TFLX/CD-inclusion-loaded liposomes (TFLX/CD-liposomes) were prepared using a passive loading technique. Conventional TFLX-loaded liposomes (TFLX-liposomes) were prepared using the dehydration-rehydration method. A lipid film composed of DPPC, CHOL, and mPEG-DSPE (6.5:3.5:1 molar ratio) was formed via evaporation. The film was hydrated with 5% dextrose containing the TFLX/CD complex (1:2.5 molar ratio) to form multilamellar vesicles, which were then extruded through 200 nm and 100 nm polycarbonate membranes using an Avanti Mini-Extruder. The liposomes were characterized in terms of encapsulation efficiency, morphology, and particle size. The release profile and in vitro antibacterial activity were evaluated.

Results and discussion: The TFLX/HP-β-CD complexation significantly enhanced TFLX's solubility. TFLX/HP-β-CD-loaded liposomes achieved a high encapsulation efficiency of 69%, compared to 17% in conventional TFLX liposomes. Transmission electron microscopy (TEM) analysis showed mostly spherical unilamellar vesicles in the size range of 100~200 nm, indicating high density and stable structure. The TFLX/CD liposomes demonstrated significantly higher encapsulation efficiency compared to conventional TFLX liposomes. These liposome-encapsulated inclusion complexes exhibited a slower release rate, ensuring sustained drug release. Additionally, the TFLX/CD liposome formulation showed a remarkable antibacterial effect compared to free TFLX and conventional TFLX-liposomes. Further studies are needed, but the CD-in-liposomes delivery system appears promising for TFLX and shows potential for treating intracellular bacterial infections.

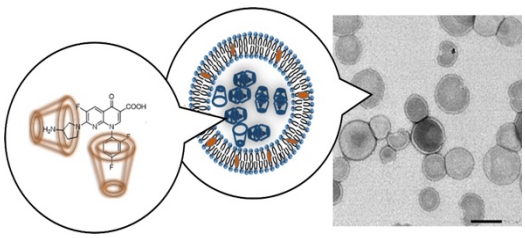


Figure 1. Scheme of the present study. TFLX/CD inclusion complex was encapsulated in liposome to improve drug solubility, encapsulation efficiency, and controlled drug release.

O-12

Preparation of gold nanostars/extracellular vesicles nanocomplex and their photothermal therapeutic effect on cancer cells

Kazuki SATO (佐藤一輝), Koki OGAWA, Tatsuaki TAGAMI, Tetsuya OZEKI
Drug Delivery and Nano Pharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University

Purpose: Gold nanostars (GNSs) are metal nanoparticles that generate high heat when irradiated by laser of specific wavelengths. GNSs by themselves have low targetability to tumor tissue. Therefore, with the aim of getting future targetability, we focused on extracellular vesicles (EVs), which are shed from cells for intercellular communication. They are then efficiently delivered to specific cells for uptake. Therefore, the aim was to prepare a complex of GNSs and model EVs.

Methods: Model EVs were recovered from the culture medium of human fetal kidney cells using an ultracentrifuge. GNSs were prepared by a general seed-mediated growth method. PEG-GNSs were prepared by modifying the particle surface with polyethylene glycol containing thiol and amino groups. Since PEG-GNSs have a positive charge and EVs have a negative charge, the desired complex, EV-GNSs was prepared by electrostatic interaction by reacting these two types of particles in a 5 % glucose solution. The particle size, zeta potential, absorption peak and morphology of each prepared particle were observed. Western blotting was also performed on the EVs and EV-GNSs solutions to check for the presence of EVs-specific membrane proteins. Furthermore, the temperature increasing of each particle when irradiated by laser was confirmed, and the cytotoxic effect when added to cells and irradiated by laser was verified.

Results and Discussion: The recovered EVs had a particle size of approximately 100 nm, a negative charge, a spherical shape and the detection of CD63, a known EVs marker. GNSs had a particle size of approximately 50 nm, a negative charge, a GNSs-specific absorption peak around 600-800 nm and a star-like protruding structure. PEG-GNSs had a particle size of approximately 100 nm, a positive charge and a black haze on the surface of the particles, which appeared to be PEG. The particles of interest, EV-GNSs, had a particle size of approximately 200 nm and a negative charge, and the detection of CD63, a known EVs marker, was confirmed. Furthermore, PEG-GNSs and EVs were observed to form a complex, with the absorption peak falling between 600-800 nm, which is characteristic of GNSs. A marked temperature increasing was observed when PEG-GNSs and EV-GNSs were irradiated by laser. EV-GNSs were added to mouse melanoma cells showed an excellent cytotoxic effect when irradiated by laser. From the above results, complexes of PEG-GNSs and EVs could be prepared by electrostatic interaction. The prepared complexes are expected to be applied to photothermal therapy.

O-13

Mitochondria-targeted Gene Delivery System

Yi WANG (王译)[1], Hulin JIANG[2]

State Key Laboratory of Natural Medicines, China Pharmaceutical University

Yi WANG: Tel (86-18551799672), e-mail (wangyi950825@163.com)

Hulin JIANG: Tel (86-18651692213), e-mail (jianghulin3@163.com)

1. Background and Purpose. Leber's hereditary optic neuropathy (LHON) is a rare inherited blindness caused by mutations in the mitochondrial DNA (mtDNA). The disorder is untreatable and tricky, as the existing chemotherapeutic agent Idebenone alleviates symptoms rather than overcoming the underlying cause. Although some studies have made progress on allotopic expression for LHON, in situ mitochondrial gene therapy remains challenging, which may simplify delivery procedures to be a promising therapeutic for LHON. Herein, a pathologically responsive mitochondrial gene delivery vector named [triphenylphosphine-terminated poly(sulfur-containing thioketal undecafluorohexylamine histamine) and Ide-terminated poly(sulfur-containing thioketal undecafluorohexylamine histamine)] (TISUH) is reported to facilitate commendable in situ mitochondrial gene therapy for LHON.

2. Method. Mitochondrial gene transfection efficiency was estimated in extracted free mitochondria. In addition, therapeutic effect of TISUH-mediated gene therapy was investigated in rotenone-induced and gene mutated LHON mouse models.

3. Results. TISUH directly targets diseased mitochondria via triphenylphosphine and fluorination addressing the decreasing MMP. In addition, TISUH can be disassembled by high mitochondrial ROS levels to release functional genes for enhancing gene transfection efficiency and fundamentally correcting genetic abnormalities. In both traditional and gene mutation-induced LHON mouse models, TISUH-mediated gene therapy shows satisfactory curative effect through the sustained therapeutic protein expression in vivo.

4. Conclusion. Pathologically responsive mitochondrial gene therapy (TISUH) in an allotopic expression-independent manner achieves in situ mitochondrial gene delivery. In addition, it is a nanomedicine to import gene into mitochondria for LHON treatment, a capability that has potential to model mtDNA mutated disorders and correct genetic abnormalities in other mtDNA mutated diseases.

Key words: Leber's hereditary optic neuropathy, mitochondrial gene therapy, fluorination

O-14

iNKT17 cells play a pathogenic role in cholestasis through CXCR3 mediated recruitment and activation

WANG Xinzhi (王欣之)

New Drug Screening and Pharmacodynamics Evaluation Center, State Key Laboratory of Natural Medicins, China Pharmaceutical University

Intrahepatic cholestasis (IHC) is a common clinical symptom of liver diseases. If patients don't receive effective treatment, IHC can develop into liver fibrosis, cirrhosis and liver cancer. IL-17 is positively related to the inflammation of IHC. Bile acid (BA) metabolites directly affect the activity of RORγt. Targeting IL-17 and RORγt improves cholestasis in mouse model. However, the source, effect, signaling pathway and potential drug target of IL-17 in IHC are still not clear. In the present study, we explored the mechanism of iNKT17 cells through CXCL9/10-CXCR3 signaling pathway in estrogen-induced IHC and demonstrated the feasibility of iNKT17 cell function regulation as a potential therapeutic target. Estrogen promoted the activation and expansion of iNKT17 cells, which contributed to a novel hepatic iNKT17/Treg imbalance. iNKT cell-deficient Jα18$^{-/-}$ mice and the RORγt inhibitor alleviated cholestatic hepatotoxicity and downregulated the IL-17 signaling pathway. In contrast, the co-administration of estrogen with recombinant IL-17 to Jα18$^{-/-}$ mice induced cholestatic hepatotoxicity and increased the infiltration of hepatic neutrophils and monocytes. The administration of IL-17$^{-/-}$ iNKT cells to Jα18$^{-/-}$ mice resulted in the attenuation of hepatotoxicity and the recruitment of fewer hepatic neutrophils and monocytes than the adoptive transfer of wild-type iNKT cells. The recruitment and activation of iNKT17 cells could be attributed to the high level of CXCR3 expression on their surface. CXCL10 deficiency ameliorated cholestatic liver damage, reduced hepatic CXCR3^{+} iNKT cells and inhibited RORγt expression. CXCR3 is functionally pleiotropic. We further demonstrated the involvement of CXCR3 in IHC in α-naphthylisothiocyanate (ANIT) and triptolide, two hepatotoxicants that induce IHC. By alleviating BA dysregulation, reducing NK/iNKT cell recruitment and endoplasmic reticulum/oxidative stress, CXCR3 deficiency ameliorated ANIT- and triptolide-induced IHC. Moreover, we screened the active compounds targeted to inhibit RORγt by big data and artificial intelligence assisted molecular docking and found that 18β-glycyrrhetinic acid alleviated estrogen-induced cholestasis via downregulating RORγt and CXCR3 signaling pathway in iNKT cells. In conclusion, the present study investigates the pathological mechanism and therapeutic target of IHC, contributing to the drug development and reasonable prevention and treatment.

O-15

In Vivo Cell Engineering for Tumor Tracking Therapy

Hao CHENG (程皓), Jianping ZHOU, Yang DING

Department of Pharmaceutics, China Pharmaceutical University, Nanjing 210009, China

Directed cell engineering renders a promising strategy for oncotherapy, but precise in vivo genome editing via systemic CRISPR/Cas9 delivery remains challenging. Herein, we developed a therapeutic approach to generate TRAIL-installed tumor cells in vivo by delivering dCas9/sgRNA with tumor targeting lipid-polymer nanoparticles (LPNPs). Therapeutic benefits of the in vivo reprogrammed TRAIL-installed tumor cells were evaluated by injecting LPNPs into a mouse model of breast cancer. Efficient delivery of TRAIL-activating dCas9/sgRNA to primary tumor was observed, leading to abundant and effective TRAIL-installed tumor cell production. The engineered tumor cells exhibited apoptosis-promoting activity via pervasive self-attack, and generated the TRAIL-rich apoptotic debris. The apoptotic debris were spontaneously spread to track the metastatic tumor lesions by self-targeting and induced the metastasis elimination. The treatment with the TRAIL-activating LPNPs enables complete self-elimination of primary and metastatic breast tumors, accompanied with negligible TRAIL variations in the normal tissues and organs. The in vivo generation of TRAIL-installed tumor cells may hold splendid potential as a therapeutic platform for metastatic cancer.

O-16

Metabolomic analysis for unveiling a novel mechanism of breast cancer formation induced by long-term estrogen exposure

Yoshinori Okamoto (岡本誉士典), Akira Aoki, Hideto Jinno

Faculty of Pharmacy, Meijo University, Nagoya, Japan

Women experience a decline in endogenous estrogen levels during menopause, leading to various menopausal symptoms. Hormone replacement therapy (HRT) is employed to mitigate these symptoms; however, numerous epidemiological studies have reported that prolonged estrogen exposure increases the risk of various malignancies, including breast cancer. Consequently, some patients avoid HRT despite experiencing menopausal symptoms. To ensure that patients can safely undergo HRT, it is crucial to elucidate the mechanisms of estrogen-induced breast carcinogenesis and develop strategies to mitigate or avoid the adverse effects of estrogen. We are investigating the mechanisms of estrogen-induced breast carcinogenesis and developing non-carcinogenic estrogen derivatives, using the estrogen-induced breast cancer model August Copenhagen-Irish/Segaloff (ACI) rats. In this presentation, we will discuss the analysis of novel carcinogenic mechanisms using metabolomic analysis. This study was conducted with the approval of the Animal Experiment Committee of Meijo University School of Pharmacy.

To elucidate the novel mechanisms of estrogen-induced breast carcinogenesis, we conducted serum metabolomic analysis using ACI rats administered with long-term 17β-estradiol (E2). The analysis revealed a significant elevation in several lysophosphatidylcholine (LPC) species. It is established that LPC is converted into the bioactive metabolite lysophosphatidic acid (LPA) by autotaxin (ATX), which exhibits phospholipase D activity. Therefore, to elucidate the effects of LPC and LPA, as well as rat serum on mammary tissue, we conducted proliferation assays using human breast cancer MDA-MB-231 cells. The results demonstrated that LPC (18:1) and LPA (18:1), as well as rat serum, induced a dose-dependent increase in cell proliferation. Furthermore, to verify the involvement of ATX and LPA receptors (LPAR) in the cell proliferation induced by rat serum, we evaluated the effects on proliferation using S32826 (ATX inhibitor) and Ki16425 (LPAR inhibitor). The results indicated that both inhibitors suppressed the cell proliferation-promoting effect of rat serum to control levels. These findings suggest that LPC in rat serum is converted to LPA by ATX, which subsequently promotes cell proliferation via LPAR. Therefore, our study suggests the involvement of lipid mediators as a novel mechanism in estrogen-induced breast carcinogenesis.

O-17

Unraveling the Mechanism of Cotinine-Induced Stabilization of Androgen Receptor Protein in Prostate Cancer Cells

Riri HAYASHI (林 莉々)[1], Yuta YOSHINO[1], Masaki SHIOTA[2], Naohiro FUJIMOTO[3], Akira IKARI[1], Satoshi ENDO[4, 5]

[1] Laboratory of Biochemistry, Gifu Pharmaceutical University,

[2] Department of Urology, Graduate School of Medical Sciences, Kyushu University

[3] Department of Urology, University of Occupational and Environmental Health

[4] United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University

[5] Center for One Medicine Innovative Translational Research (COMIT), Gifu University

Although the relationship between prostate cancer and smoking remains unclear, it has een reported that smoking habits worsen the prognosis of prostate cancer patients treated with the androgen receptor (AR) antagonist Enzalutamide (Enz). Nicotine, a major harmful component of tobacco smoke, is rapidly metabolized into cotinine in the liver after entering the bloodstream. Cotinine has a longer half-life and accumulates more than nicotine. We investigated the impact of cotinine on the treatment efficacy of prostate cancer, a hormone-dependent cancer, and explored its detailed mechanisms.

Androgen signaling, crucial for the proliferation of prostate cancer cells, is regulated by the activation of AR by androgens. In prostate cancer 22Rv1 cells, cotinine significantly increased PSA expression by enhancing AR protein stability in the presence of androgens, thereby promoting androgen signaling. To elucidate the mechanism of cotinine-induced AR protein stabilization, we focused on the AR co-regulatory factor heat shock protein (HSP), known to stabilize proteins by binding to AR. Using co-immunoprecipitation and Western blotting methods, we confirmed that cotinine treatment induced changes in the interaction between AR and HSP, suggesting that cotinine enhanced the binding affinity between AR and HSP.

Furthermore, we examined the impact of cotinine-induced AR protein stabilization on the Enz sensitivity of prostate cancer cells. In the combination group with Enz and cotinine, cell survival rates were significantly higher compared to the group treated with Enz alone. Cotinine alleviated the Enz-induced increase in Bax/Bcl2 and Bax/Bcl-xL ratios, suggesting that the restoration of mitochondrial membrane function by cotinine contributes to the reduced Enz sensitivity of prostate cancer cells.

So far, the causative agent and mechanism of malignant transformation of prostate cancer due to smoking habit have been unknown. In this study, we found that cotinine, a metabolite of nicotine, stabilizes AR protein and strengthens the binding between AR and HSP, thus enhancing androgen signaling.

O-18

Overcoming Prostate Cancer Drug Resistance by Targeting Lipid Peroxidation-Derived Reactive Aldehyde Metabolism by AKR1C3

Shinya KAWANO (河野真也)[1], Jin SEGAWA[1], Mina KAWAI[1], Atsumi OTA[1], Yuta YOSHINO[1], Masaki SHIOTA[2], Naohiro FUJIMOTO[3], Akira IKARI[1], Satoshi ENDO[4,5]

[1] Laboratory of Biochemistry, Gifu Pharmaceutical University,

[2] Department of Urology, Graduate School of Medical Sciences, Kyushu University,

[3] Department of Urology, University of Occupational and Environmental Health,

[4]United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University

[5]Center for One Medicine Innovative Translational Research (COMIT), Gifu University

In the treatment of castration-resistant prostate cancer (CRPC) unresponsive to hormone therapy, a CYP17A1 inhibitor (abiraterone) and androgen receptor (AR) antagonists such as enzalutamide and apalutamide (Apa) are used, but early acquiring resistance remains a significant problem. This study focuses on aldo-keto reductase (AKR)1C3, which is involved in androgen synthesis downstream of CYP17A1, and investigates the overcoming effects of a potent and specific AKR1C3 inhibitor **2j** on Apa resistance in Apa-resistant prostate cancer 22Rv1 (22-R) cells.

In 22-R cells, which exhibit higher AKR1C3 expression compared to 22Rv1 cells, the AKR1C3 inhibitor **2j** was found to synergistically enhance the anticancer activity of Apa. It suggests that AKR1C3 acts protectively in prostate cancer cells by reducing reactive aldehydes such as 4-hydroxynonenal (HNE), which are derived from lipid peroxidation. The combination of Apa/**2j** led to a significant increase in the levels of reactive oxygen species, lipid peroxidation, and HNE-modified proteins. On the other hand, it did not increase the levels of intracellular divalent iron. This combination also markedly reduced the levels of oxidized glutathione required for glutathione peroxidase 4 (GPx4) activity and the expression of mevalonic acid metabolizing enzymes needed for the synthesis of isopentenyl pyrophosphate, which is necessary for selenocysteine transport to GPx4. These reductions might contribute to the induction of ferroptosis. A similar phenomenon was observed when AKR1C3 was knocked down, further supporting its role in protecting from ferroptosis in 22-R cells.

In summary, our results suggest that restoring APA sensitivity in 22-R cells by AKR1C3 inhibitor **2j** involves ferroptosis induction via increased lipid peroxidation due to GPx4 dysfunction.

O-19

Disruption of intracellular iron homeostasis and mitochondrial damage by lysosomal disfunction in PARK9 model cells

Takanori MURAKAMI (村上貴規), Kazuki OHUCHI, Hisaka KURITA,
Masatoshi INDEN
Laboratory of Medical Therapeutics and Molecular Therapeutics, Gifu Pharmaceutical University

Background and Purpose: Iron accumulation in the substantia nigra (SN) may be significant in Parkinson's disease (PD), but the underlying mechanism is unclear. Although iron is an essential element, excessive amounts cause toxicity. We focused on the role of iron and ATPase cation transporting 13A2 (ATP13A2), the causative gene of PARK9 neurodegeneration with brain iron accumulation. ATP13A2 is an ATPase localized in the lysosome and maintain lysosome homeostasis by transporting cationic molecules such as polyamines, protons, and metal ions. Additionally, we reported the disruption of intracellular iron homeostasis by ATP13A2 deficiency. In patients with PD, there is a high lysosomal pH caused by loss-of-function mutations of ATP13A2, leading to proteolytic failure. Therefore, considering that mitophagy is a lysosome-mediated mechanism, decreased ATP13A2 function can result in mitophagy dysfunction. Mitochondria may be a key player in the pathogenesis of PARK9. In this study, we attempted to elucidate the relationship between mitochondria and the disruption of iron homeostasis.
Methods: We generated PARK9 model cells by alpha-synuclein (α-Syn) overexpression and ATP13A2 knockdown in human neuroblastoma cell line SH-SY5Y, and analyzed heme synthesis, IRP2 expression, mitochondrial morphology, and mitophagy. Mitochondrial morphology and mitophagy were analyzed by probes. Mitochondrial damage was estimated by mitochondrial DNA (mtDNA) leaking to cytosol.
Results and Discussion: Intracellular iron levels are maintained through an IRP2-based iron-responsive feedback system that regulates the expression of iron-related genes to prevent cytotoxicity. Therefore, we analyzed IRP2 expression with or without ferric ammonium citrate (FAC) treatment. FAC treatment decreased IRP2 levels in control SH-SY5Y cells but not in PARK9 model cells, suggesting that intracellular iron levels were increased in PARK9 model cells, but IRP2 did not respond to the upregulation of labile iron. Next, we assessed the capacity of heme synthesis, as IRP2 degradation is induced by heme upregulation in mitochondria. The results showed that heme synthesis was decreased in PARK9 model cells. Additionally, we observed abnormal mitochondrial morphology and mtDNA leakage into the cytosol in PARK9 model cell. Furthermore, the capacity of mitophagy was decreased, indicating an accumulation of damaged mitochondria. Therefore, it is possible that mitochondrial dysfunction may contribute to the disruption of intracellular iron homeostasis in PARK9 model cells. These results may contribute to the elucidation of the pathogenesis of Parkinson's disease and other neurodegenerative diseases that exhibit iron deposition, as well as to the development of drug targets.

O-20

Functional analysis of type III sodium-dependent phosphate transporter in neurons

Junya MURATA (村田潤哉), Yuto MURAYAMA, Ayane MISIMA, Kazuki OHUCHI, Hisaka KURITA, Isao HOZUMI, Masatoshi INDEN

Laboratory of Medical Therapeutics and Molecular Therapeutics, Gifu Pharmaceutical University

Background and Purpose: Primary basal ganglia calcification (PBGC) is a disease characterized by bilateral calcification in the basal ganglia and dentate nuclei. Mutations in *SLC20A2*, which is classified as a type III sodium-dependent phosphate transporter (NaPiT), have been reported as a causative gene for PBGC. NaPiT, which regulates phosphate transport in vivo, is involved in phosphorus homeostasis inside and outside the cell. In PBGC, the *SLC20A2* mutation is predicted to disrupt intracellular phosphate dynamics due to its impaired ability to transport phosphate, resulting in a disruption of phosphate homeostasis. In a previous study, neuronal cells expressed not only *SLC20A2* but also *SLC20A1*, which is also a type III NaPiT, suggesting that type III NaPiTs may be involved in phosphorus homeostasis in neuronal cells. However, the detailed molecular mechanism of phosphate homeostasis in neurons remains unclear. Therefore, the aim of this study was to analyze the function of type III NaPiT in neurons to elucidate the PBGC pathogenesis.

Methods: In human neuroblastoma cells SH-SY5Y, we transiently knocked down type III NaPiT using siRNA and measured changes in *SLC20A1* and *SLC20A2* mRNA expression and intracellular phosphorus concentration. Similarly, siRNA-treated SH-SY5Y was evaluated for phosphorus uptake using the radioisotope P-32. We also measured the effect of *SLC20A1* or *SLC20A2* siRNA on the neurites of differentiated SH-SY5Y using retinoic acid.

Results and Discussion: First, we examined gene expression changes upon transient knockdown of *SLC20A1* and *SLC20A2* by siRNA. The results showed that compensatory mechanism was not found between type III NaPiTs. In phosphorus uptake experiments using P-32, knockdown of *SLC20A1* and *SLC20A2*, respectively, resulted in decreased phosphorus uptake in both knock-downed SH-SY5Y. As for intracellular phosphorus levels, only *SLC20A2* knockdown increased phosphorus levels. In cells differentiated by retinoic acid, *SLC20A1* knockdown did not alter neurite outgrowth compared to the control siRNA-treated group, but *SLC20A2* knockdown significantly lengthened it. These results provide fundamental insights into the mechanism of phosphorus homeostasis in neuronal cells.

O-21

Mitochondrial damage activates the cGAS/STING/type I IFN pathway in neurons

Ayaka FUJIMAKI (藤牧綾香)[1], Asuka NAKAJIMA[1], Takanori MURAKAMI[1], Kazuki OHUCHI[1], Hisaka KURITA[1], Yoki NAKAMURA[2], Norimitsu MORIOKA[2], Masatoshi INDEN[1]

[1] Laboratory of Medical Therapeutics and Molecular Therapeutics, Gifu Pharmaceutical University

[2] Department of Pharmacology, Hiroshima University

Cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS), a DNA sensor, detects exogenous DNA, such as viruses, activates stimulator of interferon (IFN) genes (STING), and produces type I IFN. The cGAS/STING pathway is also activated by the mitochondrial DNA (mtDNA) leaking into the cytoplasm. This activation contributes to age-related chronic inflammation and the inflammatory pathogenesis of multiple neurodegenerative diseases, including amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease (PD). PD is characterized by the loss of dopaminergic neurons in the substantia nigra, and mitochondrial dysfunction has been implicated in PD. In previous studies, increased type I IFN was observed in postmortem PD human samples and PD model mice. One study suggested that the loss of STING prevented inflammation and dopaminergic neuron degeneration in PD model mice. Most studies on cGAS/STING pathway in the brain have focused on microglia. However, the contribution of the cGAS/STING pathway via mitochondrial damage in neurons remains unclear. In the present study, we evaluated cGAS/STING activity in SH-SY5Y cells upon exposure to rotenone, an inhibitor of complex I of the mitochondrial electron transport chain used to generate PD models. Exposure to rotenone increased mtDNA in the cytoplasm, signaling through phosphorylation of the cGAS/STING pathway was observed, and finally, type I interferon was elevated. These activations of cGAS/STING pathway by rotenone occurred at treatment concentrations of rotenone that did not cause cell death. In addition, rotenone-induced elevation of type I IFN was abolished by inhibitors of the cGAS/STING pathway. These findings indicate that mitochondrial damage in neurons causes inflammation via the cGAS/STING/type I IFN pathway. In addition, we found that certain natural compounds inhibit cGAS/STING pathway. These results suggest that inhibition of cGAS/STING/type I IFN pathway may be beneficial for PD treatment.

O-22

Effects of activated α7 nicotinic acetylcholine receptor against α-synuclein-induced neurotoxicity

Shinnosuke TAKIZAWA（滝沢進之佑）, Kazuki OHUCHI, Taisei ITO, Takanori MURAKAMI, Hisaka KURITA and Masatoshi INDEN

Laboratory of Medical Therapeutics and Molecular Therapeutics, Gifu Pharmaceutical University

Increasing evidence indicates that certain neurodegenerative diseases, including Parkinson's disease (PD), involve the loss of neuronal nicotinic acetylcholine receptors (nAChRs). Among these receptors, α7 nAChR have emerged as crucial targets in the development of PD therapeutics. Previously, we demonstrated that α7 nAChR activation protects against nigrostriatal dopamine degeneration in acute and chronic PD animal models induced by 6-hydroxydopamine and rotenone, respectively. Additionally, we revealed that α7 nAChR activation exerts multiple neuroprotective effects, including autophagy activation in a cellular model of amyotrophic lateral sclerosis. However, the precise mechanisms underlying these neuroprotective effects remain to be elucidated. Although the exact mechanisms underlying PD remain unclear, α-synuclein (α-syn, encoded by *SNCA*), a primary component of the cytoplasmic inclusions known as Lewy bodies, is a major contributor to PD pathophysiology. Additionally, *SNCA* gene mutations and multiplications can alter α-syn aggregation. These mutations cause autosomal dominant PD in a dose-dependent manner. They include amino acid substitutions, such as A53T, A30P, and E46K, as well as gene duplication and triplication events. In previous research, we successfully established novel cell lines (α-syn^{WT}-N2a, α-syn^{A30P}-N2a, and α-syn^{E46K}-N2a cells) that stably express α-syn proteins. In these cells, upregulation of wild-type α-syn or mutant-type α-syn occurs upon exposure to cumate, a chemical compound inducer. However, the effects of α7 nAChR activation on α-syn-induced neurotoxicity have not been established. In the present study, we investigated whether PNU282987, a selective α7 nAChR agonist, exerts neuroprotective effects against α-syn-induced neurotoxicity in α-syn-N2a cells. We found that α7 nAChR activation by PNU282987 promotes neuroprotection against α-syn neurotoxicity by stimulating autophagy through transcription factor EB. These results reveal a novel neuroprotective mechanism associated with α7 nAChR and offer valuable insights into the development PD therapeutic agents.

O-23

Development of a Novel Strategy to Suppress Hepatic Stellate Cell Senescence by NMN Supplementation

Tomofumi SAKA (坂 智文)[1], Riri HAYASHI[1], Rina KODERA[2], Taichi MITSUI[2], Mitsuyasu KAWAGUCHI[3], Hidehiko NAKAGAWA[3], Yuta YOSHINO[1], Akira IKARI[1], Satoshi ENDO[4,5]

[1] Laboratory of Biochemistry, Gifu Pharmaceutical University

[2] Nagaragawa Research Center, API Co., Ltd.

[3] Graduate School of Pharmaceutical Sciences, Nagoya City University

[4] The United Graduate School of Drug Discovery and Medical Information Science, Gifu University

[5] Center of One Medicine Innovative Translational Research (COMIT), Gifu University

Hepatic stellate cells are crucial for the wound-healing process in the liver. However, stressors can induce excessive activation of these cells, resulting in an inflammatory response. Cellular senescence, characterized by irreversible growth arrest due to stress, is known to be involved in the induction of this inflammatory response. Senescence is an anti-tumor mechanism that prevents the proliferation and transformation of damaged cells into cancer cells. Nevertheless, the accumulation of senescent cells enhances senescence-associated secretory phenotype (SASP) factors, such as inflammatory cytokines, leading to the induction of inflammatory responses and the development and progression of chronic tissue inflammation and age-related diseases. In the liver, cellular senescence is thought to contribute to fibrosis, cirrhosis, and hepatocellular carcinoma. Thus, developing strategies to inhibit cellular senescence in hepatic stellate cells is particularly interesting.

With increased cellular senescence, 4-hydroxy-2-nonenal (HNE) accumulates intracellularly. HNE is a lipid peroxide-derived aldehyde that causes cellular damage by binding directly to DNA, lipids, and proteins. The relationship between HNE and cellular senescence in hepatocytes remains unclear. This study investigated the effect of HNE on hepatocyte senescence and explored the anti-senescent mechanisms of NAD supplementation using nicotinamide mononucleotide (NMN), an NAD precursor that is attracting attention as an anti-aging substance. Treatment with HNE led to increased expression of SASP factors and SA-βGal activity in hepatic stellate LX-2 cells, indicating that HNE induces cellular senescence. However, pre-treatment with NMN was found to inhibit HNE-induced cellular senescence, restore mitochondrial function, and enhance Sirtuin 1 activity. Moreover, NMN also ameliorated the HNE-induced reduction in lipid metabolism and prevented intracellular lipid accumulation. These findings suggest that NMN may have an inhibitory effect on fatty liver formation.

This study could pave the way for developing novel strategies targeting the inhibition of cellular senescence in hepatocytes.

O-24

Effect of external environment pH on the characteristics of mRNA-encapsulated lipid nanoparticles

Toma SHINKAI (新海斗馬), Koki OGAWA, Maiko TSUDA, Tetsuya OZEKI

Drug Delivery and Nano Pharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University

Purpose: mRNA-encapsulated lipid nanoparticles (mRNA-LNPs) are typical delivery systems for mRNA. The administration route of mRNA-LNPs is currently limited to injection (e.g., intramuscular or intravenous). Oral administration is challenging because of harsh conditions in the gastrointestinal environment, which may decrease the functionality of mRNA-LNPs. In particular, the acidic conditions induced by gastric acid are one of the obstacles to the oral administration of mRNA-LNPs. However, the effects of the characteristics of mRNA-LNPs on the environmental pH are poorly understood. The objective of this study is to evaluate the stability and functionality of mRNA-LNPs when they are exposed to acidic condition.

Methods: mRNA-LNPs were prepared by the ethanol dilution method using a vortex mixer, followed by dialysis. mRNA-LNPs were suspended in phosphate-buffered saline (PBS) at pH values of 1, 3, 5, 7.4, and 9, and then incubated at room temperature for the determined time. After the pH of each solution recovered to neutral, the size and encapsulation efficiency of the mRNA-LNPs were measured. The integrity of mRNA was evaluated by electrophoresis. Furthermore, in vitro mRNA transfection efficiency was evaluated using a luciferase assay in HEK293 cells. Cellular uptake of mRNA-LNPs and fluorescent protein expression were measured by flow cytometry.

Results and Discussion: After the mRNA-LNPs were exposed to several pH values, there was no discernible change in size and encapsulation efficiency. The original protein expression efficiency was maintained when mRNA-LNPs were exposed to pH ≥ 3, but protein expression was markedly reduced when they were exposed to pH 1. Intracellular uptake was constant regardless of pH. Quantification of fluorescent protein expression by flow cytometry showed similar results to luciferase expression, in which only pH 1 diminished protein expression. After exposure to pH 1, the electrophoresis results showed that mRNA in mRNA-LNPs was denatured, although the detailed mechanism is unclear. These results suggest that mRNA-LNPs are stable and retain their function in acidic environments above pH 3. Collectively, we clarified the influence of acidic pH on the characteristics of the mRNA-LNPs.

O-25

Removal of microplastics and analysis of additives in plastics based on new adsorbent materials

Mengdan Zhang, Yingying Li, Sijia Zhang, Xiaohong Hou

Department of Environmental Science, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, Liaoning, China

Microplastics (MPs) were a significant environmental threat. We developed monolithic adsorbents by growing ZIF-8 nanoparticles on a sodium alginate (SA) framework, incorporating polydimethylsiloxane (PDMS) to achieve a hierarchical porous structure. These materials would promise to remove the MPs from the water environment rapidly. Additionally, we applied solution-precipitation and magnetic solid-phase extraction (MSPE) to efficiently extract four benzotriazole ultraviolet stabilizers (BUVSs) from polyester curtains for sensitive determination of BUVSs in polyester fiber samples. Based on this method, we then used a sodium alginate/MOF-derived magnetic multistage pore carbon material to extract antioxidants and UV stabilizers from polylactic acid food contact plastics. The developed approach successfully determined antioxidants and UV stabilizers in polylactic acid lunch boxes and straws. We believed that the concept of designing environmentally sustainable metal-organic frameworks and biomass composite multifunctional materials held immense potential in sample pretreatment of emerging contaminants.

O-26

A Simple and Wallet-friendly Method of α-Glucosidase Inhibitory Activity Assay

Natsuki Ichihara,[1,2] Nagisa Yamamoto,[1] Rina Shibata,[1] Chihiro Ito,[1] Yoshiaki Takaya[1]

[1]Faculty of Pharmacy, Meijo University

[2]Faculty of Pharmacy, Kinjo Gakuin University

Diabetes mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is the only hormone that can lower blood glucose levels. Recently, it is becoming more important to take measures against postprandial hyperglycemia at an early stage. Postprandial hyperglycemia is caused by the decomposition of starch in the diet, and one of the factors involved in the decomposition is α-glucosidase. The blood glucose level rises when the resulted glucose by digestion of maltooligosaccharides by α-glucosidase is taken into the blood vessels. For these reasons, foods with inhibitory activity against α-glucosidase are attracting attention in order to suppress postprandial hyperglycemia in daily life.

In order to search for α-glucosidase inhibitory compounds in plants such as fruits and vegetables, one of the most widely used methods to evaluate the amount of glucose is an enzyme-colorimetry method. The method uses the absorbance in the visible region of the final product of a series of enzymatic reactions. This method is superior in that it is simpler and enables simultaneous measurement of multiple samples. On the other hand, when the test sample is colored, the accuracy of the inhibitory activity reduces because of overlapping of absorption maximum of the product and the test sample itself. Therefore, we focused on an enzyme-electrode method since it is not affected by the color of the sample.

The enzyme electrode method uses a change in current instead of absorbance. The method commonly requires a large, expensive analyzer equipped with an enzyme electrode. However, the method was not suitable for a laboratory of natural product chemistry because of its high initial costs. On the other hand, a blood glucose meter (BGM) is very popular recently to measure the amount of glucose in blood. The BGM also uses the enzyme electrode method to quantify glucose.

After some investigation of conditions to detect glucose generated α-glucosidase digestion, BGM can measure the amount of glucose and evaluate α-glucosidase inhibitory activity. The initial cost to conduct the BGM method is only US$20 except those of reagents, and US$1 (for sensor tip) for each experiment. This method does not require any expensive spectrophotometer or glucose electrode. And the protocol is quite simple: a solution of a sample, maltose as a substrate, and α-glucosidase is incubated, and a portion of the solution is applied to BGM. With this method the α-glucosidase inhibitory activity of colored samples obtained from strawberry extract can be measured.

Exploration study for α-glucosidase inhibitor in vegetables and fruits using the new method is currently underway.

O-27

Total glucosides of paeony alleviates Sjogren's syndrome by inhibiting Th1 and Th17 responsese

Ge LIU[1], Weirong Fang (方伟蓉)[2]

[1]Department of Physiology, China Pharmaceutical University

Objective: Sjogren's syndrome (SS) is an autoimmune disease that invades exocrine glands and T helper (Th) cells play an important role in its occurrence and progress. Total glucosides of paeony (TGP) is the main effector substances in Chinese medicine Paeonia lactiflora Pall for treating autoimmune diseases including SS. This study was to define the immunologic mechanisms whether TGP modulated the differentiation and function of Th1 and Th17 cells in experimented SS.

Methods: TGP (720, 360 mg/kg) and PF (300 mg/kg) were intragastrically administered for 16 weeks for non-obesity diabetes (NOD) mice, and TGP (60, 30 μg/ml) and PF (25 μg/ml) were incubated with naive CD4+ T cells from mice spleen for 3 days. Saliva flow, tear flow, and submandibular glands (SMG) and lacrimal glands (LG) pathology were measured. Flow cytometry was used to detect the proportion of Th1, Th17 and regulatory T cells (Treg cells) in CD4+ T cells in peripheral blood and spleen naive CD4+ T cells, respectively. ELISA, RT-qPCR, Western blotting, and immunohistochemical staining were applied to analyze the expression level of Th1 and Th17 cell-related factors (Th1: IFN-γ, IL-2 and T-bet; Th17: IL-17A, RORγt and STAT3) of SMG, LG and spleen naive CD4+ T cells.

Results: Compared with NOD mice treated with vehicle, TGP treatment increased tear flow and saliva flow, improved SMG and LG pathological damage, decreased the proportion of Th1 and Th17 cells in peripheral blood, and inhibited the mRNA and protein expression of Th1 and Th17 cell-related factors in SMG and LG, while had no significant effect on the proportion of Treg cells. Furthermore, TGP could inhibit the differentiation of naive CD4+ T cells into Th1 and Th17 cells, which decreased the content of IFN-γ and IL-17A in the supernatant of cell culture medium and suppressed the mRNA and protein expression of Th1 and Th17 related factors.

Conclusion: TGP inhibited Th1 and Th17 cells differentiation and function, which contributed to improve SS symptoms.

O-28

Paeonol and its metabolites improve LPS/D-GaLN-induced acute liver injury mice by regulating Ndufs7 in Mitochondria of RAW264.7

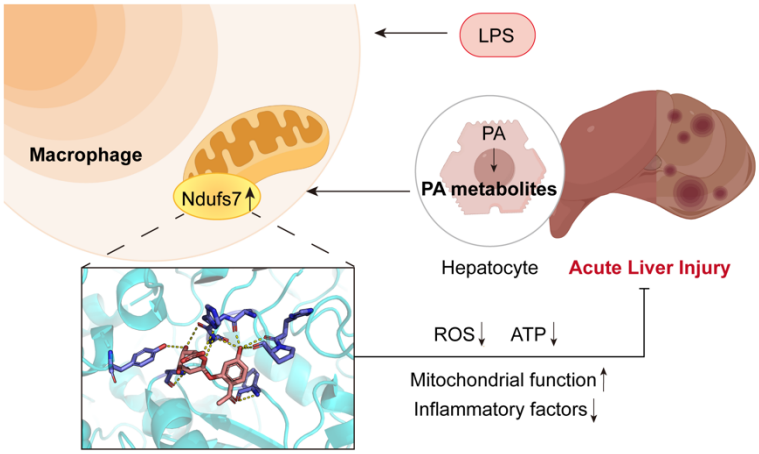
Xinru LYU (吕心如) [1a], Ziya ZHAO[1a], Na SU[1a], Guoxia LU[1], Qingqing WANG[1], Qiang FU[2], Ying PENG[1], Natalie HUGHES-MEDLICOTT[3], Guangji WANG[1], Jianguo SUN[1*]

[1]Key Lab of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing, Jiangsu, China;

[2]Department of Pharmacology of Chinese Materia Medica, China Pharmaceutical University, Nanjing, Jiangsu, China;

[3]School of Pharmacy, University of Otago, Dunedin, New Zealand

Acute liver injury (ALI) can be a fatal clinical syndrome with rapid progression and a severe systemic inflammatory response. Paeonol (PA) improved mitochondrial function and promoted expression of mitochondrial respiratory chain complex enzymes in the form of metabolites thus exerting a hepatoprotective effect by inhibiting LPS/D-GaLN-induced liver inflammation. We investigated the PA effects both in vitro and in vivo, then explored underlying mechanisms through multiple omics studies. Proteomics showed expression of mitochondrial respiratory chain complex enzymes were suppressed in a mouse model of ALI, while expression was promoted after administration of PA. Combined with the metabolomics results, PA regulated mitochondrial function and protected liver by eliminating endogenous metabolites accumulation in inflammation. AML12 experiments showed inflammatory RAW264.7 played a mediating role in liver injury and PA provided protection from this. Further studies on the macrophages indicated that mitochondria of RAW264.7 may be a new target for the treatment of liver injury. Moreover, PA metabolites bound with subunit Ndufs7 of mitochondrial complex Ⅰ better than the PA prototype. Collectively, PA and its metabolites improved ALI mice liver function by acting on Ndufs7 in RAW264.7 and reducing liver inflammation.



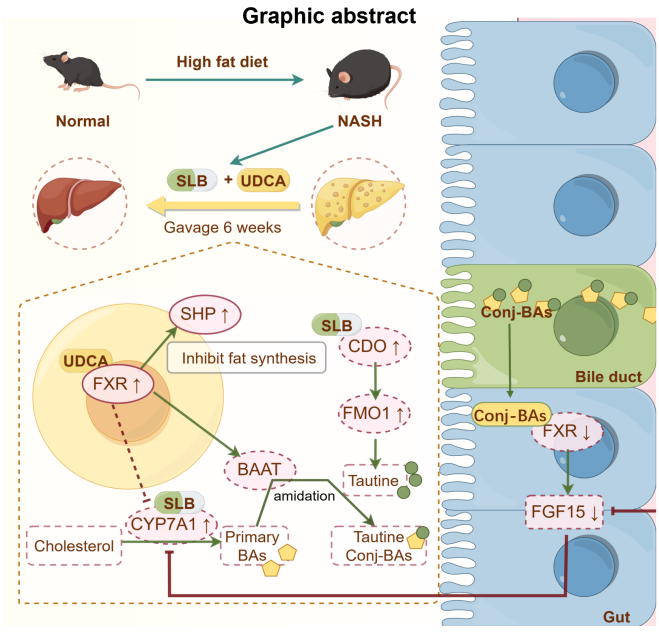
O-29

Combination therapy of silybin-ursodeoxycholic acid improved nonalcoholic steatohepatitis in mice via selectively activating hepatic farnesoid x receptor (FXR) as well as inhibiting intestinal FXR

Dongchun Liu (刘东春), Yu Fu, Xinyu Li, Linghe Zang, Nan Liu

School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University

Objective: Silybin (SLB) and ursodeoxycholic acid (UDCA) are commonly used as hepatoprotective drugs. Clinical studies found that combination therapy of SLB and UDCA exhibited more advantages in nonalcoholic steatohepatitis (NASH) than monotherapy, Therefore, the effectiveness and mechanism for oral administration of SLB-UDCA co-amorphous in NASH model mice were investigated. **Methods:** NASH model were established in C57BL/6 mice by feeding 60 kcal% fat with low methionine and without choline diet for 6 weeks. Subsequently, the mice were orally administered with SLB, UDCA, and SLB-UDCA co-amorphous (SU) respectively during the next 6 weeks. The histopathology, serum biochemical markers, as well as bile acids were observed and detected to evaluate the therapeutic effects. **Results:** SLB, UDCA or SU significantly alleviated liver fat deposition and inflammation caused by high fat diet. The SU group had better results than SLB or UDCA in improving NASH. The hepatic FXR decreased while intestinal FXR increased in model group. Compared with the model group, SLB simultaneously reversed the expression of hepatic and intestinal FXR. UDCA increased the expression of hepatic FXR, while not significantly affected the expression of intestinal FXR. However, combination therapy using SU reversed the expression of hepatic and intestinal FXR more effectively than monotherapy. In addition, the reversal of ileum FXR in SU group could be due to the increased taurine conjugated bile acids (especially T-β-MCA). SLB stimulated the synthesis of taurine-related genes *Cdo* and *Fmo1,* which leading to an increase in taurine-conjugated bile acids, while UDCA promoted *Baat*, and involved in bile acid conjugation. In SU group, the upregulation of CYP7A1 was observed which promoted the synthesis of bile acids leading to NASH improvement. The upregulation of CYP7A1 was due to the antagonistic effect of Conj-BAs on intestinal FXR signaling, despite the activation of hepatic FXR. **Conclusion:** Combining of SLB and UDCA could selectively activate hepatic FXR as well as suppress intestinal FXR, which promotes the synthesis of bile acids and their taurine conjugation, ultimately, NASH would be improved.



Keywords: Nonalcoholic steatohepatitis, Silybin, Ursodeoxycholic acid, FXR, CYP7A1

O-30

Exploring the substance basis, metabolic toxicity mechanism, and mechanism based prevention and intervention of hepatotoxicity of *Dioscorea bulbifera* L.

Zixia HU, Jiang ZHENG, Ying PENG (彭缨)

Wuya College of Innovation, Shenyang Pharmaceutical University

The uniqueness and effectiveness of herbal medicines to cure varieties of diseases are evident in their long history of practice. However, their adverse effects remain challenging problems to be solved for safer clinical applications. *Dioscorea bulbifera* L. (DBL) is a common herbal medicine widely used in China for the treatments of psoriasis, breast lumps, goiters, and lung abscesses. Unfortunately, liver injury caused by using this herb has limited its safe use; numerous hepatotoxicity cases, including death cases, have been documented. Our study is aimed to remove the toxic effect of DBL while retaining its beneficial activities by identification of the component of DBL essential for the observed liver injury; clarification of mechanisms of toxic action; and establishment of effective approaches to prevent and intervene the liver injury induced by DBL.

O-31

Preparation and application of a PEC immunosensor for pancreatic cancer Marker-CA19-9

Chunling Mao (毛春玲), Longshan Zhao*.

Department of Pharmacy, Shenyang Pharmaceutical University

In this work, CdS quantum dots were first prepared by magnetic heating stirring method, and then composite with FJU-200 to prepare CdS/FJU-200 (F&C) composite materials with 0D/1D heterostructures. The results indicate that the prepared 0D/1D heterostructure can greatly suppress the recombination of electron hole pairs, effectively improving the signal of the prepared PEC sensor. In addition, in the presence of CA19-9, the photocurrent signal of the proposed PEC sensor is selectively reduced, which may be due to the inherent insulation and steric hindrance of biomolecules, thereby interrupting the electron transfer process. Therefore, a visible light responsive PEC sensor based on 0D/1D heterostructure F&C was proposed, which utilizes antigen antibody (Ag-Ab) specific recognition reaction for quantitative analysis of CA19-9 in serum samples. Especially, this work may provide a theoretical basis for the application of HOFs materials in the field of PEC sensor construction, and has certain reference significance for the clinical analysis application of CA19-9.

O-32

Oridonin is a covalent p52 inhibitor with anti-proliferative activity of bladder cancer

Lu Zhao[1], Xialu Wang[2], Rong Zhang (张嵘)[1*]

[1]Department of Biopharmaceutics, School of Life Science and Bio-Pharmaceutics, Shenyang Pharmaceutical University, [2]Department of Biomedical Engineering, School of Medical Devices, Shenyang Pharmaceutical University

Oridonin (Ori), a bioactive diterpenoid isolated from traditional Chinese medicine Rabdosia rubescens, has been proven to possess various anti-neoplastic properties. However, the direct molecular target and underlying mechanism of Ori's anticancer effect on bladder carcinoma need to be further elucidated. p52 is a member of the NF-κB/Rel family of transcription factors that regulates a variety of genes associated with tumor malignant features. Here, we demonstrate that Ori forms a covalent bond with the Ser 3 and Cys 4 of p52, thus preventing p52 from binding to the κB sites located in the promoters of downstream target genes. Importantly, Ori suppresses bladder cancer survival by covalently targeting p52 to regulate the expression of its downstream cancer-related genes. Perspectively, we identify p52 as a direct target of Ori-mediated anti-bladder cancer effect in the present study. Ori could be proposed as a promising therapeutic agent against p52-driven diseases, particularly in the treatment of tumor diseases.

O-33 Comparison of Multiple Disease-Modifying Antirheumatic Drugs Combination Therapies with Methotrexate in Rheumatoid Arthritis: A Systematic Review and Bayesian Network Meta-Analysis of Efficacy and Safety

Linfeng LIU (刘 临风)[1,2], Kaori AMBE[1], Mayu ONISHI[1], Yuka YOSHII[1], Toshiaki MAKINO[2], Masahiro TOHKIN[1]

[1] Department of Regulatory Science, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan

[2] Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease. In the methotrexate (MTX)-intolerant population, combination therapies of biological disease-modifying antirheumatic drugs (bDMARDs) or targeted synthetic DMARDs (tsDMARDs) plus MTX are recommended as a treatment for RA. Although several kinds of bDMARDs or tsDMARDs are available now, there is little information about the comparison of each therapies. We employed to assess the efficacy and safety of combination therapy through network meta-analysis in the Bayesian method, with the aim of ranking the efficacy and safety of various b/tsDMARDs+MTX combination therapies and determining which one is both effective and safe. We systematically searched PubMed, Embase®, CENTRAL, Ichushi web, and Pharmaceuticals and Medical Devices Agency (PMDA)'s review reports and application materials up to October 2020, and found 86 randomized controlled trials involving RA patients were included. The primary efficacy outcome was the 50% improvement rate according to the American College of Rheumatology (ACR50) criteria. The primary safety outcome was the incidence of serious adverse events.

Individually, Infliximab combined with MTX had the highest efficacy ranking, while Etanercept combined with MTX had the highest safety ranking. Cluster analysis of efficacy and safety revealed that the combination of Etanercept, an Fc-fusion protein targeting TNF-α, with MTX demonstrated both high efficacy and safety.

Poster Presentations

P-1

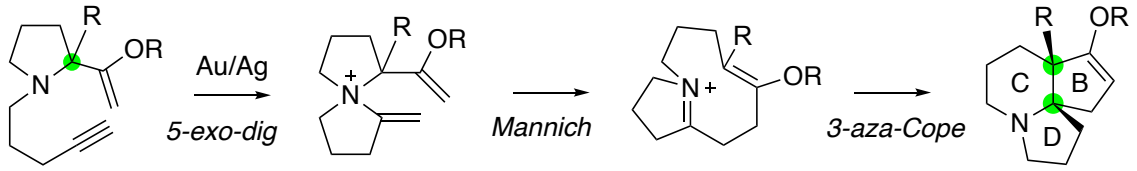
Studies toward the total synthesis of serratinines

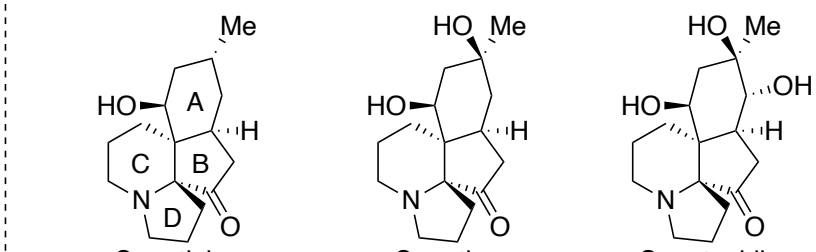
Yuto FUMIMOTO,* Yuji MORI, Takeo SAKAI

Faculty of Pharmacy, Meijo University, Nagoya, Japan

Serratinine is a tetracyclic *Lycopodium* alkaloid with a three-dimensional structure isolated from *Lycopodium serratum*. Since the first successful total synthesis was achieved by Inubushi and Harayama in 1974, many synthetic studies have been conducted based on the biosynthetic pathway involving late-stage transannular cyclization of the CD ring. Because analogs of serratinine, *viz* serratine and serratanidine, have different oxidation states of the A-ring, we envisioned that late-stage modification of the A-ring is reasonable for their unified total synthesis.

Cascade reactions allow multiple transformations in a single experimental manipulation and greatly improve synthetic efficiency. We have recently reported the synthesis of fused cyclic amines using a gold-catalyzed cyclization of tertiary amines to alkynes, 3-aza-Cope rearrangement, and transannular Mannich cyclization, which was utilized in the short step total synthesis of cephalotaxine.[1)] The successful cephalotaxine synthesis inspired us to pursue synthetic studies toward serratinines using the cascade reactions constructing the BCD-ring moiety. We herein report the synthesis of the BCD ring skeleton of serratinines via the cascade reaction and subsequent studies toward A-ring construction.





References

3) Sakai, T.; Okumura, C.; Futamura, M.; Noda, N.; Nagae, A.; Kitamoto, C.; Kamiya, M.; Mori, Y.; *Org. Lett.* **2021,** *23,* 4391-4395.

P-2

Development of FHIT Imaging Fluorescence Probes with Well-tuned Hydrophobicity for Intracellular Uptake

Mitsuyasu Kawaguchi[1], Yuri Furuse[1], Naoya Ieda[2], Yuhei Ohta[1], Hidehiko Nakagawa[1]

[1]Graduate School of Pharmaceutical Sciences, Nagoya City University

[2] Graduate School of Pharmaceutical Sciences, Hokkaido University

Cancer has long been a leading cause of death in developed countries, with an estimated 9.5 million deaths worldwide in 2018. Early diagnosis is important to improve the prognosis of cancer, and the development of technology to diagnose cancer using biological markers in urine and blood has been actively studied. In surgery to remove cancer, however, it is important to clearly distinguish the boundary region between cancerous tissue and normal tissue, which cannot be easily distinguished by the naked eye. Therefore, fluorescence probes targeting enzymes such as β-Gal and GGT, which are overexpressed in cancer cells, have been developed, and a new surgical technique called fluorescence-guided surgery has been proposed. By the way, the fragile histidine triad (FHIT) is an attractive target for early diagnosis of cancer because its expression is lost from precancerous lesions in more than half of various human cancers. FHIT is a diadenosine triphosphate hydrolase (AP_3Aase) that recognises Ap_3A and produces AMP and ADP. Although just one fluorescence probe targeting FHIT has been developed, real-time detection of FHIT activity in living cells has not been successful. This is because fluorescence probes with multiple phosphate groups have remarkably low cell membrane permeability, and thus cannot efficiently detect FHIT activity in the cytoplasm. In this study, we evaluated whether fluorescence probes with nucleotide diphosphate structures can gain membrane permeability and detect intracellular FHIT activity by logically adjusting the cLogP value.

We previously reported that **TG-AMP** has excellent reactivity with FHIT (Kawaguchi et al., *J. Med. Chem.* (2021)), but we found that it is not sufficiently reactive to detect FHIT activity at intracellular concentrations. Therefore, we thought that the reactivity could be improved by introducing a structure more similar to the biological substrate Ap_3A, and synthesized and evaluated **TG-mADP**. While **TG-mADP** was able to detect FHIT activity in cell lysate, it could not be applied to live cell imaging because its diphosphate structure makes it extremely low permeability. Therefore, we introduced various hydrophobic ester structures into **TG-mADP** and evaluated their cell membrane permeability. As a result, it was shown that the ester structures with cLogP values of 5~7 have gotten membrane permeability, and endogenous FHIT activity can be visualized. The use of the ester structure over amide one is also important in this technique. In other words, after permeation of cell membrane, the esterase hydrolyzes the ester to a carboxylic acid, which restores reactivity with FHIT and increases intracellular retention, thus achieving highly sensitive imaging. In coontrast, introduction of extremely hydrophobic structure, *e.g.* cholesterol, did not achieve detection of FHIT activity, indicating the exsistence of appropriate hydrophobicity for cellular uptake (Kawaguchi et al., *ACS Sens.* (2022)).

P-3

Effect of MPBD and DQ Alkyl Side Chain Length in *Dictyostelium discoideum* Cell Aggregation and Its Antibacterial and Antiproliferative Activity

Titah Aldila Budiastanti,[1] Salma Zulqaida,[1,2] Tamao Saito,[3] Yumiko Komori,[1] Chihiro Ito,[1] Yoshiaki Takaya[1]

[1]Faculty of Pharmacy, Meijo University

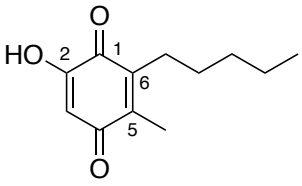
[2]Postgraduate School, Airlangga University

[3]Faculty of Science and Technology, Sophia University

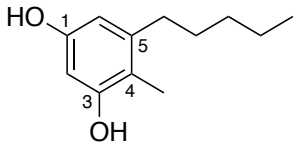
Dictyostelium discoideum has long been known as a source of natural products that possess many biological activities. MPBD (4-methyl-5-pentylbenzene-1,3-diol) and DQ (Dictyoquinone or 2-hydroxy-5-methyl-6-pentylbenzoquinone) were two polyketides isolated from D. discoideum.

They were identified to help regulate cell development in *D. discoideum*. Both compounds were able to recover the cell aggregation defect in the *D. discoideum stlA null* mutant, the mutant that can't produce MPBD. DQ activity in cell development was shown to be highly influenced by the side alkyl chain. Due to their structural similarity, the two compounds were proposed to be produced from the same biosynthetic pathway. In this study, MPBD, DQ, and their analogs containing different alkyl chain lengths, ranging from pentyl to tridecyl have been successfully synthesized. The compounds were tested for their ability for cell aggregation in *stlA null* mutant. The effect of the alkyl chain length on antibacterial and antiproliferative activity against K562 and HL60 cells was also examined.

The results showed that 5-carbon alkyl chain lengths of MPBD and DQ were important for the recovery of cell aggregation delay in *stlA null* mutant. Longer alkyl chains will lessen the compound's ability to recover the aggregation delay. MPBD and DQ analogs exhibited antibacterial activity against the gram-positive bacteria *Bacillus subtilis*, and the activity was revealed to be affected by the alkyl chain length. The best activity was exhibited by the compound with a 9 to 11 carbons alkyl chain. Lastly, DQ and MPBD analogs were able to suppress the cell growth of K562 and HL60 cells in a dose-dependent manner. Similar to the antibacterial activity, the alkyl chain length also affected cell growth inhibition. Side alkyl chains bearing 9 to 13 carbons suppress the growth of the cells better than shorter side alkyl chains.



Dictyoquinone (DQ)



MPBD

P-4

Comparison of the pharmacological activities of glycyrrhizin and its human metabolites

XIN Jingxiao (辛竞潇), SAKODA Ryota, ISHIUCHI Kan'ichiro, MAKINO Toshiaki

Department of Pharmacognosy, Nagoya City University; [2]Department of Pharmacognosy, Nagoya City University

Root of *Glycyrrhiza uralensis* (Glycyrrhiza) is not only used as a crude drug used in Japanese traditional Kampo medicine and traditional Chinese medicine but as a food additive for natural sweetener. Glycyrrhizic acid (GL) is one of the main active ingredients of Glycyrrhiza. It has anti-inflammatory and immuno-modulative function, and used as a chemical drug to improve liver function. When humans take GL, glycyrrhetinic acid (GA) is appeared in the circulation as the main metabolites because GL is hard to be absorbed from the intestine as its form, and is metabolized to GA by enterobacteria. However, in our previous studies, we found glycyrrhetinic acid-3-*O*-sulfate (GA3S) as the main metabolite of GL in not in rats but in humans[1)]. Furthermore, 3-*epi*-glycyrrhetinic acid (3-*epi*-GA), that is another metabolite of GL by enterobacteria in not rats but humans, is appeared in the circulation in humans with individual differences, and its serum concentrations are sometimes higher than GA[2)]. Therefore, it is possible that GA3S and 3-*epi*-GA may contribute to pharmacological effects of GL in humans. However, there are no reports about the pharmacological effects of GA3S and 3-*epi*-GA.

The aim of the present research is to compare the pharmacological properties among GL metabolites to find genuine active compounds of Glycyrrhiza in human. Since GA has been already reported to have the inhibitory effect on LPS-induced NO production from murine macrophage-like RAW264.7 cells[3)], we compared the anti-inflammatory effect of GL metabolites using this experimental activity.

GL, GA, GA3S and 3-*epi*-GA inhibited LPS-induced NO production in dose-dependent manners. The titers of GA and 3-*epi*-GA were comparable, and that of GL was lower than those of GA and 3-*epi*-GA . The titer of GA3S was significantly higher than those of GA and 3-*epi*-GA. Since the serum concentration of GA3S in human orally treated with Glycyrrhiza was higher than that of GA, GA3S would more contribute to anti-inflammatory effect of GL than GA.

1) Ishiuchi K, et al., Sci. Rep. 9: 1587, 2019. 2) Sakoda R., et al., Drug Metab. Dispos. (in submitting). 3) Li B, et al., Medchemcomm. 8, 1498, 2017.

P-5

One-pot Biosynthesis of (*S*)-Equol Using Enzymes Cascade

Jiaqi WANG (王珈琪)

Shenyang Pharmaceutical University

Isoflavanones are a kind of healthy natural dietary phytoestrogens which mainly exist in legumes. Among them, (*S*)-equol is a dihydric phenol with estrogen-like effects.It can prevent female osteoporosis and ameliorate female menopausal syndrome as nonsteroidal estrogen analogs. It also prevent and resist breast cancer and has a function of protecting nerves from senescence. However, (*S*)-Equol doesn't directly exist in nature. It is final metabolite of soy isoflavones. The glycosidic bond of daidzin can be resolved by endogenous microorganisms in the human intestine then transformed into daidzein absorbed into blood partly. The rest of daidzein are reduced into (*R*)-dihydrodaidzein by daidzein reductase.Then (*R*)-dihydrodaidzein transformed to (*S*)-dihydrodaidzein under the dihydrodaidzein racemase. The racemic dihydrodaidzein is reduced into four tetrahydrodaidzein. Only (3*S*,4*R*)-THD can be transformed into (*S*)-equol under the action of tetrahydrodaidzein racemase. In a word, to improve atomic economy, we design a new way to produce (*S*)-equol high-titerly reducing four steps to three steps.

P-6

Substrate specificity of anthraquinone glucosyltransferase from *Rheum palmatum*

Toranosuke Narita, Aoi Yoshino, Toshiaki Makino, Kazuyoshi Terasaka

Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University

【Background】

The rhizome of *Rheum palmatum* contains anthraquinones as active ingredients. Some anthraquinones accumulate not only as aglycones but also as glycosides in plants. However, plant secondary product glycosyltransferases (PSPGs) responsible for their glycosylation steps have yet to be well-characterized. In our previous study, 14 glycosyltransferases (*Rheum palmatum* UDP-glycosyltransferase 1-14; RpUGT1-14) were isolated and characterized from RNA-seq data. Among them, RpUGT14 glucosylated rhein and aloe-emodin, yielding rhein-8-glucoside and aloe-emodin-8-glucoside, respectively. In this study, we characterized the glucosylation activities of RpUGT13 and RpUGT14 in detail. The results provide insights into the substrate specificity of anthraquinone glucosyltransferases.

【Methods】

The recombinant proteins of RpUGT13 and RpUGT14 were expressed in *E. coli* and purified. The glucosyltransferase activity was measured in the reaction buffer containing Tris-HCl, UDP-glucose, and acceptor substrates (anthraquinones, rhapontigenin, and quercetin). The reaction products were analyzed by HPLC. To determine kinetic parameters, enzyme assays were performed in triplicate at various substrate concentrations.

【Results and Discussion】

RpUGT13 produced emodin-6-glucoside from emodin, rhaponticin from rhapontigenin, and quercetin-4'-glucoside from quercetin. The glucosylation activity of RpUGT13 toward quercetin was higher than those toward other substrates. This suggests that RpUGT13 is likely involved in the glycosylation of quercetin. RpUGT14, on the other hand, produced rhein-8-glucoside from rhein, aloe-emodin-8-glucoside from aloe-emodin, and rhapontigenin-3’-glucoside from rhapontigenin. From the kinetic parameters, RpUGT14 had a higher affinity and catalytic efficiency to the substrates aloe-emodin and rhapontigenin than rhein. This suggests that RpUGT14 is an aloe-emodin and rhapontigenin glycosyltransferase *in vitro*. However, stilbene glycosides (rhaponticin and rhapontigenin-3'-glucoside) were rarely accumulated in rhizomes and roots of *R. palmatum*, where RpUGT14 gene was highly expressed. Aloe-emodin-8-glucoside was accumulated mostly in rhizomes and roots. These suggest that RpUGT14 is an aloe-emodin glycosyltransferase in the plant and is hardly involved in the biosynthesis of rhapontigenin-3'-glucoside. The transgenic plants with overexpressing or suppressing RpUGT13 or RpUGT14 will be required for further investigation.

P-7

Tissue and Cell Dual-Penetrating Dendritic Lipopeptide Liposomes for Hypertrophic Scar Treatment

CONG Rui (丛瑞), LEI Lei, WANG Xulei, ZHOU Yinhui, TU Jiasheng*, JIANG Lei*

Department of Pharmaceutics School of Pharmacy, China pharmaceutical university

Hypertrophic scar is a complex global disease, which can lead to remarkable functional impairment, pruritus, and pain. Treating local skin diseases needs a transdermal drug delivery system that can simultaneously promote cell and tissue penetration within the hypertrophic scar. Herein, a tissue and cell dual-penetrating liposomal delivery platform consisting of arginine-based dendritic lipopeptide mimicking the pivotal amino acid sequence in viral protein transduction domains and virion-like nanostructure to enhance hypertrophic scar tissue deep penetration and cellular internalization is reported. This dendritic lipopeptide liposomal delivery platform can overcome corneum barriers to the punch on hypertrophic scar fibroblast membranes, as are natural viruses. It also can deeply deliver drugs to promote hypertrophic scar fibroblast apoptosis and collagen fiber remodeling within thickened dermis and epidermis, and finally remove the raised scars.

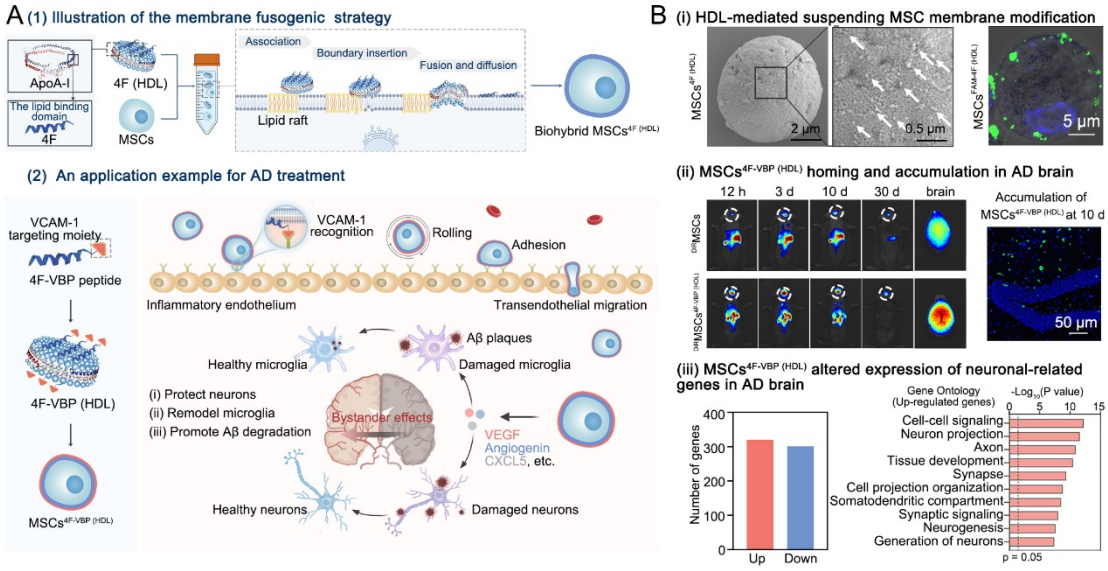
P-8

A Nanodisc-Paved Biobridge Facilitates Stem Cell Membrane Fusogenicity for Intracerebral Shuttling and Bystander Effects

DING Yang (丁杨)*, JIN Yi, ZHOU Jianping, ZHANG Huaqing*

Department of Pharmaceutics, China Pharmaceutical University

Mesenchymal stem cell (MSC) therapies experience steadfast clinical advances but are still hindered by inefficient site-specific migration. An adaptable MSC membrane fusogenicity technology is conceptualized for lipid raft-mediated targeting ligand embedding by using toolkits of discoidal high-density lipoprotein (HDL)-containing biomimicking 4F peptides. According to the pathological clues of brain diseases, the vascular cell adhesion molecule 1 specialized VBP peptide is fused with 4F to assemble 4F-VBP (HDL), which acts as a biobridge and transfers VBP onto the living cell membrane via lipid rafts for surface engineering of MSCs in suspension. When compared with the membrane-modifying strategies of PEGylated phospholipids, 4F-VBP (HDL) provides a 3.86-fold higher linkage efficiency to obtain MSCs$^{4F–VBP(HDL)}$, which can recognize and adhere to the inflammatory endothelium for efficient blood–brain barrier crossing and brain accumulation. In APPswe/PSEN1dE9 mice with Alzheimer's disease (AD), the transcriptomic analysis reveals that systemic administration of MSCs$^{4F–VBP(HDL)}$ can activate pathways associated with neuronal activity and diminish neuroinflammation for rewiring AD brains. This customizable HDL-mediated membrane fusogenicity platform primes MSC inflammatory brain delivery, which can be expanded to other disease treatments by simply fusing 4F with relevant ligands for living cell engineering.



P-9

Construction of cell membrane biomimetic nanoplatform for effective screening of active ingredients from Sanwei Tanxiang capsule

Yi Qin[a], Jiahe Ren[b], Longshan Zhao (赵龙山)[a,*],Xuefeng Guan[b,*]

[a] Department of Pharmacy, Shenyang Pharmaceutical University

[b] Department of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University

In this work, Nile Red-doped PEI functionalized magnetic fluorescent nanomaterials (CMMFNPs) disguised with Coronary Artery Smooth Muscle Cells (CASMC) were created using the covalent coupling technique. These CMMFNPs were then used to screen and enrich anti-coronary heart disease active ingredients that target CASMC from the Sanwei Tanxiang capsule. The CMMFNPs were effectively synthesized and shown great biocompatibility, good optical characteristics, and magnetic properties, as confirmed by a variety of characterization methods. Adsorption tests revealed that MFNPs treated with CASMC have superior adsorption characteristics (8.81 μg·mg^{-1}). After screening a total of six potential active ingredients from the crude extracts of the drug—N-undecanoylglycine, myrislignan, surinamensin, raphidecursinol B, dehydrodiisoeugenol, and elemicin—the pro-proliferative effects of the screened ligands on the CASMC were first assessed using the cellular imaging assay. Additionally, CCK-8 and BrdU kits were used to further validate the pharmacological actions of the screened ligands. These kits showed that the screened ligands promoted cell proliferation in a concentration-dependent manner from various angles. In conclusion, this approach, which integrated cellular imaging and cell membrane bionic screening to enable quick screening of active ingredients while also assessing the pharmacological activities of ligands, is anticipated to be a useful tool for drug discovery.

Key words: Ligand fishing; In situ imaging; Biomimetic cell membrane; Sanwei Tanxiang capsule;

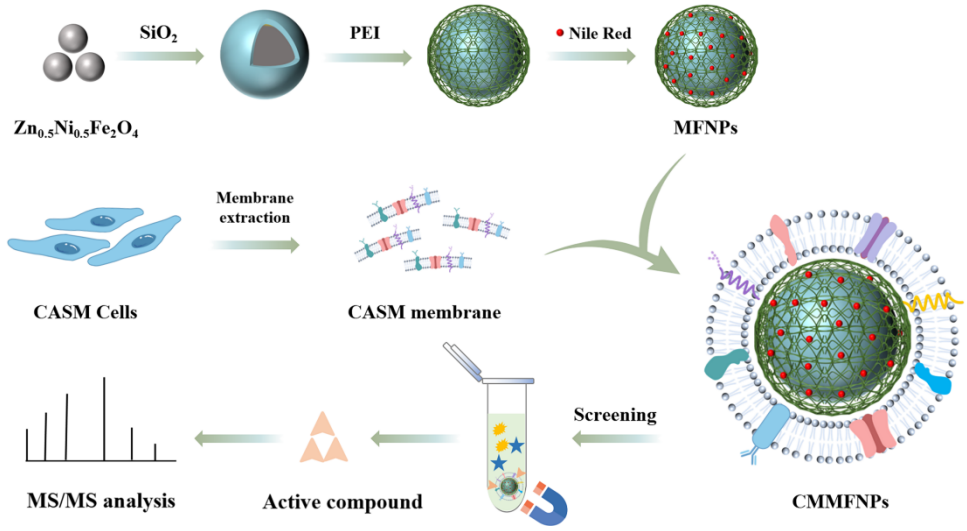


Fig. 1 Schematic diagram of ligand fishing strategy based on cell membrane camouflage MFNPs

P-10

Toward hydrogel-based photothermal therapy against skin cancer: fabrication of Ag-nanoparticles and 3D-printed hydrogel scaffold

Jayita DAS, KOKI Ogawa and TETSUYA Ozeki

Drug Delivery and Nano Pharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University

Purpose: Localized therapy with hydrogel-based drug delivery systems (DDS) is a promising approach for treating skin diseases. Therapeutic strategies involving the implantation of drug-containing hydrogels and drug release are being investigated for the treatment of skin cancer because they may minimize systemic side effects and maximize the therapeutic effect. 3D printing technology is a versatile platform that allows the fabrication of hydrogel scaffolds of any shape at the lesion site. Photothermal therapy (PTT) is an emerging cancer therapy that can kill tumours via light-induced heating. Metal nanoparticles, such as Ag nanoparticles (AgNPs), are used as light-absorbing materials for PTT. We hypothesized that localized skin cancer treatment could be achieved using hydrogel implantation and laser irradiation for skin cancer. In this study, we prepared AgNPs, and evaluated their physicochemical and photothermal properties. We then fabricated a 3D-printed hydrogel scaffold with customizable structures (Fig 1).

Methodology: AgNPs were prepared using the sodium borohydride reduction method in the presence of trisodium citrate (0.1M) and different molar concentrations of sodium borohydride (0.02M, 0.1M). The prepared AgNPs were then characterized and their photothermal activity was tested at an intensity of 1.5 W for 10 min and an on-off cycle to assess thermal stability. For the 3D printing of the hydrogel scaffold, we used gel extrusion-based 3D printing. To select the appropriate hydrogel ink, different hydrogels were formulated without the drug. The optimal formulation was then selected based on the hydrogel swelling ratio at various pH levels.

Results and Discussion: Uniform and stable AgNPs with an average diameter of 100 nm was obtained. Ultraviolet-visible spectroscopy exhibited an absorbance range of 400-420 nm, which confirmed the preparation of the AgNPs. Transmission electron microscopy images showed a homogeneous, spherical shape, which is a typical characteristic of AgNPs. They exhibited significant light-heat conversion properties, reaching temperatures of 45º-46ºC upon irradiation, making them effective for killing tumour cells. Using 3D printing, a mesh-shaped scaffold was successfully fabricated as designed (Fig. 1). When the hydrogel scaffold was exposed to pH 6.5 solution, they degraded within 60 min. In contrast, when the scaffold was exposed to pH 5.5 solution, it maintained its structure even after 200 min, which may indicate slow swelling and degradation of acidic pH in the tumour environment. Collectively, we succeeded in preparing AgNPs suitable for PTT and in fabricating hydrogel scaffolds for implantation into skin cancer. In the future, we plan to load AgNPs into hydrogels and evaluate their potential application in skin cancer therapy.

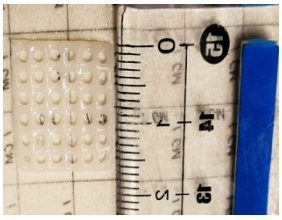


Fig 1: 3D printed gridline structure after crosslinking before drying shows successful preparation of the hydrogel scaffold

P-11

Development of Gold Nanostars Coated with Mesoporous Silica for Laser-Triggered Chemo-Photothermal Therapy

Fadilah ASRIL, Koki OGAWA, Tatsuaki TAGAMI, Tetsuya OZEKI
Drug Delivery and Nano Pharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University

Purpose: Cancer remains a significant health challenge that necessitates comprehensive treatment strategies. Combination therapy has emerged as a promising approach because of its potential to increase the effectiveness and reduce the likelihood of resistance development. Photothermal therapy (PTT), which uses light to generate heat for precise and minimally invasive cancer cell destruction, offers several significant advantages. Gold nanostars (GNS), with their multibranched structure, exhibit unique optical and colloidal properties. However, a notable challenge in using gold nanoparticles as drug carriers is their limited loading capacity. To overcome this limitation, GNS were coated with mesoporous silica (MS), allowing the effective loading of chemotherapy drugs. In this study, we developed GNS coated with mesoporous silica to facilitate a synergistic approach combining photothermal therapy and chemotherapy for more effective eradication of cancer cells.

Methods: GNS, which serves as a photothermal therapy agent, was fabricated using the seed-mediated growth method. Subsequently, the GNS were coated with an MS layer via a sol-gel process. The mesoporous silica was optimized by varying the concentration of Cetyltrimethylammonium Bromide (CTAB) as the MS template and Tetraethyl Orthosilicate (TEOS) as the MS precursor. Comprehensive characterization of both GNS and GNS@MS was performed, including examination of the size, morphology, and UV-vis spectra. Doxorubicin (DOX) was then loaded into the mesoporous layer by the passive loading method, resulting in GNS@MS-DOX. The encapsulation efficiency of GNS@MS-DOX was evaluated. The light-heat conversion efficiency was evaluated using a thermal imaging camera. The in vitro biocompatibility and cytotoxicity of GNS@MS were evaluated using B16/BL6 melanoma cells.

Results and Discussion:
GNS@MS had notable branches, forming a star shape surrounded by the MS coating. Through optimization, the formulation was achieved with 20 mL of GNS (2X concentration), 100 µL of CTAB, and 15 µL of TEOS, resulting in a GNS@MS size of 109.8 ± 15.9 nm with fewer empty silica regions. The GNS@MS exhibited a significant photothermal effect before and after mesoporous silica layering, achieving a temperature of 50.7˚C after 20-min of laser irradiation (660 nm, 1 W) with sustained temperature elevation observed over multiple irradiation cycles. Furthermore, transmission electron microscopy (TEM) imaging and optical analysis indicated negligible structural changes before and after laser irradiation, suggesting the protective role of MS against gold nanostar deformation during laser exposure. The porous structure of MS provides space for drug loading using DOX with an entrapment efficiency of approximately 77.5 %. GNS@MS was confirmed to be safe as a carrier, with 87.7% of the cells surviving after a cytotoxicity test against B16/BL6 melanoma cells. This study highlights the immense potential of the GNS@MS system as a versatile multimodal therapeutic platform for combatting cancer cells.

P-12

Establishment and application of deep eutectic solvent-assisted reverse thermal proteome profiling (DATPP)

Liu YANG, Chen-wan GUO, Wen GAO*

State Key Laboratory of Natural Medicines, School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing, 211198, China

While thermal proteome profiling (TPP) shines in the field of drug target screening by analyzing the soluble fraction of the proteome samples treated at high temperature, the counterpart, the insoluble precipitate, has been overlooked for a long time. The analysis of the precipitate is hampered by the inefficient sample dissolution reagents. Herein, we propose a novel method, deep eutectic solvent-assisted reverse thermal proteome profiling (DATPP), for drug target identification. In this study, 64 hydrophilic and stable deep eutectic solvents (DESs) were prepared. Compared with commonly used proteomic pre-treatment reagents (urea, guanidine hydrochloride), DES-63 demonstrated superior protein dissolution capability. The accuracy of the DATPP method was validated using methotrexate, cyclosporin A, geldanamycin, panobinostat, and staurosporine. The target proteins of each drug were accurately identified, demonstrating that the DATPP method is not only sensitive and reliable but also enhances protein identification coverage to a certain extent. The developed DATPP approach was applied to identify the target proteins of celastrol (CEL), a natural product known for its strong antioxidant and anti-cancer angiogenesis effect. Three target proteins were identified, including two known targets (PRDX1 and PRDX2). Among them, TOR1AIP2 was further validated for its strong affinity to CEL using the cellular thermal shift assay. DATPP is an unbiased robust method for drug target screening, filling the vacancy of stability-based target screening using a precipitate.

P-13

Establishment of Cancer Therapy Strategies Targeting a Core Autophagy Protein ATG4B

Yudai KUDO (工藤優大)[1], Yuta YOSHINO[1], Akira IKARI[1], Satoshi ENDO[2,3]

[1] Laboratory of Biochemistry, Gifu Pharmaceutical University,

[2] United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University

[3] Center for One Medicine Innovative Translational Research (COMIT), Gifu University

The induction of autophagy in cancer cells is implicated in malignant progression and the acquisition of resistance to anticancer drugs. Therefore, autophagy is considered a potential therapeutic target in cancer therapy. PI3K and lysosome inhibitors are used as autophagy inhibitors, but the point of action of these inhibitors is also vital in normal cells. In fact, clinical trials that have been conducted using these inhibitors in cancer therapy have failed due to serious side effects. Therefore, we focused on autophagosome formation, a phenomenon unique to autophagy. When autophagy is induced, autophagosomes are formed, which initiates with proteolytic cleavage of the C-terminal region of a nascent form of microtubule-associated protein 1 light chain 3 (proLC3) into LC3-I by a cysteine protease autophagy-related 4B (Atg4B). Atg4B inhibition is a potential target for autophagy inhibition because the introduction of Atg4B C74A, an inactive mutant of Atg4B, forms incomplete autophagosome membrane. Autophagy Inhibition using an Atg4B inhibitor has been reported to be particularly effective in pancreatic ductal adenocarcinoma (PDAC) with mutant K-Ras expression [Yang et al., *Cancer Discov*., 8: 276–287 (2018)]. So far, DC-ATG4in, S130, FMK9a, UAMC, and so on have been reported as Atg4B inhibitors, but little detailed bioactivity has been shown. Therefore, we synthesized Aup18, derived from the structural optimization of Aup01, an Atg4B inhibitor reported recently, which is effective against anticancer-resistant prostate and pancreatic cancer cells. In addition, we explored natural compounds based on the pharmacophore of Aup01 and revealed that long-chain fatty acids exhibited Atg4B inhibitory activity. Among them, docosahexaenoic acid (DHA) with the potent Atg4B inhibitory potency inhibited autophagy and sensitized anticancer-resistant cells through induction of mitochondrial dysfunction-mediated apoptotic cell death. In summary, we found a synthesized compound and functional ingredients targeting Atg4B. Further studies will be conducted to establish novel therapeutic strategies targeting Atg4B.

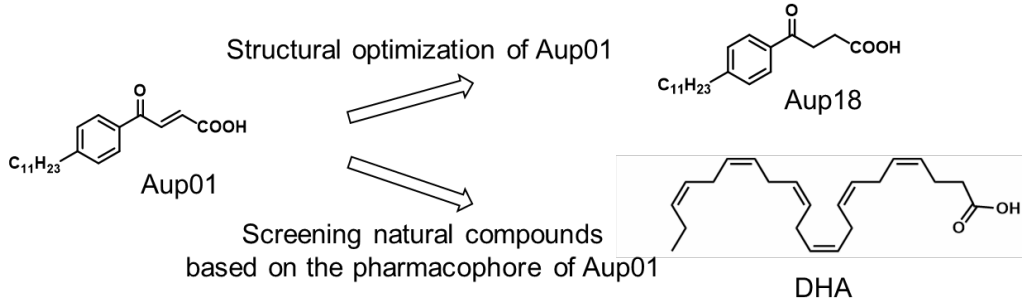


Figure 1. Discovery of novel compounds targeting Atg4B

P-14

Relationship between Anaplastic Lymphoma Kinase (ALK) Inhibitors and Epileptic Seizure Disorder: A Post-Marketing Surveillance Study.

Rikuto MASUDA (増田陸人)[1], Yoshihiro NOGUCHI[1, 2], Hiroki ASANO[3], Yuta TESHIGAWARA[1], Makiko GO[3], Michio KIMURA[1, 3], Eiseki USAMI[2, 3], Tomoaki YOSHIMURA[1, 2]

[1]Laboratory of Clinical Pharmacy, Gifu Pharmaceutical University

[2]Laboratory of Medical Collaborative Pharmacy, Gifu Pharmaceutical University

[3]Department of Pharmacy, Ogaki Municipal Hospital

Abstract

Introduction: Anaplastic lymphoma kinase (ALK) has been to be involved in the uptake and regulation of dopamine 2 receptor (D2R), a G protein-coupled receptor expressed in various brain regions. Therefore, it is crucial to understand the relationship between ALK inhibitors and seizures is an important issue. This study investigated the relationship between ALK inhibitors and seizures.

Methods: This study investigated the relationship between ALK inhibitors and seizures through a disproportionality analysis using the U.S. Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS). The target drugs were the ALK inhibitors crizotinib, ceritinib, alectinib, brigatinib, and lorlatinib. The seizures covered were defined high level group term (HLGT): "Seizures (incl subtype)" including high level term (HLT): "Seizures and seizure disorders NEC." This study used the information component (IC), a signal score, as a Bayesian statistical method for disproportionality analysis. The signal detection criteria used in this study were the same as those reported previously: a lower limit of 95% credible interval (CrI) for IC >0.

Results: The signal scores of "Seizures and seizure disorders not elsewhere classified (NEC)" for each ALK inhibitor were crizotinib (IC: -0.00052, 95%CrI: -0.38–0.27), ceritinib (IC: 1.18, 95%CrI: 0.68–1.54), alectinib (IC: 0.68, 95%CrI: 0.19–1.02), brigatinib (IC: 1.04, 95%CrI: 0.32–1.54), and lorlatinib (IC: 0.82, 95%CrI: 0.11–1.32). On the other hand, "Generalized tonic-clonic seizures", "Partial simple seizures NEC, " "Absence seizures," and "Partial complex seizures" had no or few reported cases, and no signal was detected.

Conclusion: To our knowledge, this is the first report to evaluate the relationship between ALK inhibitors and seizures using post-marketing surveillance data. These results suggest that ceritinib, alectinib, brigatinib, and lorlatinib, which are highly brain-migrating drugs, are associated with seizures.

P-15

Development of Novel Therapeutic Strategies for Refractory Cancer Targeting DDI2

Atsumi OTA (太田篤実)[1], Souta MARUYAMA[1], Yuta YOSHINO[1], Akira IKARI[1], Satoshi ENDO[2,3]

[1] Laboratory of Biochemistry, Gifu Pharmaceutical University

[2] The United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University

[3] Center for One Medicine Innovative Translational Research (COMIT), Gifu University

In cancer cells, the proteasome is upregulated to cope with excessive cell proliferation, hypoxia, and low nutritional status, which is thought to suppress cell death and to acquire anticancer drug resistance. However, proteasome inhibitors are currently only applied to hematological cancers, and there is also the problem of early resistance in blood cancers. One possible cause is the involvement of a feedback mechanism called the bounce-back response. NF-E2-related factor 1 (Nrf1), a significant regulator of the bounce-back response, is rapidly degraded by the proteasome under normal conditions. However, when proteasome activity is reduced, Nrf1 is translocated to the nucleus, promoting the transcription of proteasome components. Recently, it has been shown that the cleavage of Nrf1 by the aspartate protease DNA damage inducible 1 homolog 2 (DDI2) is essential for this reaction. Therefore, this study aimed to develop novel therapeutic strategies for refractory cancer targeting DDI2.

Nelfinavir (NFV), an HIV1 protease inhibitor, has been reported as the only DDI2 inhibitor. In bladder cancer T24 cells resistant to gemcitabine/cisplatin (GC), NFV significantly increased BTZ sensitivity when combined with the proteasome inhibitor bortezomib (BTZ), showing an effect to overcome BTZ resistance. On the other hand, NFV could not inhibit the bounce-back response due to its protease inhibitory activity.

Therefore, we searched for novel DDI2 inhibitors without protease inhibitory activity and found hexestrol (HEX). In multiple myeloma U937 cells and BTZ-resistant U937 cells, we discovered that HEX inhibited DDI2-mediated cleavage of Nrf1 and the BTZ-induced bounce-back response, thereby strengthening the anticancer potency of BTZ.

In conclusion, the combination of a proteasome inhibitor and a DDI2 inhibitor is effective in cancer treatment and presents a promising new cancer treatment strategy, even for patients who have acquired anticancer drug resistance. We plan to further optimize the structure based on the HEX pharmacophore to develop a new, more potent DDI2 inhibitor for clinical use.

P-16

Involvement of adrenergic beta2 receptors in clozapine-induced lipid droplet accumulation in 3T3-L1 cells

Ayano FUKAMI (深見彩乃)[1], Tomomi UMEMURA[1, 2], Akira YOSHIMI[1, 3], Yukihiro NODA[1, 3]

[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty of Pharmacy, Meijo University, Nagoya, Japan

[2]Department of Patient Safety, Nagoya University Hospital, Nagoya, Japan

[3]Clinical OMICs and Translation Research Center, Meijo University, Nagoya, Japan

Clozapine, a second-generation antipsychotic (SGA), cause the side effects related to metabolic syndrome. The weight gain associated with SGAs can be caused by appetite stimulation, lipid accumulation, or reduced energy expenditure. Although clozapine causes adipocyte enlargement in the 3T3-L1 cells, it is unknown its mechanism. Clozapine has a high affinity for adrenaline beta receptors, which is strongly associated with weight gain. Since adrenaline beta receptors in adipocytes are known to be involved in lipid droplet accumulation, the present study was investigated the involvement of adrenaline beta receptors in clozapine-induced liipid droplet accumulation using 3T3-L1 cells.

3T3-L1 progenitor adipocytes were cultured, and after reaching confluency, differentiation into mature adipocytes was induced. After induction of differentiation, clozapine or adrenaline beta receptor-related compounds (adrenaline beta3 receptor antagonist: L-748,337, a non-selective adrenaline beta1/2 receptor antagonist: propranolol, beta1 receptor antagonist: betaxolol, beta1 receptor agonist: dobutamine, beta2 receptor antagonist: ICI118551, beta2 receptor agonist: clenbuterol) were exposed to cells for 14 days. After compound exposures, the lipid droplet accumulation in mature adipocytes was measured by using Oil Red O staining.

The exposure to clozapine was increased in the lipid droplet accumulation in 3T3-L1 adipocytes. The similar effects were observed by propranolol (a non-selective adrenaline beta1/2 receptor antagonist) and ICI118551 (an adrenaline beta2 receptor antagonist) in 3T3-L1 adipocytes. Conversely, clenbuterol (an adrenaline beta2 receptor agonist) was decreased in the lipid droplet accumulation. Betaxolol (an adrenaline beta1 receptor antagonist), L-748,337 (an adrenaline beta3 receptor antagonist), and dobutamine (an adrenaline beta1 receptor agonist), had no affect the lipid droplet accumulation. Clenbuterol (an adrenaline beta2 receptor agonist) did not affect the lipid droplet accumulation induced by clozapine or ICI 118551 (an adrenaline beta2 receptor antagonist).

These results suggest that adrenaline beta2 receptors are involved in the mechanism in the clozapine-induced increae of lipid droplet accumulation. However, further studies are needed to clarify the relationship between the dosages of adrenaline beta receptor-related compounds and lipid droplet accumulation.

P-17

Involvement of TNF-α/TNFR1 signaling in microglia and glutamatergic neurotransmission of mice exposed to social defeat stress as juveniles

Yuya ISOZUMI (五十住優弥)[1], Mikio YOSHIDA[1], Hikari KATADA[1], Akira YOSHIMI[1], Norio OZAKI[2], Yukihiro NODA[1]

[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University, Nagoya, Japan

[2]Pathophysiology of Mental Disorders, Nagoya University, Graduate School of Medicine, Nagoya, Japan

Exposure to psychosocial stress (e.g. bullying) in juveniles is one of risk factors as development of stress-related psychiatric disorders later in life. Exposure to psychosocial stress activates microglia and causes the glutamatergic neurotransmissions. They play an important role in either exacerbating or dampening the neuroinflammation and neuronal damage. However, it is unclear whether exposure to psychosocial stress as juveniles is affected to brain immunity and glutamatergic systems. The present study was investigated the inflammatory responses and glutamatergic neurotransmission in brain of mice exposed to social defeat stress as juveniles.

Juvenile (3-week-old) male C57BL/6J mice were exposed to social defeat stres for 10 days, and then subjected to the social interaction test on the next day of the last stress exposure. R-7050, an inhibitor of tumor necrosis factor (TNF)-α signaling, was intraperitoneally administered 30 min before every stress exposure. The expression of comprehensive gene using DNA microarray analysis, expression of TNF-α, interleukin (IL)-6, or prostaglandin (PG) E_2 using ELISA, and expression intensity of Iba-1 using immunostaining were analyzed in the prefrontal cortex (PFC) of mice 2 or 3 hr after the last stress exposure. Ability to release glutamate was analyzed by *in vivo* microdialysis, on the next day of the last stress exposure. The experiments were performed in accordance with the Guidelines for Animal Experiments of Nagoya University School of Medicine and Meijo University Faculty of Pharmacy.

The mice exposed to social defeat stress showed the the impairment of social behaviors and the expression changes of inflammation- or immune system-related genes of the PFC. In the PFC of stressed mice, The expressions of TNF-α or Iba-1 and the extracellular glutamate release induced by high potassium were significantly increased and decreased, respectively, compared to those in control mice. behavioral impairment, glutamatergic transmission impairment, and Iba-1intensity change were prevented by given to R-7050 every stress exposure.

Our results suggest that the activation of microglia through TNF-α/TNFR1-madiated signaling pathway was involved in development of behavior impairment and glutamatergic neurotransmission abnormality. Activation of brain immunity may contribute to the pathophysiology of treatment-resistant stress-related psychiatric disorders in adolescents with adverse juvenile experiences.

P-18

Effect of lithium on hematopoietic toxicity induced by clozapine in HL-60 cells

Akari KATO (加藤朱莉)[1], Aya TORII[1,2], Akira YOSHIMI[1], Yukihiro NODA[1]

[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University, Nagoya, Japan

[2]Kinjo Gakuin University, Nagoya, Japan

Clozapine (CLZ) is used for patients with treatment-refractory schizophrenia, while agranulocytosis is observed in 0.8% of patients receiving CLZ. Lithium carbonate is used prophylactically and therapeutically for neutropenia induced by CLZ, though it is weak in the strength of a recommendation in the guideline. However, the mechanisms of prophylactic and therapeutic effects of lithium carbonate on neutropenia induced by CLZ have yet to be elucidated fully. This study was focused on the effect of lithium chloride (Li) on the neutropenia induced by CLZ in undifferentiated and undergoing granulocytic ATRA-differentiation HL-60 cells.

The cells were treated with CLZ (50 μM) or Li (5 mM) alone, and combination of CLZ (50 μM) with Li (1, 2 or 10 mM). The undifferentiated and undergoing granulocytic differentiation HL-60 cells were cultured for 53 hr and 120 hr, respectively. After the culture during each time, the cell viability and viable cell count were analyzed by trypan blue staining.

Treatments with CLZ (50 μM) or Li (5 mM) alone, and combination (CLZ 50 μM and Li 1, 2 or 10 mM) in undifferentiated HL-60 cells were significantly decreased the viable cell counts, compared to treatment with vehicle. The similar decrease induced by CLZ (25 μM) and Li (5 mM) alone was observed in undergoing granulocytic differentiation. The combined treatment with CLZ (50 μM) and Li at the therapeutic concentration of blood (1 mM) did not improve, rather than aggravated the hematologic toxicity in undifferentiated HL-60 cells. When Li (1 mM) was added to before or after treatment with CLZ, CLZ-induced hematopoietic toxicity was not aggravated in both cell states (viable cell count: 7.7×10^5 /mL at CLZ 50 μM vs 2.4×10^5 /mL at CLZ 50 μM and Li 1 mM).

In conclusion, these findings suggest that Li is effective in preventing and suppressing exacerbation of CLZ-induced hematopoietic toxicity. In the future, we will examine the mechanism in preventing and suppressing exacerbation of CLZ-induced hematopoietic toxicity, e.g., examining relationship between hematopoietic toxicity and Li and CLZ-related proteins, such as a brain-derived neurotrophic factor (BDNF), which plays essential roles in the development, survival or function of neurons and a B-cell lymphoma-2 (Bcl-2), which controls the intrinsic apoptosis pathway being widely known as programmed cell death eliciting no inflammatory responses.

P-19

Mice with deficiency in *Pcdh15*, a gene associated with bipolar disorders (BD), exhibit BD-like behaviors and monoaminergic properties

Takuma KATO (加藤拓真)[1], Ayaki TAKAHASHI[1], Takahiro ITO[1], Masaki KANO[1], Miyu KUSUMOTO[1], Mikio YOSHIDA[1], Daisuke MORI[2, 3], Norio OZAKI[3], Akira YOSHIMI[1], Yukihiro NODA[1]

[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University, Nagoya, Japan

[2]Brain and Mind Research Center, Nagoya University, Nagoya, Japan

[3]Pathophysiology of Mental Disorders, Nagoya University Graduate School of Medicine, Nagoya, Japan

Recent extensive genomic studies have implicated the protocadherin-related 15 *(PCDH15*) gene in the onset of psychiatric disorders, such as bipolar disorder (BD). To further investigate the pathogenesis of psychiatric disorders including BD, we developed a *Pcdh15* deficient mouse model. In the present study, we investigated whether *Pcdh15* deficiency affected BD-like behaviors such as impulsivity, cognitive or emotional behaviors and neurotransmissions using *Pcdh15* deficient mice.

The adult (8-10-week-old) male *Pcdh15* homozygous and heterozygous mice were examined the performances of impulsivity, cognitive or emotional behaviors. After all behavioral tests, the mice were sacrificed by decapitation, and then their brains were immediately removed. Several brain regions were rapidly dissected out, frozen using dry ice, and stored at −80 °C until use for analyzing the contents of monoamines or amino acids, and the levels of amino acid biosynthetic enzymes. The experiments were performed in accordance with the Guidelines for Animal Experiments of Nagoya University School of Medicine and Meijo University Faculty of Pharmacy.

PCDH15 is primarily identified as the causative gene of Usher syndrome, which presents with visual and auditory impairments, though *Pcdh15* homozygous mice did not exhibit observable structural abnormalities in either the retina or the inner ear. The *Pcdh15* homozygous mice showed the abnormal circadian rhythm (increased spontaneous activity during the dark phase), increased impulsivity (decreased cliff avoidance response), and aversive behavior (increased passive avoidance response). In the nucleus accumbens (NAc), contents of dopamine and GABA, or GAD protein expression were increased, compared to those in wild-type mice. While *Pcdh15* heterozygous mice also showed a weak increased impulsivity and dopamine content in the NAc.

These findings suggested that *Pcdh15* deficiency was involved in the development of BD-like behavioral and dopamine/GABA neuronal abnormalities. We will investigate the effects of lithium chloride on impulsivity in *Pcdh15* heterozygous mice.

P-20

Effect of clozapine on cognitive behaviors function and neurotransmitters in a schizophrenia-like mouse model

Shunya KAWAI (川合竣也)[1], Akira YOSHIMI[1], Ayaka KATO[1], Mikio YOSHIDA[1], Mizuki UCHIDA[1], Shinji KITAGAKI[2], Norio OZAKI[3], Yukihiro NODA[1]

[1]Division of Clinical Sciences and Neuropsychopharmacology, Meijo University, Faculty and Graduate School of Pharmacy, Nagoya, Japan

[2]Department of Medical Chemistry, Graduate School of Pharmacy, Meijo University, Nagoya, Japan

[3]Pathophysiology of Mental Disorders, Nagoya University Graduate School of Medicine, Nagoya, Japan

Schizophrenia is a chronic psychiatric disorder characterized by positive symptoms, negative symptoms, and cognitive impairments. Cognitive impairments, negatively affect functional capacity and community functioning, significantly reducing quality of life. Clozapine (CLZ), an atypical antipsychotic, has shown remarkable effectiveness for treatment-resistant schizophrenia, and has provided greater integration into society. However, the effects of CLZ on cognitive functions and its mechanisms remain unclear. In this study, we investigated the effect of clozapine on cognitive behaviors function and neurotransmitters in a schizophrenia-like mouse model.

Phencyclidine (PCP) (10 mg/kg, s.c.) or saline was administered once a day for 14 days to 6-week-old male ICR mice. After PCP or saline administration, CLZ (10 mg/kg, p.o.) or vehicle (10 mL/kg, p.o.) was administered once a day for 7 days. Twenty-four hours after the last administration, the novel object recognition test, the 3-chamber social interaction test, and Y-maze test were performed sequentially. The prefrontal cortex, nucleus accumbens (NAc), striatum, hippocampus (HIP), and amygdala were collected and subjected to high-performance liquid chromatography. The experiments were performed in accordance with the Guidelines for Animal Experiments of Nagoya University School of Medicine and Meijo University Faculty of Pharmacy.

PCP-administered mice showed the impairment of object recognition in the novel object recognition test and social novelty in the 3-chamber social interaction test. The impairment of object recognition, but not social novelty was improved by administration of CLZ. There were no differences in spontaneous alternation behavior in the Y-maze test among all groups. The levels of monoamines and their metabolites in the brains of PCP-administered mice did not show any significant changes across all regions. In PCP-administered mice, administration of CLZ (10 mg/kg) resulted in an increase in noradrenaline content in the NAc and a decrease in serotonin and its metabolite contents in the HIP.

These results suggest that the regulation of brain region-specific monoaminergic neural activity is involved in the improved effects of CLZ on cognitive impairments in PCP-administered mice repeatedly. However, further studies are needed to clarify the relationship between changes in monoamines in each brain region and cognitive impairments.

P-21

Influence of environment adversity during neurodevelopment on future behaviors and neuromorphogeneses in *astrotactin2* (*ASTN2*) heterozygous mice

Amane KIMURA (木村天音)[1], Mikio YOSHIDA[1], Atsuki KURODA[1], Akira YOSHIMI[1], Itaru KUSHIMA[2], Tomomi AIDA[3], Koichi TANAKA[3], Norio OZAKI[4], Yukihiro NODA[1]

[1]Division of Clinical Sciences and Neuropsychopharmacology, Meijo University, Faculty and Graduate School of Pharmacy, Nagoya, Japan; [2]Department of Psychiatry, Nagoya University, Graduate School of Medicine, Nagoya, Japan; [3]Laboratory of Molecular Neuroscience, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [4]Pathophisiology of Mental Disorders, Nagoya University, Graduate School of Medicine, Nagoya, Japan

Psychiatric disorders are common conditions that arise as a result of complex and heterogeneous combinations of genetic and environmental factors. Deletion of *astrotactin2* (*ASTN2*) in copy number variants (CNVs) has been identified in patients with schizophrenia, bipolar disorder, and autism spectrum disorder in CNV analysis. It is unclear how exposure to adverse life events during neurodevelopment affects symptoms and pathophysiology in psychiatric patients with *ASTN2* CNVs. In the present study, we investigated the behavioral performances and neurochemical events in *Astn2* heterozygous (HT) mice exposed to adverse life events during neurodevelopment.

The HT and littermate wild-type (WT) mice were injected polyinosinic-polycytidylic acid (PIC; 5 mg/kg, s.c.) during postnatal days 2–6 or were exposed to social defeat stress (DEF) for 10 consecutive days in juveniles (3-week-old). The social interaction test, novel object recognition test, elevated plus maze test, prepulse inhibition test, and swim test were subjected to adolescents (5-week-old). We also analyze morphologically the number or size of neurons using Nissl staining, and neurochemically the expression of Iba-1 using immunofluorescence or comprehensive gene using DNA microarray in the prefrontal cortex of WT, HT, HT mice injected PIC (HT/PIC), and HT mice exposed to DEF (HT/DEF). The experiments were performed in accordance with the Guidelines for Animal Experiments of Nagoya University School of Medicine and Meijo University Faculty of Pharmacy.

The HT/PIC and HT/DEF mice in adolescents showed the impaired social and cognitive behaviors, further, HT/DEF showed the depressive-like behavior. Such behavioral abnormalities were not observed in the HT mice. The decrease of neuronal size and increase of Iba-1 expression were observed in HT/DEF mice, compared to those in WT mice. In DNA microarray analysis, the expression of gene groups related to the immune system were observed in HT mice, compared to those in WT mice. The HT/PIC and HT/DEF mice exhibited the expression of genes related to the mitogen-activated protein kinase (MAPK) signaling pathway, compared to those in HT mice.

These findings suggest that adverse life events during neurodevelopment were risk factors for development of psychiatric disorders in adolescents with *ASTN2* CNVs. Further studies are needed to investigate the involvement of p38 MAPK signaling in the emotional and cognitive behaviors in *Astn2* heterozygous mice with adverse life events during neurodevelopment.

P-22

Analysis of heparan sulfate proteoglycans in mice lacking EXTL3 specifically in glomerular podocytes

Naho Matsubara (松原奈穂)[1], Rikuma Ishigaki[1], Shuji Mizumoto[1], Taiji Matsuzaka[2], Akira Sugawara[3], Shuhei Yamada[1],

[1] Department of Pathobiochemistry, Faculty of Pharmacy, Meijo University

[2] Department of Basic Medicine, Tokai University School of Medicine

[3] Tohoku University Graduate School of Medicine

[Introduction] Heparan sulfate (HS) is a linear polysaccharide, which ubiquitously distributes on cell surface and in the extracellular matrix, and is covalently linked to specific core proteins to form proteoglycans. Five EXT family glycosyltransferases are involved in the biosynthesis of HS. EXTL3 is responsible for the initiation reaction of HS synthesis. The renal glomerular basement membrane is negatively charged, forming a barrier against molecules with negative charges, and responsible for the filtration function of glomerulus. HSPGs are known to be rich in the glomerulus and considered to play the main role on this negative charge barrier. To elucidate the effects of reduction in HS on structure and functions of glomerulus, podocytes-specific *Extl3*-conditional knockout (cKO) mice were generated.

[Methods] Mice expressing glomerular podocytes-specific Cre recombinase (*Nephrin-Cre*) were crossed with *Extl3*-flox mice ($Extl3^{flox/flox}$) to generate glomerular podocytes-specific *Extl3* cKO mice. Quantitative real-time PCR using cDNA from the renal glomerulus was performed to examine the expression of core proteins of HS-proteoglycans and EXT family members. The level of HS disaccharide in renal glomerulus was analyzed by anion-exchange HPLC after digestion with a mixture of heparinases followed by a fluorophore-derivatization. Immunostaining of in the glomerulus using anti-HS antibodies was also carried out.

[Results and Discussion] The gene expression levels of the core proteins in wild-type were highest for *syndecan 4*. The expression level of *Extl3* was down-regulated by 30% in *Extl3*-cKO mice compared to wild-type mice. The analysis of the disaccharide composition of HS in wild-type mice revealed the presence of diverse structures, including HexA-GlcNAc, HexA-GlcNAc(6S), HexA-GlcN(NS), and HexA(2S)-GlcN(NS,6S) detected, where HexA, GlcNAc, 6S, NS, and, 2S represent hexuronic acid, *N*-acetylglucosamine, 6-*O*-sulfate, 2-*N*-sulfate, and 2-*O*-sulfate, respectively. Further analysis is necessary for elucidation of the role of EXTL3 on the renal glomerular basement membrane.

P-23

Involvement of nicotinic acetylcholine receptor α7 subunit in the impairment of social behaviors in mice exposed to social defeat stress as juveniles.

Kazuna MORIKAWA (森川和那)[1], Mizuki UCHIDA[1], Honami TANAKA[1], Mayu OZAKI[1], Norio OZAKI[2], Akira YOSHIMI[1], Yukihiro NODA[1]

[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University, Nagoya, Japan

[2]Pathophysiology of Mental Disorders, Nagoya University Graduate School of Medicine, Nagoya, Japan

The efficacy and safety of antidepressants for juveniles and adolescents with depressive symptoms caused by psychosocial stress is poor. Habitual smoking in mental disorders is considered to improve their own symptoms by stimulating nicotinic acetylcholine receptors (nAChRs) in the central nervous system. It is known that the nAChR α7 subunit (α7nAChR) is involved in the regulation of mental functions and/or neuroinflammation. In the present study, we investigated the involvement of α7nAChR in social behaviors in mice exposed to social defeat stress as juveniles.

The juvenile (24-day-old) male C57BL/6J mice were exposed to an aggressive male ICR mouse for 10 min each day for 10 consecutive days. A social interaction test was performed on the next day of the last stress exposure. A selective α7nAChR agonist was administered 30 min before the test. Immediately after the test, the mice were sacrificed by decapitation, and then their brains were immediately removed. The prefrontal cortex (PFC) was rapidly dissected out, frozen using dry ice, and stored at −80 °C until use for western blotting. The experiments were performed in accordance with the Guidelines for Animal Experiments of Nagoya University School of Medicine and Meijo University Faculty of Pharmacy.

The stressed mice showed the impairment of social behaviors and the decrease of α7nAChR expression in the PFC. The expressions of phosphorylated Akt and STAT3, which play the important roles in the regulation of neuroinflammation, neurodevelopment, and neurogenesis, decreased in the PFC of stressed mice. The selective α7nAChR agonist attenuated the impairment of social behaviors and the decrease of phosphorylated Akt expression.

These results suggested that neuroinflammation, neurodevelopmental and neurogenic pathways via α7nAChR-Akt and α7nAChR-STAT3 signal transduction pathways were involved in the impairment of social behaviors induced by exposure to social defeat stress as juveniles. The α7nAChR-related compounds may be one of the novel therapeutic strategies in adolescent patients with depressive symptoms, exposed to psychosocial stress as juveniles.

P-24

Involvement of serotonin transporter in chronic orofacial pain with depressive symptoms before and after duloxetine treatment

Mariko NAKAMURA (中村真理子)[1], Akira YOSHIMI[1, 2], Tatsuya TOKURA[2], Hiroyuki KIMURA[2], Shinichi KISHI[2], Tomoya MIYAUCHI[3],Kunihiro IWAMOTO[2], Mikiko ITO[4], Aiji SATO-BOKU[4], Akihiro MOURI[5], Toshitaka NABESHIMA[5], Norio OZAKI[2], Yukihiro NODA[1, 2]

[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University, Nagoya, Japan; [2]Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan; [3]Department of Psychiatry, KACHI Memorial Hospital, Toyohashi, Japan; [4]School of Dentistry, Aichi Gakuin University, Nagoya, Japan; [5]Graduate School of Health Science, Fujita Health University, Toyoake, Japan

Chronic orofacial pain (COP) is chronic nonorganic pain in the orofacial region without causative medical or dental factors and frequently causes depressive symptoms. It takes time to determine a definitive diagnosis after excluding other organic disorders. Since duloxetine (DLX) shows efficacy in COP, there is a possibility that serotonin transporter (SERT), one of DLX action sites, could be a biomarker reflecting symptoms and/or DLX therapeutic effect in COP. In this study, we examined in COP patients before and after DLX treatment as follows: 1) the association between chronic pain and comorbid depressive symptoms, and expression of platelet SERT proteins, 2) the relationship between plasma serotonin concentration and therapeutic effects on pain and comorbid depressive symptoms.

COP patients were assessed as follows:1) the severity of pain and depressive symptoms using the Visual Analog Scale (VAS) and 17-item Hamilton Depression Rating Scale (HDRS), respectively, 2) the expressions of platelet SERT proteins and plasma serotonin concentrations by Western blotting and high-performance liquid chromatography with an electrochemical detector, respectively. They were classified into two groups based on their HDRS at baseline: COP patients with (COP-D: HDRS ≥ 8) and without (COP-ND: HDRS < 8) depressive symptoms. This study was approved by the Ethics Review Committees of Nagoya University, Aichi Gakuin University, and Meijo University.

The VAS and HDRS of COP patients were decreased after DLX-treatment compared with those at baseline. Upregulation of total SERT and downregulation of ubiquitinated SERT were observed at baseline in both groups compared with controls. After DLX-treatment, there were no differences in the total SERT of both groups, and in the ubiquitinated SERT of COP-D patients compared with controls; whereas ubiquitinated SERT of COP-ND patients remained downregulated.

Our findings indicate that DLX improves not only COP, but also comorbid depressive symptoms of particularly in COP-D patients through regulation of SERT ubiquitination. These changes in platelet SERT expression could be a potential biomarker reflecting symptoms and DLX therapeutic effect in COP with/without depressive symptoms.

P-25

Measurement of the xylosyltransferase activity of mutants of XYLT1 and XYLT2 involved in glycosaminoglycan biosynthesis, that cause inherited diseases

Wakana NOHARA (野原若菜), Shinya KIYOMASU, Shuhei YAMADA, and Shuji MIZUMOTO

Department of Pathobiochemistry, Faculty of Pharmacy, Meijo University

[Introduction] Glycosaminoglycans (GAGs) are side chains of proteoglycans and bound to serine residues on specific core proteins via a common linkage tetrasaccharide, glucuronic acid-galactose-galactose-xylose (GlcA-Gal-Gal-Xyl). Xylosyltransferases (XYLTs) 1 and 2 have been identified and transfer a Xyl from UDP-Xyl to serine residues as initiation of GAG biosynthesis. Mutations in XYLT1 and XYLT2 cause hereditary disorders, Desbuquois dysplasia type 2 and spondylo-ocular syndrome, respectively (Bui *et al*., Am J Hum Genet, 94, 405, 2014; Munns *et al.*, Am J Hum Genet, 96, 971, 2015).

[Purpose] Mutations in *XYLT1* or *XYLT2*, which are responsible for the development of bone diseases, result in single amino acid substitutions in each protein. The enzyme activities of XYLTs with mutations were investigated in this study.

[Methods] Vectors containing the mutant *XYLT* genes were transfected into COS-7 cells to express recombinant proteins, XYLT1-wild-type (WT), -R481W, -R551C, and -R598C as well as XYLT2-WT, -M237R, -R387W, -R563G, -L605P, -R730X, and -D850H. Enzyme reactions were performed using UDP-Xyl as a donor substrate and synthetic peptides derived from core proteins of chondroitin-, dermatan-, and heparan sulfate-proteoglycans, aggrecan, decorin and glypican, respectively, as acceptor substrates. The reaction products were identified by matrix-assisted laser desorption/ionization time-of-flight/mass spectrometry (MALDI-TOF/MS), and quantified by a UDP detection reagent after conversion into ATP, which was measured using a luciferase/luciferin reaction.

[Results and Discussion] The XYLT activities of all mutant enzymes examined were significantly reduced compared to those of WTs, indicating that the mutations in both enzymes may lead to partial defect in the transfer of Xyl and decreased GAG biosynthesis as appeared in the patients with Desbuquois dysplasia and spondylo-ocular syndrome. Interestingly, we found the difference in the substrate preference between WT XYLT1 and XYLT2. XYLT1 transfers a Xyl residue to decorin peptide at higher velocity than other two. In contrast, glypican peptide was the best substrate for XYLT2 among the three peptides. Since aggrecan, decorin, and glypican are the typical core proteins of chondroitin sulfate, dermatan sulfate, and heparan sulfate proteoglycans, respectively, XYLT1 and XYLT2 may contribute to the sorting of the biosynthesis of distinct GAGs.

P-26

Effects of hyaluronidase 4 deficiency on a mouse model of diabetes mellitus

Seriha OGAWA (小川芹葉), Riko SUZUKI, Shuji MIZUMOTO, Shuhei YAMADA

Department of Pathobiochemistry, Faculty of Pharmacy, Meijo University

[Purpose] Hyaluronidase 4 (HYAL4) is an enzyme involved in catabolism of chondroitin sulfate (CS) and is ubiquitously expressed in various tissues. To elucidate its function *in vivo*, *Hyal4*-deficient mice have been generated using the CRISPR/Cas9 system and analyzed for phenotypes including growth and fertility. However, no characteristic abnormalities have been identified so far. Therefore, artificial pathological conditions were introduced in the *Hyal4*-deficient mice. Based on the distribution of CS in the glycocalyx of the kidney filtration barrier, we hypothesized that HYAL4 deficiency may contribute to the onset and progression of diabetic nephropathy. Thus, *Hyal4*-deficient mice with a diabetic mouse model were generated.

[Methods] Wild-type and *Hyal4*-deficient mice at 6 weeks old were fed with high-fat diet for 8 weeks. Body weight and water consumption were measured once a week during the experiments. Seven weeks after the feeding with high-fat diet, a glucose tolerance test was examined. Next week, levels of serum glucose and blood insulin were measured, and then, mice were decapitated and dissected. Wet weights of liver and kidney, visceral fat in tests, kidney and mesentery, as well as cholesterol levels in the liver were analyzed.

[Results and Discussion] There were no significant differences between *Hyal4*-deficient and wild-type mice with high-fat diet in body weight, results of glucose tolerance test, organ weights, levels of visceral fat, cholesterol contents in the liver, and concentrations of serum glucose and insulin. However, the *Hyal4*-deficient mice with high-fat diet showed a decreasing trend for the glucose tolerance test, total fat weights surrounding the epididymis and mesentery, and serum glucose and insulin concentrations, compared to the wild-type with the same diet. This suggests that HYAL4 may regulate energy metabolism and have anti-diabetic effects. To confirm these results, the repetitive experiments will be necessary increasing in the number of mice. Further analysis is required for elucidation of the molecular mechanism underlying these findings.

P-27

Comprehensive gene expression analysis of lymphoblastoid cell lines from schizophrenia patients and blood and brain samples from schizophrenia-like mouse models.

Takumi OYA (雄谷拓海)[1], Akira YOSHIMI[1, 2], Hirotake HIDA[1], Miku ASAI[1], Hiromasa ANDO[1], Souta MIZUNO[1], Shinji KITAGAKI[3], Norio OZAKI[4], Yukihiro NODA[1, 2]

[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University,

[2]Clinical OMICs and Translation Research Center, Meijo University

[3]Laboratory of Medicinal Chemistry, Faculty of Pharmacy, Meijo University

[4]Pathophysiology of Mental Disorders, Nagoya University Graduate School of Medicine

Schizophrenia (SCZ) is a complex and multifactorial disorder that involves a combination of genetic and environmental factors, leading to significant impairments in brain function. Although neuroplastic changes are significantly affected in schizophrenia at both structural and functional levels, obtaining brain biopsy samples from patients is challenging due to ethical and safety concerns. To identify novel diagnostic and therapeutic target molecules, we conducted a cross-species transcriptome analysis using lymphoblastoid cell lines (LCLs) derived from SCZ patients and controls, as well as peripheral blood and prefrontal cortex (PFC) samples from mice administered phencyclidine (PCP) or saline (SAL).

LCLs were established from the peripheral blood of SCZ patients and controls. Male ICR mice (6 weeks old) were administered PCP (10 mg/kg/day) or SAL for 14 days. Blood and PFC samples were collected 2 hours (2h) or 24 hours (24h) after the last administration. Total RNA was extracted from human LCLs and mouse samples. Gene expression was analyzed using DNA microarray and real-time PCR. This study was approved by the Ethics Review Committee and the Institutional Animal Care and Use Committee.

DNA microarray showed significant gene expression changes: 370 genes in LCL, 387 in Blood-2h, 541 in Blood-24h, 1,120 in PFC-2h, and 848 in PFC-24h. Five genes with persistent changes (at both 2h and 24h) identified in LCL were verified by PCR. Down-regulation of *ABCA7* was verified only in the Blood-2h of PCP-administered mice, and down-regulation of *SYNE1* was verified in SCZ-LCL, PFC-2h, and PFC-24h of PCP-administered mice.

ABCA7 is involved in apoptotic cell phagocytosis, and impairment of the apoptotic pathway may contribute to dysfunction of autoimmune system, which observed in schizophrenia patients. *SYNE1*, related to synaptic plasticity, showed reduced expression, potentially leading to brain dysfunction. Further verification and functional analyses are needed to investigate the molecular functions of candidate genes, which could provide insights into the pathophysiology of SCZ and potential diagnostic and therapeutic targets.

P-28

Spatial brain proteomic analysis using a schizophrenia-like model mouse treated with clozapine

Akira YOSHIMI (吉見陽)[1,2], Susumu Y. IMANISHI[3], Tomoya OGUCHI[1], Shinji KITAGAKI[4], Norio OZAKI[5], Yukihiro NODA[1, 2]

[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University, Nagoya, Japan; [2]Clinical OMICs and Translation Research Center, Meijo University, Nagoya, Japan; [3]Laboratory of Analytical Chemistry, Faculty of Pharmacy, Meijo University, Nagoya, Japan; [4]Laboratory of Medicinal Chemistry, Faculty of Pharmacy, Meijo University, Nagoya, Japan; [5]Pathophysiology of Mental Disorders, Nagoya University Graduate School of Medicine, Nagoya, Japan

Clozapine (CLZ), an atypical antipsychotic, has demonstrated remarkable effectiveness in the treatment of treatment-resistant schizophrenia, especially in cases where other antipsychotics have been ineffective. Elucidating the molecular mechanisms of clozapine can contribute to the development of effective treatment strategies. This study aims to investigate spatial alterations in the brain proteome of a schizophrenia-like mouse model administered phencyclidine (PCP) and subsequently treated with CLZ, to obtain insights into the neurobiological pathways underlying the therapeutic effects of CLZ.

PCP (10 mg/kg, s.c.) or saline (10 mL/kg, s.c.) was administered once a day for 14 consecutive days into 6-week-old male ICR strain mice. After PCP or saline administration, CLZ (10 mg/kg or 30 mg/kg, p.o.) or vehicle (10 mL/kg, p.o.) was administered once a day for 7 consecutive days. Twenty-four hours after the last administration, the prefrontal cortex (PFC), nucleus accumbens (NAc), striatum (STR), hippocampus (HIP), and amygdala (AMY) were collected and subjected to proteomic analysis. Pathway analysis was performed on differentially expressed proteins (DEPs) to confirm their biological significance.

As a result, DEPs related to the interaction of the two factors (PCP and CLZ) were associated with various pathways, including glutamatergic synapses in the PFC, retrograde endocannabinoid signaling in the STR, Alzheimer's disease in the HIP, oxidative phosphorylation in the AMY. Neuropsychiatric disorder-related proteins/pathways were identified among the overlapping DEPs across multiple brain regions affected by administration of PCP and/or CLZ. Interestingly, a common molecular pathway related to synaptic functions (such as the synaptic vesicle cycle, vesicle-mediated transport in synapses, and synaptic plasticity) was identified across five brain regions. These regions are involved in behavioral phenotypes, such as cognitive impairments and emotional disturbances.

Elucidating the functional roles of DEPs associated with common or specific pathways across various brain regions may contribute to the development of novel treatment strategies for treatment-resistant schizophrenia.

P-29

High-sensitive measurement of abasic sites in genomic DNA

Sakura Hida (樋田桜花)[1], Yoshinori Okamoto[1], Akira Aoki[1], Hideto Jinno[1]

[1]Faculty of Pharmacy, Meijo University, Nagoya, Japan

Objective: Apurinic/apyrimidinic (AP) sites are a type of DNA damage generated by various endogenous and exogenous factors, and are thought to be involved in cellular aging and carcinogenesis. AP sites are measured after derivatization of the aldehyde group derived from 2'-deoxyribose (dR) using aldehyde-reactive probes (ARP). Typically, they are quantified colorimetrically using biotinylated ARP and HRP-labeled streptavidin, but this method has low selectivity due to its reaction with aldehyde groups in non-AP sites. In this study, we developed an analytical method using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure AP sites with high sensitivity and selectivity.

Methods: This study was conducted with the approval of the Animal Experiment Committee of Meijo University School of Pharmacy. DNA was extracted from the livers of female ACI (August Copenhagen Irish) rats using a spin column method. The DNA was treated with *O*-(Pyridin-3-yl-methyl)hydroxylamine (PMOA) as the ARP to derivatize the AP sites. The PMOA-treated DNA was enzymatically digested into nucleosides, deproteinized by ethanol precipitation, and then subjected to LC-MS/MS analysis. For the preparation of standards, PMOA was mixed with dR or [$^{13}C_5$]-dR to synthesize PMOA-dR and PMOA-[$^{13}C_5$]-dR (both Z/E isomer mixtures). The LC-MS/MS (QTRAP6500+) analysis was performed under the following conditions: ESI (positive ion mode), MRM transitions (PMOA-dR, *m/z* 241.1→108.0; PMOA-[$^{13}C_5$]-dR, *m/z* 246.1→108.0). A C18 core-shell column was used for the ultra-high-performance liquid chromatography (ExionLC AD).

Results and Discussion: Analysis of PMOA-dR revealed that Z and E isomers were detected as two distinct peaks (retention times: 4.72 min and 5.00 min) with an area ratio of approximately 4:6. Quantification was performed by summing the areas of both peaks. The calibration curve showed linearity in the range of 5–1000 pM with an r^2 of 0.99850. The lower limit of quantification (LLOQ) was 0.25 fmol (on column). Measurement of AP sites in rat liver DNA showed a concentration of 7.03±2.82/107 dNs, which was about one-tenth of that obtained by conventional colorimetric quantification methods. These results indicate that our method has higher sensitivity and selectivity compared to conventional methods.

P-30

Inhalation therapy for patients with bronchial asthma during the COVID-19 pandemic: appropriate instructions amidst infectious disease spread

Masaki KANO (加納正暉)[1], Mariko NAKAMURA[1]. Noriaki MATUMOTO[2], Mikio SAKAKIBARA[2], Toshiki KANAI[3], Akira KURACHI[4], Masayuki HIRAMATU[5], Yukihiro NODA[1].

[1]Division of Clinical Sciences and Neuropsychopharmacology, Faculty and Graduate School Pharmacy, Meijo University

[2]Sugi Pharmacy

[3]Cocokarafine Healthcare Pharmacy

[4]Walnut Pharmacy

[5]Department of Chemical Pharmacology, Meijo University

The main treatment for bronchial asthma is inhalation therapy, using inhaled medications primarily. Continuous inhalation instructions by pharmacists are essential, though there is a possibility that proper face-to-face inhalation instructions may not be perfomed amidst spreading infectious diseases and climate change. To consider how to provide appropriate inhalation instructions under the circumstances of infectious disease spread, in this study, here we performed an actual survey of the current status of inhalation instruction and treatment or symptoms of relevant patients in community pharmacies during the coronavirus disease 2019 (COVID-19) pandemic.

The survey was conducted for bronchial asthma patients who visited community pharmacies and pharmacists who provided inhalation instructions in patients during the period from September 2020 to February 2021. A self-made questionnaire and the Asthma Control Test (ACT) were used as survey instrument. The survey was performed as follows : Ⅰ) for pharmacists, evaluation of the pharmacist's inhalation instructions practice and infection prevention measures. Ⅱ) for bronchial asthma patients, evaluation of anxiety, treatment, and symptoms during the COVID-19 pandemic. This study was approved by the Ethics Review Committees of Meijo University Faculty of Pharmacy; and was conducted in accordance with the Helsinki Declaration.

The survey revealed the following: 1) While taking infection control measures in community pharmacies, inhalation instructions the similar to those before the COVID-19 pandemic were performed relatively consistently; and 2) Patients with versus without deteriorating symptoms had a greater self-assessed understanding of the pathophysiology of bronchial asthma and the purpose of controller medication. These findings suggest that it is important to reconfirm patient understanding of the pathophysiology of bronchial asthma and the inhalation technique of controller medications regardless of patient self-assessments of inhalation instructions provided by pharmacists during times of infectious disease spread.

P-31

Kinetic Characterization of FLVCR2 as an Intestinal Choline Transporter in the Rat Model

Mione YAMASAKI (山﨑美音), Chitaka NAMBA, Takahiro YAMASHIRO, Tomoya YASUJIMA, Hiroaki YUASA

Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University

Choline is a nutritional compound that needs to be supplied dietarily and absorbed from the intestine. Carrier-mediated transport has been suggested to be involved in the intestinal uptake of this compound, which is otherwise only poorly permeable across the cellular membrane by simple diffusion because of its high hydrophilicity, to facilitate the process. However, the carrier-mediated mechanism has not been fully clarified. Particularly, the molecular aspects including the transporter responsible for that remain to be elucidated. To address the issue, we examined the possibility that feline leukemia virus subgroup C receptor 2 (FLVCR2/SLC49A2), which is a newly identified choline transporter and highly expressed in the intestine, might be involved in intestinal choline uptake, using the rat model. We first examined the uptake of [^{3}H]choline in MDCKII cells stably expressing rat FLVCR2 for the functional analysis. The uptake of choline was greater in FLVCR2-expressing MDCKII cells than in mock cells, indicating that rat FLVCR2 is capable of transporting choline. Kinetically, the concentration-dependent profile of the specific uptake of choline by FLVCR2 was mostly accounted for by a saturable component with the Michaelis constant (K_m) of 1.36 mM. Although it was accompanied by another component with a smaller K_m of 0.051 μM, suggesting the presence of two distinct recognition sites, the former component with the greater K_m (lower affinity) was prevailing. In addition, FLVCR2 was found to be localized to the apical membrane in polarized FLVCR2-expressing MDCKII cells, suggesting its role as an apical uptake transporter. We then evaluated the uptake of [^{3}H]choline into the everted tissue sacs prepared from the intestine (jejunum) of male Wistar rats. The uptake of choline was almost fully accounted for by a single saturable transport component with the K_m of 1.95 mM, which was comparable to that of the prevailing lower affinity component of the FLVCR2-mediated choline transport. Based on all these, FLVCR2 is likely to be responsible for intestinal choline uptake. It seems that its function for the prevailing lower affinity component is mainly involved in that, while the function for another accompanying component does not have a detectable contribution at the tissue level. Future studies are warranted to clarify the role of FLVCR2 in intestinal choline uptake in humans. It should be of help in exploring its potential role in intestinal drug absorption and also exploitation for oral drug delivery.

P-32

Functional Characteristics of SLC19A3 for the Transport of Amiloride as a Newly Found Fluorescent Substrate

Riku YAMAUCHI (山内利玖), Takahiro YAMASHIRO, Michihiro YAMAMOTO, Kaito MATSUI, Tomoya YASUJIMA, Hiroaki YUASA

Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University

SLC19A3, known as thiamine transporter 2 (THTR2), has been of increasing interest for its multispecific operation, typically in the intestinal absorption, of several basic compounds, including thiamine (vitamin B1), the originally identified substrate, and pyridoxine (vitamin B6), a more recently identified one.[1)] This transporter operates uniquely by multiple mechanisms in a substrate-dependent manner, manifested as a difference in pH dependence between thiamine transport, which favors near neutral conditions, and pyridoxine transport, which favors acidic conditions, and also manifested as an animal species difference specifically present in the pyridoxine transport function, which is absent in its rat and mouse orthologs, but not in the thiamine transport function.[2,3)] To further clarify the multiple mechanisms in the SLC19A3 operation, we examined its functional characteristics for the transport of amiloride, a newly found fluorescent substrate. We first conducted a series of experiments using MDCKII cells stably expressing human SLC19A3, evaluating the initial uptake of amiloride (10 μM) for 10 min at 37°C by fluorometric detection. SLC19A3 was found to transport amiloride in a pH-dependent manner, favoring near neutral conditions. This characteristic was similar to that of thiamine transport. Kinetically, when evaluated at pH 7.4, the K_m of SLC19A3-mediated transport of amiloride was 63.1 μM, being higher than those of thiamine and pyridoxine (2.36 μM and 18.5 μM, respectively) and, hence, indicating a low affinity characteristic. In the subsequent assessment of species differences in the amiloride transport function using transiently transfected HEK293 cells, the rat and mouse orthologs of SLC19A3 were found to lack the function, whereas human SLC19A3 was confirmed to have that. This characteristic was similar to that in the pyridoxine transport function. Thus, amiloride transport by SLC19A3 was similar to thiamine transport but not to pyridoxine transport in pH dependence, whereas it was similar to the latter but not to the former in animal species difference. This finding suggests that the substrate-dependent differences in the pH dependence and animal species difference are likely to be based on different molecular mechanisms, not associated with each other. Future studies are warranted to elucidate the underlying mechanisms. Caution may be needed when selecting model animals in studies related to SLC19A3-mediated transport, whereas amiloride could be utilized as a fluorescent probe substrate of SLC19A3.

References:
1. Yamashiro T et al.: J. Biol. Chem., 295, 16998-17008, 2020.
2. Yamashiro T et al.: Drug Metab. Pharmacokinet., 44, 100456, 2022.
3. Miyake K et al.: J. Biol. Chem., 298, 102161, 2022.

P-33

Staphylococcal superantigen-like protein 3 triggers murine mast cell adhesion and enhancement of mast cell activation by binding to CD43

Sae Kawano[1], Chisaki Noda[1], Saotomo Itoh (伊藤佐生智)[1], Ayaka Urabe[1], Chifumi Fujii[2,3,4] ,Isamu Ogawa[1], Ryo Suzuki[5], Shigeaki Hida[1]

[1]Department of Molecular and Cellular Health Sciences, Graduate School of Pharmaceutical Sciences, Nagoya City University

[2]Department of Biotechnology, Institute for Biomedical Sciences, Shinshu University

[3]Department of Molecular Pathology, Shinshu University School of Medicine

[4]Center for Medical Education and Clinical Training, Shinshu University School of Medicine

[5]Laboratory of Hygienic Chemistry, Faculty of Pharmaceutical Science, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University

S. aureus is thougt to be involved in allergic diseases, as four staphylococcal exotoxins were reported to activate a key contributor of inflammation, mast cells, in an IgE-independent manner. Although the adhesion of mast cells is an essential process for their immune responses, only a small number of exotoxins have been reported to affect the process. Here, we showed that staphylococcal superantigen-like (SSL) 3, we have previously identified as a toll-like receptor 2 antagonist, induced the adhesion of murine bone marrow-derived mast cells to culture substratum. SSL3-induced adhesion was mediated by an extracelllur matxix protein, fibronectin, in an Arg-Gly-Asp (RGD) sequence-dependent manner, suggesting that integrin adhesion receptors were involved in the process. Affinity purification of SSL3 binding protein from the mast cell lysate revealed that an anti-adhesive surface protein CD43 bound to SSL3. SSL3 trigered the adhesion of HEK293 cells expressing exogenous CD43, suggesting that CD43 is the target molecule for adhesion induced by SSL3.The interaction between SSL3 and CD43 and the induction of cel adhesion by SSL3 required the glycan binding activity of SSL3, as the introduction of mutation in its sialy-lactosamine binding motif diminished the activities. The C-terminal region of SSL3, specifically T285 and H307, were essential to induce cell adhesion. SSL3 enhanced the IL-13 production of mast cells induced by IgE-antigen complex and SSL12. These findings reveal a novel function of SSL3, triggering cell adhesion and enhancing mast cell activation. This study would clarify the correlation between *S. aureus* and the pathogenesis of allergic diseases.

P-34

Mechanisms of allergic disease suppression by molecules derived from indigenous skin bacteria

Reina MUKAINAKA (向井中玲菜), Yuma ITOH, Isamu OGAWA, Saotomo ITOH, Shigeaki HIDA

Department of Molecular and Cellular Health Sciences, Nagoya City University

In healthy skin, a variety of commensal bacteria compose the skin microbiota to maintain skin homeostasis. Skin microorganisms have important roles in the immune system and the protection against invading pathogens. However, the molecular mechanisms by which skin bacteria affect the immune system and maintain homeostasis are unclear. In this study, the effect of *Staphylococcus epidermidis* strain (ATCC12228) on the Th balance and its molecular mechanisms.

To investigate the effect of molecules from *Staphylococcus epidermidis* (*S. epidermidis*) on the type 2 Inflammation, BALB/c mice were treated with Ovalbumin (OVA) and bacterial culture supernatant. The increase in serum IgE and OVA-specific IgG1 was markedly suppressed in the *S. epidermidis* supernatant-treated group compared with the PBS-treated group. Moreover, the effect of molecules from *Staphylococcus epidermidis* on Th differentiation was also investigated using CD4+ T cells from transgenic mice with ovalbumin (OVA)-specific CD4+ T cell receptor, and differentiation into Th2 cells was suppressed in the presence of bacterial culture supernatant. These results indicated that *S. epidermidis*-derived molecules affected CD4+ T cells and innate immune cells to suppress the Type 2 Inflammation and IgE antibody production. However, many questions remain regarding the function of the skin microbiota. In our study, the identification of *Staphylococcus epidermidis*-derived molecules and their immunoregulatory mechanisms may help prevent or exacerbate allergic diseases caused by type 2 inflammation.

P-35

HMG-CoA reductase inhibitors regulate helper T cell differentiation

Ishikawa Satoshi (石川怜)[1], Murase Kanon[2], Sasano Kazuki[2], Muzuno Haruka[2], Hatanaka Honoka[2], Ogawa Isamu[1], Itoh Saotomo[1], Hida Shigeaki[1]

[1]Department of Molecular and Cellular Health Science, Graduate School of Pharmaceutical Sciences, Nagoya City University

[2]Department of Molecular and Cellular Health Science, Faculty of Pharmaceutical Sciences, Nagoya City University

Hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors, also known as “statins”, are commonly used to treat dyslipidaemia. The therapeutic potential of statins has been well recognized with its antitumor, and anti-inflammatory activities and is under clinical trial for the treatment of cancer and some inflammatory diseases.

However, the cellular mechanisms by which they exert their immunoregulatory effects remain to be elucidated. In this study, we investigated the effects of statins on immune responses in vivo and in vitro, focusing on T-cell differentiation. Statins currently approved in Japan (Atorvastatin, Fluvastatin, Rosuvastatin, Simvastatin) and Lovastatin did not significantly affect the population and total number of T lymphocytes when administered i.p. for 1 week to C57BL/6 mice. On the other hand, IL-4 but not IFNγ secretion stimulated by TCR stimulation from CD4+ helper T cell population was upregulated in the presence of statins. Furthermore, bone marrow-derived dendritic cells differentiated in the presence of certain statins showed decreased IL12 production in response to various stimuli.

These results suggest that HMG-CoA reductase inhibitors may shift the Th balance towards Th2 and that appropriate HMG-CoA reductase inhibitor selection should take into account the different underlying diseases of patients.

P-36

Development of a platelet generation evaluation system using human iPS cell-derived megakaryocytes

Tomoe Kajita, Tadahiro Hashita, Eisei Hori, Takahiro Iwao

Educational Research Center for Clinical Pharmacy, Nagoya City University

Several thousand platelets are generated by a megakaryocyte, which releases their cellular fragments into the bloodstream. Therefore, megakaryocytes play a crucial role in maintaining platelet count. Although multi-kinase inhibitors are highly effective in cancer treatment, several muti-kinase inhibitors like sunitinib cause unexpected side effects, such as thrombocytopenia. The detailed mechanisms of thrombocytopenia with multi-kinase inhibitors remain unclear. To elucidate the mechanisms, it is essential to develop an evaluation system for platelet production. However, existing static culture systems are inadequate for evaluating platelet differentiation, as blood flow is essential for this process. Therefore, we decided to develop an evaluation system for platelet production that mimics blood flow using human iPS cell-derived immortalized megakaryocytes (imMKCL).

We examined the conditions for imMKCL adhesion to cell culture inserts to facilitate platelet release. The results showed high cell viability and adhesion under four extracellular matrix conditions: fibronectin (2, 10 μg/cm²) and laminin 521 (2, 10 μg/cm²). To develop an evaluation system that mimics blood flow, we investigated the extent to which platelet production changes with shaking culture. imMKCL cells were seeded onto cell culture inserts coated with fibronectin and cultured statically for 48 hours. Subsequently, the cells were subjected to either static or shaking culture, and the platelets released from imMKCL to the bottom side under the cell culture inserts were measured by counting CD41-positive platelets using flow cytometory . As a result, increasing the number of seeded imMKCL cells led to a higher number of produced platelets. Furthermore, a comparison between shaking and static cultures revealed that the shaking culture produced a greater number of platelets.

The higher platelet count in the shaking culture compared to the static culture indicates that medium flow is necessary for efficient platelet production. The observation of a low platelet count despite an increase in the number of seeded cells suggests that many seeded cells were unable to adhere. Given that the shaking speed and direction were constant, further investigations under varying conditions are necessary.

In the future, we aim to develop a device capable of applying higher shear stress to enhance platelet production. We also plan to use this developed device to analyze platelet depletion induced by multi-kinase inhibitors.

P-37

Phospholipid flippases ATP8A1 and ATP8A2 modulate inhibitory neurotransmission in hippocampal neurons

Muneyuki KAWASE (川瀬宗之)[1], Takuto MATSUDA[1], Yuta UMEMURA[1], Hisashi OISHI[2], Takashi SAKURAI[3], Mitsuharu HATTORI[1]

[1]Department of Biomedical Science, Nagoya City University

[2]Department of Comparative and Experimental Medicine, Nagoya City University

[3]Department of Cellular and Molecular Pharmacology, Juntendo University

[Introduction] Phospholipids are asymmetrically distributed across the lipid bilayers in the plasma membrane, which is crucial for various biological phenomena. This asymmetry is regulated, at least in part, by flippases that transport phospholipids from the extracellular or lumenal side to the cytoplasmic side. Among the mammalian flippases, ATP8A2 is expressed mainly in the nervous system, and its dysfunction causes severe motor deficits and higher brain dysfunction. In addition, ATP8A1, which has a high structural homology to ATP8A2, is mostly localized in synaptic vesicles within neurons, and mice lacking ATP8A1 show spatial memory deficits. However, the expression patterns of those flippases in neurons and the molecular mechanisms by which they contribute to neural functions are largely unknown. In this study, we aim to clarify the significance of ATP8A1 and ATP8A2 in neurons.

[Methods] ATP8A1-deficient (ATP8A1 KO) and ATP8A2-deficient (ATP8A2 KO) mice were generated using the CRISPR/Cas9 system. Immunostaining for markers of pre- and post-synaptic proteins was performed on primary cultured hippocampal neurons. The exposure of phosphatidylserine (PS), which is the main transport substrate of ATP8A1 and ATP8A2, on the neuronal plasma membrane was investigated using a PS-binding probe. Surface biotinylation experiments and endocytosis assays were performed on cultured hippocampal neurons.

[Results] ATP8A1 and ATP8A2 showed different subcellular localizations, with ATP8A1 localized to excitatory presynapses and ATP8A2 mainly to inhibitory postsynapses. Under unstimulated conditions, the exposure of PS was not observed in single KO neurons but only in the neurites of ATP8A1/ATP8A2 double KO neurons. Based on these results, we hypothesized that the neuronal dysfunction caused by ATP8A1 or ATP8A2 deficiency was primarily mediated by impairment of intracellular functions such as membrane protein trafficking. Thus, we examined the surface expression levels of synaptic proteins and found that the amount of inhibitory neurotransmission receptors was selectively increased on the plasma membrane by ATP8A1 or ATP8A2 deficiency. Furthermore, ATP8A2 deficiency inhibited the endocytosis of inhibitory neurotransmission receptors, suggesting that ATP8A2 contributes to the intracellular uptake of inhibitory neurotransmission receptors. These results indicate that both ATP8A1 and ATP8A2 contribute to neural functions by modulating inhibitory neurotransmission.

P-38

Myosin Va regulates the terminal translocation of migrating neurons in the neocortex

TAKAO Kohno (河野孝夫), MONE Sato, LI Minqian, and MITSUHARU Hattori
Department of Biomedical Science, Graduate School of Pharmaceutical Sciences, Nagoya City University

Neocortex consists of six layers which is formed mainly by excitatory neurons born in the ventricular zone. The layered structure is necessary for the formation of neural circuits, and its malformation are associated with various neuropsychiatric disorders. Excitatory neurons are born from neural progenitor cells located in the ventricular zone and migrate radially to pass through already formed neuronal layers, eventually locating early-born neurons in deeper layers and late-born neurons in superficial layers. During the radial migration, neurons use multiple modes of migration, including multipolar, locomotion, and terminal translocation. However, the regulatory mechanisms of neuronal migration during postnatal stages remain poorly understood. Here we show that Myosin Va (Myo5a), a motor protein involved in the transport of membrane molecules, plays an important role in regulating layer formation in the postnatal neocortex. We identified Myo5a as one of the abundant molecules in postnatal superficial layer neurons using the proximity dependent biotin identification method. Myo5a expression increased during postnatal development and was highly expressed in superficial layers with a preference for apical dendrites. Inhibition of Myo5a function using dominant-negative form of Myo5a caused mispositioning of superficial neurons and prevented their entry into NeuN-negative regions, presumably impairing the terminal translocation step. These results suggest that Myo5a regulates the superficial layer-specific trafficking of membrane proteins required for terminal translocation.

P-39

Elucidation of the pathogenesis of neurological diseases caused by phospholipid flippase deficiency

Noritaka SASSA (佐々徳啓)[1], Yuta UMEMURA[1], Muneyuki KAWASE[1], Hisashi OISHI[2], , Mitsuharu HATTORI[1]

[1]Department of Biomedical Science, Nagoya City University

[2]Department of Comparative and Experimental Medicine, Nagoya City University

[Introduction] Phospholipids are asymmetrically distributed across the plasma membrane. Phosphatidylserine (PS) is mainly found on the cytoplasmic side. Phospholipid flippases belonging to the P4-ATPase family contribute to the maintenance of phospholipid asymmetry by translocating PS from the extracellular to the cytosolic side. Phospholipid flippases expressed in the nervous system are mainly ATP8A1 and ATP8A2, and mutations in ATP8A2 in humans cause cerebellar ataxia-mental retardation and balance disorder syndrome. Mice lacking ATP8A2 exhibit neurodegeneration. However, the mechanism by which ATP8A2 deficiency causes disease in humans and neurodegeneration in mice is unknown.

Glial cells are known to phagocytose cells with exposed PS. Therefore, I hypothesized that the deficiency of ATP8A2 prevents the translocation of PS exposed in the extracellular side to the cytosolic side, resulting in glial cells recognizing the increased exposure of PS and phagocytosing neurons excessively, leading to neurodegeneration.

[Methods] ATP8A1/ATP8A2 double-knockout (DKO) mice were generated using the CRISPR/Cas9 system. Immunostaining for markers of astrocytes and activated microglia was performed on the cerebral cortex and hippocampus and cerebellum. Fluoro-Jade C staining was performed to confirm neurodegeneration.

[Results] ATP8A1/ATP8A2 DKO mice showed growth retardation compared to wild-type mice. ATP8A1 and ATP8A2 have different expression patterns in the cerebellum, with ATP8A1 in the molecular layer and ATP8A2 in the cerebellar nuclei. Moreover, DKO mice showed neurodegeneration and glial cell activation in the cerebellum. Based on these results, we hypothesized that the loss of scramblase, an enzyme that transports PS to the extracellular space, would reduce PS exposure and ameliorate neurodegeneration. We focused on Xkr4 and TMEM16C, which are known to be highly expressed in the brain, and deleted them from DKO mice. However, there was no significant change in glial cell activation or the degree of neurodegeneration. It is speculated that neurodegeneration was not ameliorated because other scramblases expressed in the brain supplemented the function of the defective scramblase.

P-40

TREK1 channels are involved in fibrogenic matrix expression and cell proliferation in human hepatic stellate LX-2 cells

Naoki KAWATA (川田成紀), Rubii KONDO, Akari DEGUCHI, Yoshiaki SUZUKI, Hisao YAMAMURA

Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University

Liver fibrosis occurs in viral hepatitis and steatohepatitis, where repeated injury recovery, lead to excessive connective tissue accumulation, and resulted in scarring. The severity of liver fibrosis is closely associated with the prognosis of chronic liver diseases. Therefore, there is an unmet medical need for treating liver fibrosis. Under physiological conditions, hepatic stellate cells (HSCs) exhibit a quiescent phenotype and contribute to vitamin A storage. When the liver is injured, HSCs transform into an activated phenotype and are involved in liver fibrosis as the main source of connective tissue, including collagen fibers. An increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) in HSCs induces the pathogenesis of liver fibrosis, however, the molecular mechanisms remain unclear. The TREK1 channel is a type of the two-pore domain K^+ (K_{2P}) channel, which determines the resting membrane potential and $[Ca^{2+}]_{cyt}$ in several cell types. In this study, we revealed the functional roles of TREK1 channels in human hepatic stellate LX-2 cells.

Real-time PCR and immunoblot analyses revealed the expression of TREK1 channels at both mRNA and protein levels in LX-2 cells. Under whole-cell patch-clamp mode, K_{2P} channel currents were increased by the treatment with arachidonic acid (AA) in LX-2 cells. This activation was inhibited by tetrapentylammonium (TPA). AA induced membrane hyperpolarization, and TPA blocked this hyperpolarization in LX-2 cells. In LX-2 cells, siRNA knockdown of TREK1 channels decreased $[Ca^{2+}]_{cyt}$, downregulated mRNA expression of collagen type IA and platelet-derived growth factor, and inhibited cell proliferation. Our findings suggest that TREK1 channels in HSCs regulate $[Ca^{2+}]_{cyt}$ by modulating the membrane potential, contributing to the development of liver fibrogenesis. This study may help elucidate the molecular mechanism underlying liver fibrosis in HSCs and provide a potential therapeutic target for hepatic fibrosis.

P-41

Upregulated expression of two-pore domain potassium channels, KCNK1 and KCNK2, in pulmonary arterial hypertension

Taiki AMANO(天野泰樹)[1], Natsumi Shima[1], Aya Yamamura[2], Moe Fujiwara[1], Kazuyuki Matsumoto[1], Taiga Sekine[1], Haruka Okano[1], Rubii Kondo[1], Yoshiaki Suzuki[1], Hisao Yamamura[1]

[1]Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University

[2]Department of Physiology, Aichi Medical University

Pulmonary arterial hypertension (PAH) is an intractable disease in the cardiopulmonary system. Its pathogeneses involves pulmonary vascular remodeling, which results in progressive increases in pulmonary arterial pressure. Due to the recent development of PAH drugs, the five-year survival rate of PAH has increased. Nevertheless, PAH remains incurable and still has a poor prognosis. Pulmonary vascular remodeling is attributed to the excessive proliferation and migration of pulmonary arterial smooth muscle cells (PASMCs), which are induced by enhanced Ca^{2+} signaling. The two-pore domain potassium KCNK channels generate background or leak K^+ currents, thereby maintaining the resting membrane potential and cytosolic [Ca^{2+}] in several types of cells. In the present study, the functional expression of KCNK channels was examined in PASMCs from idiopathic PAH (IPAH) patients and experimental PAH animals. Expression analyses showed that the expression of KCNK1/TWIK1 and KCNK2/TREK1 channels was upregulated in PASMCs from IPAH patients and monocrotaline-induced PAH rats. The facilitated proliferation and migration of IPAH-PASMCs was suppressed by the KCNK channel blockers, quinine and tetrapentylammonium. In addition, increases in the proliferation and migration were inhibited by the siRNA knockdown of KCNK1 or KCNK2 channels. The siRNA knockdown caused membrane depolarization and subsequent decrease in cytosolic [Ca^{2+}]. The phosphorylated level of c-Jun N-terminal kinase (JNK) was elevated in IPAH-PASMCs compared to normal-PASMCs. The increased phosphorylation was reduced by the siRNA knockdown of KCNK1 or KCNK2 channels. In conclusion, these findings indicate that the upregulated expression of KCNK1 and KCNK2 channels facilitates the proliferation and migration of PASMCs via enhanced Ca^{2+} signaling and JNK signaling pathway, which is associated with vascular remodeling in PAH.

P-42

Optogenetic investigation of the role of excitation-transcription coupling in vascular smooth muscle cells.

Tsukasa KOIDE（小井手 司）[1], Yoshiaki SUZUKI[1], Rubii KONDOU [1], Gerald ZAMPONI[2], Hisao YAMAMURA[1]

[1]Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University

[2]Department of Clinical Neurosciences, Hotchkiss Brain Institute, Alberta Children's Hospital Research Institute, University of Calgary

[Background]

Chronic vascular stress induces inflammation, leading to vascular smooth muscle cell (VSMC) proliferation and vascular remodeling. Pressure overload, a major stress on blood vessels, depolarizes VSMCs by the activation of mechano-sensitive cation channels, and this increases intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) through voltage-dependent Ca^{2+} channels (VDCCs). This $[Ca^{2+}]_i$ elevation activates excitation-transcription (E-T) coupling in VSMCs, inducing inflammatory gene expression, macrophage accumulation, and medial thickening（Suzuki et al, *PNAS*, 2022）. However, it is unclear whether VDCC-mediated Ca^{2+} influx in response to membrane depolarization is sufficient for E-T coupling. To address this question, we utilized an optogenetic approach to specifically depolarize VSMCs *in vivo*.

[Aims]

This study aimed to investigate whether sustained depolarization of VSMCs directly contributes to vascular remodeling by using optogenetics.

[Methods and Results]

we developed mice expressing channelodopsin (ChR)-2 specifically in smooth muscle (SMC-ChR2). Light stimulation successfully induced an increase in $[Ca^{2+}]_i$ through VDCCs in freshly isolated VSMCs and tissue preparations from the arteries. Furthermore, light stimulation of the arteries *in vivo* induced transcription of pro-inflammatory genes (*Il-6, Cxcl1, Cxcl2, Selp*) and macrophage accumulation in the vessel wall. these responses were suppressed by VDCC inhibitors.

[Conclusion]

These results suggest that sustained depolarization specific to VSMCs can cause E-T coupling *in vivo* and trigger vascular inflammation, potentially leading to vascular remodeling.

P-43

Downregulation of Kv1.6 channel expression in chondrocytes leads to osteoarthritis via enhanced Ca^{2+} signaling.

Tomo KURATA (倉田朋)[1], Yoshiaki SUZUKI[1], Shinya TATENO[1], Shigeru MIYAKI[2], Eiva BERNOTIENE[3], Wayne GILES[4], Hisao YAMAMURA[1]

[1]Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University

[2]Department of Orthopaedic Surgery, School of Medicine, Hiroshima University

[3]Department of Regenerative Medicine, Innovative Medicine Center

[4]Department of Physiology & Pharmacology, University of Calgary

The activity of voltage-gated K^+ (K_V) channels regulates the resting membrane potentials and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in chondrocytes. It has been reported that an increase in $[Ca^{2+}]_i$ is associated with osteoarthritis (OA), which is characterized by severe pain and disorders of joint movement. However, the mechanism underlying the increase in $[Ca^{2+}]_i$ remains unclear. In the present study, we examined the pathological role of K_V channels in OA using mouse and human chondrocytes.

In mouse chondrocytes, interleukin (IL)-1β induced OA marker genes, such as IL-6, ADAMTS-5, and MMP-13, reflecting OA conditions. In IL-1β-treated chondrocytes, the resting membrane potential was depolarized due to the downregulation of K_V1.6 channel expression. In addition, the mRNA expression of voltage-dependent Ca_V1.2 channels was increased in IL-1β-treated chondrocytes. IL-1β treatment induced an increase in resting $[Ca^{2+}]_i$, loss of mitochondrial membrane potential, an increase in mitochondrial production of reactive oxygen species, and chondrocyte death. These responses were suppressed by treatment with Ca_V1.2 blocker, nifedipine.

In summary, IL-1β induces depolarization of the resting membrane potential by downregulating of Kv1.6 channel expression, thereby increasing Ca^{2+} influx via Ca_V1.2 channels in chondrocytes. Enhanced Ca^{2+} entry through Ca_V1.2 channels results in mitochondrial dysfunction and subsequent chondrocyte death. Our findings may contribute to the understanding of OA pathogenesis and the development of new treatments for OA.

P-44

Inhibitory effects of melatonin on voltage-gated K^+ (K_V4.2) channels in rat pinealocytes

Hibiki Kuzuhara(葛原響)[1], Hiroki Mishima[1], Shunsuke Ando[1], Rubii Kondo[1], Yoshiaki Suzuki[1], Hisao Yamamura[1]

[1]Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University

Melatonin is a hormone that is synthesized and secreted by the pineal glands, regulating circadian rhythms. However, the effects of melatonin on pineal ion channels remains unclear. In the present study, the effects of melatonin on voltage-gated K^+ (K_V) channels, which play a role in regulating the resting membrane potential, were examined in rat pinealocytes using whole-cell patch clamp recordings. The application of melatonin reduced pineal K_V currents in a concentration-dependent manner. Expression analyses revealed that K_V4.2 channels were highly expressed in rat pineal glands. In HEK293 cells expressing K_V4.2 channels, melatonin decreased outward K_V4.2 currents. Inhibitory effects were mediated by a shift in voltage dependence from steady-state inactivation to a hyperpolarizing direction. This inhibition was observed even in the presence of luzindole, an antagonist of melatonin receptors. Furthermore, K_V4.2 channel inhibition by 4-aminopyridine attenuated melatonin secretion induced by noradrenaline in rat pineal glands. These results suggest that melatonin directly inhibited K_V4.2 channels in rat pinealocytes, resulting in a decrease in melatonin secretion. This mechanism may function as a negative-feedback mechanism for melatonin secretion in rat pineal glands.

P-45

Pharmacologic inhibition of BMI1 activates the p53 pathway to suppress MYCN-amplified neuroblastoma

Masahiro HIRAYAMA (平山真大)[1], Eri YAMADA[1], Hiromasa AOKI[1], Kazuya IZUMI[1], Ayumi AMANO[1], Kohki TORIUCHI[1], Koichi OGAMI[2], Mai NAGASAKA[3], Yasumichi INOUE[3], Hidetoshi HAYASHI[3], Satoru TAKESHITA[1,4], Hiroki KAKITA[1,4], Yasumasa YAMADA[4], Mineyoshi AOYAMA[1]

[1]Department of Pathobiology, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Division of Molecular Oncology, Center for Neurological Diseases and Cancer, Graduate School of Medicine, Nagoya University; [3]Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University; [4]Department of Perinatal and Neonatal Medicine, Aichi Medical University

Neuroblastoma is one of the most common malignant tumors occurring in childhood, and effective therapeutic strategies have yet to be established. The amplification of the MYCN gene is associated with malignant transformation of neuroblastoma, but targeting MYCN directly is extremely challenging due to its complex regulatory mechanisms and disordered structure. BMI1 is a component of Polycomb Repressive Complexes 1 (PRC1) which epigenetically controls gene expression at the transcriptional level primarily through histone H2A Lys119 monoubiquitination. In neuroblastoma, MYCN directly binds to the promoter region of the BMI1 gene, positively controlling its expression. Currently, BMI1 inhibitors such as PTC-028, PTC-209, and PTC596 are under development, with PTC-028 showing anti-tumor effects in endometrial cancer and alveolar rhabdomyosarcoma. Therefore, we decided to investigate the efficacy of BMI1 inhibitors as a novel treatment for high-grade MYCN-amplified neuroblastoma.

We examined the effects of treatment with the BMI1 inhibitors PTC-028 and PTC-209 on MYCN-amplified or non-amplified neuroblastoma cell lines. As a result, BMI1 inhibitors strongly inhibited survival, especially of MYCN-amplified neuroblastoma. PTC-028, which exhibited toxicity at lower concentrations, induced apoptosis and cell cycle arrest due to accumulation of G1 phase and reduction of S phase cell populations in neuroblastoma cells.

To investigate the molecular mechanism by which the BMI1 inhibitor PTC-028 induces cell death in neuroblastoma, we performed a comprehensive gene expression analysis utilizing RNA sequencing. The results unveiled that treatment with PTC-028 activated the p53 signaling pathway. Indeed, PTC-028 enhanced p53 and p21 protein levels in neuroblastoma. Significantly, PTC-028 also showed antitumor effects in a mouse xenograft model of human neuroblastoma cells. These results suggest that BMI1 inhibitors, particularly PTC-028, may be potentially effective in the treatment of high-risk MYCN-amplified neuroblastoma.

P-46

Nanaomycin A exerts antitumor effect on neuroblastoma cells with DNA demethylation.

Kazuya IZUMI (泉 和弥)[1], Hiromasa AOKI[1], Kohki Toriuchi[1], Hiroki KAKITA[1,2], Satoru TAKESHITA[1,2], Hiroko UEDA[2], Yasumichi INOUE[3,4], Hidetoshi HAYASHI[3,4], Yasumasa YAMADA[2], Mineyoshi AOYAMA[1]

[1]Department of Pathobiology, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Perinatal and Neonatal Medicine, Aichi Medical University; [3]Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University; [4]Department of Innovative Therapeutic Sciences, Graduate School of Pharmaceutical Sciences, Nagoya City University

Neuroblastoma, one of the most common tumors in children, results from insufficient maturation of developing sympathetic nerves originating from the neural crest. Despite intensive multidisciplinary treatment, the current 5-year overall survival rate for children diagnosed at an advanced stage is <50%. Due to the initiation of treatment at a young age, the frequency of late complications is high. Thus, more effective treatments with fewer side effects are needed to improve the prognosis of neuroblastoma patients.

DNA methylation is a mechanism of repressive gene expression control and together with histone modification forms the epigenetic landscape. Three types of DNA methyltransferases (DNMTs) have been identified in humans: DNMT1, DNMT3A, and DNMT3B. DNMT1 is required to maintain DNA methylation patterns in genomic DNA during DNA replication; DNMT3A and DNMT3B are involved in *de novo* DNA methylation. Gene silencing via DNA methylation plays an important role during human development, and *de novo* DNA methylation catalyzed by DNMT3A and DNMT3B is essential for mammalian development. Gene silencing via DNA methylation also plays an important role in many cancers including neuroblastoma; for example, the suppression of tumor suppressor genes creates a favorable environment for tumor growth. Because genetic mutations tend to be less common in childhood cancers, epigenetic regulation via mechanisms such as DNA methylation could be more important in neuroblastoma than adult cancers.

Here, we demonstrated that nanaomycin A, a selective inhibitor of DNMT3B, decreased genomic DNA methylation levels and induced apoptosis in human neuroblastoma cells. Nanaomycin A also upregulated the expression of mRNAs for several genes related to neuronal maturation. These results suggest that nanaomycin A is an effective candidate therapeutic for treating neuroblastoma. Our findings also suggest that the inhibition of DNA methylation as a promising anti-tumor therapy strategy for neuroblastoma.

【Reference】Izumi, K. et al., Curr Cancer Drug Targets 2023;23(11):837-842. (PMID: 37221685)

P-47

Thrombopoietin Enhances Neuronal Cell Proliferation and Axonal Elongation in Intrauterine Growth Restricted Rats

Yuka SUZUKI (鈴木悠花)[1], Satoru TAKESHITA[1,2], Hiroki KAKITA[1,2], Nami NAKAMURA[2,3], Mari MORI[2], Kohki TORIUCHI[1], Hiromasa AOKI[1], Yasumichi INOUE[4,5], Hidetoshi HAYASHI[4,5], Yasumasa YAMADA[2], Mineyoshi AOYAMA[1]

[1]Department of Pathobiology, Nagoya City University Graduate School of Pharmaceutical Science

[2]Department of Perinatal and Neonatal Medicine, Aichi Medical University

[3]Department of Pediatrics, Aichi Medical University

[4]Department of Cell Signaling, Nagoya City University Graduate School of Pharmaceutical Sciences

[5]Department of Innovative Therapeutic Sciences, Cooperative Major in Nanopharmaceutical Sciences, Nagoya City University Graduate School of Pharmaceutical Sciences

Chronic hypoxia in utero causes intrauterine growth restriction (IUGR) in fetuses. IUGR infants are known to have a higher risk of neurodevelopmental disorders, but the mechanism is not elucidated. In this study, we analyzed the structure of the cerebral cortex by using an IUGR rat model produced with a reduced uterine perfusion pressure.

Compared to control rats, IUGR rats had thinner cerebral white matter and enlarged lateral ventricles. Expression of the neuronal markers Satb2, microtubule-associated protein (MAP)-2, α-tubulin, and nestin was downregulated in IUGR rats, indicating that neurons were reduced in IUGR rats at various developmental stages from neural stem cells to mature neurons. However, the number of apoptotic cells did not increase in the brains of IUGR rats. Ki67-positive cells, a marker of cell proliferation, were reduced in neurons and all glial cells of IUGR rats. In primary neuron cultures, axon elongation was inhibited under hypoxic culture conditions that mimicked the in-utero environment of IUGR infants. Thus, in IUGR rats, chronic hypoxia in utero inhibits neurons and glial cells proliferation and neuronal axon elongation, resulting in thinning of the cortex and enlarged lateral venous ventricles. The platelet growth factor thrombopoietin (TPO) suppressed the loss of neuron number and promoted axon elongation in primary neurons under hypoxic conditions. Intraperitoneal injection of TPO into IUGR rats resulted in increases in the number of NeuN-positive cells and the area coverage of Satb2.

Taken together, the inhibition of neuronal proliferation and axonal elongation in IUGR rats resulted in thinning of the cortex and enlargement of lateral ventricles. Treatment with TPO may represent a novel therapeutic strategy to treat brain dysfunction in IUGR infants.

P-48

Hypothermic culture attenuates neurotoxic activation of microglia via TRPV4 channel

Rina MIMOTO (三本里奈)[1], Naoya FUKUDA[1], Kohki TORIUCHI[1], Hiromasa AOKI[1], Hiroki KAKITA[1,2], Yoshiaki SUZUKI[3], Satoru TAKESHITA[1,2], Tetsuya TAMURA[4], Hisao YAMAMURA[3], Yasumichi INOUE[5,6], Hidetoshi HAYASHI[5,6], Yasumasa YAMADA[2], Mineyoshi AOYAMA[1]

[1]Department of Pathobiology, Nagoya City University Graduate School of Pharmaceutical Science; [2]Department of Perinatal and Neonatal Medicine, Aichi Medical University; [3]Department of Molecular and Cellular Pharmacology, Nagoya City University Graduate School of Pharmaceutical Sciences; [4]Department of Anesthesiology and Intensive Care Medicine, Nagoya City University Graduate School of Medical Sciences; [5]Department of Cell Signaling, Nagoya City University Graduate School of Pharmaceutical Sciences; [6]Department of Innovative Therapeutic Sciences, Cooperative Major in Nanopharmaceutical Sciences, Nagoya City University Graduate School of Pharmaceutical Sciences

Hypoxic-ischemic encephalopathy (HIE), which occurs following cardiac arrest or neonatal asphyxia, results in severe neurological deficits. Therapeutic hypothermia (TH) provides neuroprotection against HIE, however, the cellular mechanisms underlying the neuroprotective effects of TH are not fully elucidated. Transient receptor potential vanilloid 4 (TRPV4), a nonselective cation channel, is activated by temperature stimulus at 27–35 °C. Although it is speculated that TRPV4 is associated with the neuroprotective mechanisms of TH, the role of TRPV4 in the neuroprotective effects of TH is not well understood. Microglia, immune responsible cells in the brain, are closely related to neuroinflammation after acute brain injury. In the present study, we investigated whether hypothermia suppresses microglial activation via TRPV4 channel.

Microglia were cultured under normothermic (37°C) or hypothermic (33.5°C) conditions following lipopolysaccharide (LPS) stimulation. LPS stimulation activated AMP-activated protein kinase (AMPK)-NF-κB signaling, however, its activation was suppressed under hypothermic conditions. Moreover, TRPV4 inhibitor suppressed AMPK-NF-κB signaling and the expression of proinflammatory cytokines and inducible nitric oxide synthase. In contrast, TRPV4 agonist treatment under hypothermic culture counteracted the inhibitory effect of hypothermic culture. Furthermore, TRPV4 inhibitor suppressed phagocytosis of microglia to fluorescent beads.

These results suggest that therapeutic hypothermia inhibits neurotoxic activation of microglia via the TRPV4-AMPK-NF-κB pathway. Inhibition of TRPV4 may lead to a novel therapy that mimics TH.

P-49

The activity of Pinellia tuber raphides onto TRPA1 and the effect of ginger, organic acids

Itsuki NOSE (能瀬逸紀)[1], Taiki AMANO[2], Tsukasa FUEKI[1,3,4], Hisao YAMAMURA[2], Toshiaki MAKINO[1]

[1]Dept. of Pharmacognosy, and [2]Dept. of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University

[3]Department of Traditional Medicine, Toho University School of Medicine

[4]Matsuya Pharmacy

【Introduction】Pinellia tuber (the dried tuber of *Pinellia ternata*) is a crude drug used in traditional Chinese medicine (TCM) and Japanese Kampo medicine. The unprocessed Pinellia Tuber causes very strong acridity at oral and laryngopharynx mucosa when taken orally, which is known to disappear by heating and various detoxification methods. Needle-like crystals composed of calcium oxalate and protein, called raphides, are known to cause the acridity[1)]. We found that dried ginger (the rhizome of *Zingiber officinale*) extract (DG) reduces the content of *Pinellia ternata* lectin, denatures the Pinellia tuber raphides, and reduces the acridity. The active ingredients in DG extract were organic acids such as oxalic acid, malic acid, citric acid, tartaric acid[2–4)]. However, the mechanism of acridity generation and detoxification remains unclear. In this study, we investigated the activity of Pinellia tuber raphides onto transient receptor potential ankyrin-1 (TRPA1), a pain-related ion channel, and the effect of ginger, as well as organic acids.

【Method】 Pinellia tuber raphides were prepared by petroleum ether extraction (PEX) method[2)]. hTRPA1 stable expressing HEK293 cells were loaded with Fluo4-AM for 15 min, then transferred to a chamber containing extracellular fluid 2 ml. PEX raphides suspension (0.4 mg/ml, 2 ml) was added into the chamber, and the intracellular calcium change was monitored for 12 min by fluorescence of 525-555 nm excited with 488 nm laser, using confocal laser microscope (AR1; Nikon). For detoxification, PEX raphides were treated with DG or organic acids at 40°C for 90 min.

【Results and Discussion】Intracellular fluorescence increased by adding PEX raphides suspension, and this increase was inhibited by A-967079 (TRPA1 antagonist) pre-treatment, indicating that PEX raphides stimulated TRPA1. The TRPA1 agonistic activity of PEX raphides was significantly suppressed by the treatment with organic acids. DG treatment did not suppress the TRPA1 agonistic activity of PEX raphides because of its own TRPA1 agonistic activity.

【Conclusion】Activation of TRPA1 would be one of the mechanisms of Pinellia tuber raphides causing acridity, and the traditional detoxification methods of Pinellia tuber influence its TRPA1 agonistic activity.

【References】(1) Zhong L., et al. *Chin. J. Chin. Materia Medica*. 31, 1706–1710, 2006. (2) Fueki T., et al. *Acupunct. Herb Med*. 2, 33–40, 2022. (3) Liu Y., et al. *J. Nat. Med.* 77, 761–773, 2023. (4) Nose I., et al. 20th International Congress of Oriental Medicine, PS-06, Seoul, 2023.

P-50

TMEM55B promotes calcium induced calcium release between the endoplasmic reticulum and lysosomes

Nagi MUKAE (向江 凪), Keitaro YAMAMOTO, Wakana OKUDA, and Michiko SHIRANE
Department of Pharmacy, Nagoya City University

Membrane contact sites (MCSs) are intracellular microregions, in which different organelles are closely apposed to communicate with each other, play an important role in intracellular Ca^{2+} signalling. Calcium-induced calcium release (CICR) at the MCSs between the endoplasmic reticulum (ER) and endolysosomes (LE/Ly) has been recently focused on the intracellular Ca^{2+} signaling, but the molecular mechanism underlying that process remains unclear. CICR is a process in which Ca^{2+} release from lysosomes (Lys) is followed by enhanced Ca^{2+} release from the ER through ryanodine receptors and inositol triphosphate receptors. We identified TMEM55B as a component of the Protrudin-PDZD8 complex at the ER-LE/Ly MCSs in neuronal cells by proteomic analysis. We then showed that TMEM55B regulates V-ATPase, which is an ATP-driven lysosomal transmembrane proton pump (Hashimoto, ***Gene to Cells***, 2018). These results have led to the hypothesis that TMEM55B might be involved in the regulation of CICR at the ER LE/Ly-MCSs, so that we investigated that possibility in this study.

The human cervical carcinoma cell line HeLa cells were transfected with control siRNA or TMEM55B siRNA as well as cDNA coding G-CEPIA1er (Ca^{2+} indicator for lumenal side of the ER) or Ly-GG (Ca^{2+} indicator for outside Ly). Subsequently, the cells were stimulated with ATP to induce CICR, and time-lapse Ca^{2+} imaging was performed by confocal live cell fluorescence imaging analyser. As a result, Ca^{2+} release from Lys as well as from the ER was significantly decreased in TMEM55B-depleted cells compaired to control cells. These results indicate that the Ca^{2+} signaling through CICR was reduced in TMEM55B-depleted cells.

In conclusion, this study has suggested that TMEM55B plays an important role in promoting CICR. As CICR is supposed to be associated with various pathophysiological processes, such as myocardial contraction, T-cell activation, and neuronal excitation, our results may potentially inform the development of therapeutic strategies for these conditions. Further studies are needed to elucidate the mechanism underlying the enhanced CICR by TMEM55B.

P-51

The mechanism of inflammation related to Alzheimer's disease in the brain of PDZD8-deficient mice

Honoka MAKI (眞木穂香), Mariko WADA, Hikari KITANO, Yumi MORISUGI, and Michiko SHIRANE

Department of Molecular Biology, Graduated School of Pharmaceutical Sciences, Nagoya City University

As risk factors for Alzheimer's disease (AD), lipoprotein regulator APOE4 and inflammation regulator TREM2 have been identified. Furthermore, accumulation of cholesteryl esters (CEs) has been reported in the brains of ApoE knockout (KO) or TREM2-KO mice as well as in AD patient-mimic iPSC-derived neurons. Thus, abnormal lipid metabolism is strongly suggested in the brain of AD.

We have identified a lipid transfer protein PDZD8 in the protein complex of ER-endosome membrane contact sites (Shirane, et al, ***Nat Commun***, 2020). We then performed lipidome analysis of PDZD8-KO mice and found abnormal accumulation of CEs in the brain (Morita, et al. ***iScience***, 2022). We also performed a behavioral battery analysis of PDZD8-KO mice and found abnormalities in brain functions such as cognitive function and emotional control (Kurihara, et al, ***Mol Brain***, 2023). Thus, PDZD8-KO mice exhibit phenotype mimic to AD in the CE accumulation in the brain and brain dysfunction.

Increased inflammation is one of the pathologies of AD, and its underlying mechanism has been suggested to be microglial activation due to the accumulation of amyloid-β (Aβ). However, the associated mechanism between dyslipidemia and inflammation in the brain of AD has not been well understood. In this study, we have focused on the mechanism of inflammation caused by CE accumulation in the brain using PDZD8-KO mice to elucidate AD pathophysiology.

First, we induced inflammation in wild-type (WT) and PDZD8-KO mice by intraperitoneal administration of lipopolysaccharide (LPS), and performed the RNA-seq analysis of the brain to extract genes with aberrant expression. As a results, several genes associated with AD and/or inflammation were identified to be significantly different in KO mice. The reproducibility of RNA-seq results was then confirmed by qRT-PCR analysis to select genes whose expression was reliably abnormal in KO mice. Furthermore, we observed microglia in the brain by immunohistochemistry (IHC) and found accumulation of microglia in specific regions in the brain.

In conclusion, we found enhanced inflammatory responses in the brain of PDZD8-KO mice and identified a set of its associated genes. These results suggest that PDZD8 plays a role in the suppression of inflammation in the brain.

The causative mechanism underlying the inflammation triggered by the accumulation of CEs as well as the abnormal gene expression in the brain of PDZD8-KO mice need to be further investigation to elucidate the pathophysiology of AD.

P-52

Elucidation of molecular mechanisms involved in proteasome inhibitor resistance in multiple myeloma

Airi NAKAGAWA (中川愛理), Shogo YAMANAKA, Chiharu MIYAJIMA, Hodetoshi HAYASHI, Yasumichi INOUE

Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University

【Objective】Multiple myeloma is a refractory malignancy of antibody-producing plasma cells in the bone marrow, causing renal failure, osteolysis, and a variety of other symptoms. Currently, proteasome inhibitors (PIs) are the mainstay of therapy, and although PIs have been shown to be highly effective, the development of resistance to PIs in myeloma cells is a major cause of poor prognosis. The mechanism by which PI resistance occurs has not been fully elucidated; however, it has been suggested that the integrated stress response (ISR), which acts to maintain cellular homeostasis, is involved. It was hypothesized that the ISR may enable multiple myeloma cells to survive by reducing the stress caused by PI. In this study, we aimed to elucidate the molecular mechanisms involved in PI resistance and to establish novel therapeutic strategies for multiple myeloma by combining PI and ISR inhibitors.

【Results】In multiple myeloma cells, treatment with bortezomib (BTZ), one of the PIs, activated the ISR kinases HRI, PERK, and GCN2. Therefore, we combined BTZ with ISRIB, an ISR inhibitor, and measured cell death using flow cytometry, and found that ISRIB enhanced BTZ-induced cell death. We also confirmed the cleavage of caspase 3 by the combination of BTZ and ISRIB. In addition, the combined use of ISRIB reduced the expression of the stress response transcription factor ATF4 downstream of the ISR induced by BTZ.

【Discussion】We revealed that inhibition of the BTZ-induced ISR can enhance the BTZ sensitivity of multiple myeloma cells and strengthen the anti-tumor effects of BTZ. Mechanitically, ISRIB reduced expression of ATF4, which acts in a cell-protective manner in the ISR. We believe that this study will help to elucidate the molecular mechanisms involved in PI resistance in multiple myeloma and will lead to the establishment of combination therapy with PI and ISR inhibitors.

P-53

Mechanism of induction of stress-responsive transcription factor ATF4 through HRI activation by compounds from Myanmar plant

Shogo YAMANAKA (山中翔悟)[1], Kotaro HIRAMARU[1], Yuya MIZUNO[1], Chiharu MIYAJIMA[1], Kan'ichiro ISHIUCHI[2], Toshiaki MAKINO[2], Michiyo MATSUNO[3], Hajime MIZUKAMI[3], Yasumichi INOUE[1], Hidetoshi HAYASHI[1]

[1]Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Nagoya City University; [3]The Kochi Prefectural Makino Botanical Garden

【Objective】

The integrated stress response (ISR) is triggered by phosphorylation of eIF2α (eukaryotic initiation factor 2α) by various stresses and reduces cellular stress by suppressing cap-dependent mRNA translation. On the other hand, translation of the transcription factor ATF4 (activating transcription factor 4) is rather promoted during ISR, reducing stress by inducing expression of ATF4 target genes. Under severe stress, however, ATF4 has a role in activating the transcription of apoptosis-inducing genes and eliminating abnormal cells. Some cancer cells can utilize ISR to enable cell survival under stressful conditions due to unregulated proliferation. Therefore, we hypothesized that further induction of ATF4 expression could selectively induce cell death in cancer cells with constitutively active ISR.

The myanmar plant had been collected under MTA between The Ministry of Natural Resources and Environmental Conservation of the Republic of the Union of Myanmar and The Kochi prefectural Makino Botanical Garden. Using a Myanmar plant extract library, we isolated and purified compound X, which induces ATF4, from *Ellipeiopsis cherrevensis* stem extract. The aim of this study was to elucidate the molecular mechanism by which compound X induces ATF4 and to apply it to ISR-related diseases such as cancer.

【Methods and Results】

In human prostate cancer PC3 cells, knockdown of each of the four eIF2α kinases attenuated the induction of ATF4 and its target gene TRB3 (tribbles homolog 3) by compound X treatment only when HRI (heme-regulated inhibitor of translation) was knockdowned. HRI is known to be activated by heme deficiency and mitochondrial stress. Therefore, we pretreated PC3 cells with hemin, a type of heme iron, and treated them with compound X under heme iron-rich conditions, but the induction of ATF4 and TRB3 was hardly affected. On the other hand, HRI knockdown suppressed the induction of ATF4 and TRB3 by the mitochondrial depolarizing agent CCCP (carbonyl cyanide 3-chlorophenylhydrazone) in PC3 cells similar to that of compound X. These results suggest that activation of HRI is required for induction of ATF4 by compound X, and that mitochondrial stress may contribute to this activation.

P-54

The deubiquitinating enzyme USP2 stabilizes TAZ to mediate cancer cell proliferation

Takuto FUJIWARA(藤原巧斗), Chiharu MIYAJIMA, Yasumichi INOUE, Hidetoshi HAYASHI

Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University

Transcriptional co-activator with PDZ-binding motif (TAZ) is transcription mediator in the Hippo pathway and regulates cell proliferation as a co-factor of transcription factors. It has been reported that TAZ is highly expressed in various human cancers, including breast and colorectal cancers, and that abnormal activation of TAZ contributes to the uncontrolled growth of cancer cells. Although stabilization of TAZ protein has been suggested to be responsible for the high expression of TAZ in cancer cells, the mechanism is not yet well understood. We hypothesized that the abnormal stabilization of TAZ protein in cancer cells involves a dysregulation of ubiquitination and identified ubiquitin-specific protease 2 (USP2) as a deubiquitinating enzyme that targets TAZ protein. In this study, we investigated the mechanism of TAZ protein stabilization by USP2 and its effect on cancer progression.

To analyze whether USP2 is involved in TAZ protein stabilization, TAZ and USP2 were co-expressed in COS7 cells. The results showed that TAZ protein was stabilized in a manner dependent on USP2 expression and its enzymatic activity. The binding of TAZ to USP2 was examined using immunoprecipitation and found to form a complex. We also investigated whether USP2 contributes to the deubiquitination of TAZ, and found that the ubiquitination of TAZ is reduced in an enzyme activity-dependent manner. Then, we examined the contribution of USP2 to the regulation of endogenous TAZ stabilization. In human breast cancer MCF7 cells and human osteosarcoma U2OS cells, USP2 knockdown induced reduced TAZ protein levels and decreased expression of target genes downstream of TAZ. As it was suggested that USP2 regulates TAZ stabilization, its physiological effects on cancer cells were investigated. Indeed, knockdown of USP2 resulted in reduced cancer cell proliferation.

Collectively, these results suggested that USP2 regulates cancer cell proliferation via stabilization of TAZ protein.

P-55

ID3 is a target gene of p53 and modulates lung cancer cell metastasis

Sakura HASHIGUCHI (橋口咲良)[1], Mai NAGASAKA[1], Chiharu MIYAJIMA[1], Hiromasa AOKI[2], Kohki TORIUCHI[2], Mineyoshi AOYAMA[2], Hidetoshi HAYASHI[1], Yasumichi INOUE[1]

[1]Department of Cell Signaling, Graduate School of Pharmaceutical Sciences, Nagoya City University; [2]Department of Pathobiology, Graduate School of Pharmaceutical Sciences, Nagoya City University

The tumor suppressor gene *TP53* plays a central role in tumor suppression, but genetic mutations are found in more than half of all human tumors. p53 functions mainly as a transcription factor and regulates the transcription of target genes, thereby performing a variety of biological functions such as cell cycle arrest. The multifunctionality of p53 is attributed to the diversity of its target genes, and many p53 target genes have been identified to date. However, complex p53 functions have not yet been fully elucidated. In this study, we searched for novel p53 target genes by RNA-seq aimed at further elucidating the physiological functions of p53. Here, we identified *inhibitor of DNA-binding/differentiation 3* (*ID3*) as a p53 target gene.

We reanalyzed public ChIP-seq data in the sequence read database SRA. We identified a p53-responsive element (*ID3*-p53RE) approximately 33 kb upstream of the transcription start site of *ID3*. We examined chromatin immunoprecipitation, and confirmed that indeed p53 does bind. Reporter assays using *ID3*-p53RE showed increased transcriptional activation by p53. Furthermore, deleting the *ID3*-p53RE by CRISPR in MCF7 cells attenuated the induction of ID3 expression by Nutlin-3 treatment, it indicates that *ID3* is a novel p53 target gene.

Knockdown of ID3 in the human lung cancer cell line A549 did not affect A549 cell proliferation, but decreased expression of the epithelial marker E-cadherin and increased cell migration ability. In addition, knockdown of ID3 significantly increased the number of lung metastatic nodules formed in a mouse lung metastasis model. A long-term prognostic analysis in cohorts of lung cancer patients showed that overall survival outcomes were less favorable in the group with lower ID3 expression. These results indicate that ID3 contributes to the suppression of lung cancer metastasis, and is expected to be a useful predictor of prognosis.

P-56

Physiological roles of nicotinic acetylcholine receptors in human pulmonary arterial smooth muscle cells.

Koya NAKAHAMA (中浜光哉)[1], Aya YAMAMURA[2], Rubii KONDO[1],
Yoshiaki SUZUKI[1], Hisao YAMAMURA[1]

[1]Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University

[2]Department of Physiology, Aichi Medical University

In pulmonary arterial smooth muscle cells (PASMCs), the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) is mainly regulated by the activity of ion channels. Physiological elevation of $[Ca^{2+}]_{cyt}$ is involved in many cellular functions, such as contraction, proliferation, migration, differentiation, and apoptosis. However, excessive $[Ca^{2+}]_{cyt}$ elevation due to the upregulation of Ca^{2+}-permeable channel expression induces pulmonary vasoconstriction and vascular remodeling, resulting in the development of pulmonary arterial hypertension. Limited information is currently available on the physiological role of nicotinic acetylcholine receptors (nAChRs) in PASMCs. Quantitative real-time PCR analysis revealed that the α5 and α9 subunits of nAChR were expressed at the mRNA levels in human PASMCs. The treatment with nicotine (≧100 μM) increased $[Ca^{2+}]_{cyt}$ in a concentration-dependent manner in PASMCs loaded with Ca^{2+}-sensitive fluorescent indicator, fluo4-AM. Under whole-cell patch-clamp mode, nicotine evoked inward currents in PASMCs and HEK293 cells transfected with the α5 subunits. The nicotine-induced $[Ca^{2+}]_{cyt}$ increase was inhibited by the nAChR antagonist, hexamethonium (1 mM). Taken together, nicotine activates nAChRα5 channels and promotes Ca^{2+} signaling in PASMCs.

P-57

Exploration of Novel CaMKK2 Inhibitors Using a FRET-based Assay System

Haruya TANABE (田邊晴也)[1], Yoshiaki SUZUKI[1], Itsuki OKADA[1], Nanaho MICHIGAMI[1], Takashi MURAYAMA[2], Rubii KONDO[1], Hisao YAMAMURA[1]

[1]Department of Pharmaceutical Sciences, Nagoya City University

[2]Department of Medicine, Juntendo University

Background: Calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) is a kinase expressed in various tissues, including the brain, adipose tissue, and blood vessels. It phosphorylates CaMK1, CaMK4, and AMPK. CaMKK2 is involved in a variety of physiological functions, such as memory formation, gluconeogenesis, and vascular remodeling. In addition, CaMKK2 has been reported as a contributing or exacerbating factor in various diseases, including atherosclerosis, diabetes, and cancer. Therefore, the development of specific inhibitors for CaMKK2 is promising as new therapeutic agents for these diseases. However, drugs that specifically inhibit CaMKK2 are still scarce, and there are no methods to measure CaMKK2 activity in live cells. In this study, we developed a method to measure CaMKK2 activity using AMPK AR-EV, a FRET probe of AMPK, that generates FRET upon activation by AMPK, a downstream molecule of CaMKK2.

Objective: The objective was to establish an assay system for CaMKK2 activity using AMPK-AR-EV and to discover novel CaMKK2 inhibitors using a compound library.

Results: The human CaMKK2 and AMPK AR-EV genes were introduced into HEK293A cells using baculovirus vectors. These cells were seeded on 96-well plates, and 2-deoxy-D-glucose was added to activate the CaMKK2/AMPK pathway and induce FRET via AMPK AR-EV. The FRET efficiency was measured before and 20 minutes after the addition of compounds. First, the validity of the assay system was verified using a well-known CaMKK2 inhibitor (ALK-IN-1), resulting in a Z' value of 0.69, indicating the assay system was appropriate. Next, a screening of 400 compounds was conducted, resulting in finding for five hit compounds. Reproducibility of the effects of these compounds was confirmed, showing concentration-dependent inhibitory effects in three of the five hit compounds.

Discussion: The assay system developed in this study suggests its utility as a screening method for CaMKK2 inhibitors. Further studies are needed to verify the specificity and potency of the hit compounds.

P-58

Paxilline blocks calcium-activated chloride TMEM16A channels

Taiga Sekine (関根大雅)[1], Rubii Kondo[1], Akane Suzukawa[1], Yoshiaki Suzuki[1], Aya Yamamura[2], Hisao Yamamura[1]

[1]Department of Molecular and Cellular Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University

[2]Department of Physiology, Aichi Medical University

［Background］Pulmonary arterial hypertension (PAH) is a progressive and fatal disease characterized by vasoconstriction and remodeling of the pulmonary artery. Narrowing of the pulmonary arteries induces right ventricular hypertrophy and eventually right heart failure. Despite the recent development of specific PAH drugs, which has increased the five-year survival rate of PAH after its diagnosis to approximately 70%, PAH remains incurable and still has a poor prognosis. Calcium-activated chloride (Cl_{Ca}) channel is predominantly composed of the TMEM16A protein and plays important roles in various physiological functions. Interestingly, it has been reported that the expression of TMEM16A channels upregulated in pulmonary artery smooth muscle cells (PASMCs) from PAH patients. Therefore, the inhibition of TMEM16A-mediated Cl_{Ca} channels is important for the drug development for PAH. In the present study, the effects of paxilline, which is widely used as a blocker of large-conductance calcium-activated potassium (BK_{Ca}) channels, on TMEM16A-mediated Cl_{Ca} channels were examined using human TMEM16A-expressing HEK293 cells and PASMCs.

［Method］Cells used in this study were HEK293 cells stably expressing TMEM16A and PASMCs. For functional analysis of TMEM16A channels, whole-cell patch clamp configuration was used. Cell viability was assessed using WST assay.

［Results］In HEK293 cells stably expressing TMEM16A, a slowly activating outward current in response to depolarization and an inward tail current during repolarization were observed under whole-cell patch clamp configuration. The application of paxilline (BK_{Ca} channel blocker) blocked TMEM16A-mediated Cl_{Ca} currents in a concentration-dependent manner. On the other hand, other BK_{Ca} channel modulators (tamoxifen and 17β-estradiol) did not affect the activity of TMEM16A channels. Furthermore, PASMCs from PAH patients exhibit excessive cell proliferation compared to healthy individuals, and TMEM16A channels are known to be involved in cell proliferation. Therefore, cell viability assay was performed to investigate whether paxilline inhibit the proliferation of PASMCs from PAH patients. The treatment with paxilline attenuated the excessive proliferation of PASMCs from PAH patients.

［Conclusion］Paxilline (BK_{Ca} channel blocker) inhibits TMEM16A-mediated Cl_{Ca} currents. This is useful finding for the drug development targeting on TMEM16A-mediated Cl_{Ca} channels.

P-59

Ameliorative effects of sweetening agents on jet lag

Jiayi LI（李佳憶）, Tianxiang GAO, Kazuhiko KUME

Department of Neuropharmacology, Nagoya City University

Jet lag is a condition that causes physical abnormalities due to the shift between the biological clock and the environmental time. When traveling rapidly by airplane to an area with a time difference of several hours or more, the biological clock, which entrained to the time of departure place, is greatly shifted from that of arrival place, resulting in jet lag. It occurs typical symptoms include malaise during daytime, insomnia, chronic fatigue, and headaches. Previous research has shown that food intake has an effect on recovery from jet lag. In addition, a previous study in our laboratory using drosophila showed that sleep is decreased in a fasted state and sweetening agents, such as sucrose, or even non-nutritive sweetening agents such as sucralose, which is known as an artificial sweetener, have an effect on increasing sleep. However, similar effects have not yet been examined in mammals.

In this study, we examined a 6-hour phase advanced jet lag model using mice. Acceleration and temperature sensors (nanotag) were implanted in the abdominal cavity of each mice to enable continuous measurement of activity and temperature changes. The mice were also given a running wheel that could measure the count of rotations, and acclimated to active running during the activity phase (light off phase).

We also quantified rest time using our own definition. In this model, when sucrose or sucralose, an artificial sweetener without nutrition, was given in addition to normal nutrition, the ameliorative effect from jet lag was observed. Therefore, we are seeking to solutions from jet lag by the food intake and conditions that improve sleep disorders.

P-60

Enhanced Sleep in *Drosophila melanogaster* under the Presence of Predators

Haruki KATO (加藤遥輝)[1], Namiki SHIMA[1,2], Yoshinori SUZUKI[1], Jun TOMITA[1], Kazuhiko KUME[1]

[1]Department of Neuropharmacology, Nagoya City University

[2]Department of Informatics, Nagoya University

Sleep is considered as a disadvantage for survival due to immobility with reduced reactivity to external stimuli, which increase the risk of predation and potential disruption of reproductive chances. Various proactive roles such as memory consolidation have been reported in response to the question "Why do we sleep?".

However, recent research has revealed the preservation of sleep even in species lacking a central brain such as hydra (Kanaya et al., 2020) or jelly fish (Nath et al., 2017), suggesting physiological significance for sleep beyond advanced brain functions. We hypothesized that a state of quiescence is an adaptive behavior for small organisms during periods not dedicated to activities like feeding and reproductive behavior, and immobility itself constitutes a part of the functions of sleep. While proposed as the "immobility hypothesis" (Meddis et al., 1975), this hypothesis has seen little empirical experimentation to date.

The aim of this study is to uncover the role of immobility during sleep in *Drosophila melanogaster* using jumping spider, *Hasarius adansoni*, a natural predator of the fruit fly.

Measurements were conducted using the Drosophila activity monitor (DAM, Trikinetics). In light and dark condition, flies exposed to the spider increased sleep at dusk. Notably, these responses were not observed when we presented flies or objects of similar size instead of the spider. These results suggest that flies visually recognize the spider and increased sleep. Additionally, short sleep mutant, *fmn* increased sleep during not only daytime but also nighttime. Furthermore, recently we found that similar phenotype was also observed by spider smell. These data indicate that flies do not simply response the spider movement but recognize the predator cue and increase its immobility.

Besides, to explore the impact of the sleep-wake states of flies on their susceptibility to predation, we present spiders with flies in which mobility or immobility is optogenetically induced, recording changes in predatory behavior.

P-61

Regulation of sleep by *dAWP1*, a homolog of the anesthetic sensitivity gene *AWP1*

Ryo FURUKAWA（古川稜）[1)],Taro UENO [2)], Yusaku MORI [1)], Jun TOMITA [1)], Shoen KUME [3)], Kazuhiko KUME [1)]

[1)] Department of Neuropharmacology, Nagoya City University

[2)] Toho University

[3)] Tokyo Institute of Technology

Sleep is a physiological state involving a reversible loss of consciousness, while general anesthesia is a drug-induced reversible state involving unconsciousness. General anesthesia has been used in surgery since 1842, but its mechanism is still unknown. A new body of evidence indicates that sleep and general anesthesia share not only behavioral characteristics but also neural circuits and molecular mechanisms. In a previous study, *dAWP1* was discovered as a Drosophila orthologue of the anesthetic-sensitive mammalian gene *AWP1*. Using Drosophila melanogaster as a model for sleep studies, we found that both pan-neuronal and pan-glial knockdown of *dAWP1* resulted in a reduced sleep phenotype. Knockdown of *dAWP1* in neurons resulted in hypersensitivity to sevoflurane. Knockdown screening of *dAWP1* in specific glial cells also resulted in decreased sleep in specific glial species. Thus, *dAWP1* is a novel sleep-regulating gene that functions in both neurons and glia. These results suggest that similar mechanisms for anesthesia and sleep are conserved in Drosophila and that specific glial cell types are involved in sleep.

P-62

Identification of Novel HDAC6-selective Inhibitor as Potential Treatment of Triple-negative Breast Cancer through Induction of PANoptosis

Siyuan WANG (王思远), Meidi LUO, Dan LIU

Department of Medicinal Chemistry, Shenyang Pharmaceutical University

HDAC6-selective inhibitor ACY-1215 is being evaluated in phase I clinical trial as a potential treatment against triple-negative breast cancer (TNBC) [1]; However, the suboptimal on-target activity and promiscuity of ACY-1215 may weaken its clinical efficacy and give rise to potential toxic side effects.

PANoptosis, a newly identified form of cell death that integrates features of apoptosis, pyroptosis, and necroptosis, presents several distinctive advantages over conventional chemotherapy. PANoptosis may trigger inflammatory responses and immune system activation, thereafter potentiating immune cell recognition and decreasing cancer cells proliferation. Additionally, PANoptosis holds promise in circumventing resistance mechanisms encountered with therapies targeting single cell death pathways, as concurrent targeting of multiple death routes diminishes opportunities for tumor cell evasion [2,3].

Recently, our research has identified a highly active and selective HDAC6 inhibitor. Through cell proliferation assays on (non-)sensitive breast cancer cell lines, compound **B6** demonstrated improved anti-proliferative effects and specificity over ACY-1215. Phenotypic experiments using YO-PRO-1/PI staining revealed that compound **B6** effectively induces PANoptosis. In xenograft models, **B6** caused significant antitumor efficacy, with a tumor growth inhibition rate (TGI) of 82.18% at a dose of 40 mg/kg. Immunohistochemical analyses of the anatomical tumoral tissue further validated the strong PANoptosis-inducing ability of **B6**.

Collectively, our study provided a novel drug candidate for TNBC, but also unmasked the mode of action of HDAC6 inhibitor in inducing PANoptosis in TNBC.

[1] Zeleke TZ, Pan Q, Chiuzan C, et al, Network-based assessment of HDAC6 activity predicts preclinical and clinical responses to the HDAC6 inhibitor ricolinostat in breast cancer, Nature Cancer. 4 (2023) 257-275.

[2] Zheng M, Karki R, Vogel P, et al, Caspase-6 Is a key regulator of innate immunity, inflammasome activation, and host defense, Cell. 181 (2020) 674-687.

[3] Lee S, Karki R, Wang Y, et al, AIM2 forms a complex with pyrin and ZBP1 to drive PANoptosis and host defence, Nature. 597 (2021) 415-419.

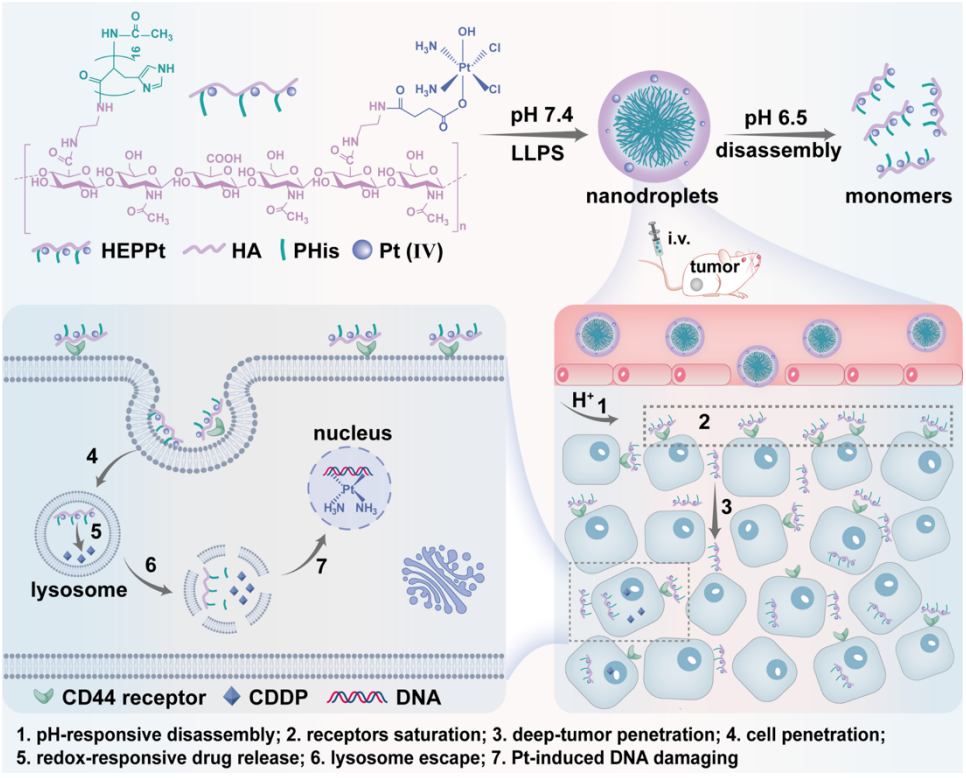
P-63

LLPS-inspired engineering of phage-separating Pt(IV)-graft-glycopeptides sequentially sensing pH and redox for deep penetration and targeting chemotherapy of breast cancer

Dali CHEN (陈大力)[1], Yunai DU, Jiasheng TU*, Chunmeng SUN*

NMPA Key Laboratory for Research and Evaluation of Pharmaceutical Preparations and Excipients, and Department of Pharmaceutics, School of Pharmacy, China Pharmaceutical University

Active-targeting nanomedicines have been widely employed in cancer treatment for increasing therapeutic index. However, the limited permeability caused by the binding site barrier (BSB) and size hindrances compromises their clinical anti-tumor efficacy in patients. Herein, learning from the liquid-liquid phase separation (LLPS) of bio-macromolecules, we report tumor-penetrating glycopeptides (HEP) from polyhistidine (PHis) grafted hyaluronic acid (HA) that sense the tumor extracellular pH by LLPS that concomitantly overcome size and BSB dilemmas for enhanced tumor penetration. HEP is aggregated into nanodroplets in solutions at neutral pH, while once reaching into tumor extracellular acidic environment, the pH-responsive PHis triggers the phage transition of the coacervates nanodroplets into monomeric glycopeptides. This makes glycopeptides conjugated with platinum prodrug (HEPPt) deeply penetrating into tumors due to tackling the BSB effect of the nanodroplets-CD44 interaction and the decreased size. Moreover, HEPPt in monomeric states displays promoted cellular uptake after pH triggered phage separation. Subsequently, the continuously redox-activated release of Pt(II) improves the anti-tumor effects. The phase-separating glycopeptides represent a promising platform for increasing the tumor penetration and the intracellular delivery of therapeutic agents.



P-64

Does long-term care insurance increase social participation among the younger elderly? Quasi-experimental evidence from China

Yifan Chen (陈一凡)[1], Xia Hu2[2]

[1]School of International Pharmaceutical Business, China Pharmaceutical University

[2]School of International Pharmaceutical Business, China Pharmaceutical University

Background: Enhancing social participation (SP) among the elderly and addressing the long-term care (LTC) needs of disabled older adults are two hot issues in dealing with population aging. Since the implementation of Long-Term Care Insurance (LTCI) pilot policy in China, it has been proven that it has solved the care dilemma of the disabled elderly in the pilot areas to a certain extent. However, little is known about the positive effects of this policy on the SP of the younger elderly.

Methods: Utilizing the China Health and Retirement Longitudinal Study (CHARLS) database from 2011 to 2020 across five waves, we treat the LTCI pilot policy as a quasi-experiment. Employing difference-in-differences (DID) and triple-differences (DDD) methods, along with a series of robustness tests, this study investigates the impact of LTCI on enhancing SP among the younger elderly individuals and explores its underlying mechanisms.

Results: We find that LTCI significantly increases an amount of time spent in SP among the younger elderly population. Heterogeneity analysis indicates that LTCI enhances the informal SP of the elderly at an early age. The increase in SP time is more pronounced among males, younger individuals, those with chronic diseases, and those with higher levels of education. This promotional effect is more pronounced in situations where single-service payment is the primary mode of reimbursement and the coverage extends to a wider range of insured individuals. Mediation analysis indicates that the LTCI pilot policy has a positive impact on the SP of younger elderly individuals by enhancing their self-rated health.

Conclusion: LTCI has played a positive role in promoting the SP of the younger elderly individuals. China should further advance LTCI program, optimize the payment method of care services, expand medical insurance coverage, and achieve nationwide promotion of LTCI at the earliest. Additionally, LTCI could explore collaborative models for mutual assistance in elderly care among the younger elderly individuals, establish community caregiving service teams, and enhance the caregiving service supply capacity.

P-65

Scutellarein protects angaint cardiac hypertrophy though peroxiredoxin 3 mediated mitochondrial quality control in heart failure

Xiaobing LIN (蔺小兵), Wen GAO*

State Key Laboratory of Natural Medicines, School of Traditional Chinese Pharmacy, China Pharmaceutical University

The mitochondria-specific peroxidase Peroxiredoxin 3 (PRDX3) plays a protective role against mitochondrial dysfunction by removing mitochondrial reactive oxygen species. Accumulating evidence has recently revealed mitochondrial quality control mediated by PRDX3 plays a pivotal effect on cardiac hypertrophy and heart failure (HF). Accordingly, the discovery of PRDX3 activators and elucidating their underlying mechanisms of HF should be urgently needed. Herein, we identified that scutellarein targetly binds to PRDX3, and promoted its activity through inhibiting the K254 acetylation. Scutellarein was shown to alleviate TAC-induced cardiac hypertrophy and myocardial cardiac dysfunction *in vivo* mice models. And scutellarein can suppress cardiac hypertrophy and myocardial fibrosis through regulating mitochondrial dysfunction in ISO-induced cardiomyocytes. Importantly, Prdx3 knockdown inhibit the effect of scutellarein against cardiac hypertrophy and mitochondrial dysfunction *in vitro*. Mechanically, scutellarein or over-expression of PRDX3 can promote mitochondrial dynamics and PINK-1-mediated mitophagy to improve cardiac hypertrophy and mitochondrial dysfunction *in vitro*. Furthermore, the acetylation of PRDX3 can promote mitochondrial dysfunction, and the deacetylation of PRDX3 can improve mitochondrial injury through regulating mitochondrial dynamics and mitophagy in ISO-induced cardiomyocytes. Collectively, these results suggest that the deacetylation of PRDX3 plays a role in regulatingcardiac hypertrophy through mitochondrial quality, and inhibition of its acetylation by scutellarein could be a promising treatment for HF.

P-67

A novel anti-inflammatory oxaliplatin (IV) prodrug nanomedicine to enhance colorectal cancer therapy

Lei Xing (邢磊)[1], Wen-Jia Wang[2], Hu-Lin Jiang[3]

State Key Laboratory of Natural Medicines, China Pharmaceutical University

Background and Purpose. Colorectal cancer (CRC) is the third most common malignancy and the third most common cause of cancer-related death worldwide. Oxaliplatin (Oxa) is the first-line chemotherapeutic drug for the treatment of colorectal cancer (CRC). However, long-term Oxa chemotherapy can induce inflammation and increase the levels of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2), which can promote tumor metastasis. Moreover, high glutathione (GSH) levels in CRC cells significantly reduce Oxa sensitivity and seriously restrict the clinical application of Oxa. Here, we constructed a GSH-depleted cyclodextrin pseudo-polyrotaxane nano-system to deliver anti-inflammatory Oxa (IV) prodrug for CRC treatment.

Method. The DN-Pt (IV) prodrug was obtained by oxidation and esterification. And we assembled α-cyclodextrin-itaconic anhydride (α-CD-IAn) and methoxy poly(ethylene glycol)-poly(L-lactide-co-glycolide) (mPEG-PLGA) into pseudo-polyrotaxane (PPRI) by host – guest interaction. The anti-inflammatory Oxa (IV) prodrug (DN-Pt)) was loaded into the PPRI nano-system by nanoprecipitation to form the final preparation DNPt@PPRI. In vitro and in vivo anti-tumor and anti-metastasis of DNPt@PPRI were evaluated in CT26 cells and tumor-bearing mice, respectively.

Results. The relesae of DN from DNPt@PPRI can reduce the level of PGE2 to inhibit inflammation and tumor metastasis by decreasing COX-2 protein, and also synergize with oxaliplatin to inhibit tumor. More importantly, GSH depletion can reduce the detoxification of Oxa and further enhance chemotherapy-induced apoptosis.

Conclusion. We have rationally designed a GSH-depleting cyclodextrin pseudo-polyrotaxane nano-system, which not only overcomes the inflammatory defect induced by Oxa chemotherapy and plays the dual anti-tumor effect of Oxa and COX-2 inhibitor, but also overcomes the limitation of easy detoxification of Oxa.

Keywords: oxaliplatin, glutathione depletion, tetravalent platinum prodrug, synergistic therapy, colorectal cancer

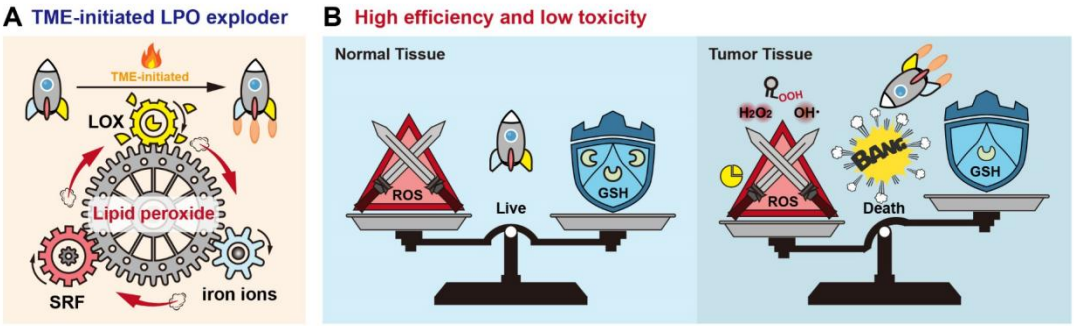
P-68

Tumor microenvironment-initiated lipid redox cycling for efficient triple-negative breast cancer therapy

Tian-Jiao Zhou (周天娇)[1], Hu-Lin Jiang[2]

State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University, Nanjing 210009, China

The use of overwhelming reactive oxygen species (ROS) attack has shown great potential for treating aggressive malignancies; however, targeting this process for further applications is greatly hindered by inefficiency and low selectivity. Here, a novel strategy for ROS explosion induced by tumor microenvironment-initiated lipid redox cycling was proposed, which was developed by using soybean phosphatidylcholine (SPC) to encapsulate lactate oxidase (LOX) and sorafenib (SRF) self-assembled nanoparticles (NPs), named LOX/SRF@Lip. SPC is not only the delivery carrier but an unsaturated lipid supplement for ROS explosion. And LOX catalyzes excessive intratumoral lactate to promote the accumulation of large amounts of H_2O_2. Then, H_2O_2 reacts with excessive endogenous iron ions to generate amounts of hydroxyl radical for the initiation of SPC peroxidation. Once started, the reaction will proceed via propagation to form new lipid peroxides (LPO), resulting to devastating LPO explosion and widespread oxidative damage in tumor cells. Furthermore, SRF makes contribution to mass LPO accumulation by inhibiting LPO elimination. Compared to normal tissue, tumor tissue has higher levels of lactate and iron ions. Therefore, LOX/SRF@Lip shows low toxicity in normal tissues, but generates efficient inhibition on tumor proliferation and metastasis, enabling excellent and safe tumor-specific therapy. This work offers new ideas on how to magnify anticancer effect of ROS through rational nanosystem design and tumor-specific microenvironment utilization.



P-69

Improving effects of additional administration of brexpiprazole to antipsychotics on cognitive function in schizophrenia patients - A pilot study -

Yuma SHIMIZU (清水侑真)[1], Ippei TAKEUCHI[2,3], Manako HANYA[1,2], Kiyoshi FUJITA[3], Hiroyuki KAMEI[1,2]

[1]Office of Clinical Pharmacy Practice and Health Care Management, Graduate School of Pharmacy, Meijo University, [2]Office of Clinical Pharmacy Practice and Health Care Management, Faculty of Pharmacy, Meijo University, [3]Department of Psychiatry, Okehazama Hospital

In addition to positive and negative symptoms, cognitive dysfunction is observed in schizophrenia patients. The present study examined the attenuating effects of the additional administration of brexpiprazole on cognitive dysfunction in schizophrenia patients.

This observational study included schizophrenic patients aged 18-65 years who had attended Okehazama Hospital (Aichi, Japan) as outpatients with a negative score of ≥20 on the Positive and Negative Syndrome Scale (PANSS) and had started additional treatment with brexpiprazole. Two neuropsychological tests and PANSS were conducted on subjects at 0, 4, 8 and 16 weeks: Trail Making Test, and Word Fluency Test to assess cognitive function and PANSS to evaluate severity.

Between February and August 2023, 19 patients started to receive additional treatment with brexpiprazole. In Part A of the Trail Making Test, the time required was 122.8±54.4 sec at the start of brexpiprazole treatment and significantly decreased to 106.4±57.8 sec after 16 weeks. On the other hand, changes in PANSS scores did not significantly differ in any period and did not correlate with cognitive function.

Thus, the present results demonstrated that the additional administration of brexpiprazole achieved favorable outcomes for cognitive function, particularly information processing speed, in schizophrenic patients with negative symptoms. This is the first study to investigate the effects of the additional administration of brexpiprazole on the attenuation of cognitive dysfunction. Further studies are needed on methods to assess cognitive function and recovery in schizophrenia patients.

P-70

In vivo monitoring of pancreatic acetylcholine using microdialysis in rats

Arisa Hayashi (林亜里紗)[1], Tokina Imura[1], Yoshinori Okamoto[1], Akira Aoki[1], Hideto Jinno[1]

[1]Faculty of Pharmacy, Meijo University, Nagoya, Japan

Objective: The pancreas has both exocrine functions, which secrete digestive juices, and endocrine functions, which secrete hormones such as insulin and glucagon, playing a crucial role in digestion and blood glucose regulation. One of the mechanisms for the secretion of digestive enzymes and hormones involves the activation of efferent vagus nerves (efferent VN) projecting to the pancreas. Therefore, elucidating the relationship between dietary habits and efferent VN activation could contribute to the prevention or treatment of lifestyle-related diseases, including diabetes. This study aims to develop a method for measuring acetylcholine (ACh) in the local pancreas of rats using the microdialysis technique.

Methods: This study was conducted with the approval of the Animal Experiment Committee of Meijo University School of Pharmacy. To determine the perfusion rate suitable for ACh recovery, we examined the relationship between perfusion rate and absolute recovery rate. *In vitro*, dialysis samples were collected at various perfusion rates (1-5 μL/min) by immersing a tissue dialysis probe in Ringer's solution containing ACh (1-10 nM), and ACh levels were measured. *In vivo*, a dialysis probe was inserted along the splenic artery in the pancreas of male ACI (August Copenhagen Irish) rats under isoflurane anesthesia, and dialysate was collected using the optimized conditions. The ACh concentration in the dialysate was measured using a QTRAP6500+ triple quadrupole mass spectrometer with ultra-high-performance liquid chromatography in positive ion mode.

Results and Discussion: *In vitro*, when Ringer's solution containing ACh (10 nM) was perfused at various rates, the absolute recovery rate increased with the rise in flow rate, reaching a plateau at 3 μL/min (81.3±0.5%). At all examined ACh concentrations (1, 2, 5, 10 nM), a consistent absolute recovery rate (mean 90.4±8.5%) was achieved at 3 μL/min. Therefore, the perfusion rate was set to 3 μL/min. *In vivo*, the baseline ACh concentration in pancreatic dialysate was 0.6±0.2 nM. Furthermore, when the efferent VN projecting to the pancreas was severed at the head side and electrically stimulated, the ACh concentration in the dialysate increased to 116.2±4.2% of the baseline. These findings suggest that it is feasible to monitor ACh in the pancreas using the microdialysis technique.

P-71

Evaluation of hot flashes using non-invasive continuous measurement of tail skin temperature in ovariectomized rats

Shin-nosuke Hayashi (林信之介)[1], Yoshinori Okamoto[1], Akira Aoki[1], Hideto Jinno[1]

[1]Faculty of Pharmacy, Meijo University, Nagoya, Japan

Objective: Hot flashes are reported as one of the symptoms of menopausal disorders. In rodents such as rats, heat dissipation from the tail skin surface is a primary mechanism for heat loss, and measuring tail skin temperature (TST) during the active phase (dark phase) can observe hot flash-like symptoms. Previous methods for measuring TST often involve highly invasive procedures, such as implanting transmitters, or are unsuitable for long-term continuous measurement, like thermography. Therefore, this study aimed to develop a non-invasive method for continuous TST measurement.

Methods: This study was conducted with the approval of the Animal Experiment Committee of Meijo University School of Pharmacy. A thermocouple data logger thermometer was used to measure TST, which was fixed 4 cm from the base of the tail with surgical tape. The experiment consisted of four phases. Phase 1: TST of 7-week-old female ACI (August-Copenhagen-Irish) rats was measured for 3 days. Phase 2: After a 1-week recovery period following ovariectomy (OVX), TST was measured for 3 days. Phase 3: A homemade sustained-release tablet containing 17β-estradiol (E2) was subcutaneously administered, and TST was measured for 3 days starting 4 days after administration. Phase 4: One week after removing the administered E2 tablet, TST was measured for 3 days. Surgical procedures were performed under isoflurane anesthesia.

Results and Discussion: The average TST during the active phase (dark phase) was calculated and compared. In Phase 1 (pre-OVX), the average TST was 28.4°C, which increased by approximately 2.3°C to 30.7°C in Phase 2 (post-OVX). Therefore, the OVX procedure was deemed to have successfully induced hot flash-like symptoms. In Phase 3 (E2 administration), the average TST decreased by about 4.7°C to 26.0°C. Finally, in Phase 4 (E2 removal), the average TST increased again by about 4.4°C to 30.4°C. These results suggest that the decrease in average TST in Phase 3 was due to the supplementation of E2 in E2-deficient rats, which alleviated the hot flash-like symptoms. This study successfully established a non-invasive measurement method applicable to evaluating compounds that alleviate hot flash-like symptoms in rodents.

P-72

In silico analysis for prediction of metabolites from halogenated estrogens

Kohki Kurihara (栗原皓紀)[1], Yoshinori Okamoto[1], Akira Aoki[1], Hideto Jinno[1]

[1]Faculty of Pharmacy, Meijo University, Nagoya, Japan

Objective: Estrogen is hydroxylated by cytochrome P450 (CYP) to form catechol estrogen, which has been reported to exhibit carcinogenic initiation effects. We synthesized 17β-estradiol (E2) derivatives halogenated at the 2 or 4 positions of the A-ring and evaluated their carcinogenicity, finding that halogenation at the 2 position significantly reduces the carcinogenicity of E2. However, the reason for the difference in carcinogenicity based on the modification position remains unclear. This study aims to predict the metabolites of halogenated E2 using *in silico* analysis and to explore the factors contributing to estrogen-induced carcinogenesis.

Methods: Metabolite prediction was performed using GLORYx (https://nerdd.univie.ac.at/gloryx/), a tool capable of calculating the metabolites and their probabilities generated by phase I and phase II reactions. For docking simulations, we used the molecular visualization software Chimera and the docking simulation software Autodock Vina. The docking simulation displays the docking poses of the receptor (CYP in this study) and the ligand (E2 and its derivatives) in order of stability. The crystal structure of human CYP1B1 was obtained from PBD (PDB ID: 3PM0) for the simulation.

Results and Discussion: The metabolite prediction scores focused on catechol estrogen. For E2, the 2-OH and 4-OH forms were predicted to be produced in similar proportions. In contrast, for E2 halogenated at the 2 or 4 positions, the probabilities of forming the 2-OH and 4-OH forms decreased, with a marked reduction in hydroxylation at the halogenated positions. Therefore, oxidative dehalogenation by CYP1B1 is considered unlikely. Docking simulations showed that all halogenated E2 compounds adopted poses with the A-ring oriented towards the heme, with no significant differences observed based on the substitution position. These results suggest that catechol forms are expected to be produced to a similar extent regardless of the halogen substitution position. Therefore, to understand the differing carcinogenicity of halogenated E2, new perspectives beyond metabolic patterns are required.

P-73

Association between the daily intake of herbal drugs and adverse events: a retrospective study using the Japanese Adverse Drug Event Report database

Koumi MIYASAKA[1], Keita OURA[1], Yamato KATO[1], Mika MAEZAWA[1], Sakiko HIROFUJI[1], Moe YAMASHITA[1], Nanaka ICHIHARA[1], Yuka NOKURA[1], Kana SUGISHITA[1], Tomofumi YAMAZAKI[1], Satoshi NAKAO[1,2], Hirofumi TAMAKI[3], Kazuhiro IGUCHI[3], Jun LIAO[4], Mitsuhiro NAKAMURA[1]

[1]Laboratory of Drug Informatics, Gifu Pharmaceutical University

[2]Department of Pharmacy, Kyushu University Hospital

[3]Laboratory of Community Pharmacy, Gifu Pharmaceutical University

[4]Department of Information Science and Information System, China Pharmaceutical University

Japanese herbal medicines “Kampo” is formulated from natural agents, and in Japan, 148 “Kampo extract formulations for prescription” have been approved for ethical use. This study examined the association between the daily intake of herbal drugs and adverse events of drug-induced interstitial lung disease (DIILD) and pseudoaldosteronism.

Adverse events in the Japanese Adverse Drug Event Report database were defined using codes based on the terminology used in the Medical Dictionary for Regulatory Activities. This study evaluated the adverse event indicators of DIILD following the daily intake of herbal drugs (Scutellariae radix [“ogon” in Japanese], Bupleuri radix [“saiko” in Japanese], and Pinelliae tuber [“hange” in Japanese]). The relationship between licorice dosage and pseudoaldosteronism was evaluated.

For DIILD, according to the receiver operating characteristic curves for Scutellariae radix (AUC=0.63572), Bupleuri radix (AUC=0.5685), and Pinelliae tuber (AUC=0.5705), the cut-off values (g/day) were 0.96, 3.30, and 1.60, respectively. It was suggested that even low doses of licorice (<2.5 g) can induce pseudoaldosteronism.

We demonstrated that the daily intake of herbal drugs influenced the risk for adverse events in a dose-dependent manner. This research was partially supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number, 17K08452, 21K06646 and 20K10408.

1. Kato Y et al. Analysis of licorice-induced pseudoaldosteronism in the Japanese Adverse Drug Event Report database. Traditional & Kampo Medicine, 2016, 3, 63-70.
2. Oura K et al. Analysis of drug-induced interstitial lung disease caused by herbal medicine using the Japanese Adverse Drug Event Report database. BMC Complement Med Ther, 2024. 24, 121.

P-74

The incentive mechanism for fostering industry-university-research collaboration in pharmaceutical innovation

Xing Wang, Xiaoliang Yuanc (袁小量)*

School of Business Administration, Shenyang Pharmaceutical University, Shenyang, Liaoning, China

The incentive mechanism for promoting industry-university-research collaboration in pharmaceutical innovation is crucial for enhancing efficiency and facilitating the successful translation of research outcomes. However, it is imperative to address potential issues related to free-riding behavior during this collaborative process in order to prevent significant losses for all involved parties. In this study, we employ a quantum game analysis approach to investigate the incentive mechanism governing industry-university-research collaboration in pharmaceutical innovation under government supervision. We develop a tailored quantum game model specifically designed for collaborative innovation between pharmaceutical enterprises, universities, and research institutions within the sector. Through comparing strategies and benefits among these entities operating under stringent governmental oversight, our findings demonstrate that effective governmental supervision mitigates free-riding concerns by ensuring that lack of effort from one party does not impose negative consequences on the other.

Acknowlegdements

The Organizing Committee of the 9th Nagoya / Gifu / Nanjing / Shenyang Symposium of Pharmaceutical Sciences, 2024 Nagoya are grateful to the following organizations and companies for supporting the congress.

Special Research Grant of Nagoya City University

Daiko Foundation

Qol Co., Ltd.

FREUND CORPORATION

ROHTO Pharmaceutical Co., Ltd.

Sun Ten Pharmaceutical Co., Ltd.

Kyoto Pharmaceutical Industries, Ltd.

PLCM Research Group.

Fuji Chemical Industries Co., Ltd.

TSUMURA & Co.

ZAISEIDO Pharmaceutical Co., Ltd.

Powrex Corporation

Tomita Pharmaceutical Co., Ltd.

Tokai Kampo Kyogikai.

Chubu Yakuhin Co., Ltd.

Pharmedico Co., Ltd.

OHKAWARA KAKOHKI Co., Ltd.

Kobayashi Pharmaceutical Co., Ltd.

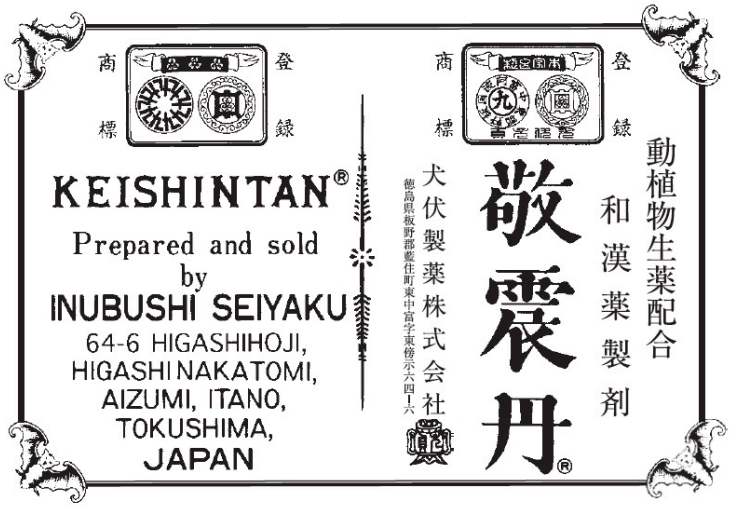
Minophagen Pharmaceutical Co., Ltd.

INUBUSHI SEIYAKU Co., Ltd.

JPS Pharaceutical Co., Ltd.

Stec Co., Ltd.

香りで気を回(めぐ)らせるくすり



第2類医薬品

麝香・牛黄・サフラン・沈香
人参・香附子・竜脳・甘松
桂皮・牛胆・丁子・木香
甘草・生姜

下記書籍にてご紹介

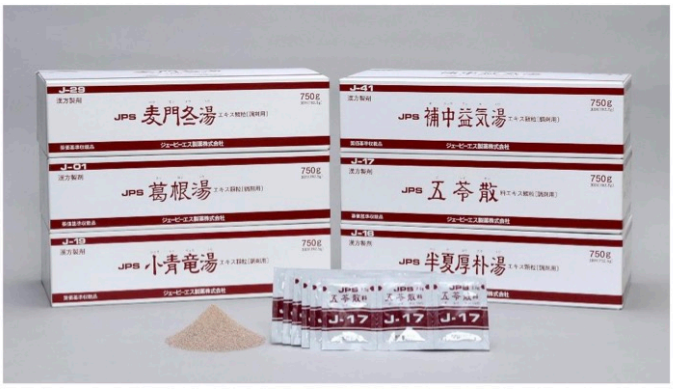
『日本の名薬』『日本の伝統薬』
『妙薬探訪』『文藝春秋 Special』
『ファルマシア（日本薬学会会報誌）』

文政年間（1818～1830年）創製

犬伏製薬株式会社

徳島県板野郡藍住町東中富字東傍示64-6
〒771-1220　電話（088）692-2302

自然の恵みを大切に活かし、人々の健康と社会に貢献する



ジェーピーエス製薬は、
『人間は自然と共にあり自然に生かされているという摂理に従い、病める人の身体の調和（バランス）を求めることによって病を治す』
という漢方理論に基づいて医薬品の提供を行っています。すべての人々の健康で幸せな毎日を願い、常に信頼される製品を提供することで皆様の暮らしにより一層の貢献をしてまいります。
また自然の恵みである生薬を大切に活かし、サステナビリティの実現に向けて、様々な取り組みを行っていきます。

薬価基準収載　漢方製剤

効能・効果、用法・用量、使用上の注意等などは電子化された添付文書をご覧ください。

資料請求先

ジェーピーエス製薬
公式キャラクター
『ジェイくん』

ジェーピーエス製薬

〒224-0023　神奈川県横浜市都筑区東山田4-42-22
TEL: 045-593-2060　FAX: 045-330-4130
https://www.jps-pharm.com

漢方製剤にとって「良質」とは何か。その答えのひとつが「均質」である、とツムラは考えます。自然由来がゆえに、ひとつひとつに個性がある生薬。漢方製剤にとって、その成分のばらつきを抑え、一定に保つことが「良質」である。そう考える私たちは、栽培から製造にいたるすべてのプロセスで、自然由来の成分のばらつきを抑える技術を追求。これからもあるべき「ツムラ品質」を進化させ続けます。現代を生きる人々の健やかな毎日のために。自然と健康を科学する、漢方のツムラです。

良質。均質。ツムラ品質。

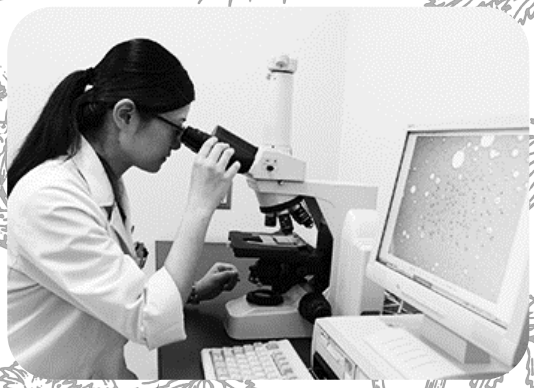
ツムラ

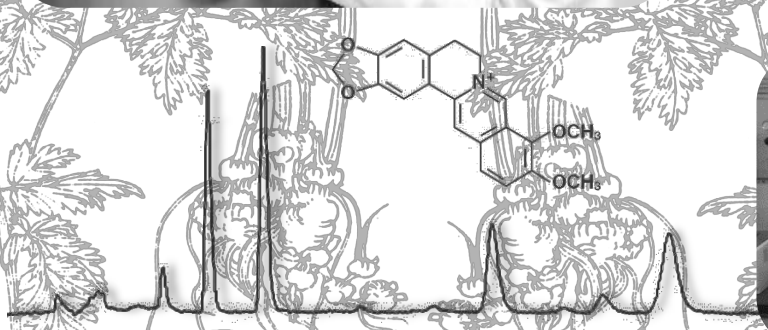
株式会社ツムラ https://www.tsumura.co.jp/　資料請求・お問合せは、お客様相談窓口まで。
医療関係者の皆様 tel.0120-329-970　患者様・一般のお客様 tel.0120-329-930
受付時間 9:00～17:30（土・日・祝日は除く）

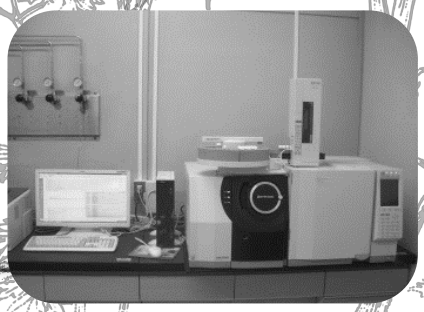
2021年4月制作 審

＝ 科学的根拠に基づき、確かな品質を ＝

現代人に合わせた漢方・生薬製剤の研究開発・製造に挑戦し続けます！







～薬学部生、大学院生の皆様へ～

漢方・生薬製剤の研究・開発などに興味をお持ちの方、
随時、会社見学を受け付けておりますので、
お気軽にお問い合わせください。

【お問い合わせ先】総務部 亀田［E-mail］jinji@zaiseido.co.jp

ホノミ漢方
剤盛堂薬品株式会社

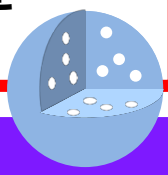
〒640-8323 和歌山県和歌山市太田二丁目8番31号
TEL 073-472-3111 URL: https://www.zaiseido.co.jp/

▼詳しくは当社ホームページをご覧ください

ざいせいどう　検索

富士化学工業の高機能性医薬品用添加剤
―製剤のニーズにこたえる機能性添加剤―

富士化学工業の無機合成技術とスプレードライ技術を組み合わせた高機能性添加剤

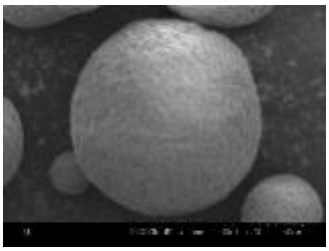


Neusilin®

メタケイ酸アルミン酸マグネシウム
JP, USP/NF, EP, CP 登録申請中

＜特徴＞

複数の問題を解決する多機能性をする添加剤（中性品：US2, UFL2）
- 成形性・崩壊性の改善
- 流動性改善・固結防止



NeusilinUS2

用途と推奨使用量（%）

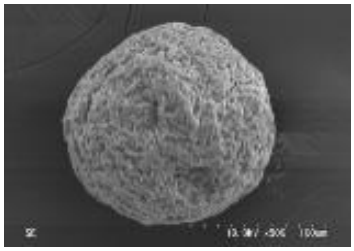
グレード	US2 (中性品)	UFL2 (中性品)
タブレット－バインダー, 崩壊助剤, 硬度上昇	1-10	1-10
流動促進剤	-	0.5-5
潮解性薬剤の安定化	5-15	5-15
賦形剤	30-90	30-90
Solidification 液体薬剤の固定化	30-50	30-50
Solid Dispersion 個体分散体の担体、SMEDDS	20-50	20-50

Fujicalin®

無水リン酸水素カルシウム
JP, USP/NF, EP, CP 収載

＜特徴＞

従来のリン酸水素カルシウムにはない特性を持つ直打用添加剤
- 高い成形性と流動性
- 低含量製剤での含均性向上



Fujicalin SG

フジカリンと従来のDCPA/DCPDとの物性比較

物性	DCPA		DCPD
	フジカリン	従来品	
平均粒子径（μm）	120	45	127
嵩比容積、疎充填（mL/g）	2.3	1.3	1.2
嵩比容積、密充填（mL/g）	2.0	0.9	1.1
安息角（°）	30	42	35
BET 比表面積 (m^2/g)	40	0.7	0.57
吸油量（mL/g）	1.1	0.5	0.2

富士化学工業株式会社
Fuji Chemical Industries Co., Ltd.
[富山] 富山県中新川郡上市町郷柿沢1 TEL076-472-5555
[東京] 東京都港区芝公園1-8-21 TEL03-3437-2350

PLCM研究会第17回シンポジウム

2024.2. 22　参加者　約250名

- 中国からの参加者
- 胡　富強　浙江大学･薬学院
- 崔　福徳　瀋陽薬科大学
- 朴　洪宇　瀋陽薬科大学
- 劉　東春　瀋陽薬科大学
- 朱　長軍　大連業建貿易
- 徐　輝　　深圳華溶機器
- 小　嘉　　深圳華溶機器
- 祝　綿敏　深圳華溶機器

委員

- 砂田久一　名城大学
- 竹内洋文　岐阜薬科大学
- 丹羽敏幸　名城大学薬学部
- 山本浩充　愛知学院薬学部
- 並木徳之　帝京平成大･薬
- 橋都なほみ　じほう社
- 中道孝一　Nakamiti Juku
- 増田義典　精薬研究所
- 槙野 正　マキノ製剤技術研究所
- 岩本 清　ツキオカフィルム
- 児玉雅志　パウレック社
- 真榮田篤　アステラス製薬
- 鵜野沢一臣　フロイント産業

PLCM研究会第18回シンポジウム
2025年2月28日（金）名城大学･薬学部

作成：安永峻也（愛知学院･薬）

第７回PLCM（耕薬）実習講習会のご案内

日時：　2024年9月19-20日（2日間）
場所：　フロイント産業（株）技術開発研究所（浜松市）
定員：　24名　　募集開始：５月初頭

【概要・特徴】

経験豊かな講師陣

- ＯＤ錠の３つの基本プロセス（造粒、打錠、微粒子コーティング）の体験
- ＯＤ錠独自の評価基準と評価法
- 少人数グループ制、受講者の経験年数に合わせた細かな指導。十分な受講時間
- 事前の導入レクチャー
- 実習後の総合討論

その他

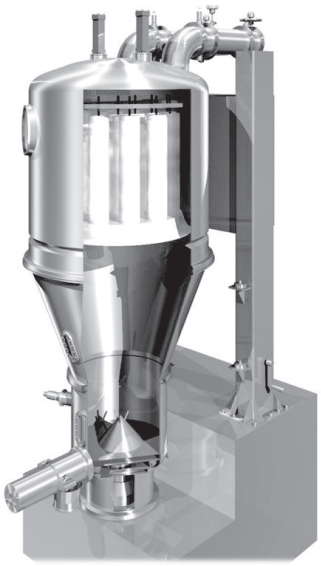
- ◆ 施設内の見学ツアー
- ◆ 懇親会による受講者間、受講者-講師間の交流促進
- ◆ 全員宿泊可能なホテルを用意
- ◆ その他、昼食、送迎バス、傷害保険加入等

識る、観る、触る”ＯＤ錠技術！

OD錠に活用される微粒子コーティング

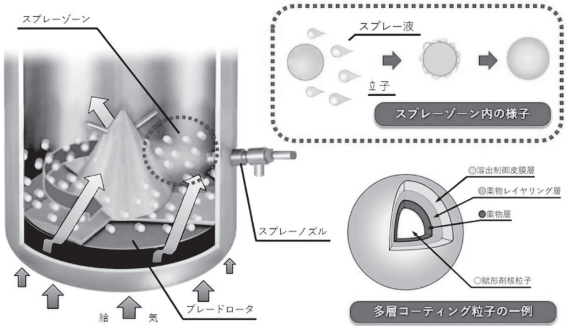
転動流動層造粒・コーティング機
MP（マルチプレックス）

詳細はこちら！



様々な用途に使用可能!!
OD錠用コーティング顆粒の調製 / 薬物レイヤリング / 苦味マスキング / 腸溶性コーティング / 胃溶性コーティング / 微粒子コーティング / 溶出制御

流動層装置の原理をベースとし、底部にブレードロータを搭載したことで、強制的に流動を促進します。



多種多様な粉体の研究開発に。

FD-MP-01 E 型
（タッチパネル搭載）

FD-MP-01 D 型
（デジタル表示）

オプションユニットを載せかえることで、様々なプロセスに対応可能！

造粒　乾燥　直接顆粒化
粒子コーティング　攪拌混合造粒

株式会社パウレック　https://www.powrex.co.jp

ISO 9001:2015 認証取得　ISO 14001:2015 認証取得

本社・イノベーションセンター	〒664-0837 兵庫県伊丹市北河原 5-5-5	TEL 072-778-7304	FAX 072-778-7314
東京支店	〒101-0041 東京都千代田区神田須田町 1-9 神田須田町プレイス 6F	TEL 03-3518-5144	FAX 03-3518-5261
富山営業所	〒930-0083 富山県富山市総曲輪 1-5-24	TEL 076-433-6330	FAX 076-433-6331
湘南アイパークイノベーションセン	〒 251-8555 神奈川県藤沢市村岡東2-26-1	2022年10月に開設	

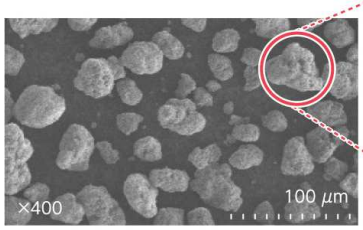
多機能型粉体
フローライト®シリーズ

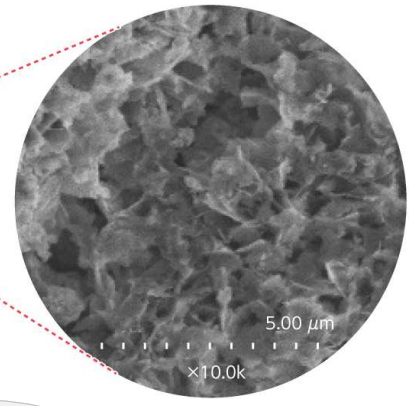
富田製薬株式会社
https://tomitaph.co.jp

フローライト®は、医薬品、化粧品、食品、工業製品など幅広い分野で優れた機能を発揮します

フローライト®の独特な結晶構造*

粒子内に大きな細孔
容積を形成した独自の
花弁状結晶構造*





高い吸液・吸油能
自重の3～5倍量*の液体を
吸収し、液体、油状物質の
粉末化が可能
*番手によって異なる

優れた成形性
低打圧でも錠剤を高硬度に、
ラミネーション等の打錠障害を
低減し、直接打錠を実現

崩壊性の向上
錠剤内部に水を引き込み、
高硬度でも
高い崩壊性を実現

FLORITE®

有効成分安定化
有効成分や香料成分を
細孔内に保持し、
光や酸素から保護

溶出特性の制御
香料の徐放化
被膜用ポリマーとの組み合わせで
有効成分溶出速度の調整や
香料の徐放化が可能

難溶性成分の
溶出性改善
難溶性成分を細孔内で
非晶質化し、溶出性を改善

FLORITE® R, PS-200, H, PS-10

Innovative excipients for various pharmaceuticals

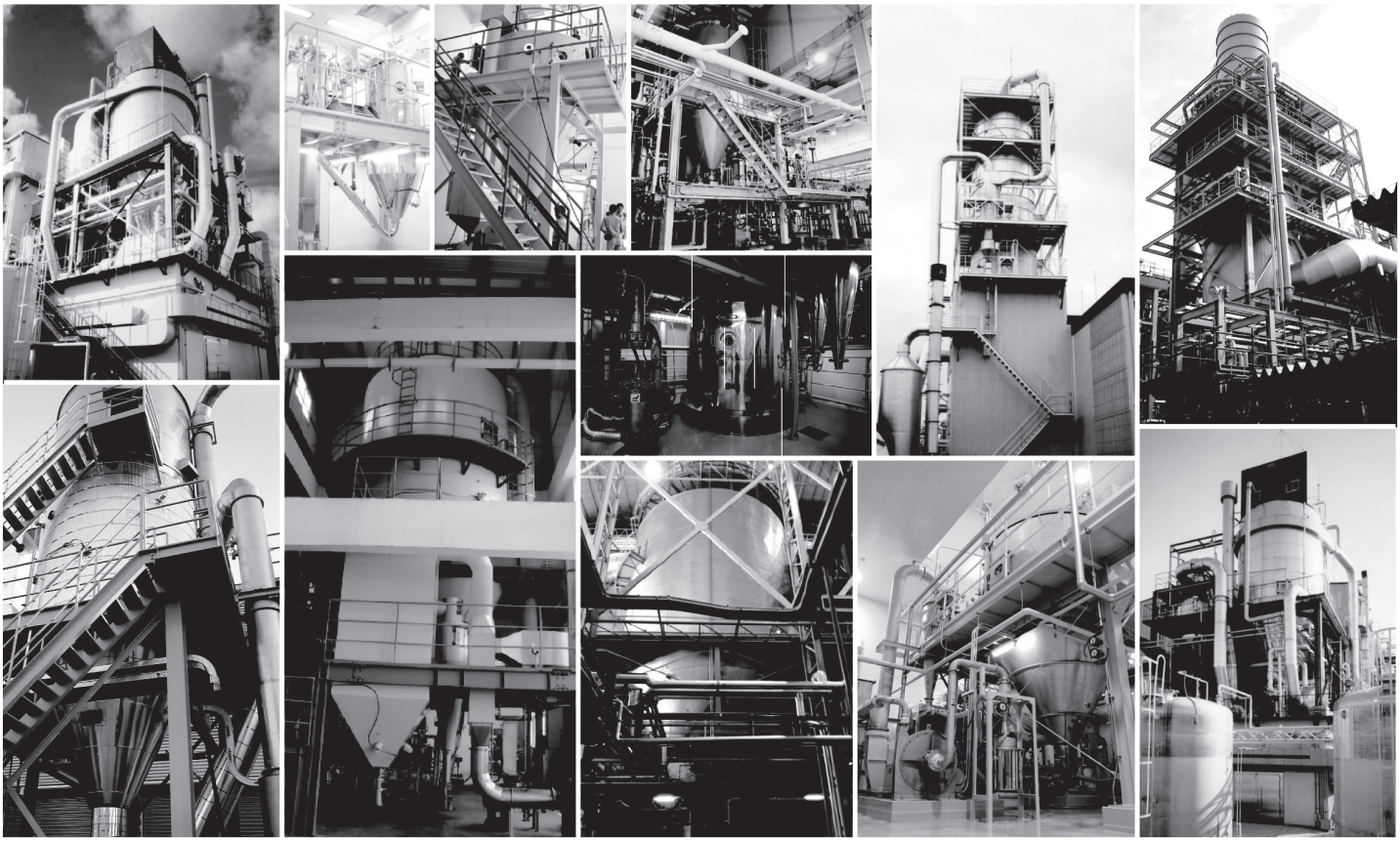
特徴的な花弁状結晶構造がもたらす様々な機能を
製剤開発にお役立てください！

SPRAY&DRY

ミルクから電子部品材料まで

大川原化工機は2100基を超える納入実績と、
30年余以上の経験に裏付けられたノウハウから
常に最新鋭のスプレードライヤをご提案します。
お客様がどのような粉体製品をお求めなのか?
それを達成するにはどのようなシステムがベストなのか?
当社は繰り返し考えます。
研究設備から大型生産設備まで、あらゆるニーズに
粒子づくりのエンジニアリングでお答えします。

研究開発用スプレードライヤ　L-8i



大川原化工機株式会社
http://www.oc-sd.co.jp
本社営業部　〒224-0053 横浜市都筑区池辺町3847 TEL:045-932-4111
大阪営業部所 〒531-0072 大阪市北区豊崎3-4-14 TEL:06-6375-3211

愛される医療人に。

ドラッグストア薬剤師は、オーダーメイドな服薬指導、
未病・予防のサポート、在宅医療などを通して患者様の
健康管理をトータルサポートできる医療人。
ですが、それだけがV・drugの目指す薬剤師ではありません。

薬剤師は、いつでも気軽に相談できる医療人に
薬剤師は、患者様の生活にまで深く関われる医療人に
薬剤師は、みんなの笑顔を1つでも増やせる医療人に

患者様からもスタッフからも「愛される医療人」。
それがV・drugで働く薬剤師です。

V drug
VALOR DRUG STORE

中部薬品株式会社
〒507-0078 岐阜県多治見市高根町4丁目29番地
TEL.0572-27-3436／FAX.0572-27-3912

エントリーはこちら

新卒採用HP
Here for entry!

からだの中から、ごきげんさん。

和漢箋 わかんせん®

からだのなかから、ごきげんな自分へ。

いつまでも気力体力のある大人でいたい。

ロートの漢方は、そんなあなたが

快活な生活を送るための、身近な選択肢。

漢方の力で内側から元気を高め、

治してゆくのです。

時々訪れる不調とも、うまく付き合いながら。

これからの身体と、

ずっと仲良くいられる自分へ。

第2類医薬品

〈販売名〉新・ロート防風通聖散錠満量

【効能・効果】体力充実して、腹部に皮下脂肪が多く、便秘がちなものの次の諸症：高血圧や肥満に伴う動悸・肩こり・のぼせ・むくみ・便秘、蓄膿症（副鼻腔炎）、湿疹・皮膚炎、ふきでものにきび、肥満症

第2類医薬品

〈販売名〉ユクリズム

【効能・効果】体力中等度以下で、心身が疲れ、血色が悪く、ときに熱感を伴うものの次の諸症：貧血、不眠症、精神不安、神経症

第2類医薬品

〈販売名〉ラクリア

【効能・効果】体力中等度以下で、疲れやすく、汗のかきやすい傾向があるものの次の諸症：肥満に伴う関節の腫れや痛み、むくみ、多汗症、肥満症（筋肉にしまりのない いわゆる水ぶとり）

第2類医薬品

〈販売名〉ツラレス

【効能・効果】体力に関わらず使用でき、筋肉の急激なけいれんを伴う痛みのあるものの次の諸症：こむらがえり、筋肉のけいれん、腹痛、腰痛

ロート製薬の
漢方のこだわり

3つのこだわり

飲みやすさ・続けやすさ

「苦い薬はいやだ」「漢方は味が苦手」そんな人も
多いのではないでしょうか。
和漢箋は、錠剤タイプ。
一部商品はフィルムコーティングを実施し、
飲みやすさにこだわっています。
適切に服用できてこそ、
薬本来の効き目が発揮できるのです。

効き方へのこだわり

同じ漢方でも、生薬（漢方薬を構成する原料）の
産地や抽出方法により得られる効果は
大きく変わります。
和漢箋は、中国現地での
生薬の仕入れ・精製度にもこだわっていて、
国内研究所と現地工場が連携し、
漢方エキスの抽出方法の検討を続けています。

届けたい人への想い

年齢による体の悩みは出てくるもの。
一方で、老若男女問わず自分らしい生活や
社会で活躍ができる現代。
そうありたいと目指す社会。
和漢箋は、不調に負けず快活な生活を
送っていただくには何が必要かを考え、
開発に取り組んでいます。

Moving the Heart ROHTO

写真はイメージです。

あなたの、いちばん近くにある安心。

QOLとは、クオリティ・オブ・ライフという意味。
クオールの、社名の由来です。

クオールは、一人ひとりの患者さまに信頼される調剤薬局として、
あらゆる地域社会の健康で豊かな生活に貢献します。

これからも私たちは、安心・快適にご利用いただける薬局をめざし続けます。

くらしを支える、良質な地域医療をすぐそばで。

クオール薬局 グループ
Quality Of Life