JSTP

The 29th Annual Meeting of the Japanese Society of Toxicologic Pathology

- Future of Toxicologic Pathology in the Post-Genomic Era -

January 31st [Thursday] - February 1st [Friday], 2013

The Okura Frontier Hotel Tsukuba

The 29th Annual Meeting of the Japanese Society of Toxicologic Pathology

～ Future of Toxicologic Pathology in the Post-Genomic Era ～

President Takanori Harada (The Institute of Environmental Toxicology)

Date January 31st (Thursday) - February 1st (Friday), 2013

Venue The Okura Frontier Hotel Tsukuba
1-1364-1, Azuma Tsukuba-city, Ibaraki 305-0031, Japan
TEL: +81-29-852-1112

Organizing Committee

Drs. Dai Nakae(Chair), Katsumi Imaida, Yuji Oishi, Kumiko Ogawa,
Makoto Sibutani, Kinji Shirota, Munehiro Teranishi, Hiroyuki Nakayama,
Ryo Fukuda, Satoshi Furukawa, Satoru Hosokawa, and Katsuhiko Yoshizawa

Secretariat of the 29th Annual Meeting of the Japanese Society of Toxicologic Pathology

The Institute of Environmental Toxicology
4321 Uchimoriya-machi, Joso-shi, Ibaraki 303-0043, Japan
TEL: 0297-27-4521

[Secretariat during the conference]
The Okura Frontier Hotel Tsukuba Annex 2F Room "Yubae"
TEL：029-852-1112

[Operating Office]
Procom International Co., Ltd.
TEL: 03-5520-8821
URL: http://www.procomu.jp/jstp2013

The 29th Annual Meeting of the Japanese Society of Toxicologic Pathology

Table of Contents

Greetings

It is our honor and pleasure to welcome you to the 29th Annual Meeting of the Japanese Society of Toxicologic Pathology (JSTP) to be held at Okura Frontier Hotel Tsukuba on January 31- February 1, 2013.

The Organizing Committee consisting of 12 members (Chair, Dr. Dai Nakae) from academia, CROs, public and industrial research institutes has proposed the main topic of this meeting, "The Future of Toxicologic Pathology in the Post-Genomic Era", which provides a key stone to the next door in this field. From this point of view, the 29th JSTP scientific sessions will address new approaches in defining the future of toxicologic pathology in the post-genomic era and the program includes a special lecture on "The Role of the Toxicologic Pathologist in the Post-Genomic Era" and symposia on "Contemporary Models and Strategies for Use in Toxicity and Carcinogenicity Testing" and "New Trends with *in vivo* Experimental Models and Alternatives".

We have organized a pleasant social event for you. On the evening of January 31 (Thursday) from 18:00 to 20:00, we would like to invite you to join the Welcome Reception at the Banquet Hall "Jupiter" (Main building, 3F) of Okura Frontier Hotel Tsukuba. This is a buffet style reception and gives you the opportunity to meet old and new friends and to promote friendship among the members of different STPs. And please do not miss the Annual General Assembly and Council of the JSTP. The Assembly and Council of the JSTP membership will take place on February 1 (Friday) from 14:45 to 16:15 in the main session hall "Subaru" at the Okura Frontier Hotel Tsukuba. The results of the elections in the JSTP Board of Directors and our business activities will be presented, and a discussion with audience on the future of the JSTP will be addressed. IFSTP meeting will be held on Friday lunch time at Hokuto room.

Prior to this annual meeting, the following events will be held at Nova Hall close to the hotel: The 1st Joint JSTP/NTP Satellite Symposium on January 29 (Tuesday) from 12:00 to 18:50 and a meeting to explain qualifying examination problems for diplomates of the JSTP and Slide Conference on January 30 (Wednesday) from 10:00 to 17:30. In addition, IATP lecture will be given to you on Wednesday evening from 17:45-18:45.

Finally, we greatly appreciate various donations including luncheon seminars and exhibition booths from many companies and CROs to support this meeting. Their donations make it possible for the JSTP to continue offering high quality science.

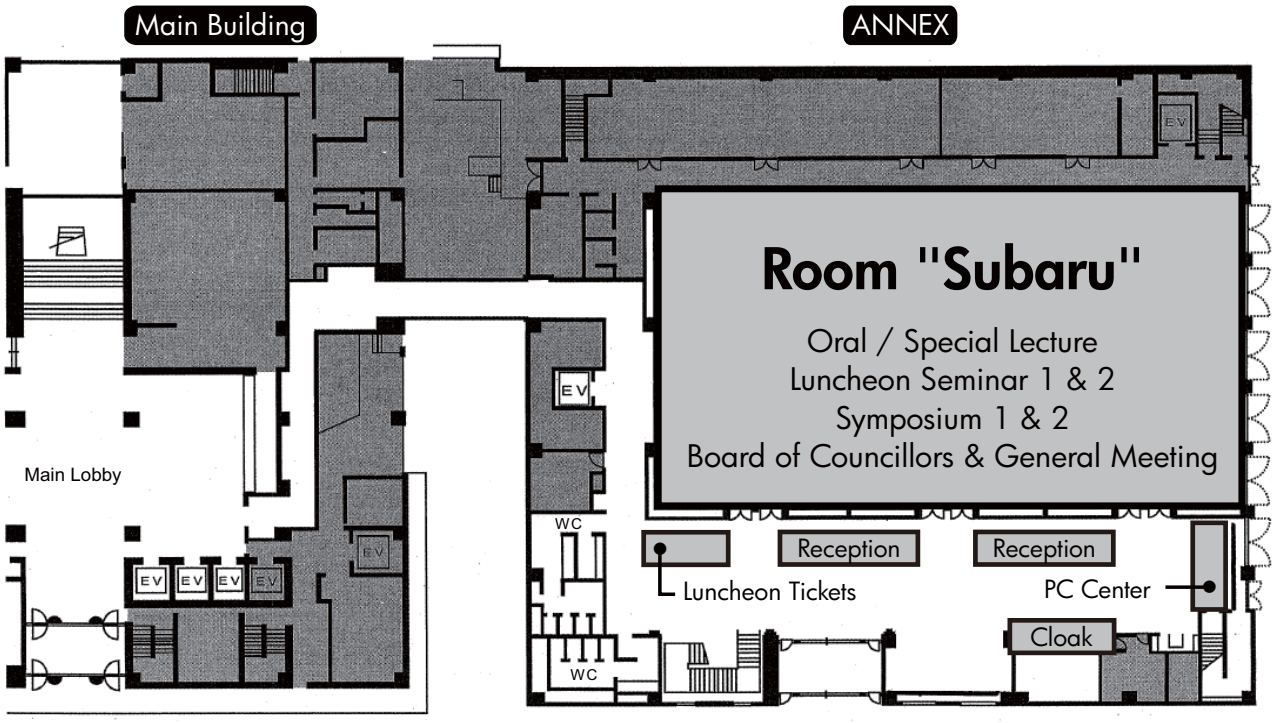
We hope you enjoy the scientific sessions and also other events in Tsukuba, Japan.

Takanori Harada, DVM, PhD

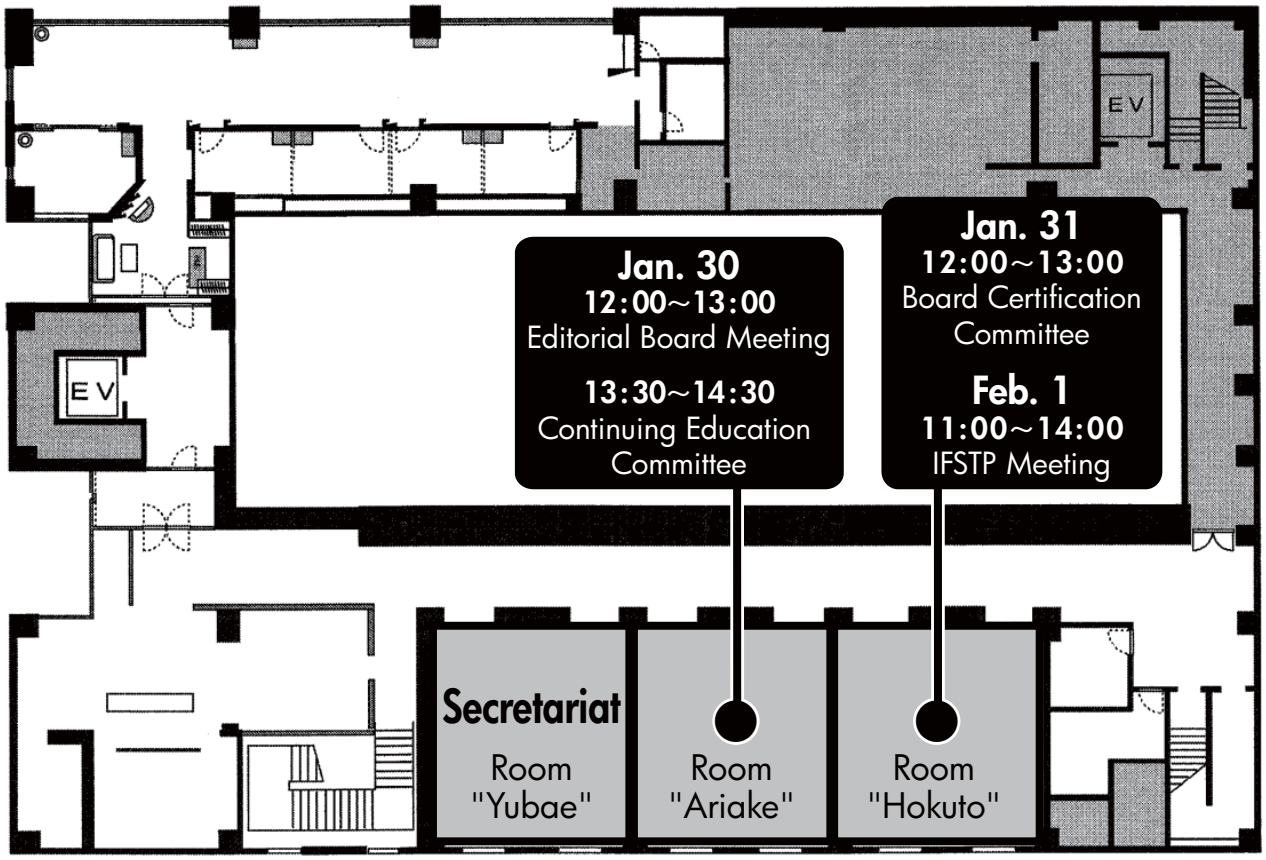
President of the 29th JSTP Annual Meeting

Floor Plan

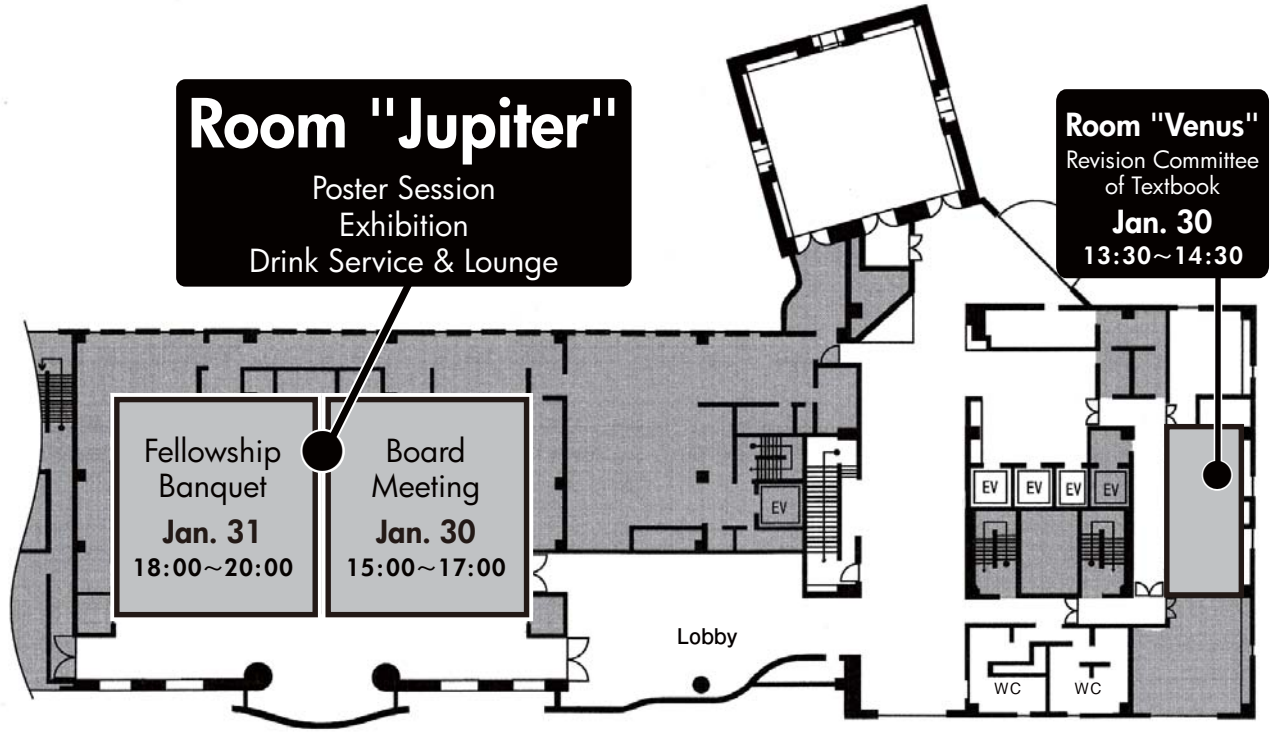
❑ Room "Subaru" The Okura Frontier Hotel Tsukuba ANNEX [1F]



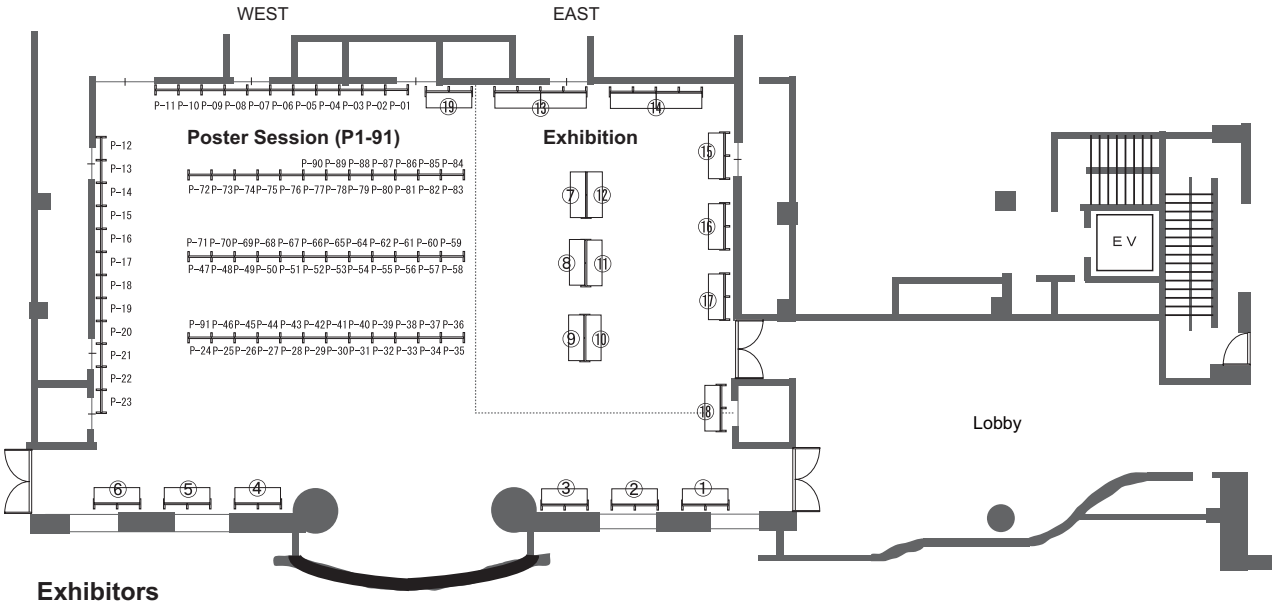
❑ Room "Ariake", Room "Hokuto" & Room "Yubae" The Okura Frontier Hotel Tsukuba ANNEX [2F]



❑ Room "Jupiter" & Room "Venus" The Okura Frontier Hotel Main Bldg. [3F]



Room "Jupiter" - Poster Session / Exhibition



Exhibitors

① Japan SLC, Inc.
② Visions Co., Ltd.
③ H & T Corporation
④ Histo Science Laboratory Co., Ltd.
⑤ Hamamatsu Photonics K.K.
⑥ Kurabo Industries Ltd.
⑦ Safety Research Institute for Chemical Compounds Co., Ltd.
⑧ KAC Co., Ltd.
⑨ Shiraimatsu Co., Ltd.
⑩ Bozo Research Center Inc.
⑪ Fujitsu Limited
⑫ CLEA Japan, Inc.
⑬ Media Services, Ltd.
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⑮ Charles River Laboratories Japan, Inc.
⑯ DIMS Institute of Medical Science, Inc.
⑰ Sakura Finetek Japan Co., Ltd.
⑱ Harlan Laboratories Japan, Co., Ltd.
⑲ Aperio Technologies, KK.

❑ Nova Hall

JSTP/NTP Satellite Symposium
Explanation of the Test for Diplomates of JSTP
The 25th Slide Conference
IATP Lecture

To Participants

1. Main Reception Desk

   The registration/information desk is located in the lobby of the Banquet Hall “Subaru” in the Okura Frontier Hotel Tsukuba Annex.

   Dates and Times: January 31 (Thursday) 8:00 a.m. -
   February 1 (Friday) 8:00 a.m. -

2. Early-Bird Registration

   Early-bird registrants should bring their conference program, book of abstracts and name badge to the venue, and wear their name badge at all times. Name holders will be available at the reception desk.

3. On-Site Registration

   On-site registrants must fill out a registration form and pay their fees. At registration, participants will be given a badge on which to write their name and affiliation. These badges should be worn at all times during the conference. Students must present their student ID cards at registration.

4. Registration Fees

   JPY 12,000 (Students JPY 6,000; Non-Members JPY 17,000)

   Extra copies of the conference program and the book of abstracts are available at JPY 2,000 (Students JPY 2,000; Non-Members JPY 5,000) per issue.

5. During question-and-answer and discussion sessions, please follow the chairman's directions and state your name and affiliation when you first approach the microphone.

6. Smoking is not permitted in any of the conference venues.

7. Mobile phones should be turned off or in silent mode.

8. Lunch will be provided at luncheon seminars. Participants also may dine at the hotel's restaurants and coffee shops.

9. Cloakroom

   Location: The lobby of the "Subaru" Banquet Hall in the Okura Frontier Hotel Tsukuba Annex

   Dates and Times: January 31 (Thursday) 8:30 - 18:00
   February 1 (Friday) 8:30 - 17:00

   * Please note that valuables will not be accepted.

10. Paging Service and Message Board

    No paging service is available except for an emergency. The message board is located near the registration desk in the lobby of the Banquet Hall “Subaru” in the Okura Frontier Hotel Tsukuba Annex.

11. The recording of all or part of any scientific presentation or poster presentation is prohibited.

12. General Meeting and Board of Councilors

    Place: Banquet Hall “Subaru” in the Okura Frontier Hotel Tsukuba Annex
    Date and Time: February 1 (Friday) 14:45 - 16:00

13. Fellowship Banquet

Place: Banquet Hall "Jupiter", Okura Frontier Hotel Tsukuba Main Building
Date and Time: January 31 (Thursday) 18:00 -

* The Fellowship Banquet generally requires pre-registration, but a small number of participants can register at JPY 9,000 (student JPY 7,000) at the main reception on February 1. These registrations will be accepted on a first-come, first-served basis.
* The registrant's name badge must be stamped as proof of pre-registration.

14. Luncheon Seminar

Luncheon Seminar 1

Place: Banquet Hall "Subaru", Okura Frontier Hotel Tsukuba Annex
Date and Time: January 31 (Thursday) 12:00 - 13:00
Speaker: Dr. Hijiri Iwata (Harlan Laboratories, Ltd., Switzerland)
Title: Target sites of tumor development in long-term carcinogenicity studies with mice
Chair: Dr. Michihito Takahashi (Pathology Peer Review Center)
Co-sponsor: Harlan Laboratories Japan, Co., Ltd.

Luncheon Seminar 2

Place: Banquet Hall "Subaru", Okura Frontier Hotel Tsukuba Annex
Date and Time: February 1 (Friday) 11:55 - 12:55
Speaker: Dr. Dianne Creasy (Huntingdon Life Sciences)
Title: Are environmental chemicals causing a decline in human reproductive health?
Chair: Dr. Michihito Takahashi (Pathology Peer Review Center)
Co-sponsor: Huntingdon Life Sciences, Inc.

* Luncheon tickets will be distributed each day at 8:30 a.m. in the lobby of the Banquet Hall "Subaru" in the Okura Frontier Hotel Tsukuba Annex.
(*The desk will close when the last ticket has been given out*.)
* Luncheon tickets will be available on a first-come, first-served basis.

To Chairpersons

❑ For chairpersons of the Special Lecture, Symposium and General Oral Presentations

Chairpersons should be seated in the designated next-chairperson's seat in the session room 15 minutes before the session starts.

❑ For chairpersons of the Poster Sessions

Chairpersons should arrive at the poster session reception desk, located in the lobby of the Jupiter Banquet Hall in the Okura Frontier Hotel Tsukuba Main Building, at least 15 minutes before their session starts.

To Speakers

For speakers for the Special Lecture, Symposium and General Oral Presentations:

1. PC Center (Presentation Data and PC Reception Desk)

   Speakers who will be using USB memory sticks should report to the PC Center at least 60 minutes before their presentation is scheduled to start.

   Speakers with their own PCs should deliver them to the PC operators seated to the left at the front of the session room at least 30 minutes before their session is scheduled to begin, after checking their PCs in at the PC Center.

2. Allotted Presentation Time

   The allotted time for each General Oral Presentation is 12 minutes; 8 minutes for the presentation and 4 minutes for discussion.

   The light on the time-keeping device will change to yellow after 7 minutes; at 8 minutes the light will switch to red.

3. Only PCs may be used for oral presentations; a slide projector will not be available.

4. Guidelines for General Oral Presentations

   1) Presenters should be seated in the next-speaker's seat soon after the previous presentation concludes.
   2) A monitor display and a wireless mouse will be on the podium. Please operate PCs with the wireless mouse and monitor.
      Two large screens will be at the front of the session rooms. Please use the wireless mouse cursor as a pointer. A laser pointer will not be available.

   <Windows>

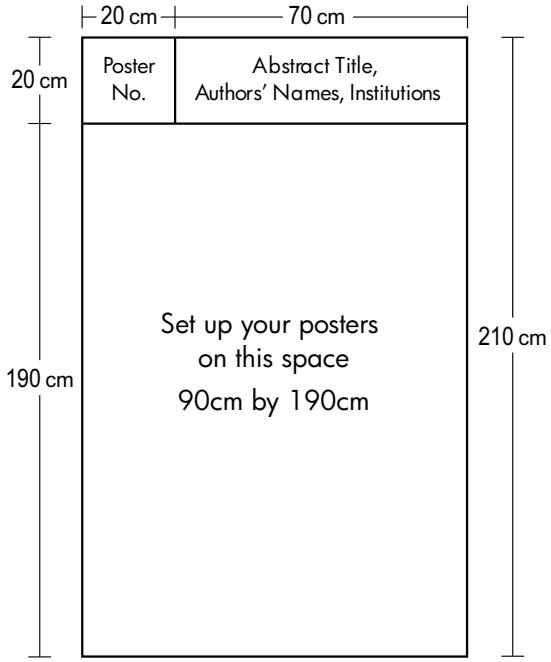
   1) You are encouraged to bring a USB memory stick or your own PC to avoid interface difficulties.
   2) Windows7 and PowerPoint 2007 or 2010 are available.
   3) Fonts
      Please use the following fonts to avoid character corruption:
      Century, Century Gothic, Times New Roman, Arial
   4) USB memory sticks should be in a Windows-readable format.
   5) Presentations should be saved on the USB stick with only the presentation date.
   6) The Secretariat will delete all presentation data after the conference.

   <Macintosh>

   1) Those who wish to use Macintosh software should bring their own Macintosh PC.
   2) Please bring an AC adaptor and connector to mini D-sub (15pins).
   3) Please bring a back-up of your presentation in the event of PC/technical difficulties.
   4) Videos (movies) and audio cannot be accommodated.
   5) After presenting, speakers can pick up their PCs from the PC operator in the session room.

❑ For Poster Session Speakers

1. Poster Board Size
   90cm wide by 210cm high, including 20cm for title, name and affiliation.
   In preparing your presentation, we ask that you keep your name, title, and affiliation at 70cm wide by 20cm high. The poster number (20cm by 20cm) will be added by the Secretariat.
   The poster display space is 190cm high by 90cm wide. If more space is needed, use the space allotted for the name, title, and affiliation, as information in the lower portion of the poster board will be difficult to see.



2. Poster session speakers should arrive to set up for the session at the reception desk in the Jupiter Banquet Hall in the Okura Frontier Hotel Tsukuba Main Building.
3. Ribbons and push-pins for the speakers will be at the poster session reception desk. Speakers also should wear their ribbons during the question-and-answer session.
4. Poster boards should be moved temporarily after 15:30 on January 31 (Thursday).
   Please note: Speakers should not leave personal belongings near the poster boards.
5. Please proceed with each session according the chairperson's instructions.
   Note: The allotted time for each poster session presentation is 8 minutes; 5 minutes for the presentation and 3 minutes for discussion.

Presentation Date & Time	Reception & Set-up	Presentation / Q&A	Removal
January 31 (Thur)	All Posters Jan. 31 (Thur) 9：00～10：00	**13：20～13：44** P1－P3 **13：20～13：52** P8－P11, P16－P19 P24－P27, P32－P35 P40－P43 **13：55～14：27** P4－P7 **14：00～14：32** P12－P15, P20－P23 P28－P31, P36－P39 **14：05～14：37** P44－P46, P91	All Posters Feb. 1 (Fri) 15：00～16：30
February 1 (Fri)		**10：30～10：54** P47－P49, P54－P56 P84－P86 **10：30～11：02** P61－P64, P68－P71 P76－P79 **11：05～11：37** P50－P53, P57－P60 P87－P90 **11：10～11：34** P65－P67 **11：10～11：42** P72－P75, P80－P83	

Program at a Glance

	Jan. 29 (Tue)	Jan. 30 (Wed)			
	Nova Hall	ANNEX 2F Room "Ariake"	Main Bldg. 3F Room "Jupiter"	Main Bldg. 3F Room "Venus"	Nova Hall
9:00					
10:00					10 : 00 Explanation of the Test For Diplomates of JSTP 11 : 00
11:00					11 : 15 The Slide Conference 12 : 15
12:00	12 : 00	12 : 00 Editorial Board Meeting 13 : 00			12 : 15 Lunch 13 : 00
13:00				13 : 30 Revision Committee of Textbook 14 : 30	13 : 00 The 25th Slide Conference 17 : 30
14:00		13 : 30 Continuing Education Committee 14 : 30			
15:00	**JSTP/NTP Satellite Symposium**		15 : 00 **Board Meeting** 17 : 00		
16:00					
17:00					17 : 45 **IATP Lecture** 18 : 45
18:00	18 : 50	19 : 00 CEAH Board Meeting 21 : 00			

Program at a Glance

Jan. 31 (Thur)

ANNEX 1F Room "Subaru"

8 : 00~Reception opens

9 : 00 **Oral Presentation** O-01～O-08

10 : 40

10 : 50 **Special Lecture**

11 : 50

12 : 00 **Luncheon Seminar 1**

13 : 00

14 : 45 **Oral Presentation** O-09～O-11

15 : 25

15 : 30 **Symposium 1**

17 : 30

Main Bldg. 3F Room "Jupiter"

8 : 30 Open

9 : 00 Poster Set-up

10 : 00

10 : 00 Poster Exhibition

13 : 20

13 : 20 **Poster Discussion**

14 : 40

14 : 40 Poster Exhibition

15 : 30

18 : 00 **Fellowship Banquet**

20 : 00

ANNEX 2F Room "Hokuto"

12 : 00 Board Certification Committee

13 : 00

Feb. 1 (Fri)

ANNEX 1F Room "Subaru"

8 : 30 Open

9 : 00 **Oral Presentation** O-12～O-17

10 : 20

11 : 55 **Luncheon Seminar 2**

12 : 55

13 : 00 **Symposium 2**

14 : 40

14 : 45 Board of Councillors General Meeting

16 : 00

16 : 00～ Award Ceremony of Excellent Papers

16 : 15～ Closing Remarks

Main Bldg. 3F Room "Jupiter"

8 : 30 Open

9 : 00 Poster Exhibition

10 : 30

10 : 30 **Poster Discussion**

11 : 50

11 : 50 Poster Exhibition

15 : 00

15 : 00 Poster Removal

16 : 30

ANNEX 2F Room "Hokuto"

11 : 00 **IFSTP Conference**

14 : 00

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(Listed in random order)

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❑ Sponsor of Name Badge Holders

Ina Research Inc.

List of Chairpersons

Date	Time	Session		List of Chairpersons	Organization	Room
Jan. 31	10:50～11:50	Special Lecture		Takanori Harada	The Institute of Environmental Toxicology	ANNEX 1F Room "Subaru"
Jan. 31	15:30～17:30	Symposium 1		Dai Nakae	Tokyo Metropolitan Institute of Public Health	
				Kumiko Ogawa	National Institute of Health Sciences	
Feb. 1	13:00～14:40	Symposium 2		Makoto Shibutani	Tokyo University of agriculture and Technology	
				Munehiro Teranishi	Daiichi Sankyo Company, Limited	
Jan. 31	9:00～ 9:50 (O-01～0-05)	General Oral Presentation I	Biomarker	Yuji Oishi	Astellas Pharma Inc.	
				Ryo Fukuda	Takeda Pharmaceutical Company Limited	
	9:50～10:40 (O-06～O-08)		Animal models/ Nervous system	Hiroyuki Nakayama	The University of Tokyo	
				Satoru Hosokawa	Eisai Co., Ltd.	
	14:45～15:25 (O-09～O-11)		New animal models/ Carcinogenicity	Katsumi Imaida	Kagawa University	
Feb. 1	9:00～ 9:40 (O-12～O14)	General Oral Presentation II	Stem cells	Kinji Shirota	Azabu University	
	9:40～10:20 (O-15～O-17)		Animal models/ Alternatives	Satoshi Furukawa	Biological Research Laboratories, Nissan Chemical Industries, Ltd.	
Jan. 31	13:20～13:44 (P-01～P-03)	Poster Session I	Hepatobiliary system	Atsushi Watanabe	Asahi Kasei Corp.	Main Bldg. 3F Room "Jupiter"
	13:55～14:27 (P-04～P-07)		*In vitro* genotoxicity/ Kidney toxicity	Masami Suzuki	Fuji Gotemba Research Labs., Chugai Pharmaceutical Co., Ltd.	
				Akihito Shimoi	Ina Research Inc.	
	13:20～13:52 (P-08～P-11)		Prostate carcinogenesis	Naoki Yoshimi	University of the Ryukyus	
				Osamu Sawamoto	Research and Development Center, Otsuka Pharmaceutical Factory, Inc.	
	14:00～14:32 (P-12～P-15)		Large intestine	Itaru Yamaguchi	Kyowa Hakko Kirin Co., Ltd.	
				Yoshiaki Tagawa	Sanwa Kagaku Kenkyusho Co., Ltd.	
	13:20～13:52 (P-16～P-19)		Respiratory system	Kazuo Hakoi	Tokushima Research Center, Taiho Pharmaceutical Co., Ltd.	
				Mayumi Kawabe	DIMS Institute of Medical Science, Inc.	
	14:00～14:32 (P-20～P-23)		Nanomaterial toxicity	Hiroshi Onodera	Pharmaceuticals and Medical Devices Agency	
				Shigetoshi Aiso	Japan Bioassay Research Center	
	13:20～13:52 (P-24～P-27)		Subchronic toxicity studies	Shim-mo Hayashi	San-Ei Gen F.F.I.,Inc.	
				Shogo Iwasaki	Kyorin Pharmaceutical Co.,Ltd.	
	14:00～14:32 (P-28～P-31)		Nervous system	Mitsuru Kuwamura	Osaka Prefecture University.	
				Kosei Inui	Central Research Institute, Ishihara Sangyo Kaisha, Ltd.	
	13:20～13:52 (P-32～P-35)		Strain differences	Toshio Imai	National Cancer Center Research Institute	
				Toru Hoshiya	Bozo Research Center Inc.	
	14:00～14:32 (P-36～P-39)		Immune system/Bone marrow	Kiyokazu Ozaki	Setsunan University	
				Mikinori Torii	Drug Development Research Laboratories, Shionogi & Co., Ltd.	
	13:20～13:52 (P-40～P-43)		Case reports	Kazumoto Shibuya	The Nippon Institute for Biological Science	
				Kimiaki Hirakawa	Shin Nippon Biomedical Laboratories, Ltd.	
	14:05～14:37 (P-44～P-46, P91)		Case reports/ Carcinogenicity	Yumi Wako	Mitsubishi Chemical Medience Corporation	
				Osamu Fueki	Pharmaceuticals and Medical Devices Agency	

Date	Time	Session		List of Chairpersons	Organization	Room
Feb. 1	10:30～10:54 (P-47～P-49)	Poster Session II	Hepatocarcinogenesis	Keisuke Izumi	Institute of Health Biosciences The University of Tokushima Graduate School	Main Bldg. 3F Room "Jupiter"
	11:05～11:37 (P-50～P-53)		Renal toxicity/ mutagenicity	Hideki Wanibuchi	Osaka City University Medical School	
				Takashi Umemura	National Institute of Health Sciences	
	10:30～10:54 (P-54～P-56)		Urinary bladder	Yasushi Kurata	Meiji Seika Pharma Co., Ltd	
	11:05～11:37 (P-57～P-60)		Skin/Mammary gland carcinogenesis	Osamu Katsuta	Santen Pharmaceutical Co., Ltd.	
				Makoto Ueda	Nippon Shinyaku Co., Ltd.	
	10:30～11:02 (P-61～P-64)		Cell cycle/Signal transduction	Satoru Takahashi	Nagoya City University Graduate School of Medical Sciences	
				Takuji Tanaka	The Tohkai Cytopathology Institute : Cancer Research and Prevention	
	11:10～11:34 (P-65～P-67)		*In vitro* models	Jyoji Yamate	Osaka Prefecture University	
	10:30～11:02 (P-68～P-71)		Nanomaterial toxicity	Midori Yoshida	National Institute of Health Sciences	
				Hiroshi Satoh	FUJIFILM Corporation	
	11:10～11:42 (P-72～P-75)		Toxicological studies	Atsushi Shiga	Public Interest Incorporated Foundation BioSafety Research Center (BSRC)	
				Kochi Kakimoto	JapanTobacco Inc.	
	10:30～11:02 (P-76～P-79)		Implantatiom tests/ Animal ethics	Yoshimasa Okazaki	AnaPath GmbH	
				Shiro Fujihira	Safety Research Institute for Chemical Compounds Co., Ltd.	
	11:10～11:42 (P-80～P-83)		Musculoskeletal/ Thyroid	Kaori Miyata	Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.	
				Kaoru Toyosawa	Dainippon Sumitomo Pharma Co., Ltd.	
	10:30～10:54 (P-84～P-86)		Case reports	Toshihisa Fujiwara	Safety Research Laboratory, Mitsubishi Tanabe Pharma Corporation	
	11:05～11:37 (P-87～P-90)		Case reports/ Developmental toxicity	Hideshi Kaneko	Teijin Pharma Limited	
				Maki Kuwahara	The Institute of Environmental Toxicology	

Program

Special Lecture

Jan. 31 (Thur) 10 : 50～11 : 50 ANNEX 1F Room "Subaru"

Chair : Takanori Harada (The Institute of Environmental Toxicology)

"The Role of the Toxicologic Pathologist in the Post-genomic Era" 35

Dr. Robert R. Maronpot Raleigh, North Carolina

Luncheon Seminar 1

Jan. 31 (Thur) 12 : 00～13 : 00 ANNEX 1F Room "Subaru"

Chair : Dr. Michihito Takahashi (Pathology Peer Review Center)
Co-sponsor : Harlan Laboratories Japan, Co., Ltd.

"Target sites of tumor development in long-term carcinogenicity studies with mice"

Dr. Hijiri Iwata Harlan Laboratories, Ltd. Switzerland

Luncheon Seminar 2

Feb. 1 (Fri) 11 : 55～12 : 55 ANNEX 1F Room "Subaru"

Chair : Dr. Michihito Takahashi (Pathology Peer Review Center)
Co-sponsor : Huntingdon Life Sciences, Inc.

"Are environmental chemicals causing a decline in human reproductive health?"

Dr. Dianne Creasy Huntingdon Life Sciences

Symposium 1

Jan. 31 (Thur) 15 : 30～17 : 30 ANNEX 1F Room "Subaru"

Chair : Dai Nakae (Tokyo Metropolitan Institute of Public Health)
Kumiko Ogawa (National Institute of Health Sciences)

Symposium 2

Feb. 1 (Fri) 13 : 00～14 : 40 ANNEX 1F Room "Subaru"

Chair : Makoto Shibutani (Tokyo University of agriculture and Technology)
Munehiro Teranishi (Daiichi Sankyo Company, Limited)

General Oral Presentation I

Jan. 31 (Thur) 9 : 00～10 : 40 ANNEX 1F Room "Subaru"

Biomarker

Chair : Yuji Oishi (Astellas Pharma Inc.)
Ryo Fukuda (Takeda Pharmaceutical Company Limited)

Animal models/Nervous system

Chair : Hiroyuki Nakayama (The University of Tokyo)
Satoru Hosokawa (Eisai Co., Ltd.)

General Oral Presentation I

Jan. 31 (Thur) 14 : 45～15 : 25 ANNEX 1F Room "Subaru"

New animal models/Carcinogenicity

Chair : Katsumi Imaida (Kagawa University)

General Oral Presentation II

Feb. 1 (Fri) 9 : 00～10 : 20 ANNEX 1F Room "Subaru"

Stem cells

Chair : Kinji Shirota (Azabu University)

○Ayako shiraki[1,2], Hirotoshi Akane[1], Fumiyo Saito[3], Yumi Akahori[3], Shinya Imatanaka[3], Megu Itahashi[1,2], Liyun Wang[1], Takumi Ohish[1], Kazuhiko Suzuki[4], Kunitoshi Mitsumori[1], Makoto Shibutani[1]

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○Atsuko Murai[1], Akihiro Hirata[2], Manabu Takamatsu[3], Toshiya Kuno[3], Tokuma Yanai[1], Akira Hara[3]

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○Shotaro Yamano, Min Wei, Masaki Fujioka, Anna Kakehashi, Hideki Wanibuchi

Department of Pathology, Osaka city university graduate school of Medicine, Osaka, 545-8585, Japan

Animal models/Alternatives

Chair : Satoshi Furukawa (Biological Research Laboratories, Nissan Chemical Industries, Ltd.)

○Masatoshi Furukawa[1], Masumi Rokukawa[1], Takashi Sakakibara[1], Kota Ito[1], Shiro Fujihira[1], Takeo Hiraga[2], Hajime Kojima[3], Masao Matsuura[1]

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○Tadashi Itoh, Shinsuke Suzuki, Hisami Matsusita, Ken-ichi Yoshijima, Tsuneo Koike, Shin-ya Yabuki, Jun Imai, Hitoshi Kimura

Nihon Bioresearch Inc.

○Etsuko Fujii[1], YuJau Chen[2,3], Atsuhiko Kato[1], Takeshi Watanabe[1], Chie Kato[1], Akio Miyoshi[1], Shuji Hayashi[1], Koichi Matsubara[2,3], Yasuyuki Onishi[4], Masami Suzuki[1]

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Poster Session I

Jan. 31 (Thur) 13 : 20～14 : 40 Main Bldg. 3F Room "Jupiter"

Hepatobiliary system

Chair : Atsushi Watanabe (Asahi Kasei Corp.)

In vitro genotoxicity/Kidney toxicity

Chair : Masami Suzuki (Fuji Gotemba Research Labs., Chugai Pharmaceutical Co., Ltd.)
Akihito Shimoi (Ina Research Inc.)

Prostate carcinogenesis

Chair：Naoki Yoshimi（University of the Ryukyus）
Osamu Sawamoto（Research and Development Center, Otsuka Pharmaceutical Factory, Inc.）

Large intestine

Chair：Itaru Yamaguchi（Kyowa Hakko Kirin Co., Ltd.）
Yoshiaki Tagawa（Sanwa Kagaku Kenkyusho Co., Ltd.）

Respiratory system

Chair : Kazuo Hakoi (Tokushima Research Center, Taiho Pharmaceutical Co., Ltd.)
Mayumi Kawabe (DIMS Institute of Medical Science, Inc.)

Nanomaterial toxicity

Chair : Hiroshi Onodera (Pharmaceuticals and Medical Devices Agency)
Shigetoshi Aiso (Japan Bioassay Research Center)

Subchronic toxicity studies

Chair：Shim-mo Hayashi（San-Ei Gen F.F.I.,Inc.）
Shogo Iwasaki（Kyorin Pharmaceutical Co.,Ltd.）

○Saeko Onami[1)], Young-Man Cho[1)], Takeshi Toyoda[1)], Midori Yoshida[1)], Akiyoshi Nishikawa[2)], Kumiko Ogawa[1)]

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○Takeshi Toyoda[1)], Young-Man Cho[1)], Saeko Onami[1)], Yasuko Mizuta[1)], Jun-ichi Akagi[1)], Isamu Suzuki[1)], Akiyoshi Nishikawa[2)], Kumiko Ogawa[1)]

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○Kazuhiko Suzuki[1)], Eriko Taniai[2,3)], Reiko Morita[2,3)], Atsunori Yafune[2,3)], Kunitoshi Mitsumori[4)], Makoto Shibutani[2)]

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○Myoung Jun Kim[1)], Yong-Hoon Lee[1)], Duyeol Kim[1)], Sun Hee Park[1)], Hye-Yeong Lee[1)], Mi-Young Lee[1)], Mi Ju Lee[1)], Beom Seok Han[2)], Min Kwon[2)], Woo Chan Sohn[3)], Ji Hyeon Seok[4)], Jong Kwon Lee[4)], Jayoung Jeong[4)], Jin Seok Kang[5)], Jongkoo Kang[1,6)]

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Nervous system

Chair：Mitsuru Kuwamura（Osaka Prefecture University.）
Kosei Inui（Central Research Institute, Ishihara Sangyo Kaisha, Ltd.）

○Katsumi Soma, Naofumi Takahashi, Yuko Shimada, Aya Koyama, Maki Kuwahara, Yutaka Komatsu, Hideaki Fujie, Atsuko Motomura, Yasufumi Shutoh, Toshinori Yoshida, Nobuaki Nakashima, Hiroaki Aoyama, Takanori Harada

The Institute of Environmental Toxicology, Ibaraki, 303-0043, Japan

○Ryuichi Nakamura, Tomonari Nishimura, Taehito Ochiai, Shuichi Koda, Hiroyuki Ogasawara

Asubio Pharma Co., Ltd., Hyogo 650-0047, Japan

○Hirotoshi Akane[1)], Fumiyo Saito[2)], Ayako Shiraki[1,3)], Megu Itahashi[1,3)], Liyun Wang[1)], Kazuhiko Suzuki[1)], Kunitoshi Mitsumori[1)], Makoto Shibutani[1)]

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Strain differences

Chair：Toshio Imai（National Cancer Center Research Institute）
Toru Hoshiya（Bozo Research Center Inc.）

Immune system/Bone marrow

Chair：Kiyokazu Ozaki（Setsunan University）
Mikinori Torii（Drug Developmental Research Laboratories, Shionogi & Co., Ltd.）

Case reports

Chair：Kazumoto Shibuya（The Nippon Institute for Biological Science）
Kimiaki Hirakawa（Shin Nippon Biomedical Laboratories, Ltd.）

Case reports/Carcinogenicity

Chair：Yumi Wako（Mitsubishi Chemical Medience Corporation）
Osamu Fueki（Pharmaceuticals and Medical Devices Agency）

Poster Session II

Feb. 1 (Fri) 10 : 30～11 : 50 Main Bldg. 3F Room "Jupiter"

Hepatocarcinogenesis

Chair : Keisuke Izumi (Institute of Health Biosciences The University of Tokushima Graduate School)

Renal toxicity/mutagenicity

Chair : Hideki Wanibuchi (Osaka City University Medical School)
Takashi Umemura (National Institute of Health Sciences)

Urinary bladder

Chair：Yasushi Kurata（Meiji Seika Pharma Co., Ltd）

Skin/Mammary gland carcinogenesis

Chair：Osamu Katsuta（Santen Pharmaceutical Co., Ltd.）
Makoto Ueda（Nippon Shinyaku Co., Ltd.）

Cell cycle/Signal transduction

Chair：Satoru Takahashi（Nagoya City University Graduate School of Medical Sciences）
Takuji Tanaka（The Tohkai Cytopathology Institute : Cancer Research and Prevention）

P-61＊ Cellular Distribution of Proliferation, Apoptosis, and Cell Cycle-related Markers at the Early Stage of Tumor Promotion in Rat Two-stage Carcinogenesis Models 91

○Eriko Taniai[1,2], Atsunori Yafune[1,2], Reiko Morita[1,2], Megu Itahashi[1,2], Kazuhiko Suzuki[3], Kunitoshi Mitsumori[4], Makoto Shibutani[1]

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P-62＊ Expression Characteristics of Cell Cycle-related Proteins at the Early Stage of Tumor Promotion in Rat Two-stage Carcinogenesis Models 91

○Atsunori Yafune[1,2], Eriko Taniai[1,2], Reiko Morita[1,2], Hirotoshi Akane[1], Liyun Wang[1], Kazuhiko Suzuki[1], Kunitoshi Mitsumori[3], Makoto Shibutani[1]

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P-64＊ Activation of the Canonical Wnt Signaling Maintains Normal and Neoplastic Gastric Epithelial Cells in Undifferentiated and Proliferative States 92

○Akihiro Hirata[1], Yasuhiro Yamada[2], Hiroyuki Tomita[3], Toshiya Kuno[3], Atsuko Murai[4], Tetsuya Tsukamoto[5], Masami Yamamoto[5], Akira Hara[3]

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In vitro models

Chair：Jyoji Yamate（Osaka Prefecture University）

P-65 Soluble VEGFR-3 Decoy but not SATB1 siRNA Suppresses Metastasis in a Highly Metastatic Mouse Mammary Cancer Model 92

○Masa-Aki Shibata[1], Junji Morimoto[2], Eiko Shibata[3], Shigekazu Fujioka[1], Mariko Harada-Shiba[3]

[1]Lab. Anat & Histopathol., Osaka Health Sci. Univ., Osaka, 530-0043, Japan, [2]Lab. Animal Center, Osaka Med. Coll., Osaka, 599-8686, Japan, [3]Dept. Mol. Innovation in Lipidol., Natl. Cerebral & Cardiovasc. Center Res. Inst., Osaka, Japan

P-66 Autophagy signalings in human breast cancer cell MCF-7 were up-regulated by allil isothiocyanate (AITC) and induced the cell death. 93

○Makoto Asamoto, Aya Naiki-Ito, Satoru Takahashi

Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, 1-Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan

P-67＊ Epithelial-mesenchymal Transition in Mice Bearing Human Lung Cancer Cell Lines. 93

○Yoshika Yamakawa[1], Takahisa Noto[1], Mihoko Ono[1], Kenjiro Tsubota[1], Masamichi Mori[2], Yoko Ueno[2] Shunji Nakatsuji[1], Masahiro Matsumoto[1]

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Nanomaterial toxicity

Chair : Midori Yoshida (National Institute of Health Sciences)
Hiroshi Satoh (FUJIFILM Corporation)

Toxicological studies

Chair : Atsushi Shiga (Public Interest Incorporated Foundation BioSafety Research Center (BSRC))
Kochi Kakimoto (JapanTobacco Inc.)

Implantatiom tests/Animal ethics

Chair：Yoshimasa Okazaki（AnaPath GmbH）
Shiro Fujihira（Safety Research Institute for Chemical Compounds Co., Ltd.）

Musculoskeletal/Thyroid

Chair：Kaori Miyata（Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.）
Kaoru Toyosawa（Dainippon Sumitomo Pharma Co., Ltd.）

Case reports

Chair：Toshihisa Fujiwara（Safety Research Laboratory, Mitsubishi Tanabe Pharma Corporation）

Case reports/Developmental toxicity

Chair：Hideshi Kaneko（Teijin Pharma Limited）
Maki Kuwahara（The Institute of Environmental Toxicology）

Abstracts

Special Lecture

Symposium

Oral Presentation

Poster Session

Special Lecture

The Role of the Toxicologic Pathologist in the Post-genomic Era

Dr. Robert R. Maronpot

Raleigh, North Carolina

An era can be defined as a period in time identified by distinctive character, events, or practices. We are now in the genomic era.

The pre-genomic era

There was a pre-genomic era. It started many years ago with novel and seminal animal experiments, primarily directed at studying cancer. It is marked by the development of the two-year rodent cancer bioassay and the ultimate realization that alternative approaches and short-term animal models were needed to replace this resource-intensive and time-consuming method for predicting human health risk. Many alternatives approaches and short-term animal models were proposed and tried but, to date, none have completely replaced our dependence upon the two-year rodent bioassay. However, the alternative approaches and models themselves have made tangible contributions to basic research, clinical medicine and to our understanding of cancer and they remain useful tools to address hypothesis-driven research questions. The pre-genomic era was a time when toxicologic pathologists played a major role in drug development, evaluating the cancer bioassay and the associated dose-setting toxicity studies, and exploring the utility of proposed alternative animal models. It was a time when there was shortage of qualified toxicologic pathologists.

The genomic era

We are in the genomic era. It is a time when the genetic underpinnings of normal biological and pathologic processes are being discovered and documented. It is a time for sequencing entire genomes and deliberately silencing relevant segments of the mouse genome to see what each segment controls and if that silencing leads to increased susceptibility to disease. What remains to be charted in this genomic era is the complex interaction of genes, gene segments, post-translational modifications of encoded proteins, and environmental factor that affect genomic expression. In this current genomic era, the toxicologic pathologist has had to make room for a growing population of molecular biologists. In this present era newly emerging DVM and MD scientists enter the work arena with a PhD in pathology often based on some aspect of molecular biology or molecular pathology research. In molecular biology, the almost daily technological advances require one's complete dedication to remain at the cutting edge of the science. Similarly, the practice of toxicologic pathology, like other morphological disciplines, is based largely on experience and requires dedicated daily examination of pathology material to maintain a well-trained eye capable of distilling specific information from stained tissue slides - a dedicated effort that cannot be well done as an intermezzo between other tasks. It is a rare individual that has true expertise in both molecular biology and pathology. In this genomic era, the newly emerging DVM-PhD or MD-PhD pathologist enters a marketplace without many job opportunities in contrast to the pre-genomic era. Many face an identity crisis needing to decide to become a competent pathologist or, alternatively, to become a competent molecular biologist. At the same time, more PhD molecular biologists without training in pathology are members of the research teams working in drug development and toxicology. How best can the toxicologic pathologist interact in the contemporary team approach in drug development, toxicology research and safety testing? Based on their biomedical training, toxicologic pathologists are in an ideal position to link data from the emerging technologies with their knowledge of pathobiology and toxicology. To enable this linkage and obtain the synergy it provides, the bench-level, slide-reading expert pathologist will need to have some basic understanding and appreciation of molecular biology methods and tools. On the other hand, it is not likely that the typical molecular biologist could competently evaluate and diagnose stained tissue slides from a toxicology study or a cancer bioassay.

The post-genomic era

The post-genomic era will likely arrive approximately around 2050 at which time entire genomes from multiple species will exist in massive databases, data from thousands of robotic high throughput chemical

screenings will exist in other databases, genetic toxicity and chemical structure-activity-relationships will reside in yet other databases. All databases will be linked and relevant information will be extracted and analyzed by appropriate algorithms following input of the latest molecular, submolecular, genetic, experimental, pathology and clinical data. Knowledge gained will permit the genetic components of many diseases to be amenable to therapeutic prevention and/or intervention. Much like computerized algorithms are currently used to forecast weather or to predict political elections, computerized sophisticated algorithms based largely on scientific data mining will categorize new drugs and chemicals relative to their health benefits versus their health risks for defined human populations and subpopulations. However, this form of a virtual toxicity study or cancer bioassay will only identify probabilities of adverse consequences from interaction of particular environmental and/or chemical/drug exposure(s) with specific genomic variables. Proof in many situations will require confirmation in intact *in vivo* mammalian animal models. The toxicologic pathologist in the post-genomic era will be the best suited scientist to confirm the data mining and its probability predictions for safety or adverse consequences with the actual tissue morphological features in test species that define specific test agent pathobiology and human health risk.

Symposium

1 & 2

SS-1-1 Transgenic Animals for Evaluation of Carcinogenicity

○Gary A. Boorman, DVM, PhD[1)], Victoria Laast[2)]

[1)]Covance Laboratories Inc., Chantilly Virginia, USA,
[2)]Covance Pharmaceutical R&D (Shanghai) Co., Ltd., Shanghai, China

Two-year rodent carcinogenesis bioassay has been used to evaluate carcinogenetic potential of chemicals for decades. Some chemicals and prospective drugs demonstrate carcinogenic potential in rodents while many do not cause increased cancer rates even with two years of exposure. Most known human carcinogens cause cancer in one or more sex/species combination providing some confidence about the relevance of this test system. Generally, chemicals that do not increase tumors rates in rodents with long-term exposure are considered safe while those causing increased cancer rates are considered potentially hazardous. However, phenobarbital exposure increases liver tumors in both rats and mice while humans treated with phenobarbital for decades have no increase in tumors. There are many other examples of rodent specific carcinogens.

While two-year rodent carcinogenicity studies have been considered the gold standard for compound evaluation, these studies also have limitations. Rodent carcinogenicity studies take three or four years to complete, are costly and utilize hundreds of animals. More importantly considering the resources utilized, these studies provide very little information on potential mechanisms for tumor increases that may result in additional years of effort to determine potential human relevance of positive study findings.

Development of genetically modified rodents especially those with alterations found in pathways leading to cancer attracted the attention of the scientific community as an alternative to two-year rodent carcinogenesis bioassays. Hundreds of chemicals have been evaluated in 6-month transgenic mouse studies. More than twenty years of evaluating chemicals in transgenic mouse models and multiple symposia evaluating the results is leading to a greater understanding of which models best detect potential carcinogens. These models use fewer animals and reduce the, cost time to complete. The most widely used models are the RasH2, Trp53+/-, and Tg.AC models.

Scientists and regulatory agencies have become comfortable with transgenic models that may replace the mouse two-year bioassay. An emerging challenge is long-term toxicity and carcinogenicity evaluation of human proteins, gene products and other novel biologicals that offer promise for the treatment of human diseases. There is little experience on the relevance of two-year rodent for this newer types of therapeutics. This presentation provides an overview of the advantages and disadvantages of various bioassays. The newer emerging therapeutics that are increasing a part of the toxicologic pathologist daily routine will require that models to assess toxicity, potential carcinogenicity and risk assessment also evolve to meet this challenge.

SS-1-2 Contemporary NTP Toxicity and Carcinogenicity Testing Strategies

○Susan A. Elmore, MS, DVM, DACVP, DABT, FIATP

National Toxicology Program/National Institute of Environmental Health Sciences

The National Toxicology Program (NTP) was established in 1978 as a cooperative interagency federal resource designed to evaluate environmental agents of public health concern. To date, over 600 chronic studies have been performed as well as thousands of toxicology studies. The NTP uses rodent models for studies and protocols specifically designed to fully characterize the toxic potential of selected chemicals. The scope and types of studies performed are flexible and dictated to a large degree by the data needs for the specific test articles nominated for study. In addition to animal bioassays, genetic studies, chemical disposition and toxicokinetic studies as well as toxicogenomic studies are used to fully characterize the chemical effects. Some of the most recent NTP efforts and updates have occurred in the areas of 1) perinatal exposure, 2) neuropathology, 3) immunotoxicology, 4) reproductive and developmental studies, and 5) high throughput screening.

Toxicology/Carcinogenicity studies generally fall into three main categories: Fourteen day dose range-finding prechronic toxicity studies, 90-day subchronic bioassays to confirm doses for the chronic study and to determine potential organ toxicity, and 2-year toxicology and carcinogenesis studies. Perinatal exposure in the rat is one of the newest additions to these study protocols. Perinatal exposure occurs during gestation (in utero via the placenta) and lactation (via mother's milk). Since chemical exposure during the perinatal period occurs during critical periods of in-utero and postnatal development, this can result in differences in toxicity and/or carcinogenicity as compared to exposure starting only from adulthood.

The evaluation of reproductive toxicity is another focus of the NTP. Reproductive Assessment by Continuous Breeding (RACB) is a study that has been used by the NTP for over 15 years, and the design and endpoints have constantly evolved. This is a two-generation study, using mostly rats, and is designed to identify potential hazards and toxic effects on male and/or female reproduction, to characterize that toxicity, and to define the dose-response relationships for each compound. Developmental endpoints, lesions and malformations in offspring are also characterized. In 2011 the NTP began the pathology evaluation of tissues for RACB studies. The Modified One-Generation (MOG) studies were begun in 2012 and are primarily developmental studies that have evolved from the NTP's multi-generational RACB studies. These studies produce cohorts that may be used to populate 90-day, 2-year, immunotoxicity, neurotoxicity or RACB studies.

In 2007 the NTP began a focused "enhanced histopathology" evaluation of immunotoxicity studies. The testing strategies for immunology include tests for immunomodulation (studies of altered hematopoietic or immunologic events associated with exposure of humans and animals to chemicals) and hypersensitivity (studies of immune-mediated hypersensitivity resulting from exposure to environmental chemicals or therapeutics). Enhanced histopathology uses the evaluation of individual lymphoid organ compartments combined with descriptive rather than interpretive diagnostic terminology to report the lesions.

To improve our neuropathology evaluations, the number of sections of brain for routine histological evaluation was increased from 3 to 7 in 2012. There is now a focus on the correlation of brain anatomy with functional endpoints. The Functional Observational Battery (FOB) is also used when needed to evaluate various neurobehavioral and activity related parameters.

"Tox21" is the High Throughput Screening (HTS) Initiative that was begun in 2011 and is part of the NTP's new toxicology testing strategy. The HTS program approach to toxicological testing screens for mechanistic targets active within cellular pathways considered critical to adverse health effects such as carcinogenicity, reproductive and developmental toxicity, genotoxicity, neurotoxicity, and immunotoxicity in humans. The NTP recognized that the dramatic technological advances in molecular biology and computer science offered an opportunity to use in vitro biochemical- and cell-based assays and non-rodent animal models for toxicological testing. The goal of this initiative is to move toxicology from a predominately observational science to a predictive science focused on a broad inclusion of target-specific, mechanism-based, biological observations.

SS-1-3 Genomics Strategies in National Toxicology Program Toxicity and Carcinogenicity Testing

○Mark J. Hoenerhoff, DVM, PhD, DACVP

Investigative Pathology Group, Cellular and Molecular Pathology Branch
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health

The development of toxicity or carcinogenicity in mammalian models following exposure to various compounds provides evidence that humans exposed to these or similar compounds may be at an increased health risk. While toxic and neoplastic lesions in rodents may be morphologically similar to those that may develop in humans, the underlying mechanisms of lesion development, including carcinogenesis, are often unknown and may be different between the species. Therefore, the relevance of an animal tumor response to a human health hazard should also consider their underlying comparative molecular pathogenesis. The molecular mechanisms of carcinogenesis are complex, and involve multiple genetic and epigenetic events. With a better understanding of the underlying mechanisms leading to a carcinogenic response, one may extrapolate a human health hazard from rodent bioassay data with greater confidence.

Genomic technologies provide a powerful tool to characterize the molecular changes that occur during toxicity and carcinogenesis. By evaluating changes across the genome of thousands of genes simultaneously, one can gain great insight into the major genetic pathways involved in a toxic or cancer response to chemical exposure. Cancer is a complex disease, involving the interplay between altered growth pathways, oncogenes, tumor suppressor genes, hormonal influences, and epigenetic factors, among others. Alteration in the expression of any of these pathways provides information on pathogenesis, targets for diagnosis or therapeutic intervention, in addition to providing information for the prediction of hazardous or carcinogenic substances. Mechanistic genomic strategies identify and define mechanisms of toxicity or tumorigenesis of hazardous exposures in rodent models, providing data on their potential effects in human populations.

Traditionally, the National Toxicology Program (NTP) evaluates compounds for their potential human carcinogenic risk through the rodent bioassay using pathology endpoints. Concurrent with the gold-standard pathology assessment, the Investigative Pathology Group in the Cellular and Molecular Pathology Branch investigates the molecular end points of carcinogenicity in the rodent bioassay for alterations in cancer genes and epigenetic events associated with human cancer. These include targeted and global gene expression profiling, global methylation and microRNA profiling, DNA mutation analysis, and protein expression analysis, retrospectively through tissue from the NTP rodent bioassay, or in in vitro model systems. These molecular studies have identified several genetic alterations in chemically induced rodent neoplasms that are important in human cancer, and have added value to the NTP bioassay by providing relevant comparative molecular endpoints that aid in risk assessment.

SS-1-4 Regulatory Requirements, Advantages, Disadvantages, and Relevance of Dogs, Monkeys and Small Models in Toxicity Testing

○Klaus Weber, PhD, DVM, MSBiol

AnaPath GmbH, Buchsweg 56, 4625 Oberbuchsiten, Switzerland

Applied research and safety testing includes testing in genetics, developmental biology, biomedical research, xenotransplantation, drug and toxicology testing. For chemicals, a number of OECD guidelines determine the different test conditions including the appropriate test species (daphnia, enchytraeids, fish, bees, earthworm, rabbits, rodents, non-rodents etc).

The basics on testing for pharmaceuticals are ruled in directives an guidelines. For safety testing on pharmaceuticals, the choice of species is ruled namely in the M3 Guidance for Industry (Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals), ICH S4 (Duration of chronic toxicity testing in animals (Rodent and Nin Rodent Toxicity Testing), ICH S1B (Testing for carcinogenicity of Pharmaceuticals), and ICH S6 Preclinical Safety Evaluation of biotechnology-derived pharmaceuticals.

All these guidelines define criteria on species requirements that can be summarized as a need in the most appropriate animal species gives the most important information. This information is obtained by short term mechanistic studies and should under classical conditions determine the appropriate species and dose for an oncogenicity study. The testing should be performed generally in a rodent and a non-rodent species. A long-term carcinogenicity study in two rodent species is still considered acceptable, whereby the rat is the species of choice, and the mouse or another rodent species could be justified.

An alternative is described in the ICH S1B regarding the short and medium term rodent assays when the data indicate that the alternative testing could provide information that is not likely obtained from traditional testing. Namely, the rasH2 model is currently under use. Other models are helpful in mechanistically explanations (Tg.AC, Pim1, XPA etc).

The conventional approaches may not be appropriate in testing biologicals that is mainly due to biological properties and structures, as well as species specificity. The standard test species are often not appropriate. The relevant species is one '...in which the test material is pharmacologically active due to the expression of the receptor or an epitope…'. For monoclonal antibodies, there should be a similar expression of the desired than for human tissues. For hormones, mediators or cytokines, the test item should be functionally active in the test species. It may be possible to justify the use of only one species for subsequent long-term toxicity studies. When no relevant species exists, the use of relevant transgenic animals expressing the human receptor is the alternative (e.g. for Alzheimer's disease, Sickle cell disease, Poliovirus etc.).

It should be considered, that the discussion of the appropriate strain models for toxicity studies in rodents and non-rodents is ongoing and published. Differing data on these strains, depending on their source, feed and housing conditions have been made available. Generally, before starting a toxicology program in rodents, it should be considered that long term an oncogenicity studies will be performed for the majority of compounds. Survival, body weight development, the incidence, type and time of onset of age-dependent lesions and neoplasms and some special considerations of the rodent and non-rodent strain selected may be decisive. Therefore, knowledge of the historical background data is needed. An overview on the most currently used rodent and non-rodent strains will be presented. The use of primates and transgenic animal models will be explained on examples.

SS-2-1 Environmental Response Study Using Model Animal System

○Dr. Masayuki Yamamoto

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Our body equips a cytoprotective system that senses environmental insults and activates cellular defense enzyme genes. Toxic chemicals (often electrophiles) and reactive oxygen species (ROS) activate expression of detoxifying and antioxidant genes through antioxidant/electrophile responsive element (ARE/EpRE) and transcription factor Nrf2 is essential for the coordinated induction of cellular defense enzymes through ARE/EpRE. This notion is best demonstrated in animal models, showing that Nrf2-null mice are sensitive to a wide variety of electrophiles and ROS.

Keap1 is a component of ubiquitin-E3 ligase and degrades Nrf2 constitutively. Electrophiles and ROS liberate Nrf2 from the repression by Keap1 and provoke nuclear accumulation of Nrf2. Keap1 possesses reactive cysteine residues that act as sensors for xenobiotic and oxidative stresses. We refer this system to as the Cysteine Code. Mouse and zebrafish models demonstrate that multiple sensor functions reside within Keap1. The Hinge and Latch model proposed for the Keap1-Nrf2 system describes the mechanism of nuclear accumulation of Nrf2 in a Cul3-Keap1 E3 ubiquitin ligase-dependent manner. We have verified this model through structure biology, mouse/zebrafish genetics, and human cancer analyses.

In human cancers missense mutations have been identified in *KEAP1* and *NRF2* genes. These mutations disrupt the KEAP1-NRF2 complex and result in constitutive activation of NRF2. Elevated expression of NRF2 target genes confers advantages on cancer cells. Transgenic mouse models provide evidence that mutated form of Keap1 analogous to cancer genotypes lose the ability to repress Nrf2 *in vivo*. Thus, the Keap1-Nrf2 system opens a new avenue to the understanding of the signal transduction and regulatory processes underlying the stress response and cancer progression.

SS-2-2 Development of a humanized liver model using immunodeficient mice

○Dr. Hiroshi Suemizu

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The liver, which is the central organ to perform detoxification, drug metabolism, and excretion *in vivo* is an indispensable research subject for drug discovery and development. However, since there are species differences in the characteristics of the drug-metabolizing enzymes between human and rodent such as a mouse and rat, there is a limit to be used the rodents as a substitute for human. Since we also cannot fully address with rodents for the study of viral hepatitis that is highly host-specific for some primates, such as chimpanzees, and humans, we have felt the limited usefulness of mice and rats as laboratory animals. Experimental animal models having reconstituted liver with human hepatocytes (chimeric mice) were developed to overcome such problems more than 10 years ago. However, the chimeric mice have not been widely used in the research fields yet. We have developed an immunodeficient NOG mouse that is expressing herpes simplex virus thymidine kinase type 1 gene (HSVtk) in specifically in the liver (TK-NOG), and established an inducible liver injured model that the liver is selectively destructed by administration of ganciclovir (GCV). When commercially available human hepatocytes were transplanted into the liver of GCV administered TK-NOG mouse via the portal vein from the spleen, the transplanted cells were successfully engrafted without exogenous drug treatment. The reconstituted humanized liver could be stably maintained in these mice with a high level of synthetic function for a prolonged period (8 months). The 'humanized liver' was shown to be a mature and functioning "human organ" that had zonal position-specific enzyme expression and a global gene expression pattern representative of mature human liver; and could generate a human-specific profile of drug metabolism. This novel *in vivo* system provides an optimized platform for studying human liver physiology, including drug metabolism, toxicology, or liver regeneration.

SS-2-3 Hair Follicle Aging with Genotoxic Stress through Stem Cell Regulation

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Multicellular organisms senesce with the expression of various aging phenotypes characterized by functional tissue decline and organismal changes with decreased regenerating capabilities. Hair loss and hair graying are typical aging phenotypes in mammals, but the underlying mechanisms of aging are still largely elusive in most tissues. In recent decades, some signaling pathways which determine organismal lifespan and molecules responsible for progeroid syndromes have been identified in some organisms, but the underlying cellular mechanisms of aging-associated tissue decline and diseases are still largely unknown. We have studied the mechanisms of aging-associated hair graying and hair loss by focusing on adult stem cells, which have been identified in many tissues and play key roles in tissue turnover and homeostasis. We analyzed aging-associated changes in hair follicle stem cells (HFSCs) and in melanocyte stem cells (McSCs), of which coordinated activation are essential for hair follicles to grow pigmented hair every hair cycle. Our studies revealed that these stem cells show sustained DNA damage response with aging. The fate analysis of those stem cells with sustained DNA damage showed that the defective renewal of HFSCs and McSCs cause hair loss and hair graying, typical aging phenotypes. Chronological analysis of McSCs demonstrated that McSCs differentiate into pigment-producing melanocytes within the niche without renewing themselves under excessive genomic stress or with aging. Strikingly, HFSCs with sustained DNA damage switch their fate and locally differentiate into epidermal keratinocytes within the niche to be desquamated from the skin surface. The stem cell fate changes with aging and the underlying mechanisms of tissue aging will be illustrated and discussed.

SS-2-4 New International Development of Alternatives to Animal Experiments (3Rs)

○Dr. Tsutomu Miki Kurosawa, DVM, M.Phil, Ph.D, DVCS, DJCLAM, FBB

Osaka University Medical School (Past-President of JSAAE)

Alternatives to animal experiments is becoming one of International "must" for animal experimentation as 3Rs tenet. In early 1980s, the research group of alternatives was incorporated by Prof. Sugawara at Kyoto University Medical School and then Japanese Society for Alternatives to Animal Experiments was founded in 1989. Its journal "AATEX" has published in 1990. The annual meeting has been held regularly and the 25th meeting has been just held in Tokyo. JSAAE is regularly present World Congress of Alternatives and we have invited the WC6 2007 in Tokyo. JaCVAM which was founded with support of JSAAE is getting activated in particular the international relations. Several new test methods were already proposed to OECD Testing Guideline by JaCVAM as a member of ICATM (International Cooperation on Alternative Test Methods) with enormous information exchange. Although the activities in Replacement of 3Rs are significant with many researchers developing new testing methods, Reduction and Refinement are not so active in Japan. The result of JSAAE international activities and JaCVAM international information exchange with the prospect of future 3Rs will be discussed.

The year of 2010 was an epoch making year in Alternatives research. Firstly OIE (World Animal Health Organization)published laboratory animal welfare code as a first international standard at the general assembly held in May 2010. The code is based upon 3Rs and veterinary care is emphasized. Secondly, EU Parliament amends laboratory animal protection directive in October 2010. The volume of Directive is enormous but more importantly the animal experimentation is restricted for animal protection. The experiment on Apes is practically prohibited and the research on NHP is restricted. Other remarkable revision include that it asks member countries to establish regal regulations and they should be enforced by January 2013. The stringency of this directive appears in the amendment of Animals Act of UK which is believed to be the most stringent animal restriction regulation should have been revised. Finally the ILAR Guide, a bible for animal experiment in US was revised January 2011 after 14 years passed since the last revision which was translated to more than 11 languages was published. The Guide is a prerequisite of application for government research fund and is a basic literature for AAALAC International accreditation. The recent application to AAALAC by Japanese farms may be influenced by the revision. The Guide emphasizes the importance of veterinary care as refinement. Furthermore, OECD prohibits LD50 of general toxicity test and approves many alternative testing methods. Eye irritation and corrosion test is re-revised October 2012 to emphasize Replacement and Refinement.

Oral Presentation

O-01～O-17

O-01＊ Investigation of renal biomarkers in the urine, blood, and renal tissues in rat models of drug-induced nephrotoxicity.

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<Objective> Recently, renal biomarkers (BMs) were used clinically and non-clinically in early detection of renal injury, but there were a few reports that compared changes of BMs in the urine, blood, and renal tissues in detail. In this study, we investigated the time course of BMs changes in the urine, blood and renal tissues in rat models of Gentamicin- or Cisplatin- induced nephrotoxicity.
<Material and method> Gentamicin was dosed intraperitoneally to 6-week old male Crl:CD(SD) rats for 4 days at a dose level of 240 mg/kg. Cisplatin was dosed intraperitoneally once at a dose level of 5 mg/kg. Rats were necropsied on Day 2 (the next day of initial dosing), 5, or 12. At each time point, histopathological examination of kidney and immunohistochemical examination for Kim-1, NGAL, Clusterin, Cystatin C, and β2-microglobulin (β2MG) were performed, and these BMs in urine and blood were measured.
<Result> [On Day 2] In the Gentamicin-treated group, urinary NGAL, Cystatin C, and β2MG, and blood β2MG were increased, while no histopathological finding was observed in the kidney. In the Cisplatin-treated group, nuclear change in the proximal tubular epithelium in the kidney was observed histopathologically, and immunohistochemical expressions of Kim-1 and NGAL in the kidney, and urinary Kim-1 were also increased. [On Day 5 and 12] In both groups, degeneration/necrosis and regeneration of renal tubular epithelium were observed, and immunohistochemical expressions of all renal BMs except for β2MG were changed. Moreover, all renal BMs in the urine and blood were increased except for blood NGAL and Cystatin C.
<Conclusion> In both Gentamicin and Cisplatin treated-groups, renal BMs were changed earlier than injurious histopathological findings. In the Gentamicin-treated group, the increase in Cystatin C and β2MG, which were excreted into urine by inhibition of absorption, were observed from Day 2 and considered to reperesent functional disorder. In the Cisplatin-treated group, the increase in Kim-1, which expression correlated with renal tubular injury, was observed from Day 2 and considered to represent slight renal injury. From these results, the use in combination of several renal BMs with different characters was considered to be helpful for early detection of renal toxicity and provide useful information to understand toxicity mechanism.

O-02＊ Surfactant protein D is a useful biomarker for cyclophosphamide-induced interstitial pneumonia in rats

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<Background> Interstitial pneumonia (IP), a progressive and life-threatening interstitial lung disease, is one of the serious adverse effects of anticancer drugs. It is desirable to predict and avoid the risk of IP during the early stages of drug development processes. In nonclinical toxicity studies, there are few useful biomarkers that are used to detect IP. However, in clinical practice, surfactant protein D (SP-D) has been widely used to detect IP. In this study, we assessed SP-D levels in the serum, bronchoalveolar lavage fluid (BALF), and pleural effusion (PE) in rats with cyclophosphamide (CPA)-induced IP.
<Materials and Methods> CPA was intraperitoneally administered to 6-week-old male Crl:CD(SD) rats at 10 mg/kg/day daily for 3 weeks. Lung, blood, BALF, and PE samples were collected 1, 2, and 3 weeks after the first CPA injection and 7 and 10 days after the last CPA injection. Histopathological examinations of the lung were performed to confirm the stage of IP. SP-D levels in the serum, BALF, and PE were measured by EIA (rat/mouse SP-D kit YAMASA EIA, Chiba, Japan). The traditional biomarkers of lung injury, including the WBC count and LDH activities in blood, were also examined.
<Results> Macrophage infiltration into the alveolar lumen, hyperplasia of type II alveolar cells, and thickening of the alveolar wall were observed from 2 weeks after the first CPA injection. These changes became marked 3 weeks after the first CPA injection. SP-D levels in the serum and BALF increased in accordance with IP progression, while the levels in PE did not change. During the 3-week CPA treatment, the WBC count decreased and LDH activities did not change in the blood.
<Conclusion> SP-D levels in the serum and BALF increased in the subacute stage of IP characterized by the proliferation of type II alveolar cells. The changes of in WBC count and LDH activities in the blood did not correlate with IP until 3 weeks during CPA treatment. Thus, we conclude that serum and BALF SP-D are useful biomarkers for anticancer drug-induced IP in rats.

O-03 Identification of MicroRNA Markers for Predicting Bladder Carcinogenicity of Chemicals in Rats

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MicroRNA (miRNA) has been known to be involved in development of various cancers. The purpose of the present study is to examine expression of miRNA in rat bladder carcinogenesis. miRNA microarray analyses of 12 N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN)-induced rat bladder cancers revealed that 6 miRNAs (miR-30d, miR-125a, miR-125b, miR-126, miR-143 and miR-203) were commonly down-regulated in all cancers compared to control bladder urothelium. Two of above 6 miRNAs (miR-125a and miR-125b) were found to be also down-regulated in dimethylarsinic acid (DMA)-induced bladder cancers. Then, expression levels of the two miRNAs were evaluated in bladder urothelium of rats treated with 7 bladder carcinogens (BBN, DMA, 2-acetylaminofluorene, sodium o-phenylphenol, phenethyl isothiocyanate, benzyl isothiocyanate, uracil) and 3 non-bladder carcinogens (diethylnitrosamine, N-ethyl-N-hydroxyethylnitrosamine, 1.2-dimethyhydrazine), respectively, for 4-weeks. Both miR-125a and miR-125b was found to be down-regulated in all urothelium treated with any of bladder carcinogens but not in any of non-bladder carcinogens. These findings indicate that miR-125a and miR-125b are potential early markers of rat bladder carcinogenesis.

O-04 A serum tumor marker for preclinical trials of rat lung cancer model

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Lung cancer is the leading cause of cancer death worldwide. The 5-year survival rate for lung cancer is only 15%. This is mainly because most lung cancer patients are diagnosed at advanced stages. Animal models can be used to explore screening and therapeutic approaches are clearly desirable. We established transgenic (Hras250) rats in which expression of a human *Ha-ras*^{G12V} oncogene is regulated by the Cre/loxP system. Targeted lung activation of the transgene was accomplished by injection of Cre recombinase-carrying adenovirus into the lung. Four weeks after injection, grossly visible nodules were observed throughout the lung in all Hras250 rats. Histological examination determined that these nodules were adenocarcinomas and squamous cell carcinoma. The establishment of the rat lung cancer serum biomarker will greatly facilitate both basic and preclinical research on lung cancer. We utilized this model to identify biomarkers to detect lung cancer. Serum levels of N-ERC are significantly higher in rats bearing lung cancer than in controls. Serum N-ERC measurements might be useful to identify rats with tumors or to monitor response to therapy.

O-05＊ Bone Formation with the Marrow Tissue Observed in the Liver of KK-A^y Mice Used for NASH Model Examination Experiment

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KK-A^y mice feed a high fatty choline-deficient L-amino acid-defined [CDAA (HF)] diet and a high fatty choline-supplemented L-amino acid-defined [CSAA (HF)] diet to make NASH models in our study. Occasionally, bone formation with the marrow tissue was observed in the liver of KK-A^y mice fed these diets. This report describes histopathological characteristics and mechanisms of the lesion in detail.

Male KK-A^y mice (7 weeks old) were given following 3 different diets for 9~12 weeks: normal diet (CRF-1), CDAA (HF) diet, and CSAA (HF) diet. There were 21, 26, and 22 mice in the CRF-1, CDAA (HF), and CSAA (HF) groups, respectively. The animals were necropsied after the food consumption period for histopathological examinations of their livers.

Macroscopically, livers showed discoloration and swellings. In the CRF-1 group, centrilobular microvesicular steatosis of the hepatocytes was found in 20 animals, and focal inflammatory cell infiltration was found in some animals. In the CDAA (HF) and CSAA (HF) groups, these changes were increased in their intensity, and inflammatory cell infiltration and microvesicular/macrovesicular steatosis of the hepatocytes occurred diffusely in all 48 animals. Furthermore, in 28 out of 48 animals there was the formation of bone tissues. The bone tissues showed lamella structures and contained osteocytes inside, and osteoblasts lined close to the bone tissues. Also, 15 out of the 28 animals had bone-marrow tissues around the bone tissues. The bone marrow tissues composed of hematopoietic cells and vascular structures, which were different from the existing ones.

It was considered that the formation of bone tissues were associated to the increase of inflammation and steatosis of the hepatocytes since it was not found in the CRF-1 group but found in the CDAA(HF) and CSAA(HF) groups. The bone marrow tissues were also associated to the bone formation since they were only formed around the bone tissues. Further research will be done about the mechanism of the lesion in the future.

O-06＊ Clinical course and pathology of multiple sclerosis model in rats

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【目的】多発性硬化症 (MS) は中枢神経を侵す炎症性脱髄疾患であり、再発・寛解を繰り返す自己免疫疾患として知られている。病理組織学的には、はじめに中枢神経の小静脈周囲に単核細胞浸潤が認められ、その後脱髄が認められて病態が悪化していくと報告されている。臨床の病態を正確に反映した動物モデルは、多発性硬化症治療薬の開発に有用であることから、今回、より臨床病態に近い炎症および脱髄を誘発する実験的自己免疫性脳脊髄炎 (EAE) モデルを探索し、病理組織学的検討を実施した。具体的には、いくつかの系統、抗原及びその接種法について検討し、症状観察による麻痺の重篤度判定、病理組織学的解析を実施した。

【方法】ラットはLewis、Brown Norway (BN)、Dark Agouti (DA) の雌雄 (系統によっては片性のみ) を使用し、接種抗原はミエリン塩基性蛋白質 (MBP) またはミエリンオリゴデンドロサイト糖蛋白質 (MOG) を使用した。抗原は蹠底皮下または尾基部皮下に単回接種した。麻痺の重篤度を症状観察によりスコアで判定し、発症後に動物を剖検した。剖検後、脊髄 (頚髄、胸髄、腰髄) を採取した。常法に従いパラフィン包埋切片を作製し、HE染色を施し、光学顕微鏡で観察した。脱髄の評価のためルクソールファーストブルー LFB染色を実施した。炎症細胞の種類の確認のため、抗CD3抗体および抗ED1抗体を用いた免疫組織化学的染色を実施した。

【結果】DAラット雌にMOGを蹠底皮下に接種した条件のEAEモデルで、脊髄への広範な炎症細胞浸潤と脱髄が認められた。CD3陽性細胞は脊髄に散在性に分布し、ED1陽性細胞は脱髄領域に集簇して認められ、ミエリンの貪食が示唆された。この条件下では、慢性的持続的な麻痺の症状が認められた。高用量では再発寛解型の経過が認められ、症状発現経過においてもヒトのMSに近い経過をたどった。一方、その他の条件では、血管周囲の炎症細胞浸潤が主な所見で、脱髄性病変は極軽度であった。麻痺の症状は一過性であった。

O-07＊ Inhibitory Effect of Postnatal Treatment with Cyclopamine, a Hedgehog Signaling Inhibitor, on Medulloblastoma Development in Ptch1 Heterozygous mice

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<Background> *Ptch1* encodes a receptor of Sonic Hedgehog (Shh), and is one of the major genes related to the formation of medulloblastoma (MB) in humans. The Shh signaling pathway is important for the development of not only cerebella but also MBs. MBs are derived from granule cell precursors in the external granular layer (EGL) of the developing cerebellum. Heterozygous Ptch1 knockout (Ptch1) mice develop MBs that resemble human MBs with activation of Shh signaling pathway. This study was conducted to clarify the effects of postnatal treatment with cyclopamine, a hedgehog signaling inhibitor, on MB development in Ptch1 mice.
<Materials and methods> Ptch1 mice and wild-type littermates were dosed daily with cyclopamine at 40 mg/kg bw (sc) or vehicle from postnatal day (PND) 1 to PND14. Cerebella at PND14, 21 and postnatal week 12 (W12) were examined for histopathology and immunohistochemistry. Cerebella at PND7 were also examined for the effects of cyclopamine on development.
<Results> Proliferative lesions in the cerebellum, MBs and their preneoplastic lesions, were detected in Ptch1 mice only. At PND14 and 21, cyclopamine treatment decreased the incidence and/or area of proliferative lesions. The decrease persisted at W12, but not significantly. At PND7, cyclopamine treatment reduced the width of EGL regardless of genotype. Ki-67 staining also revealed the decrease of proliferating cells in the EGL in the cyclopamine group at PND7.
<Discussion> These results indicate that postnatal treatment with cyclopamine has inhibitory potential on proliferation of EGL and MB development in Ptch1 mice, and that this effect persisted.

O-08 Sampling Practices for Brain Evaluation in Adult Rats (Concepts for the Analysis of the Central Nervous System)

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We are presenting practical methodology for neuropathological examination of the CNS in perfusion fixed adult rats. This methodology considers cost and time effectiveness; retention of structural integrity during brain removal; avoiding artifacts during processing; and consistent trimming with use of a brain matrix. Sectioning of the brain with a specific matrix is necessary for accurate and consistent brain sections. This presentation is focused on trimming details and specimen preparation for high quality neuropathological evaluation of coronal (cross) sectioning of the CNS. Each brain level is placed rostral surface down in the cassette and submitted for paraffin wax embedding as 6 μm sections. H&E staining is generally sufficient for recognition of neuropathological changes in the CNS. Areas represented within coronal sections provide a practical screen of rat brain, to recognize key structures when using rat brain stereotaxic atlas (Paxinos and Watson, 6th edition). If a compound is known or suspected to have neurotoxic or specific CNS site effects, the method of perfusion fixation for evaluation of the brain should be considered. Tissue samples for neurohistopathology are only taken from animals where the whole-body perfusion is considered to have been successful. Organs of the perfusion fixed animals are stored in the fixative for 48 hours. The brains are then carefully dissected out. The special techniques, such as recording changes of behaviour by Functional Observation Batteries, monitoring locomotor activity, grip strength or clinical signs can provide important additional information to histopathological evaluation.

O-09＊ Identification of gene marker sets that could predict the hepatocarcinogenicity and toxic phenotype in the rat liver.

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Many of non-genotoxic hepatocarcinogens are taken for long time, generally half a year or more, to be observed the precancerous lesion or tumor in the rat liver.

The purpose of this study is to identify gene marker sets that can discriminate the toxic phenotype (necrogenic phenotype or enzyme inducer or PPAR alpha agonist) and predict the dose of non-genotoxic hepatocarcinogenesis within a month repeated dose study.

We used gene expression data from Open TG-GATEs (http://toxico.nibio.go.jp) which is known as public gene expression data base of the rat liver. In the selection of gene marker sets, we chose followed non-genotoxic hepatocarcinogens as a training compound: thioacetamide and methapyrilene as necrogenic phenotype compounds, hexachlorobenzene and phenobarbital as enzyme inducers and WY-14643 and clofibrate as PPAR alpha agonist. Up or down regulated gene at dose more TD_{50} (mg/kg/day) reported as the dose of observed hepatocarcinogenesis in rat on CPDB (The Carcinogenoic Potency Database) were selected in each compounds. In the test step after selection of gene marker sets, principal component analysis was applied used selected gene marker sets, other non-genotoxic hepatocarcinogens and non-hepatocarcinogens in the Open TG-GATEs.

As a result of selection of gene marker sets, 83 probe sets were selected as gene marker sets. These sets could distinctly discriminate the three type of toxic phenotype and the dose of non-genotoxic hepatocarcinogenesis with training compounds In the results of principal component analysis, gene marker sets could discriminate the three types of toxic phenotype and detect the dose of hepatocarcinogenesis with 70% sensitivity and 95% specificity.

In conclusion, we could identify gene marker sets and the analysis method that could predict the dose of hepatocarcinogenesis of non-genotoxic hepatocarcinogens and also could discriminate the three types of toxic phenotype (necrogenic phenotype or enzyme inducer or PPAR alpha agonist). It might be useful for the screening of hepatocarcinogenisity of compounds and predict the toxic phenotype.

O-10＊ Examination of *in vivo* mutagenicity and carcinogenicity in the gpt delta rat with 2-AAF

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Gpt delta rats have be widely used to determine the genotoxicity of chemicals and clarify whether its genotoxicity is involved in carcinogenicity in the target organs. 2-Acetylaminofluorene (2-AAF) reported that 2-AAF has carcinogenicity in liver of rats. The purpose of this study is to simultaneous detection for carcinogenicity and mutagenicity of 2-AAF in rats used medium-term bioassay system (Ito-test).

6 week-old male gpt delta F344 rats were divided into 4 groups (Group 1, 2, 5, 6) and F344 rats were divided into 2 groups (Group 3-4). Group 1-4 was given a single i.p. injection of DEN (200 mg/kg) dissolved in 0.9% NaCl to initiate hepatocarcinogenesis. After 2 weeks on basal diet, group 2 and 4 received test compounds in the basal diet. Animals were subjected to partial hepatectomy at week 3. Group 5 were received basal diet. After 2weeks basal diet, group 6 were received test chemicals in the basal diet.

At 8 weeks after starting the experiment, quantitative analysis of GST-P, which are preneoplastic lesions in the rat liver and mutation assays were performed. Both numbers and area of GST-P positive foci were significantly increased in 2-AAF treatment groups. These findings indicated that 2-AAF exerts promotion effect on liver carcinogenesis used this model. There was significantly increased in 2-AAF group compared to control group.

O-11* Development of A Medium-Term Animal Model Using *gpt* delta Rats to Evaluate Chemical Carcinogenicity and Genotoxicity

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In this study, the potential for development of an animal model capable of rapidly detecting chemical carcinogenicity and the underlying mechanisms of action was examined in *gpt* delta rats using a reporter gene assay to detect mutations and a medium-term rat liver bioassay to detect tumor promotion. Briefly, *gpt* delta rats were exposed to various chemicals for 4 weeks, followed by a partial hepatectomy (PH) to collect samples for the *in vivo* mutation assay. The mutant frequencies (MFs) of the reporter genes were examined as an indication of tumor initiation. A single ip injection of 10 mg/kg DEN was administered in rats 18 h after the PH to initiate hepatocytes. Tumor promoting activity was evaluated based on the development of glutathione S-transferase placental form (GST-P) positive foci at week 10. The genotoxic carcinogens 2-acetylaminofluorene (2-AAF), 2 - amino - 3 - methylimidazo [4,5-f] quinolone (IQ) and safrole (SF), the non-genotoxic carcinogens piperonyl butoxide (PBO) and phenytoin (PHE), the non-carcinogen acetaminophen (APAP) and the genotoxic non-hepatocarcinogen aristolochic acid (AA) were tested to validate this model. The MFs of the *gpt* genes were significantly increased in *gpt* delta rats treated with 2-AAF, IQ, SF and AA. Spectrum analysis in the *gpt* mutant colonies revealed that GC:TA and AT:TA transversions were predominant in mutations induced by 2-AAF or IQ and AA, respectively, which is in agreement with the previous reports. The number and area of GST-P positive foci were increased in the livers of rats treated with 2-AAF, IQ, SF, PBO or PHE, and APAP treatment inhibited the development of GST-P positive foci as in the conventional medium-term bioassay. The validation results show the possibility of developing a new animal model using *gpt* delta rats. However, a possible limitation of this protocol is that the test chemicals are co-administered with DEN. Because this regimen may modify the detoxification or metabolic activation of DEN, we are working toward improving the timing of the regimen to avoid the mutual effects.

O-12* Identification of Gene Expression Profile Related to Aberration in Neuronal/Glial Development Induced by Maternal Exposure to 6-propyl-2-thiouracil in Rats.

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It is reported that developmental hypothyroidism of rat causes aberrations in neurogenesis and glial differentiation in the hippocampal dentate gyrus and cingulum, respectively. However, global gene expression changes related to these aberrations have not been elucidated. This work aimed at identification of gene expression profile of different brain region, hippocampal dentate gyrus, corpus callosum, cingulate gyrus, and cerebellum, related to aberration in neuronal/glial development induced by hypothyroidism after maternal exposure to PTU in rats. Pregnant SD rats were treated with 0, 1, 3, 10 ppm of PTU in the drinking water from gestational day 6 to postnatal day (PND) 21. Pups were killed on PND 21 and PND 77. Brain samples of pups at PND 21 were subjected to analysis of gene expression microarray and gene-ontology-based clustering. Gene clusters related to neurogenesis, neuron differentiation, and neuronal function were significantly changed in all four brain regions. In the corpus callosum and cingulate gyrus, gene clusters related to glial development/differentiation showed expression down regulation. Characteristically, down regulation of *Ephb1*, *Ephb2*, *Grin2a*, *Gria3* and up regulation of *Efnb3*, *Reln*, and *Sdc2* were commonly found in each brain region. In addition, down regulation of *Mbp*, *Plp1*, *Hdac11* and up regulation of *Vim* and *Gfap* were found in the corpus callosum and cingulate gyrus. These changes in gene expression were validated by real-time RT-PCR analysis. Immuohistochemistry on brain sections revealed increase in vimentin- and glial fibrillary acidic protein-positive cell number in the corpus callosum. In addition to involvement in the neuron proliferation, migration, and plasticity of glutamatergic synapse, ephrin-Eph signaling has shown to interact with reelin and syndecan 2. Therefore, these molecules are possible markers reflecting permanent aberration in neuronal development induced by developmental hypothyroidism. We also identified gene expression profile related to aberration in glial development in the corpus callosum, where glial differentiation is continued through to the adult stage. Therefore, we judge it may be possible to identify responsible molecular targets specific to the disruption of neurogenesis or gliogenesis.

O-13＊ Roles of the Colonic Stem cells in the Process of Epithelial Regeneration in Dextran Sulfate Sodium-Induced Colitis

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<Objective> Intestinal stem cells reside at the basal crypts and generate all types of colonic epithelial cells. Identification of stem cell markers, such as Lgr5, has revealed several roles of intestinal stem cells. However, it remains unknown about roles of intestinal stem cells in the process of epithelial regeneration. In the present study, we evaluated the roles of Lgr5+ intestinal stem cells in the crypt regeneration after dextran sulfate sodium (DSS)-induced colitis.

<Materials and Methods> C57BL/6J mice and Lgr5-GFP knock-in mice (*Lgr5-EGFP-IRES-creERT2* mice), in which GFP gene is regulated by endogenous *Lgr5* promoter, at ages 5 weeks were used. These mice were treated with 2% DSS in the drinking water for 5 to 7 days, and then sacrificed at days 3 to 21. Colons were subjected to histological and immunohistochemical analysis.

<Results> Histologically, degeneration and loss of the colonic epithelial cells of basal crypts were found with mixed inflammatory infiltration, such as macrophages and neutrophils, in the lamina propria after DSS treatment. Surface epithelium covered the areas of crypt loss, and blanching crypts were observed adjacent these areas. Immunohistochemically, many Ki-67+ cells were found at the regenerative branching-crypts. GFP+ Lgr5 stem cells were also detected at the bases of branching-crypts in Lgr5-GFP knock-in mice. Interestingly, crypt regeneration was found at surface epithelium and Ki-67+ cells were found in the lower parts of regenerative crypts.

<Discussion> The present study suggested that crypt regeneration after DSS treatment occurs at (I) adjacent the areas of crypt loss and (II) surface epithelium. In addition, our findings clearly indicated that Lgr5+ stem cells involves in the crypt regeneration after DSS treatment. We are trying to confirm whether Lgr5+ stem cells actually regenerate crypts by genetic lineage tracing method using *Lgr5-EGFP-IRES-creERT2* mice carrying *Rosa26-lacZ* reporter allele.

O-14＊ Possibility of Cancer Initiating Cell of Bronchiolar Alveolar Stem Cell for Mice Lung SCC

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The cell of origin of human lung squamous cell carcinoma (SCC) has not been clearly identified. It was proposed that the cancer initiating cells (CICs) for lung adenocarcinoma are putative bronchiolar alveolar stem cell (BASC) at the bronchiolar alveolar duct junction but not identified for lung SCC. The aim of the present study was to identify the involvement of BASC for early stage of carcinogenic process in mice lung SCC.

Sixteen A/J female mice were treated topically with NTCU in acetone twice a week for 4 weeks and 16 mice were used as a control and treated topically with acetone. All mice were sacrificed after 8 weeks and histopathological analysis, flow cytometry , microarray analysis and proteome analysis were carried out using the mice lung samples.

Histopathologically, BASC were significantly increased in NTCU-treated group compared with control group. Ki-67^{pos} and $p63^{pos}$ BASC were only identified in NTCU-treated group. Using flow cytometry, we are sorting Sca-$1^{pos}CD31^{neg}CD45^{neg}$ fraction containing BASC (BASC fraction) in both groups. Cancer stem cell marker sca-1, CD133, CD44, c-kit, c-myc and klf4 were overexpressed and activated p38/ERK/MAPK pathway in NTCU-treated BASC fraction compared with Acetone-tread same fraction. Moreover we identified 11 proteins only overexpressed in NTCU-treated BASC for proteome analysis and Rev1 as known TLS polymerase was overexpressed NTCU-treated BASC compared with control BASC for immunohistochemistry.

The present study suggensted that NTCU-treated BASC might become the cancer initiating cell of mice lung SCC.

O-15 The Bovine Corneal Opacity and Permeability (BCOP) Test Method for Assessment of The Ocular Irritation: Histopathological Examination

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<Background> As a replacement for the ocular irritation test using rabbits (Draize rabbit eye test), a test method which had been conducted for safety assessment of chemical substances, various alternative test methods have been investigated from the standpoint of animal protection. The Bovine Corneal Opacity and Permeability (BCOP) test in an isolated bovine cornea, as a test method for identifying ocular corrosives and severe irritants, had been adopted as OECD Guideline for the Testing of Chemicals (TG) 437 in 2009. The BCOP test is an established testing method conducted in contract laboratories in other countries, while it has not been conducted in Japan. We recently started the BCOP test for the first time as a contract laboratory in Japan and have microscopically investigated the morphological changes in corneas.
<Methods> The cornea dissected from cattle eyes was mounted on a corneal holder, and the holder was filled with the medium, warmed, and equilibrated. Following exposure to the test substance, corneal opacity was measured with an opacitometer (BASF) and corneal permeability was determined with a spectrophotometer using sodium fluorescein. In addition, *in vitro* irritation scores (IVISs) were calculated from the opacity and permeability values to assess corrosive or irritating intensity of the chemical substance. After the opacity and permeability determinations, all corneas were fixed in 10% neutral buffered formalin for at least 24 hours, and then stained with hematoxylin and eosin according to the ordinary method. The corneal epithelium, stroma and endothelium were separately assessed for histopathological changes, using a standard grading system (1= minimal, 2 = mild, 3 = moderate, 4 = severe).
<Results and discussion> We have examined several substances recommended in OECD TG 437 and those that had been already demonstrated to have ocular irritancy potential *in vivo*. The IVISs calculated from the results are highly consistent with the publicly-available results from the *in vitro* tests, which proved high detection accuracy of ocular corrosives and severe irritants. Histopathological examination of the corneas revealed various morphological changes, i.e. vacuolization, nuclear changes and cell loss. This raises expectations for improvement of detection accuracy of ocular irritancy potential of new chemical compounds.

O-16* The Ratio of the Number of Atrophied Seminiferous Tubules and the Ratio of the Area of Leydig Cells in Minipigs

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<Objective> Minipigs preserve anatomical and physiological resemblances to human beings. Additionally, the body weights of adult minipigs and the ratio of each organ weight to the body weight are similar to human beings. Thus non-clinical studies on pharmaceutical agents and medical devices in minipigs have increased in recent years, and the background data including histopathological findings have been reported by some laboratories. Atrophy of seminiferous tubules and hyperplasia of Leydig cells were reported in these background data. We also have detected atrophy of seminiferous tubules in control animals in previous histopathological examinations. Besides, we have noted that the Leydig cells in minipigs were more distinct than those in other experimental animals. To clarify the histological features of testis in minipigs, we calculated the ratio of the number of atrophied seminiferous tubules and the ratio of the area of Leydig cells in control minipigs.
<Method> The testes in control animals in previous studies conducted in our laboratory were examined. We used Göttingen minipigs (Ellegaard Göttingen Minipigs A/S; import agent: Oriental Yeast Co., Ltd.) aged 7 and 15 months and NIBS minipigs (Nisseiken Co., Ltd.) aged 8 months. The testes were fixed in Bouin's solution overnight and then fixed again in 90% ethanol. The transverse sections of the fixed specimens were embedded in paraffin, and H. E. stained specimens were prepared. We calculated the ratio of the number of atrophied seminifeous tubules to the number of all seminiferous tubules and the ratio of the area of Leydig cells to the area of the transverse section.
<Results and conclusion> Atrophy of seminifeous tubules were detected in the testes in the control animals, and there were individual differences in the ratio of the number of atrophied seminiferous tubules. In addition, the ratio of the area of Leydig cells was much higher than any other experimental animal. Knowing the physiological range of the ratio of the number of atrophied seminiferous tubules and the ratio of the area of Leydig cells is important to assess the testicular toxicity in non-clinical studies in minipigs. Hence accumulation of background data is valuable. In this meeting, we will also report the data of the study in progress.

O-17＊ Utilizing the NOD/Shi-scid, IL-2Rgamma null mouse transplanted with human thyroid tissue

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<Objective> The thyroid has become an organ of interest because of the recent highlights on radiation exposure and endocrine disrupting chemicals. Thus a model that is relevant to human is in demand. In the present study, we have attempted to refine the current scid mouse model that can be constructed by transplantation with human thyroid tissue but requires serial passage for long-term observation. We have aimed to do this by utilizing the NOD/Shi-*scid*, IL-2Rγ^{null} (NOG) mouse. The NOG mouse was thought suited for the construction of a long-term model due to its characteristics as an excellent recipient for human tissues and also as a stable strain.

<Materials and Methods> Tissues from 9 cases of human adenomatous goiter that were surgically resected for therapeutic purposes was transplanted into the subcutis of 1 to 2 NOG mice. Three to 4 transplanted tissues were collected every 5-6 weeks up to 44 weeks after implantation, and examined histopathologically.

<Results> Positive intake was observed in 11/13 mice with follicular structures containing colloid substances up to 44 weeks. The pre-transplant tissues of intake-positive cases contained few degenerative changes and were judged to have higher integrity compared to unsuccessful cases. Blood vessels that were positive for human-specific vimentin were only observed in intake-positive cases. In the cases with positive intake, expression of functional proteins such as Thyroglobulin (IHC, ISH) and TPO (IHC) was observed up to 44 weeks.

<Conclusions> We were able to construct a model that retains the morphology and function of human thyroid tissues for a long term by utilizing the NOG mouse.

Poster Session

P-01～P-91

P-01 Mode of action of ethyl *tertiary*-butyl ether tumorigenicity in the rat liver: Evidence for oxidative stress induction via activation of CAR, PXR and PPARs

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To elucidate the possible modes of action (MOAs) and human relevance of the hepatotumorigenicity in rats for ethyl *tertiary*-butyl ether (ETBE) which is a non-genotoxic chemical and gasoline oxygenate synthesized from bioethanol and isobutane, the formation of oxidative stress, cytochrome P450, hydroxyl radicals and 8-hydroxy-2'-deoxyguanosine (8-OHdG) induction, DNA repair, alteration to protein expression by LC-MS/MS, apoptosis and cellular proliferation in the rat livers were investigated after 1 and 2 weeks of ETBE administration to rats at doses of 300 and 2000 mg/kg/day by gavage and compared with effects induced by non-genotoxic carcinogen phenobarbital (PB, 500 ppm in diet) served as a reference material. Slight centrilobular hepatocellular hypertrophy of the liver was found in rats given 2000 mg/kg/day ETBE, but not 300 mg/kg/day ETBE at week 2. Immunohistochemical ssDNA labelling indices were also significantly increased in rats given 2000 mg/kg/day ETBE, but not 300 mg/kg/day ETBE at week 2. Significant increase of P450 total content, generation of hydroxyl radicals by weeks 1 and 2 of 300, 2000 mg/kg/day ETBE and PB treatments accompanied the accumulation of P450 isoenzymes CYP2B1/2, CYP3A1/2 and CYP2C6 in the cytoplasm of hepatocytes. Up-regulation of CYP2E1 and CYP1A1 was obvious in the high dose ETBE-administered animals. Conspicuous elevations of 8-OHdG and apoptosis in the liver tissue observed in 2000 mg/kg/day ETBE and PB groups were associated with suppression of cell proliferation (Ki-67), cyclin D1 mRNA expression and decreased mRNA expression of 8-OHdG repair enzyme, DNA glycosylase 1 (Ogg1) after 2 weeks of application. Results of QSTAR LC/MS/MS, ProteinPilot and Ingenuity Pathway analyses of alterations to rat liver proteomes indicated that upstream regulators of gene expression altered by ETBE included CAR, PXR and PPARs leading to formation of oxidative stress and activation of fatty acid metabolism. These results indicated that MOA of ETBE hepatotumorigenicity in rats is related to conspicuous alteration to DNA oxidative base modifications in the nuclear of hepatocytes via generation of oxidative stress due to activation of CAR, PXR and PPARs with a sequence of events leading to cell cycle arrest, apoptosis and apparent accommodation, which presumably lead to regenerative cell proliferation. The MOA of ETBE hepatotumorigenicity in rats is indicated to be not relevant to human.

P-02 Different Macrophage Populations in GST-P-Positive/Negative Liver Lesions in Cirrhosis Induced in Rats by Repeated Injections of Thioacetamide (TAA)

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After acute hepatocyte injury, a variety of macrophage populations appear; the macrophages may have functions to stimulate injury or to repair lesions, indicating their importance. However, macrophage properties have not yet been decided in progressive hepatic lesions such as cirrhosis. The relationship between macrophages and GST-P-positive/negative hepatic lesions was investigated in developing cirrhosis induced by TAA (100 mg/kg BW, twice a week for 32 weeks). Pseudolobules (PLs) separated by collagen bridges consisting of α-SMA-positive myofibroblasts were completely formed at week 15, and thereafter the myofibroblasts gradually decreased. In contrast to controls, mRNA expressions of MCP-1 and TGF-β1 were consistently increased. GST-P-positive foci began to be seen at week 10; at week 25, GST-P-positive and negative PLs were clearly distinguished. BrdU-positive proliferating cells were less in GST-P-positive than-negative PLs. The numbers of macrophages reacting to CD68, CD204, Gal-3, Iba1 and MHC class II gradually increased with the maximum at weeks 15; $CD163^+$ macrophages showed a consistent increase. Interestingly, the numbers of $CD68^+$, $CD163^+$, $CD204^+$, Gal-3^+ and $Iba1^+$ macrophages were more increased in GST-P-positive PLs, whereas MHC class II^+ macrophage number was more predominant in GST-P-negative PLs. It was found that TAA-induced cirrhosis at advanced stages consisted of GST-P-positive/negative PLs, and there were differences in macrophage populations between these PLs.

P-03* Comparisons of Macrophages and Myofibroblasts Properties between Acute and Chronic Biliary Lesions Induced by α-naphthylisothiocyanate (ANIT) in Rats

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Macrophages play important roles in development of hepatic lesions by hepatotoxicants. To compare macrophage properties, cholangiocyte injury was induced in F344 rats by single (acute) or repeated (chronic) injections of ANIT. In acute study, biliary lesions (consisting of cholangiocyte necrosis/regeneration, cell reaction and slight fibrosis) were greatest on day 2-3 of injection, which recovered by day 12. In chronic study, by contrast, biliary lesions gradually progressed until week 19 with advancing fibrosis. In both studies, MHC class II (OX6; reflecting antigen presentation) expressing macrophages consistently increased almost throughout the experiment period, followed by increment of CD68 (ED1; reflecting phagocytosis)- and CD204 (SRA-E5; reflecting lipid metabolism)-positive macrophages. The MHC II+ macrophages were seen exclusively in the affected Glisson's sheath and CD204+ macrophages along the sinusoids in the vicinity of the Glisson's sheath. Interestingly, CD68+ macrophages were distributed in the Glisson's sheath as well as in the vicinity. Appearance of macrophages reacting to CD163 (ED2; reflecting proinflammatory cytokine production) was transient (mid stage) in acute study and delayed (late stage) in chronic study. Vimentin expressed in myofibroblasts at early stages in acute and consistently in chronic study. Although α-smooth muscle actin (α-SMA) expressed at late stages in both studies, desmin expressing cells were fewer in chronic study. Taken together, MHC class II+ macrophages seem to have central roles in induction of myofibroblasts in biliary fibrosis.

P-04 *In vivo* mutagenicity of potassium bromate in the kidneys of *gpt* delta mice: effects of combined treatment with nitrilotriacetic acid, a renal tumor promoter

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Renal carcinogenesis mediated by potassium bromate ($KBrO_3$) likely involves gene mutations via oxidative DNA damage. Unlike the rat kidney, $KBrO_3$ is not carcinogenic in mouse kidneys despite causing oxidative DNA damage. In the present study, to clarify the contribution of oxidative DNA damage to $KBrO_3$–mediated carcinogenesis, we examined reporter gene mutations in the kidneys of *gpt* delta mice given $KBrO_3$ for 13 weeks and conducted 8-hydroxydeoxyguanosine (8-OHdG) analysis. Additionally, we investigated the effects of combined treatment with nitrilotriacetic acid (NTA), a renal tumor promoter.

Six-week-old male B6C3F$_1$ *gpt* delta mice were randomly divided into four groups consisting of five animals each and treated with basal diet, 0.15% $KBrO_3$ in the drinking water, 1.5% NTA in the diet and combination of 0.15% $KBrO_3$ plus 1.5% NTA, respectively. All mice were sacrificed 13 weeks after the start of the treatment and histopathological examination, 8-hydroxydeoxyguanosine (8-OHdG) analysis, *gpt* and Spi$^-$ mutation assays were performed in the kidneys.

8-OHdG levels were significantly increased in the kidneys of mice given $KBrO_3$ compared with basal diet. *gpt* and Spi$^-$ mutant frequencies (MFs) in the mice treated with $KBrO_3$ were significantly elevated. Analysis of the mutation spectra of *gpt* mutants revealed that the frequency of single base pair (bp) deletions was significantly increased. Also, frequencies of frameshift mutations derived from single bp deletions in the *gam* gene and over two bp deletions were elevated in the Spi$^-$ mutants. Combined effects of NTA treatment were not observed in these changes caused by $KBrO_3$ administration.

$KBrO_3$ caused oxidative DNA damage and *in vivo* mutagenicity characterized by deletion mutations. Since 8-OHdG is capable of causing deletion mutations during DNA repair process, these results implied that $KBrO_3$ induced gene mutations via oxidative stress even in the kidneys of mice at non-carcinogenic sites. There were no promoting effects of NTA on any alterations induced by $KBrO_3$, indicating that increased cell proliferation did not affect $KBrO_3$ genotoxicity in the mouse kidney.

P-05 Chemical structure-related mechanisms underlying *in vivo* mutagenicity induced by nitrofurantoin

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<Background> Some nitrofuran antimicrobials having a basic nitrofuran structure were prohibited for use because of their carcinogenic activity and residual presence in food. Although their carcinogenic mechanism remains unclear, new nitrofuran agents have been synthesized with various hydrazide derivatives at the side chain. To clarify the chemical structure-related carcinogenesis of these hydrazide side groups, a reporter gene mutation assay was performed in kidneys of *gpt* delta rats given nitrofurantoin (NFT), a known renal carcinogen in male rats, or its metabolites.

<Methods> NFT, 5-nitro-2-furaldehyde (NFA, a metabolite of NFT with a nitrofuran structure) and 1-aminohydantoin (AHD, an NFT metabolite with a hydrazide group) were administered to male F344 *gpt* delta rats by gavage for 4 or 13 weeks. The NFT dose was at carcinogenic levels, while doses of the metabolites were the maximum tolerated based on dose-finding tests with the same mole concentrations as the NFT dose. NFT was also administered for 13 weeks to female *gpt* delta rats at the same dose used for the males. At necropsy, the kidneys were extirpated and subjected to a *gpt* assay to determine 8-hydroxydeoxyguanosine (8-OHdG) levels in kidney DNA, as well as western blotting analysis and histopathologic examination (including immunostaining of α_{2u}-globulin).

<Results and discussion> In the NFT-treated group, *gpt* mutant frequency (*gpt* MF) was increased from 4 weeks, and the increase was statistically significant at 13 weeks. In spectrum analysis of *gpt* mutants, the incidence of GC-TA transversion mutations was significantly elevated. In the NFA-treated group, the *gpt* MF was also significantly increased at 13 weeks. 8-OHdG levels in NFT-treated rats were significantly raised at 4 weeks, and were increased by over 3-fold compared to controls at 13 weeks. Histopathologically, NFT caused increases in the number of hyaline droplets in the proximal tubules, which showed positive immunostaining for α_{2u}-globulin. Likewise, elevation of α_{2u}-globulin protein levels was confirmed by western blotting analysis. Thus, these results suggest the involvement of a genotoxic mechanism associated with oxidative DNA damage in NFT carcinogenesis. In addition, it is highly probable that NFA contributes to NFT-induced genotoxicity. The effects of α_{2u}-globulin accumulation on NFT carcinogenesis will be discussed with results in female rats.

P-06＊ Effects of Renin Inhibition on Glomerular Podocyte Injury in Osborne-Mendel Rats

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<Background> Osborne-Mendel (OM) rats develop renal injury with mild hypertension and proteinuria at young age. We have showed that podocyte injury is important for development of the nephropathy in OM rats, and the renin-angiotensin system (RAS) plays a significant role in the pathogenesis. (Pro) renin, one of the RAS factors, contributes not only to induction of the RAS activity, but also to activation of intracellular signaling without dependence on angiotensin II (AII). Thus, activation of the RAS might have an impact on various cells *via* prorenin receptor (PRR). In this study, we examined the role of (pro)renin for podocyte injury in OM rats.

<Materials and Methods> Male OM rats were orally administered direct renin inhibitor (DRI) (aliskiren, 30mg/kg/day) or tap water (control group) from 3 weeks of age. These rats were sacrificed and kidneys were collected to examine glomerular damages at 13 or 20 weeks of age, and the results were compared with the groups administered angiotensin conversion enzyme inhibitor (ACEi) (lisinopril, 5mg/kg/day) and AII receptor blockers (ARB) (losartan, 27mg/kg/day).

<Results> In the DRI group, systolic blood pressure and urinary protein level were lower than those in control rats and glomerular sclerosis was significantly inhibited. Expression of desmin, a marker of podocyte injury, and reduction of nephrin expression seen in controls, were significantly suppressed in DRI group at 13weeks of age. Ultrastructually, diffuse global fusion and effacement of podocyte foot processes were observed in controls, while these podocyte changes were infrequent in DRI group. As compared to the DRI, ACEi and ARB were more effective for inhibiting these podocyte injuries.

<Discussion> It has been shown that the podocyte expresses both AII type1 receptor (AT1R) and PRR, and secretes AII. Podocyte injury of OM rat was inhibited by DRI treatment but the effect was less than that in ACEi or ARB groups. These results suggest that AII-independent intracellular signaling triggered by renin-PRR binding might have a little contribution to local activation of RAS, and AII plays an important role for podocyte injury in OM rats.

P-07＊ Evaluation of Wilms' tumor 1 negative podocyte in Puromycin aminonucleosido nephrosis rats

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Wilms' tumor 1 (WT1) is a specific nuclear protein of mature podocytes in the renal glomeruli. Decreasing and disappearance of WT1 in the podocytes have been reported under pathologic conditions. However, the pathological significance of WT1-negative podocytes have not been clarified. The purpose of this study is to identify of WT1-negative podocyte in the glomeruli of puromycin aminonucleosido (PAN) nephrosis rats, and to examine the expression of nephrin, a component of slit diaphragm, in WT1-negative podocytes. PAN nephrosis was induced in male F344 rats at 11 weeks of age by intravenous injection of PAN of 8 mg/100 g body weight. At 0 and 10 days post injection (DPI), urinary protein was measured, and both kidneys were removed. Glomeruli were isolated from kidneys, and immunofluorescence for WT1 was performed using these isolated glomeruli. The consecutive images of immunofluorescence were obtained by confocal laser scanning microscope (CLSM). A series of images were reconstructed and WT1-positive nuclei were counted. The epoxy resin-embedded cortical tissues were serially sectioned at 1μm and stained with toluidine blue. Serial images were reconstructed and the cells covering the glomerular capillaries were counted as podocytes. Double immunofluorescence for WT1/nephrin and immunohistochemistry for WT1 were performed using frozen sections and paraffin ones, respectively. Proteinuria was observed in PAN-treated at 10 DPI. In CLSM analysis, the number of WT1-positive cells per glomerulus was significantly decreased at 10 DPI (52.4±23.7 cells/glomerulus) as compared to that at 0 DPI (95.8±17.6 cells/glomerulus). On the other hand, the number of podocytes was not decreased at 10 DPI in morphological examination on semi-thin sections. The immunofluorescence revealed double negative areas of nephrin and WT1 in the glomeruli at 10 DPI. Also, WT1-negative podocytes were observed at 10 DPI by immunohistochemistry. In this study, two quantitative methods for evaluation of the number of podocytes were developed. The difference in the number of podocytes between immunohistological and morphological examinations indicated the presence of WT1-negative podocytes in the glomaruli at 10 DPI. Expression of nephrin was decreased in these WT1-negative podocytes. These results show that WT1-negative podocytes may be involved in developing proteinuria.

P-08 Anti-carcinogenesis effect of apocynin, NADPH oxidase inhibitor, on rat prostate.

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【目的】活性酸素は前立腺癌の増殖や進展に関与する報告があり、活性酸素の産生抑制は発癌抑制にも繋がると考えられる。そこで、我々は内因性の活性酸素誘導酵素であるNADPH oxidaseに着目し、その阻害剤であるapocyninの前立腺発癌に対する抑制効果について、前立腺癌好発トランスジェニックラット (Transgenic Rat for Adenocarcinoma of Prostate, TRAP) を用い検討した。
【対象および方法】6週齢TRAPラットにapocynin を100および500mg/Lの濃度で飲料水投与し、8週間の投与後、屠殺・剖検した。前立腺、肝臓、腎臓および精巣を採取し、病理組織学的に検討した。前立腺組織は各葉に分けて検討し、免疫組織学的解析も行った。また、屠殺時に採血を行い、血清中のテストステロンおよびエストラジオールを計測した。
【結果】実験期間中にapocynin 500mg/L投与群において1割程度の飲水量低下を認めたが、体重および諸臓器重量に群間で差は見られなかった。血中テストステロン濃度は500mg/L投与群でわずかに有意な上昇を認めたが、エストラジオールに変化は見られなかった。前立腺腹葉における癌の発生頻度は100%で群間差を認めなかった。前立腺側葉では対照群および100mg/L投与群で9/11匹 (82%) であったのに対して、500mg/L投与群では6/11匹 (55%) と低下を観察したが、有意差は見られなかった。一方、単位面積あたりの癌病巣数は、腹葉、側葉のいずれにおいてもapocynin濃度依存性に有意に低下した。細胞増殖指標としてKi67、酸化ストレス指標として8-oxo-dGの免疫染色を、アポトーシス指標としてTUNEL染色を行ったが、いずれにおいても、apocynin濃度依存性に低下するとともに有意な減少を認めた。前立腺腹葉でタンパク発現を検討した結果、p44/42 MAPKリン酸化抑制およびcyclin D1発現低下を認めた。
【結論】TRAPラットにおいて、apocyninによる発癌抑制が認められ、前立腺癌化学予防剤としての可能性が示された。現在、その抑制機序について詳細に検討している。

P-09* Inhibitory effect of OBP-801 on Prostate Carcingenesis

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<Introduction> Histone acetylation plays an important role in chromatin remodeling and gene expression. Histone deacetylase inhibitor (HDAC inhibitor) promotes histone acetylation and regulates many cellular functions. HDAC have been reported to suppress growth of several malignancies including lymphoma, and is expected to prevent cancer. We examined the effect of HDAC inhibitor, OBP-801, on prostate carcinogenesis using Transgenic Rat for Adenocarcinoma of Prostate (TRAP) rat.

<Materials & Methods> OBP-801 was generously gifted from Oncolys Biopharma. Thirty-four TRAP rats were divided into 3 groups: control, low dose (0.5mg/kg of OBP-801), and high dose (2mg/kg of OBP-801). OBP-801 was given intravenously once a week for 8 weeks.

<Results> Body weight loss was observed in treated groups. Incidences of adenocarcinomas in ventral and lateral lobe of prostate were not significantly different among control, low dose, and high dose groups. Relative number of carcinoma acini in lateral prostate in treated groups was significantly decreased compared with that of control group. Relative number of carcinoma acini in ventral prostate in high dose groups was significantly decreased compared with that of the control group.

<Conclusion> Present data suggests that OBP-801 may be a good candidate for preventing prostate carcinogenesis, although side effects such as body weight loss may occur. We are intending to clarify how HDAC inhibitor may contribute to the inhibition of prostate carcinogenesis.

P-10 Modifying effect of castration on PhIP-induced prostate carcinogenesis by Nakagama method treatment

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In recent years, prostate cancer increases rapidly in Japan and is get a lot of attention as Japan is an aged society. The cause of prostate cancer is not clear. Broiled meat has been reported to be one of the causes of prostate cancer. From pathological findings, it has been suggested that the prostate cancer is related with prostatitis. Several epidemiological data also support these findings. On the other hand, it is not clear that the relation between prostate carcinogenesis and inflammation in animal studies. In this study, we investigated the effects of castration on PhIP-induced prostate carcinogenesis by Nakagama method with administration of testosterone propionate (TP).

Prostatitis was observed in rats treated with TP. Most of the lesions were in the lateral lobes. Prostate neoplasms were found in the PhIP + TP treated, PhIP treated and non-treated groups. All of them were PIN. No neoplasms were found in the other groups. Prostate neoplasms were 3 of 11 rats in the PhIP + TP treated group, 9 of 11 rats in the PhIP treated group and 1 of 7 rats in the non-treated group. Any prostate neoplasma were not found in any castrated rats, although prostate neoplasms was observed only in non-castration groups.

From these results, the origin of prostate cancer was assumed to be lost by castration. TP had suppressive effects on PhIP-induced prostate carcinogenesis. The lobes with inflammation were different from those with PIN. The modifying effect of inflammation on prostate carcinogenesis is not clear in this study. The findings that prostate tumorigenesis was suppressed by TP exposure, suggesting possibility that changes in the dose of TP affected carcinogenesis.

P-11 The relationship between the tissue morphology and the quality of RNA in the PFA-AMeX processed samples: The application for LCM and DNA microarray analysis.

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Recently, large-scale cancer gene expression profiling was often performed in combination with Laser-capture microdissection (LCM) and DNA microarray analysis, since the RNA source could be obtain without contamination of interstitial components of the cancer. In this technique, RNA samples are often extracted from unfixed fresh frozen (F-F) or formalin-fixed paraffin embedded (FFPE) samples. However, both samples had its own drawbacks in morphological preservation and RNA quality, respectively. In the present study, we investigated the application of the PFA-AMeX method (fixation using paraformaldehyde (PFA) followed by embedding in paraffin by AMeX) to DNA microarray combined with LCM, using 30 human prostate tissues (BPH, 17; AC, 13). Morphologically, the loss of basal cells in prostate adenocarcinoma surrounded by atypical adenomatous hyperplasia was identified in PFA-AMeX as well as in FFPE, but it was hardly distinguishable in some of F-F samples. As for quality of RNA which determined by the amount of 5' amplicons of beta-actin and ratios of 3' to 5' amplicons of beta-actin, it was considered the RNA quality were higher in PFA-AMeX than that of FFPE. Based on this result, the samples were assessed with DNA microarray (Human X3P GeneChip® arrays). Once applied the generally accepted quality criteria for genechip data (%Pcall ≥30, SF ≤1.5), acceptance rate of PFA-AMeX was well over 80%, whereas 25% in FFPE samples. In conclusion, the PFA-AMeX method is a promising tool for DNA microarray combined with LCM, because the method well balance the trade-off between the preservation of tissue morphology and RNA quality.

P-12 Intestinal crypt response in X-irradiated mice

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放射線被曝によるリスク発症に関しては、様々な疫学研究とともに、分子レベルあるいは細胞レベルでの多数の知見が報告されてきている。しかし、生体内では、どのような細胞が放射線に応答するのか、そのような細胞が時間的空間的にどのような動態を示すのかという検証はほとんどなされていない。そこで、X線照射したマウスから、大腸陰窩を腺管分離して、X線照射に対して発現量が変動するタンパクの同定を試みた。大腸分離腺管から蛋白質を抽出し、得られた可溶化蛋白質を二次元電気泳動法により分離した。蛍光色素で染色後、イメージスキャナーで得られたゲル内の全スポットを非照射群と照射群で比較した。発現量に差異のあるスポットから蛋白質成分を抽出・酵素処理後、MALDI-TOF/TOFタンデム質量分析計によって解析した。その結果、X線照射によって発現量の増加あるいは減少するタンパクが同定された。増加するものとして、ezrin、radixin、moesin family、減少するものとして、high mobility group grotein box 1が得られた。タンパクレベルの大きな変動を同定できれば、遺伝子障害作用に対する早期の鋭敏な反応として検出できる可能性が考えられた。

P-13* Microarray Analysis of Liver and Colonic Mucosa in LETO and OLETF Rats Treated with Caloric Restriction and High Fat Diet

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We showed recently that diabetic and mildly obese Otsuka Long-Evans Tokushima Fatty (OLETF) rats were more susceptible to azoxymethane-induced carcinogenesis than control Long-Evans Tokushima Otsuka (LETO) rats, and carcinogenesis was inhibited by 20% caloric restriction (CR) and enhanced by 10% high fat diet (HFD, control diet with 10% safflower oil). To elucidate the mechanism of cancer susceptibility, we examined the serum biochemical analysis and microarray analysis of the liver and colonic mucosa.

Male LETO and OLETF rats were given control diet, 20% CR diet or HFD for 5 weeks, and killed at 24 weeks of age (n=5). In both strains, blood glucose, serum triglyceride, total cholesterol, insulin and leptin levels decreased by 20% CR, and increased by HFD. The liver of HFD-treated OLETF rats showed severe fatty change histologically, and it was improved by 20% CR. Microarray analysis showed that 20% CR up-regulated metallothionein 1A gene, and down-regulated heat shock 105kDa/110kDa protein 1 (Hsph1) and heat shock 70kDa protein 1B (Hspa1b) genes in both strains. There were no histological differences in the colonic mucosa in both strains and the effect of the dietary difference was not found. Microarray analysis showed that 20% CR down-regulated Hsph1 and Hspa1b genes, and HFD up-regulated fatty acid binding protein 2 in both strains.

These data suggest that hyperinsulinemia, hyperlipidemia and oxidative stress exert significant role in cancer susceptibility in OLETF rats.

P-14 Effects of High Fat Diet on the Polyp Formation in the Intestine of Min mice

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<Objective> The consumption of the high fat diet has considered the risk factor of the colorectal carcinogenesis. Min (Multiple intestinal neoplasia) mice, which has truncating mutation in the APC (Adenomatous polyposis coli) homolog, has been known as the model for colorectal polyposis. This study was conducted to confirm the effects of high fat diet on polyp formation in the intestine of Min mice.

<Method> Groups of 10 male and female mice at the age of 7 weeks were given basal diet (fat; 4.3 gm%) or high fat diet (fat; 34.9 gm%) for 7 or 11 weeks and the intestine was examined anatomically. Intestines were inflated and fixed with 10% buffered formalin. After that, intestines were opened and sandwiched in filter paper, and preserved in 10% buffered formalin solution. After fixation, the number of polyps in the small and large intestines were counted using transmissive stereomicroscope and classified by size.

<Results> There were no high fat diet treatment-related changes in general condition, body weights, food consumption and gross pathology other than coloring of feces. The number of surviving animals in the basal diet groups of both sexes after 7 weeks was 9 mice each, and those in the high fat diet groups of male and female were 9 and 10 mice, respectively. The number of surviving animals in the basal diet groups of both sexes after 11 weeks was 6 mice each, and those in the high fat diet groups of male and female were 8 and 9 mice, respectively, and survival rate was slightly high in the high fat diet group as compared with the basal diet group. The cause of death was considered the hemorrhage in the polyps.

The mean total numbers of intestinal polyps in the basal diet groups of male and female after 7 weeks were 52.4 and 55.2, respectively, and those in the high fat diet were 52.0 and 50.5, respectively. After 11 weeks, those were 51.5 and 66.8 in the basal diet groups against 60.9 and 57.1 in the high fat diet groups. No significant difference was observed between basal and high fat diet group. Moreover, no significant difference of the polyp size and site of occurrence was observed.

<Conclusion> No significant effect of the high fat diet treatment on the intestinal polyp formation was found after 7 or 11 weeks treatment.

P-15＊ Impacts of High Fat Diet-induced Obesity on Spontaneous Reporter Gene Mutations in *gpt* delta Mice

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Epidemiologically, obesity has been suggested to be associated with increased risk of several human cancers, including cancers of the liver, kidneys, and colon. In several rodent models, genetically induced obesity or high fat diet (HFD)-induced obesity has been shown to enhance carcinogenesis in the liver and colon after treatment with a regimen of respective tumor initiators. However, the factors responsible for the obesity-related progression of carcinogenesis, especially the initiation phase, are not fully elucidated. To reveal the effects of obesity induced by an HFD on spontaneous gene mutations, we performed reporter gene mutation assays in liver, kidney, and colon tissues from obese mice fed an HFD. Six-week-old male and female C57BL/6 *gpt* delta mice were fed either an HFD or a standard diet (STD) for 13 or 26 weeks. At the end of the experimental period, reporter gene mutation assays in liver kidney, and colon tissues were performed. Final body weights of male and female mice fed an HFD for 13 weeks were significantly increased compared with those fed an STD. *gpt* and Spi- mutant frequencies in the liver, kidneys, and colon of male mice fed an HFD for 13 weeks were not significantly different from those fed an STD. These results implied that HFD-induced obesity does not influence the spontaneous frequencies of somatic gene mutations, indicating that obesity may affect the tumor promotion phase rather than the tumor initiation phase to enhance carcinogenesis. Further data from the organs of mice fed an HFD for 26 weeks will be presented to evaluate the effects of long-term consumption of an HFD on spontaneous mutagenicity *in vivo*.

P-16＊ Prenatal Exposure of PFOS Modulate NNK-Induced Rat Lung Carcinogenesis

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Perfluorooctane sulfonate (PFOS) is fluorinated organic com- pounds that have been used in a variety of consumer and industrial applications. Although there have been produced for many years, only recently have reports surfaced suggesting widespread exposure in wild life and humans. PFOS is used for surfactant in applications ranging from oil and water repellent to specialty chemical applications such insecticides and fire fighting foams. Moreover, PFOS have been reported to influence for the lung development, but the detail is unclear. The aim of this study has been to investigate the dose-response relationship prenatal exposure of PFOS for 4 - methylnitrosamino-1 - (3-pyridyl) -1- butanone (NNK) induced rat lung carcinogenesis.

<Materials and Methods> Pregnant Spague-Dawley (slc) rats were given 3mg, 1mg, 0.5 mg/kg/day PFOS or vehicle (i.g.) on day 6th though 15th after conception. The offspring rats were analysed number of each sex, and body weights. For the male rats offspring, lung carcinomas were initiated with 20ppm NNK in the drinking water from 3 weeks old to 12 weeks old, and then dissected lung at 90 weeks old.

<Results> The incidences of lung tumors of the PFOS-groups were relatively higher, but that was not significantly difference to that of the vehicle-group. But, the size of lung tumors of the 3 mg and 1mg PFOS-groups were significantly higher than that of the 0.5 mg PFOS-group and the vehicle group, and the biological character of the tumors of the PFOS-groups were different from that of the vehicle-group.

<Discussion> It has been suggested that the prenatal high dose PFOS- exposure modulated NNK induced rat lung carcinogenesis.

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P-17＊ Modifying effect of ATI receptor blocker Losartan in mice lung carcinogenesis

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Recent clinical studies have reported that ATI receptor blockers are associated with a slightly increased risk of lung cancer. The aim of the present study was to evaluate modifying effects of Losartan on mice lung carcinogenesis. Eighty female A/J mice at 6 weeks of age were divided into 4 groups randomly. Groups 1 and 2 were administrated 4-(methylnitrosamino)-1-(3-pyridyl)-1 -butanone by a single i.p. injection at the start of the experiment to induce adenocarcinoma, and were given Losartan (200 mg/kg BW/day) or vehicle by gavage from week 2 for 19 weeks. Groups 3 and 4 were treated topically with N-nitroso-tris-chroloethylurea in acetone twice a week for two weeks to induce squamous cell carcinoma (SCC), and given Losartan (200 mg/kg BW/day) from week 3 for 16 weeks. Histopathological analyses of lung at the end of the treatment found that incidences of adenoma, adenocarcinoma and total tumor were 71, 6, 71% and 83, 17, 83% in groups 1 and 2, respectively. In groups 3 and 4, incidence of squamous dysplasia and SCC were 25, 8% and 31, 8%, respectively. The present study indicated that Losartan has not promoting effect on lung adenocarcinoma and SCC carcinogenesis in mice.

P-18 Nasal Lesion in Rats and Mice Exposed to Methylamine for 13 Weeks

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<Introduction> Methylamine is used as a raw material for synthetic medical products and agrochemicals, paints, etc., and there is a concern for its health effects in the work environment because it is a strong irritant. We report on the lesions in the nasal cavity observed in our 13-week whole-body inhalation study in rats and mice.
<Methods> F344 rats and B6D2F1 mice of each sex (n=10 for each group) at 6 weeks of age were employed and were whole-body exposed to 0, 10, 20, 40, 80, 160 ppm (v / v) methylamine, 6 hours/day, 5 days/week, for 13 weeks. The nasal cavity was fixed with 10% buffered formalin and decalcified with formic acid-formalin solution, and then the nasal cavity of rats was cut into 4 cross-sections and that of mice was cut into 3 cross-sections. Thereafter, the sections were embedded in paraffin and stained with H&E for a histopathological examination.
<Results> In rats, inflammation and/or ulcer of squamous epithelium were observed in the respiratory area of nasal cavity in males of the 80 ppm or higher and females of the 160 ppm groups. In addition, in the respiratory epithelium, inflammation was found in males of the 80 ppm or higher and females of the 160 ppm groups, ulcer was observed in both sexes of the 160 ppm groups, and squamous metaplasia was observed in a male of the 160 ppm group. Furthermore, atrophy of olfactory epithelium was observed in males of the 80 ppm or higher and females of the 160 ppm groups. In mice, in the respiratory epithelium, inflammation and eosinophilic changes (eosinophilic globules) were observed in males of the 20 ppm or higher and females of the 10 ppm or higher groups, and ulcer was observed in males of the 20 ppm and both sexes of the 80 ppm or higher groups. Moreover, in the olfactory epithelium, atrophy was observed in both sexes of the 80 ppm or higher groups, respiratory metaplasia was observed in males of the 160 ppm and females of the 80 ppm or higher groups, and eosinophilic changes were observed in females of the 80 ppm or higher group. The lesions were observed mainly in the frontal part of the nasal cavity in both rats and mice as described above.
<Conclusions> Methylamine is known to dissolve well in water and to be a strong alkaline. It is reported that water-soluble irritating chemicals are likely to deposit in the nasal cavity, causing injury to the site of deposition. Thus, it is considered that the lesions in the nasal cavity observed in this study were histopathological changes caused by a strong alkaline and irritation. In addition, from adverse effects on the nasal cavity, it is concluded that NOAEL/NOAEC is 40 ppm in rats and that LOAEL/LOAEC is 10 ppm in mice. (The study was carried out on a commission from the Ministry of Health Labour and Welfare of Japan.)

P-19 Nasal Lesion in Rats and Mice Exposed to Methylamine for 104-weeks

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Methylamine is an irritating gas that dissolves in water in high rate, and its aqueous solution is strongly basic. In the present study, nasal lesions were examined by inhalation exposure of methylamine to male and female F344 rats and B6D2F1 mice for 104 weeks.

<Methods> The 104-week study consisted of 3 exposed groups and one control group, each comprising of 50 animals of both sexes (starting at 6 weeks of age), and the methylamine concentrations used were 5, 20 or 80 ppm (v/v) in rats and 5, 15 or 45 ppm (v/v) in mice. The nasal cavity was fixed in 10% buffered formalin and was decalcified in formic acid-formalin solution. The nasal cavity was trimmed at three levels, and embedded in paraffin. The sections were stained routinely with hematoxylin and eosin.

<Results> No increased in tumors was indicated in the rats and mice. In the rats, inflammation and squamous metaplasia in the respiratory epithelium were increased in both sexes exposed 80 ppm, and hyperplasia and ulcer in the transitional epithelium were observed in both sexes exposed 80 ppm. In the mice, inflammation and hyperplasia of the transitional epithelium were increased in 45 ppm exposed males and 15 and 45 ppm exposed females, and squamous metaplasia of transitional epithelium and eosinophilic change of olfactory epithelium were increased in 45 ppm exposed females. Nasal lesions were observed in frontal part of the nasal cavity in both rats and mice.

<Conclusion> 104-weeks inhalation study of methylamine observed the lesions in the respiratory epithelium such as inflammation, squamous metaplasia and transitional cell hyperplasia. Cytotoxic changes of the respiratory epithelia were observed followed from 13-week inhalation exposure. Hyperplasia of the transitional epithelium was found in 104-week inhalation exposure study. In the nasal cavity, adverse effects of methylamine were observed at lower concentrations in mice than rats. In mice, the effect was observed at lower concentrations in females than males. No-Observed-Adverse-Effect Level / No-Observed-Adverse-Effect Concentration (NOAEL / NOAEC) of methylamine were 20 ppm in the rats and 5 ppm in the mice. (The study was carried out on a commission from the Ministry of Health Labour and Welfare of Japan)

P-20 Influence of the fiber length of multi-wall carbon nanotube on its ability to induce mesothelioma in rats

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<Introduction> We previously reported that mesothelioma is induced in F344 rats by an intrascrotal or intraperitoneal administration of multi-wall carbon nanotube (MWCNT). Characteristics of MWCNT has been thought to be largely involved in its ability and magnitude to induce mesothelioma. In this context, we compared inducibility of mesothelioma in rats by MWCNTs with different fiber lengths.

<Methods> MWCNTs from W-company (2 types: WS-CNT, 0.5-2 mm in length, 40-70 nm in diameter, Fe content 0.046%; and WL-CNT, 0.5-10 μm in length, 85-200 nm in diameter, Fe content 6.503%), and from S-company (2 types: V-CNT, 8 μm in length, 150 nm in diameter, Fe content 0.031%; and VX-CNT, 3 μm in length, 10-15 nm in diameter, Fe content 1.233%) were intraperitoneally administered to male F344 rats (15 rats per group) with a single dose of 1 mg/kg body weight. Rats were then kept for up to 52 weeks.

<Results and disccusion> All WL-CNT rats were autopsied due to death or being moribund within 26-43 weeks. In V-CNT group, 1 and 9 rats died and became moribund, respectively, within 29-50 weeks, while 4 rats survived for 52 weeks. All rats of WS- and VX-CNT groups were alive at the end of the scheduled 52-week observation period. In all 14 and 12 rats in WL- and V-CNT groups, respectively, intraperitoneal tumor nodules were detected, whereas no mesothelioma-related gross findings were observed in WS- or VX-CNT groups. Histologically, in all animals of WL- and V-CNT group, mesotheliomas were detected, and MWCNT was observed in the submetothelial area as single fibers form or fine agglomerates of fibers. In WS- and VX-CNT groups without mesothelioma, MWCNT was mostly encapsulated in granulomas and not freely observed the submesothelial area. It is thus suggested that the fiber length and the resultant way of presence in the submesothelial area of MWCNT is one of the most critical factors for its carcinogenicity in rats.

P-21 Pulmonary Toxicity in Rats Exposed to Multi-Walled Carbon Nanotube (MWCNT) for 13-Weeks

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The long fibers such as straight type of MWCNT have been demonstrated the hypothesis for similar risk to asbestos-induced inflammation and mesothelioma in the pleura. We conducted 13-week inhalation study of rat for rangefinding study of carcinogenicity study of MWCNT by whole body exposure. This study presents the data of pulmonary toxicity in 13-week inhalation study of rat.

<Methods> MWCNT employed MWNT-7 (Hodogaya Chemical, Co. Ltd.). A dry aerosol generator by cyclone sieve method was used in whole-body inhalation exposure system. F344 rats of both sexes were exposed by inhalation to MWCNT aerosol for 6 h/day, 5 day/week and 13 weeks at doses of 0.2, 1 or 5 mg/m^3. Complete necropsy was preceded, and organ weight, microscopy and bronchoalveolar lavage fluid (BALF) were examined.

<Results> The mass concentration in the inhalation chambers of MWCNT-exposed groups were controlled to the each target concentration throughout 13 week exposure period. Lung weights increased in the rats exposed to 1 and 5 mg/m^3. Histo- pathologically, granuromatous changes were observed in all MWCNT-exposed males and in 1 and 5 mg/m^3 exposed females. Localized fibrosis of the alveolar wall was shown in male and female rats exposed to 1 and 5 mg/m^3. MWCNTs were deposited in the lung of male and female rats in the all MWCNT-exposed group. Goblet cell hyperplasia in the nasal cavity was observed in the 1 and 5 mg/m^3 exposed rats. In cytological analyses of BALF, the number of neutrophils and lymphocytes were increased in the 1 and 5 mg/m^3 exposed male and female rats. In BALF analyses, concentration-related significant increase occurred for ALP and LDH activity and total protein and albumin concentration in the all MWCNT-exposed rats. Number of neutrophils and lymphocytes were increased in the 1 and 5 mg/m^3 exposed rats.

<Conclusion> Concentration-related pulmonary toxicity in the BALF analysis and histopathological examination were observed to the 0.2 mg/m^3 MWCNT-exposed rats in the MWCNT 13-week inhalation study by whole-body exposure. Therefore, No-Observed-Adverse-Effect Level (NOAEL) was not determined, and Low-Observed-Adverse-Effect Level (LOAEL) was determined at 0.2mg/m^3 for those changes in BALF and histopathological findings.

P-22 Effects of multiwall carbon nanotubes on the lung at 2 and 52 weeks after pulmonary instillation

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Using a sieve, MWCNT was separated into3 fractions; the remaining (R), flow through (FT) and whole (W). Effects of each fraction on the lung, cell proliferation, and gene expression were examined. Each fraction (1 mg) was administered into rat lung and the rats were sacrificed at 2 and 52 weeks after administration. Mean length of FT and W was 2.6 and 4.2 mm, respectively. After exposure of each fraction to cultured macrophage, the conditioned medium increased the growth of A549 human lung carcinoma cells. Four genes (Csf3, IL6, Cxcl2, Ccl4) were highly expressed by microarray analysis. mRNA and protein expression of 4 molecules were increased in all 3 fractions. In the lung tissue, histological examination displayed existence of MWCNT, inflammation and granulation at 2 experimental weeks. MWCNT was seen and granulation remained at 54 experimental weeks. Mean areas of inflammation in FT was significantly greater than R and W at 2 experimental weeks. Length of MWCNT affected the area of inflammation in short-term experiment. Once MWCNT was exposed to the lung, MWCNT and inflammatory reaction remain at least 52 weeks after exposure.

P-23 Carcinogenic Potential of the Lung in Long Term after Inhalation of Carbon Nano Tubes

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Risk of asbestos inhalation such as lung cancer, malignant mesothelioma and fibrosis are well known to be induced in more than 30 years after the first exposure. Because carbon nanotubes (CNT) have a fibrous structure similar to asbestos, there is a strong concerns that inhalation of CNT may induce such risk in long-term after the exposure.

The present study was conducted to examine carcinogenic potential in the lung in long-term after the exposure. Male F344 rats were treated with five times over a 9-day period by intra pulmonary spraying (IPS) of 0.5 mL of 250 ug/mL suspensions of single or multi-walled carbon nanotubes (SWCNT-N, MWCNT-N, MWCNT-M) or crocidolite (CRO). The rats were killed in six hours, and in 30 days after the last IPS, respectively, and inflammation, macrophage induction and CD163 positive macrophages in the lung were examined at each time point.

Relatively strong inflammation was observed in the lung of every treatment group in 6hrs after IPS. Such inflammatory changes were relieved in MWCNT-M and CRO group in 30 days after IPS although such lesions were persisted in SWCNT-N, MWCNT-N group. The number of macrophage was increased in every group in 6hrs after IPS. However, 58%, 56% and 58% of the macrophages were remained in the lung in vehicle, MMWCNT-M and CRO group while 82% and 77% were observed in SWCNT-N and MWCNT-N, respectively in 30days after IPS. IHC study of CD163, which is the marker of M2 type macrophages, revealed that small number of CD163 positive macrophages were observed in every group in 6hrs after IPS, while very few was observed in every group in 30days after IPS.

These results indicate that it would be necessary to evaluate risk of nano materials in long term after the exposure, because activity of macrophages in long term exposure is different from that just after exposure.

P-24* A 13-week Repeated Dose Study of 3-MCPD Esters in Rats.

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3-Monochloropropane-1,2-diol (3-MCPD) is regarded as a rodent renal and testicular carcinogen. Furthermore, 3-MCPD esters may be generated in various foods and food ingredients as a result of food processing, raising the question of human exposure. Since there are limited reports about toxicity of these compounds, we conducted the present 13-week subchronic toxicity study of 3-MCPD esters (3-MCPD palmitate diester, 3-MCPD palmitate monoester, 3-MCPD oleate diester) in rats.

We orally administered a carcinogenic dose (3.6×10^{-4} mol/kg bw) of 3-MCPD or these esters at the equivalent molar concentrations and two 1/4 lower doses 5 times a week for a total of 13 weeks to 6-week-old F344 male and female rats (10 animals/group).

In both males and females, there were no significant differences in final body weights among the groups. The absolute and relative kidney weights were significantly increased in rats treated with the esters at medium and high doses as compared to the vehicle control group. Relative liver weights were significantly increased in rats treated with the esters at high dose, except for females treated with the 3-MCPD palmitate monoester. On histopathological analysis, a significantly increased incidence of renal mineralization in all high dose ester-treated females, and of apoptotic epithelial cells in the epididymis of high dose ester-treated males were observed as compared to the vehicle controls. In the 3-MCPD group, 1 male and 5 females died by week 4 with renal tubular necrosis. At week 13, significant increase of the absolute and relative kidney weights and apoptotic epithelial cells in the epididymis were also observed in 3-MCPD-treated rats.

The results suggested that these 3-MCPD fatty acid esters have potential for toxicity to the rat kidneys and epididymes at high dose, similar to 3-MCPD. NOAELs of 3-MCPD palmitate diester, 3-MCPD palmitate monoester and 3-MCPD oleate diester were suggested to be 14, 8 and 15 mg/kg/day, respectively.

P-25＊ A 13-Week Repeated Dose Toxicity Study of Glycidol Fatty Acid Esters in F344 Rats

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Glycidol fatty acid esters (GEs) have been recently identified as food process contaminants in refined edible oils. Although there is toxicological concern arising from potential release of glycidol from parent esters during digestion in the gastrointestinal tract, little is known about *in vivo* toxicity of GEs. In the present study, subchronic toxicity of two types of GEs, oleate and linoleate esters, was investigated with administration at concentrations of 0, 225, 900 and 3600 ppm (equivalent molar concentration to 800 ppm glycidol) in drinking water for 13 weeks to male and female F344 rats. For comparison, treatment with 200 and 800 ppm of glycidol was also performed. Body weight gain of both sexes was markedly reduced with 800 ppm glycidol compared to the controls. Hematological data showed significant increase of MCV in 800 ppm glycidol females and decrease of WBC in 3600 ppm oleate ester females. In serum biochemistry, increase of total cholesterol, increase of potassium, and decrease of ALT were detected in 800 ppm glycidol males, 3600 ppm linoleate ester males, and 800 ppm glycidol females, respectively. Serum creatinine levels in both sexes were decreased in the 800 ppm glycidol group. Relative weights of kidney and spleen were significantly increased in 200 and 800 ppm glycidol males and 800 ppm females. In addition, increase of relative kidney weights was also found in 3600 ppm oleate ester males. On histopathological assessment, increased cell debris was observed in the epididymal ducts of 800 ppm glycidol males, but not in ester groups. There were no significant lesions in the kidney of any groups. Although more detailed analysis will be needed to clarify any testicular toxicity of glycidol and *in vivo* genotoxicity of GEs, these results indicate that oleate and linoleate esters might be less toxic to F344 rats than glycidol itself.

P-26＊ Dose Effect Relationship between Co-exposed Phthalate Esters on the Liver and Male Genital System after 90 Days Repeated Oral Administration in Rats

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In the last annual meeting of this society, we reported the effect of co-exposure to different phthalate esters on the liver and male genital system after 90 days repeated oral administration in rats. As a result, di(2-ethylhexyl)phthalate (DEHP) exerted antagonistic activity on PPARa-independent hepatocarcinogenic response induced by dihepthyl phthalate (DHP). On the other hand, DHP exerted additive or enhancing effect on PPARa activity-related testicular atrophy of DEHP. The present study was performed to examine the dose effect relationship of co-administration to the two phthalate esters on the rat liver and male genital system. For this purpose, male F344 rats were administered DEHP-alone (12,000 or 24,000 ppm), DHP-alone (10,000 or 20,000 ppm), DEHP + DHP (12,000 ppm + 10,000 ppm, 12,000 ppm + 5,000 ppm, 12,000 ppm + 2,500 ppm, 12,000 ppm + 1,250 ppm, 6,000 ppm + 10,000 ppm, 3,000 ppm + 10,000 ppm or 1,500 ppm + 10,000 ppm) for 90 days. Histologically, hepatocyte hypertrophy with increased cytoplasmic eosinophilia was observed with DEHP-alone at ≥ 12,000 ppm. On the other hand, swelling and vacuolar degeneration of hepatocytes were observed with DHP-alone at ≥ 10,000 ppm. DHP-induced histopathological liver cell changes and the increases in GST-P-positive liver cell foci and Ki-67-positive cells were dose-dependently weakened by DEHP co-exposure from 1,500 ppm, and were entirely disappeared at 12,000 ppm. In the male genital system, decreased testicular absolute weight and diffuse seminiferous tubular atrophy characterized by shedding of germ cells were observed with DEHP-alone at 24,000 ppm and co-exposure of DEHP at 12,000 ppm and DHP at 10,000 ppm. Slight and focal seminiferous tubular atrophy dose-dependently increase with co-exposure of DEHP at 12,000 ppm and DHP at 1,250 to 5,000 ppm, but was lacking with DHP-alone and co-exposure of DHP at 10,000 ppm and DEHP at 1,500 to 6,000 ppm. These results suggest that DEHP exerted dose-dependent antagonistic activity on PPARa-independent liver changes induced by DHP from low doses. In male genital system, DHP exerted enhancing but not additive effect on the DEHP-induced testicular atrophy at higher doses.

P-27＊ 90-day Repeated Dose Toxicity and Genotoxicity Tests of Coptis chinensis in F344 Rats

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Coptis chinensis has been widely used in oriental medicine and it has been known to have some pharmacological effects such as antibacterial, antiviral, anti-inflammatory activities. However, its toxicity has been yet fully elucidated. In the present studies, we carried out a 90-day repeated dose toxicity study of *Coptis chinensis* (orally five times per week at doses of 25, 74, 222, 667 and 2,000 mg/kg) using in F344 rats and its mutagenic potential in genotoxicity studies (bacterial reverse mutation test in *Escherichia coli* WP2*uvrA, Salmonella typhimurium* TA98, TA100, TA1535 and TA1537, chromosome aberration test using Chinese Hamster Lung cell, micronucleus test using ICR mice and mammalian cell gene mutation test using the L5178Y/TK$^{+/-}$ mouse lymphoma). Decreased body weights in males of 2,000 mg/kg group during 9 to 12 week ($P<0.005$), decreased food consumption in both sexes of 2,000 mg/kg group on 1 week ($P<0.005$), increased N-Acety1-β-Glucosaminidase (NAG) in males of 2,000 mg/kg group ($P<0.001$), and increased creatinine in both sexes of 2,000 mg/kg group ($P<0.005$) were relevant. For genotoxicity study, the bacterial reverse mutation test was positive at doses of 313 ug/plate in TA, and 625, 1,250, 2,500 ug/plate in WP2*uvrA,* However, it did not have any evidence for chromosome aberration and micronucleus formation.

Thus, the no-obsevered-adverse-effect level (NOAEL) of *Coptis chinensis* in F344 rats is considered 667 mg/kg. For genotoxicity study, *Coptis chinensis* did not show the mutagenic potential, chromosome aberration and micronucleus formation.

P-28＊ Historical Data on Neuropathological Examination in Acute and Repeated Dose Oral Neurotoxicity Study of Rats

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Neurotoxicity study is conducted to confirm neuronal effects which are suggested by evidence in preceding general toxicity studies. Neuropathologists require training on differential diagnosis to be divided into two distinct categories: artifacts and neuronal changes. They also need knowledge and experience on historical background data from several strains of rats and typical neuronal changes induced by positive neurotoxicants. The study is designed to establish well-organized background data in acute and repeated dose 28 day or 90 day oral neurotoxicity studies of Wistar, F344, and SD rats. Some of the studies were conducted using neurotoxicants such as trimethyl tin, hexachlorophene, acrylamide. Rats were housed in wire-meshed cages, and freely given water and feed. During the study, rats were subjected to detailed clinical observation and functional observation according to the guidelines. The whole body of each rat was perfused with heparinized 0.1M phosphate buffer followed by a mixed solution of phosphate-buffered 1% glutaraldehyde and 2% paraformaldehyde mixed solution using tubing pump. CNS, eye and calf muscle were processed routinely, embedded in paraffin, and the sections were stained with H&E, and PNS was embedded in epon and the sections were stained with toluidine blue. In the CNP, there were higher incidences of neural fiber (axonal) degeneration in the pons (trapezoid body) and spinal cord (cervical and lumber swellings) than other sites. The lesions in the pons were lower in Wistar rats than the other strains. In the spinal cord, the lesions were commonly observed in each strains of rats; those were frequently observed in the cervical swelling than lumber swelling. Retinal changes including hypoplasia and dysplasia were observed in the eye; the incidences of the lesions were relatively lower in SD rats than the other strains. In the PNS, neural fiber (axonal) degeneration with myelin bubble was observed in the sciatic and tibial nerves. Positive neurotoxicants induced typical changes such as neuronal degeneration and necrosis, demyelination in the white matter, degeneration in the PNS. As whole body perfusion sometimes induces artifacts at a certain part of the nervous tissues, neuropathologists need to distinguish the artificial changes with specific neuropathological changes. They should work through continuous education and observation using the sections from rats treated with positive control substances.

P-29＊ Ultrastructural Analysis of Demyelination and Remyelination Induced by Cuprizone in Mice

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<Purpose> Cuprizone (CPZ), a copper chelating agent, induces central nervous system (CNS) demyelination resulted from functional impairment of oligodendrocyte. We analyzed ultrastructurally CNS demyelination and remyelination induced by CPZ in mice with time.
<Material and method> C57BL/6 mice were fed diet containing 0.2% CPZ for 5 weeks. After necropsy, their brains were analyzed sensitive lesions of demyelination stained with Kluver-Barrera (KB) and myelin basic protein (MBP) by imuunohistochemistry. In addition, we evaluated the process of demyelination and remyelination in the brain with KB staining using mice fed diet containing 0.2% CPZ for 1 to 4 weeks and for 5 weeks followed by 0 to 14 days recovery period over time. Furthermore, we also analyzed axon, myelin sheath and glial cells with transmission electron microscopy in this model.
<Result and Discussion> Caudal corpus callosum (CC), rostral CC and cerebellar nucleus were sensitive lesion of demyelination when the mice were fed diet containing 0.2% CPZ for 5 weeks. Next, when we evaluated demyelination and remyelination of caudal CC with KB staining, demyelination and infiltration of glial cells in caudal CC were observed at day 0 of recovery, and tendency of remyelination occurred after day 10 of recovery. In ultrastructural examination, swelling of mitochondria in axon and formation of space between axon and myelin sheath were observed at day 0 of recovery, and multiply-twined myelin sheath was also observed after day 10 of recovery. We also show the results of demyelination process in mice fed 0.2% CPZ containing diet for 1 to 4 weeks, and summarized ultrastructural features of demyelination and remyelination induced by CPZ in mice.

P-30＊ Effect of Glycidol on Adult Neurogenesis in the Hippocampal Dentate Gyrus after 28 Days Repeated Administration in Rats

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Glycidol is generated during digestion of glycidol fatty acid esters, food process contaminants contained in refined edible oils. Recently, we revealed that developmental exposure to glycidol through maternal drinking water at 108.8 mg/kg resulted in the aberration of late-stage neurogenesis in the hippocampal dentate gyrus of rat offspring, while maternal animals developed axonopathy involving both central and peripheral nervous systems. In the present study, to examine the possibility whether the similar effect suggestive of developmental neurotoxicity could be detected in a scheme of 28-day toxicity study, we examined the effect of glycidol on adult neurogenesis in the hippocampal dentate gyrus using young adult rats. Glycidol was administered to five-week-old male Sprague-Dawley rats repeatedly for 28 days by oral gavage at 0, 30 or 200 mg/kg. The brains, trigeminal and sciatic nerves were examined histopathologically. Immunohistchemistry was performed on the brain with antibodies against neurofilament-L (NF-L), dpysl3 (TUC-4), doublecortin (DCX), NeuN, reelin, calretinin, proliferating cell nuclear antigen (PCNA), as well as apoptosis assay using TUNEL method. At 200 mg/kg, animals revealed abnormal gait and histopathological and immunohistochemical changes suggestive of axonal injury as evidenced by generation of $NF\text{-}L^+$ spheroids in the cerebellar granule layer, central chromatolysis in the trigeminal nerve ganglion cells and axonal degeneration in the sciatic nerves. At the same dose, animals revealed aberration in neurogenesis as evidenced by decreases of TUC-4- and DCX-positive cells in the subgranular zone (SGZ) of the dentate gyrus and increases of NeuN-, calretinin- and reelin-positive cells in the dentate hilus. There were no differences in the number of PCNA- or TUNEL-positive cells in the SGZ. These results suggest that glycidol causes aberration in adult neurogenesis in the SGZ at the late stage involving the process of neurite extension through targeting immature granule cells and probably type-3 progenitor cells in a scheme of 28-day toxicity study similar to the developmental exposure study of glycidol.

P-31＊ Similar Expression Change of Midline1 on Neuronal Stem/Progenitor Cells Between Developmental and Adult-stage Hypothyroidism in the Hippocampal Dentate Gyrus in Rats

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We previously clarified different impact on neurogenesis in contrast to similar distribution changes of GABAergic interneurons in the hippocampal dentate gyrus between developmental and adult-stage hypothyroidism induced by exposure to methimazole (MMI) in rats. We found a reduction of paired box 6-positive stem or early progenitor cells and a transient reduction of doublecortin-positive late-stage progenitor cells in the developmental hypothyroidism with MMI, while these cells were unchanged in the adult-stage hypothyroidism. In another study of developmental exposure to manganese (Mn) in mice, we identified *Mid1* showing hypermethylation of the promoter region in the dentate gyrus of mice offspring suffering permanent disruption of neurogenesis targeting late stage granule cell lineage. Interestingly, midline1-positive cells distributed in both of the hilar interneurons and subgranular zone stem/progenitor cells with right side predominance in untreated controls; however, Mn-exposure canceled this asymmetry to the adult stage. In the present study, we examined the cellular distribution of midline1-expressing cells in the dentate gyrus after developmental and adult-stage hypothyroidism induced by MMI-exposure in rats. Exposure to MMI at 50 and 200 ppm in the drinking water was performed using pregnant rats from gestation day 10 to postnatal day (PND) 21 (developmental hypothyroidism) and adult male rats from PND 46 to PND 77 (adult-stage hypothyroidism). Offspring with developmental hypothyroidism at PND 21 or PND 77, and animals with adult-stage hypothyroidism at PND 77 were immunohistochemically examined. As well as in mice, rats also exhibited expression of midline1 in the subgranular zone stem/progenitor cells with right side predominance in untreated controls. By both developmental hypothyroidism and adult-stage hypothyroidism, midline1-positive cells significantly decreased with MMI at both 50 and 200 ppm, and MMI-exposure canceled this asymmetry to the adult stage. These results suggest that developmental hypothyroidism causes permanent cancellation of bilateral difference of neurogenesis probably through disruption of epigenetic gene control of midline1-expression. Such risk also could appear in the stem cell population by adult-stage hypothyroidism.

P-32 Strain differences in pleural mesothelial cell reactions induced by TISMO fibers infused directly into the thoracic cavity.

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The development of approaches for therapy of malignant mesothelioma is an urgent priority, and an appropriate animal model is necessary for this purpose. Although we have previously reported that the fiber-shaped TISMO, morphologically similar to asbestos, can induce a severe mesothelial reaction in A/J mice, it is important to clarify any strain differences.

In the present study, female A/J, C3H/HeN, ICR and C57BL/6 mice were employed as test strains. Potassium octatitanate fibers, trade name TISMO and chemical formula $K_2O \cdot nTiO_2$, were supplied by Otsuka Chemical Co., Ltd. (Osaka) with dimensions mostly shorter than 50μm in length and less than 2μm in width. At the beginning of the experiment, all mice underwent a left thoracotomy and direct administration of 3mg of TISMO particles suspended in 0.2 ml saline into the left thorax. Each mouse was given an intraperitoneal injection of 0.2 ml pentobarbital sodium (Somunopentyl, Kyoritsu Seiyaku Co., Tokyo) with 10 times dilution (0.06-0.1ml /10 g body weight). Under deep anesthesia, a skin incision (about 7mm) was performed on the left axilla. After confirmation of the location of the thoracic wall, thoracotomy was completed with an incision (approximately 5mm) between ribs. The left lung was observed directly through the opened hole and atelectasis was confirmed. After infusion of TISMO solution into the left thoracic cavity, the skin was clipped together to close the thorax. The experiment was terminated after 21 weeks and all groups were sacrificed and the mesothelium and main organs were examined histopathologically. To contribute to mechanistic analysis, iron staining with Berlin blue and Turnbull's blue, and immunostaining for calretinin were also performed.

The present experiment demonstrated only minor strain differences in the degree of pleural reaction to TISMO. However, there was clear variation in the iron and lymphocyte accumulation in the pleura and in the liver. This difference in response to TISMO fibers *in vivo* is important information when considering the development of mesothelioma as an animal model and the extrapolation to human risk from such animal studies.

P-33* Effects of chronic inflammation by quartz on DHPN–induced lung carcinogenesis in F344 and Wistar-Hannover rats – strain difference studies-

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It is well known that intratracheal instillation (i.t) of fine particles of quartz induces severe chronic inflammation in the lung. We have reported the effects of chronic inflammation induced by quartz on lung tumorigenesis initiated by N-bis (2-hydroxypropyl) nitrosamine (DHPN), focusing on lung adenoma as the end-point marker in F344 and Wistar-Hannover male rats for 25 weeks (28th JSTP, 2011). In this study, the same experimental design with the longer for 52 weeks, focusing on lung tumors (adenoma and adenocacinoma), was examined.

Male F344 (Groups 1, 3, 5 and 7) and Wistar-Hannover rats (Groups 2, 4, 6 and 8) were maintained in the Kagawa University Animal Facility according to the Institutional Regulations for Animal Experiments. On week 0, the animals in Groups 1, 2, 3 and 4 were initiated for lung tumorigenesis by 0.1% DHPN in drinking water for 2 weeks, and 2 mg quartz/rat was administrated to animals in Groups 1, 2, 5 and 6 by i.t. on week 4. On week 52, all rats were sacrificed after sampling their blood and bronchoalveolar lavage fluid (BALF). Carcinogenic potential was analyzed by comparing the incidences and areas of their tumors for each histopathological lung proliferative lesions. And lung inflammation was evaluated by hematological analysis, Interleukin-6 (IL-6) in the serum and BALF, inflammatory cellular fraction in BALF and histopathological parameters.

IL-6 in BALF of the quartz i.t. groups (Groups 1, 2 and 5) were significantly increased. The percentage of neutrophils and eosinophils in BALF were increased significantly in both strains with DHPN + quartz i.t. (Groups 1 and 2). Histopathologically, severe infiltration of inflammatory cells was observed in the groups of quartz i.t., and those inflammatory changes in F344 rats showed stronger than in Wistar-Hannover rats. Incidences of adenocarcinoma in DHPN + quartz i.t. (Groups 1 and 2) were significantly increased in both strains. Furthermore, the area of lung tumors in F344 rats was larger than in Wistar-Hannover rats.

This experiment indicated that chronic inflammation by quartz i.t. trends to have a potential of promoting effect on lung carcinogenesis in both F344 rats and Wistar-Hannover rats. In addition, F344 rats have stronger sensitivity for induction of inflammation by quartz, and stronger promoting effects on DHPN-lung carcinogenesis, than Wistar-Hannover rats.

P-34 Mouse Colon Cancer Model Using Benzo[*a*]pyrene and Dextran Sulfate Sodium: Strain Difference in Tumor Incidence

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<Introduction> Benzo[*a*]pyrene (BP) is highly mutagenic but not carcinogenic to the colon by oral exposure in mice. We showed previously that it takes only 4 weeks for tumors to develop in the colon when $CD2F_1$ mice are treated orally with BP followed by 4% dextran sulfate sodium (DSS) in drinking water. In the present study, CD-1 mice were given BP/DSS to investigate the strain difference in this model.

<Materials and Methods> Exp.1: Male CD-1 mice at the age of 7 weeks (5 mice/group) were treated with 3, 4, or 5% DSS in drinking water for 7 days to examine the tolerability for DSS.

Exp.2: On the basis of the results of Exp.1, 7-week old male CD-1 mice (5 mice/group) were treated with oral doses of BP at 125mg/kg for 5 days, and subsequently with 2, or 2.5% DSS in drinking water for 7 days. Mice were necropsied at 4 weeks after cessation of DSS treatment, and subjected for histological examination of the colon.

<Results and Discussion> Exp.1: Because of death at all doses (1-2 mice/dose, 3%, 4% and 5%) of DSS, the concentration of 2% and 2.5% was chosen for the following studies.

Exp.2: In the BP/2% DSS group, neoplastic lesions (adenoma and adenocarcinoma) were present in the colonic mucosa in 2/5 mice with mild colitis and dysplasia. In the BP/2.5% DSS group, 2/5 mice died after DSS treatment due to severe colitis. In survivors, at 4 weeks after DSS treatment, neoplastic lesions were observed in addition to dysplasia and colitis in one of three mice. The other 2 mice had severe colitis, with few dysplastic lesions in one of them.

The present results indicate that this colonic cancer model is valid also in CD-1 mice, with suitable concentration of DSS being 2%.

Compared with $CD2F_1$ mice, CD-1 mice appear to be more sensitive to DSS treatment, and have lower tumor incidences in the BP/DSS model.

P-35 Examination of Formation and Development of Ovarian Follicles among the Different Strains of Rats

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In reproduction studies with rats, strain differences are observed in the number of primordial follicles in the ovary in addition to reproductive parameters including number of corpora lutea and pups delivered. Also in general toxicity studies, histopathological lesions associated with aging are varied among rat strains. In order to understand the strain difference, the number of follicles in each developmental stage was counted on rats of 3 different strains at the age of 4 days, 17 weeks, 1 year and 2 years. At 4 days of age, serial sections were prepared from ovaries of Wistar and SD rats. The numbers of primordial and primary follicles were counted on the section in which the longest diameter was included, and percentage of the number of follicles in each developmental stage against the total number of follicles was calculated. At 17 weeks of age, the number of primordial follicles was counted on ovary sections from Wister, SD and F344 rats prepared by the method required in the reproduction study. At 1 and 2 years of age, a section in which the longest diameter was included was prepared from each ovary of 3 strains of rat, and the numbers of small, medium and large follicles in each sex cycle were counted. As the result, although total follicular number was comparative among Wister and SD rats of 4 days of age, the ratio of primary follicles in SD rats was more than in Wistar rats. It was suggested that the follicular development was preceded in SD rats. At 17 weeks of age, the number of primordial follicle was comparative between Wistar and F344 rats, but was 1.5 times more in SD rats. At 1 year of age, the number of primordial follicles in F344 rats was twice as much as those in Wistar and SD rats. Likewise, the number of residual primordial follicles in F344 rats was largest among 3 strains at 2 years of age. The strain difference in the number of primordial follicles at 17 weeks of age was considered to depend on the difference of efficiency of follicular consumption because there was no clear difference in the size of follicular pool at 4 days of age. Histopathlogical valuation on morphological difference such as atretic follicles and small follicles without oocyte are needed to evaluate the strain difference observed in rats at 1 and 2 year of age, because the strain difference of ovarian atrophic process associated with aging was deeply related at the ovarian phase.

P-36* Spontaneous megakaryocytic hypoplasia in a SD rat

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巨核球系細胞の自然発生病変は実験動物において報告が少なく、その発生機序についても不明な点が多い。今回我々は、SDラットにおいて自然発生性の巨核球低形成の一例を認めたため報告する。SDラットにおける同様の病変の自然発生は、現在までに報告がなされていない。

【症例】本例は10週齢時に剖検された雌のCrl:CD (SD) ラットであり、体重、摂餌量、摂水量に異常は認められなかった。剖検時、肺に暗赤色巣が認められ、血液学的検査では血小板数の顕著な低値 ($59\times10^3/\mu L$) が認められた。器官重量、血液化学的検査では、同週齢のSDラットと比較して明らかな差は認められなかった。

【病理学的特徴】骨髄および脾臓では、同週齢のSDラットと比較して巨核球数が明らかに少なかった。骨髄についてvon Willebrand factorに対する免疫染色を実施したところ、陽性を示す巨核球が少ないことが確認された。認められた巨核球についてはPCNA陽性率に明らかな異常は認められなかったものの、細胞径が小さく、核の分葉も乏しい傾向が認められた。骨髄における巨核球以外の造血細胞では、細胞密度、M/E比および成熟度に明らかな異常は認められなかった。この他の臓器では、肺暗赤色巣部および腺胃粘膜において出血が認められた。

【まとめ】本例では造血組織において巨核球のみ細胞数が明らかに少なかったことから、巨核球低形成と診断し、血小板数の低値はこれに起因するものと判断した。巨核球の細胞径が小さく、核の分葉も乏しいことから、低形成の原因として分化・内分裂の障害が考えられた。分化・内分裂障害の原因としてThrombopoietinあるいはその受容体の異常が疑われたため、これらの可能性について現在検討中である。

P-37＊ Th2-based host immune response promote the development of cryoglobulinemia in mice infected with *Capillaria hepatica*

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The detailed pathogenesis of type II mixed cryoglobulinemia (CG) has not yet been understood. In this study, we aimed to elucidate the host immunologic factors of CG caused by *Capillaria hepatica* infection in mice.

We used ICR, C57BL, and BALB/c strains of mice, which were known to exhibit different immune response. Mice orally infected with 5,000 *C. hepatica* eggs were sacrificed on days 20 and 24 post-infection. Cryoglobulin formation in the serum and histological and immunofluorescence findings of the liver, kidney, and spleen were examined. The immune phenotypes of splenic lymphocytes were evaluated by flow cytometry and confocal microscopy based on expression of IL-4, IL-5, CD4, CD5 and CD45R/B220. IL-5 level in the serum was measured by ELISA.

Infected mice of all strains developed type II CG. CG was more severe in BALB/c mice than in ICR and C57BL mice. The $IL\text{-}4^{+}CD4^{+}$ T cells were significantly increased in the spleen of infected BALB/c mice. Furthermore, these mice showed increased $IgM^{+}\kappa^{+}$ B cells in the spleen, severe eosinophilic infiltration in the liver and spleen, and elevated serum IL-5 level. Flow cytometry revealed that the increased B lymphocytes in the spleen were B-1a cells, which expressed IL-5 receptors.

These results suggest that severe CG in BALB/c mice might be induced by a marked Th2-type immune response due to infection with *C. hepatica*. It is possible that the expansion of B-1a cells by the stimulation of IL-5 is a key event in the development of CG following *C. hepatica* infection under Th2-based host immune status.

P-38＊ A Four-week Repeated Study of Intravenous Toxicity of Recombinant Human Interleukin-2 in Sprague-Dawley Rats

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Interleukin-2 (IL-2) is a lymphokine with a potential role in cancer therapy. Many clinical trials of recombinant human IL-2 (rhIL-2) have been conducted to treat malignant renal carcinoma, melanoma, leukemia, lymphoma, multiple myeloma. BMI Korea has developed a method to manufacture rhIL-2 in bulk using Escherichia coli as a biosimilar. Prior to conducting human clinical trials, 4-week repeated toxicity study of rhIL-2 was conducted. In this study, rhIL-2 was administered intravenously to rats at doses of 9×10^6, 18×10^6, and 36×10^6 IU/kg/day over a period of 4 weeks. Adverse effects were observed in RBC, HGB, HCT, reticulocyte, mesenteric lymph node from middle dose, and changes of total bilirubin, femoral bone marrow, thymus, and clinical signs were observed at high dose. Local irritation was determined at low dose of female rats and at middle dose of male ones. Taken together, no observed adverse effect levels (NOAEL) was determined at dose of 9×10^6 IU/kg/day in male, and NOAEL was determined under the dose level in female rats. It suggests that present rhIL-2 is less toxic prior produced rhIL-2 and may be contribute more effective cancer-treatment strategy in human.

P-39 Spontaneous Thymoma Observed in Wistar Han rats

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Wistar Han rats are an appropriate model for toxicity and carcinogenicity studies in rodents and spontaneous thymoma is sometimes recorded in carcinogenicity studies of this strain of rats. The incidence of thymoma in historical control data of Harlan Laboratories is as follows: benign thymoma 0.64% for males, 2.39% for females; malignant thymoma 0.50% for males, 0.63% for females. The incidence of benign thymoma is higher in females with a range of 0% to 17.02% in these data. Histologically, these tumors commonly appear as solitary lesions with expansive growth, consisting of a mixture of thymic epithelial cells and lymphocytes with medullary differentiation. It was not always clear and requires careful consideration to distinguish between hyperplastic lesions and benign thymoma, and also between benign and malignant thymoma for many cases. In this report, we introduce typical hyperplastic lesions, thymoma and also the rarer epithelial cell type thymoma observed in Wistar Han rats.

P-40 Ophthalmologic and Histopathological Examinations in the Aged Buphthalmic Rabbits

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<Purpose> To evaluate functional and structural changes in the eyes of aged New Zealand White (NZW) rabbits with buphthalmos (congenital glaucoma).
<Methods> Four eyes from 3 male and a female buphthalmic NZW rabbits (3.2 to 3.8 years old) were analyzed in this study. Intraocular pressure (IOP), electroretinography (ERG) and spectral-domain optical coherence tomography (OCT) were measured before the animals were sacrificed. The eyes were enucleated and processed for light microscopic evaluation. In addition, immunohistochemical examinations for GFAP, neurofilament and vimentin were performed.
<Results> IOP in three male buphthalmic rabbits was found to be significantly higher than in normal rabbits (22.7±1.5 and 16.8±0.4 mmHg, respectively), and mean amplitude of ERG a- and b-wave was reduced significantly. In addition, glaucomatous cupping of the optic nerve and thinning of retinal nerve fiber layer were observed by OCT and the histopathological examinations. Absence and incomplete formation of trabecular meshwork, retinal ganglion cell loss and dilatation of choroidal vessels were observed in the buphthalmic eyes. Immunolocalization of GFAP and neurofilament was decreased in older buphthalmic rabbit eyes, although there was no change in the expression of vimentin between older and younger buphthalmic rabbit eyes. In contrast, IOP in a female buphthalmic NZW rabbit was found to be comparable to normal in association with the disappearance of ERG a- and b-wave, severe retinal atrophy and fibrosis.
<Discussion> These results might be suggest the uveoscleral pathway of aqueous outflow has been vicariously enhanced in aged buphthalmic rabbit eyes, and lead to a result of the failure of compensatory function.

P-41＊ Histopathological Analysis of the Ocular Lesions in Experimental Autoimmune Uveoretinitis in C57BL/6 Mice

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Experimental autoimmune uveoretinitis (EAU) is used as an experimental model for human autoimmune ocular disease such as non-infectious uveitis. Several EAU models are reported so far, and the character of each model is different based on its retinal antigen, animal species and strain. In this study, we conducted histopathological analysis of ocular lesion in EAU induced by IRBP (interphotoreceptor retionid-binding protein) immunization in C57BL/6 mice.

<METHODS> Female C57BL/6NCr Slc mice aged 10 weeks were immunized with subcutaneus injection of human IRBP peptide emulsified with Freund's complete adjuvant at the base of the tail, and then were administered an additional intraperitoneal injection of *Bordetella pertussis* toxin. Non-treated animals were used as a negative control. On day 17 post-immunization, all animals were euthanized by exsanguination under isoflurane anesthesia after ophthalmologic examination. Eyes were removed and fixed with glutaraldehyde and paraformaldehyde mixtures, and examined histopathologically.

<RESULTS> In IRBP-immunized mice, inflammatory changes in the retina (white spots), the optic disc (swelling and obscure vessels) and the retinal vessels (engorgement or perivascular cuffing) were detected ophthalmologically. Histopathological findings in animals immunized with IRBP were characterized with inflammatory cell infiltration in the uvea (iris, ciliary body, choroid), the retina and the vitreous, and retinal perivasculitis (mainly in the central retinal artery and vein). In the severe lesion, retinal folds or detachment and Dalen-Fuchs nodules were also observed. These retinal and choroidal lesions were prominent in the posterior pole centered around the optic disk. In the mild lesion, retinal or vitreous cellular infiltration and perivasculitis were limited in the optic disk and the juxtapapillary retina.

<CONCLUSION> EAU was induced by immunization of IRBP in C57BL/6 mice, and we confirmed that the ocular inflammatory lesion spread from the vessels of the optic disk and developed into retinal structural damage.

P-42＊ Spontaneous Immune-Mediated Glomerulonephritis in a Hatano Rat

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<Background> Immune-mediated glomerulonephritis can be experimentally induced in rats but spontaneous cases are rarely reported. In this report, we present a typical case of spontaneous immune-mediated glomerulonephritis in Hatano rat, which is of strain originally selected and bred from Sprague-Dawley (SD) rats based on shuttle-box tasks.

<Animal> A male Hatano rat had a tumor mass (7 mm in diameter) on the right auricle of ear and sacrificed at 41 weeks of age. Urinary screening by reagent strips revealed proteinuria (500 mg/dL). At necropsy, there were no significant gross lesions on the kidneys except several V-shaped infarct scars in the left kidney. The other organs were grossly normal and pleural or peritoneal effusion was not detected.

<Results> Histologically, irregularly sized eosinophilic hyaline materials were frequently present on the capillary wall and in the mesangium in the glomeruli. These materials were stained as red by Masson's trichrome stain. Several glomeruli included swollen podocytes with periodic acid Schiff-positive droplets, thick glomerular basement membrane (GBM), increased mesangial matrix, adhesion between the tuft and Bowman's capsule, or infiltration of ED1-positive macrophages. Immunofluorescence assay revealed granular deposits of IgG, IgM, and C3 in the glomeruli, which were coincident with those of the red materials stained with Masson's trichrome stain. By electron microscopy, subepithelial dense deposits were observed accompanied by effacement of the foot process of the podocytes and occasional irregular thickening of the GBM. On the left kidney, dilation of the tubules with interstitial infiltration of lymphocytes was present in focal areas. Antinuclear antibody test targeting normal rat liver tissue using the serum of this rat was negative. The rat also developed lymphocytic pancreatitis and mass at the right auricle of ear was diagnosed as fibrosarcoma.

<Discussion> The SD rat develops progressive chronic nephrosis with age which is characterized by increased density in the mesangium and thickened basement membrane of the capillary loops and Bowman's capsule. The chronic progressive nephrosis lacks the formation of dense deposits and is not immune- mediated. The glomerulopathy observed in this case was considered as early stage of membranous glomerulonephropathy (stage I) but further examinations were required to clarify its pathogenesis.

P-43＊ Mesangial erythrophagocytosis in renal glomeruli in aged B6C3F1 mice

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<Background> We have found frequently trapped erythrocytes in the glomerular mesangial area, while investigating various lesions of progressive glomerulonephropathy associated with aging in mice. It has been reported that anti-erythrocyte autoantibody contributes to the progress of glomerulonephropathy in mice of some strains; however, the trapped erythrocytes in the mesangial area have not been clearly described.
<Materials and Methods> The renal tissues of 220 B6C3F1 mice (approximately 110 weeks old, 110 males and 110 females) were used, and the trapped erythrocytes were observed on hematoxylin and eosin staining sections. In 14 selected cases, the sections were stained with periodic acid-methenamine-silver and Berlin blue, followed by immunohistochemistry using anti-Iba1 antibody. The ultrastructural changes were examined in 4 cases by transmission electron microscopy.
<Results> The trapped erythrocytes were observed in 208 cases (94.5%) and mesangial proliferation was noted in a number of cases. In the milder cases, erythrocytes were rarely seen in the mesangial area of a few glomeruli, while in severe cases, a number of erythrocytes were seen in the mesangial area of 20% to 30% glomeruli in the specimen. The trapped erythrocytes were more clearly demonstrated by periodic acid-methenamine-silver staining, and Berlin blue staining elucidated no reaction in glomerular mesangium. In some cases, Iba1 positive cells were occasionally seen in the mesangial area; however, the number was not associated with the frequency of the trapped erythrocytes. Ultrastructurally, phagocytic erythrocytes and its fragments were evidenced in the cytoplasm of mesangial cells and the products of lysis were not seen. In other organs including spleen and bone marrow, there was no increasing erythrophagocytosis by macrophage.
<Discussion> We revealed erythrocytes are frequently phagocytosed by glomerular mesangial cells in aged B6C3F1 mice. The mesangial erythrophagocytosis may be involved with the pathogenesis of progressive glomerulonephropathy in mice.

P-44 Cytoplasmic Vacuoles Detected in the NNK-induced Lung Tumors of A/J Mice

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The vacuoles, which are various sizes and occurs by various causes, frequently observed in cytoplasm. In this study, the property of large vacuoles detected intracellularly in the NNK-induced mice lung adenocarcinomas, were examined histopathologically.

Female 7-week old A/J mice were administered at a single dose of NNK (2 mg/0.1 ml saline/mouse, i.p.), then maintained without additional treatment until sacrifice at week 52. The 2 adenocarinomas, formalin-fixed paraffin-embedded (FFPE) lung tissue, were analyzed by light and electron microscopy. They were stained with H.E., PAS and dPAS. The expressions of perilipin and adipophilin, a member of lipid droplet-associated proteins, were examined by immunohistochemical staining.

Mucous vacuoles were not observed in the large vacuoles by PAS and dPAS stains. The perilipin expression was not detected in large vacuoles, except for adipocyte in normal tissue, immunohistochemically. Similarly, the adipophilin expression was not detected in the majority of large vacuoles. In contrast, there were adipophilin positive small vacuoles in the cytoplasm of the adenocarcinomas strongly, compared with normal cells and adenomas. Furthermore, electron microscopy showed the small empty vacuoles in the cytoplasm.

Although mucous and lipid accumulation in large vacuoles were not observed in the adenocarcinomas, these results suggest that small lipid droplets exist in the cytoplasm of adenocarcinoma. Adipophilin will be useful for the specification of lipid droplet on the FFPE tissues, those are unable to perform fat stain.

P-45 Mesothelioma of Thoracic and Abdominal Cavity in a B6C3F$_1$ Mouse

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Mesotheliomas have been repeatedly reported in human, cattle and laboratory rats, but spontaneous cases are relatively rare in mice. We encountered a case of mesothelioma with uncommon morphologic feature appeared multiply in thoracic and abdominal cavity of a male B6C3F$_1$ mouse from middle dosage group of a 104-week carcinogenicity study for certain chemical.

The affected mouse was found moribund at 103th week and was sacrificed by exsanguination under deep anesthesia immediately, then necropsied. Necropsy revealed a pulmonary nodule sized 5x4x3 mm, multiple nodules sized up to 3 mm in mediastinum, multiple nodule up to 10x9x5 mm in abdominal cavity, enlargement of intestinal, jejunal-pancreatic and renal lymph nodes up to 15x12x9 mm, a hepatic nodule sized 18x15x10 mm and a nodule of jejunal lumen sized 7x5 mm.

Histopathologically, the hepatic nodule was hepatocellular carcinoma and jejunal nodule was adenocarcinoma. Others were neoplastic nodules of same morphologic character. These nodules consisted of neoplastic cells with round or cuboidal cells with epithelial appearance in the surface area and spindle cells with sarcomatous appearance in the nodules of organs in thoracic cavity (heart, lungs, and aorta), and mediastinum, omentum, peritoneal surface, lymph nodes. Occasionally, the round or cuboidal cells filled with cytoplasmic pale eosinophilic substance. Some tumor cells were positive for alcian blue for hyaluronic acid by special staining and positive for cytokeratin (AE1/AE3) and positive in superficial area for podoplanin by immunohistochemistry.

Morphologic, histochemical and immunohistochemical characteristics of the tumor cells strongly suggested that this case is a mesothelioma (mixed type), however the primary site of tumor was unclear. This tumor was thought to be spontaneous, because only two cases of mesothelioma were detected middle dosage group of the carcinogenicity study. We are currently studying the differential diagnosis from other epithelial tumors and mesenchymal tumors.

P-46* The State of The Occurrence of Amyloidosis and The Correlation between Amyloidosis and Excoriation in Carcinogenicity Studies in ICR Mice

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<Background and purpose> Amyloidosis accompanied by excoriation is a histopathological change which is frequently observed in carcinogenicity studies in ICR mice and one of the important measuring factors in the discussion of the effects of the test substance or in the determination of the cause of death. Therefore we made investigation into the state of the occurrence of amyloidosis and the correlation between amyloidosis and excoriation.

<Materials and methods> We investigated the data of control group animals in the background data collection studies or carcinogenicity studies (total 7 studies) that were finalized in the year of 2007 or thereafter. Animals are ICR strain SPF mice (Crj:CD1 (ICR), Charles River Laboratories Japan, Inc.) which were housed individually in bracket type stainless-steel wire mesh cages. The length of the study period was 91-104 weeks, and the number of animals was 580 of each sex.

<Results and summary> All the animals were divided into groups: group A that had amyloidosis and group A (–) that had no amyloidosis, and then group E that had excoriation and group E (–) that had no excoriation. The incidence of A was 27% in males and 21% in females in all animals, and the incidence of AE was 69% in males and 43% in females in group A. The incidence of A by organs was 85% or higher for the spleen, liver, kidney and thyroid in AE, while it was 84% for the kidney, 74% for the spleen, 61% for the liver and 59% for the thyroid in males and 66% for the kidney, 56% for the spleen and 49% for both the liver and thyroid in females in AE (–). The fatality/moribundity rate caused by amyloidosis was 75% for AE and 52% for AE (–) in males, and 57% for AE and 32% for AE (–) in females. For the incidence of amyloidosis and excoriation, AE, AE(–), A(–)E, A(–)E(–) were 19%, 8%, 12% and 61%, respectively, in males and 9%, 12%, 3% and 76%, respectively in females in all animals. Therefore, it was suggested that amyloidosis correlated with excoriation in male ICR mice but not clearly correlated in females because only few animals had excoriation (12% of all female animals). After all, in the long-term studies in ICR mice, there was a relationship between amyloidosis and excoriation in male mice, moreover it was indicated that the incidence of amyloidosis by organs and the fatality/moribundity rate caused by amyloidosis would be changed depending on the presence/absence of excoriation.

P-47＊ Analysis of Cytokeratin8/18 and Cytokeratin 19 Expression in Mouse Liver Tumors

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<Introduction> Previously, we established a suitable Diethylnitrosamine (DEN) treatment range for postweaning period C57BL/6 mice models and indicated immunohistochemically stained cytokeratin 8/18 (CK8/18) and cytokeratin 19 (CK19)-positive foci to be appropriate for evaluation as preneoplastic liver marker lesions. In the present study, we tried to clarify the relationship between CK8/18 and/or CK19 expression and liver neoplastic lesions not only in C57BL/6 but also C3H mice.
<Methods> we modified our postweaning DEN-initiation model for C57BL/6 mice. Postweaning males were treated with intraperitoneal injections of DEN at the dose of 50 mg/kg 8 times (C57BL/6) or 4 times (C3H) (weekly at the commencement) and analyzed for CK8/18 and CK19 expression in liver proliferative lesions at weeks 60 or 31, respectively.
<Results> We could obtain not only hepatocellular preneoplastic, but also neoplastic lesions. CK8/18 and CK19 were found to be expressed in hepatocellular adenomas and carcinomas in both strains. Almost all hepatocellular carcinomas were positively stained for CK8/18 or CK19, but the intensity of CK19 expression varied greatly. Interestingly, strong CK19 expression was observed in the single hepatoblastoma induced.
<Discussion> Our previous and this study suggest that both the background C57BL/6 strain and other transgenic mouse models derived from it have potential for postweaning period models, and using CK8/18 and CK19 might facilitate assessment of cancer risk for the evaluation of chemical safety using mouse models.

P-48＊ Study on Modification of Liver Tumor Promotion in Rats Subjected to Co-Administration of Phenobarbital and Orphenadrine

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Phenobarbital (PB) and orphenadrine (ORPH) are cytochrome P450 (CYP) 2B inducers and have liver tumor promoting effects in rats. In this study, we investigated the co-promoting effects of PB and ORPH in rats. Six-week old male rats were subjected to partial hepatectomy, and given 60 or 120 ppm PB in drinking water, 750 or 1500 ppm ORPH in basal diet or 60 ppm PB+750 ppm ORPH for 6 weeks after *N*-diethylnitrosamine initiation.

The relative liver weight increased in the PB+ORPH group as well as the High PB or High ORPH groups compared with the DEN-alone group. The number of glutathione *S*-transferase placental form-positive foci, the mRNA levels of *Cyp2b1/2*, *Gstm3* and *Gpx2* and the oxidative stress markers, thiobarbituric acid-reactive substance and microsomal reactive oxygen species (ROS), significantly increased in the PB+ORPH group as well as the High PB or High ORPH groups compared with the DEN-alone group.

The results of our study suggest that the co-administration of PB and ORPH results in additive effects in the liver tumor promotion in rats which are attributable to the oxidative stress resulting from the enhanced ROS production.

P-49＊ The role of gap junctional intercellular communication on apoptotic signaling in rat hepatocellular carcinoma cells

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Acetaminophen (APAP) is commonly used as antipyretic and analgesic agent. Connexin (Cx) are components of gap junction channels, and Cx32 is a major gap junction protein in the liver. We previously established Cx32 dominant negative transgenic rats (Tg), which have much decreased capacity for gap junctional intercellular communication (GJIC). Tg rats are less sensitive to APAP-induced apoptosis.

In this study, to investigate the function of Cx32 on APAP-induced cell death, rat hepatocellular carcinoma cell line (C6), having low expression level of Cx32, was transfected with a Cx32 expression vector (C6-Cx32). C6-Cx32 and control cell (C6-Mock) were treated with APAP.

C6-Cx32 showed increased expression of Cx32 and functional recovery of GJIC compared to mC6-Mock cells. APAP induced apoptosis with activation of caspase 3 in rat HCC cells, and its sensitivity was increased in C6-Cx32 compared to C6-Mock. These results indicated that Cx32 promoted the induction of caspase3-dependent apoptosis signaling induced by APAP. This phenomenon corresponded to the results *in vivo*.

In conclusion, Cx32 regulates apoptotic signaling in both normal liver tissue and hepatocellular carcinoma cells.

P-50 Differences in nephrotoxicity according to polymixin B administration route

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<Objective/Method> Polymixins have conventionally been used as the most effective antibacterial agents against multidrug-resistant *Pseudomonas aeruginosa*, but when administered to patients with renal disorders, disease status reportedly worsened. We reported at the 28th Annual Meeting of the Japanese Society of Toxicologic Pathology that polymixin B nephrotoxicity was induced in rats only when polymixin B was administered as repeated subcutaneous doses. In the present study, to clarify the differences in nephrotoxicity among polymixin B administration routes, polymixin B was repeatedly administered intravenously at a dose of 2.5 mg/kg or subcutaneously at a dose of 20 mg/kg. Urine and blood samples were collected in a time-course dependent manner for evaluation of renal functions and for blood drug concentration determinations.

<Results/Conclusion> On urinalysis, the urinary volume tended to increase and the urine specific gravity tended to decrease in the subcutaneous administration group. On blood biochemistry, BUN and CRE levels were transiently increased from Day-1 to Day-2 and then increased again on Day-8. In the histopathological examinations, desquamation/necrosis and basophilic change (regeneration) of the proximal tubular epithelium suggesting renal damage were seen in the subcutaneous group. On the other hand, rats given intravenous administration showed no clear changes on urinalysis, biochemical, or pathological examinations. As to blood concentrations of polymixin B in rats receiving subcutaneous administration, the respective T-max and AUC were 4 hours and 87000 mg/mLh after the first dose, 1 hour and 110000 mg/mL･h after the 14th dose, and 2 hours and 210000 mg/mL･h after the 28th dose. Thus, the T-max was shortened when the administration period was prolonged. Furthermore, the blood concentrations of polymixin B at 4 hours after daily doses were increased on Day-9. With intravenous administration, neither nephrotoxicity nor alterations of blood concentration profiles caused by repeated dosing were seen.

Although the mean AUC of the subcutaneous administration group with nephrotoxicity was 10 to 20 times higher than that of the intravenous administration group, mean C-max values were comparable between the subcutaneous and intravenous administration routes. These results suggest AUC to be strongly involved in the onset of nephrotoxicity due to polymixin B. The levels of renal damage markers, BUN and CRE, were increased from Day-8 onward, in parallel with rising blood concentrations.

P-51＊ Histopathological Evaluation of Temporal Changes in Renal Toxicity of Colistin Sodium Methanesulfonate in Mice

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It has been known that colistin (polymyxin E), cationic polypeptide antibiotic, causes renal toxicity in proximal tubules although colistin sodium methanesulfonate ("CMS" hereafter) which is a prodrug of colistin is used in clinical practice. However, the mechanism of renal toxicity of colistin remains unclear. Therefore, in the present study, we have histopathologically evaluated the early renal toxicity with CMS in mice to clarify the mechanism of renal toxicity of colistin.

Eight hundred mg/kg of CMS was injected into the dorsal subcutaneous tissue of mice, and the animals were euthanized under anesthesia 1, 2, 4, 8, 24 and 48 hours after administration. In this study, blood chemistry examination (48 hours after administration), analysis of tissue concentration of colistin and histopathological examination were conducted. Additionally, immunohitochemical examination using anti-4-Hydroxynonenal (4-HNE) antibody, marker of lipoperoxidation, was performed to evaluate the relationship between early change of toxicity and reactive oxygen species (ROS).

As a result, Cmax in kidney tissue was reached 2 hours after administration, and micro-vacuolization was observed at luminal side in proximal tubular epithelium cells at that time. An increase in the number and/or size of the micro-vacuole was noted from 4 hours after administration onwards, and nuclear degeneration and necrosis were noted from 8 hours after administration onwards. Significant necrosis in the kidney was observed 24 hours after administration onwards. In blood chemistry examination, significant increases in BUN and creatinine were observed. Moreover, immunohistochemical examination using anti-4-HNE antibody revealed positive reactions to micro-vacuoles, and some vacuoles or whole cytoplasm from 2 and 4 hours after administration onwards, respectively.

Thus it was found that renal toxicity progressed with increasing tissue concentration of colistin, and necrosis in proximal tubule occurred following the development of micro-vacuolization and nuclear degeneration. The results of immunohistochemical examination suggested that ROS was involved in renal toxicity of colistin from the early stage onwards.

P-52＊ Effects of *p53* knockout on OTA-induced *in vivo* mutagenicity, apoptosis, and karyomegaly in the kidney, a carcinogenic site

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Ochratoxin A (OTA), a mycotoxin and food contaminant found in cereals and agricultural products, has been shown to be a renal carcinogen, targeting the straight segment of the proximal tubule epithelium (S3) in the outer stripe of the outer medulla (OSOM). In the 27th and 28th Annual Meetings of JSTP, we reported that OTA increased the mutant frequency (MF) of the reporter gene, suggesting large deletion mutations and fluctuated mRNA levels in genes associated with DNA double-stranded break (DSB) repair, particularly homologous recombination (HR) repair, and transcribed by p53 at the target site of the kidneys using *gpt* delta rats. In the present study, we investigated the effects of p53 knockout on OTA-induced mutagenicity, apoptosis, and karyomegaly in the renal tubular cells using *p53*-proficient or -deficient *gpt* delta mice. Groups of 5 male *p53*-proficient or -deficient *gpt* delta mice were given OTA at concentrations of 0 (control), 1, or 5 mg/kg for 4 weeks. p53 protein levels, *in vivo* reporter gene mutations, and the incidences of apoptosis and karyomegaly in renal tubular cells were analyzed. Significant increases in Spi$^-$ MFs were observed in the kidneys of *p53*-deficient *gpt* delta mice given 5 mg/kg OTA (control group: $0.14 \pm 0.09 \times 10^{-5}$; 5 mg/kg OTA group: $0.36 \pm 0.09 \times 10^{-5}$, $P < 0.01$), but not of *p53*-proficient *gpt* delta mice, although there were no changes in *gpt* MFs in both genotypes. Western blotting analysis demonstrated that p53 protein levels in the kidneys of *p53*-proficient *gpt* delta mice given 5 mg/kg OTA were significantly increased by approximately 17-fold ($P < 0.05$) compared with the control. Incidences of apoptosis and karyomegaly in the OSOM in *p53*-deficient *gpt* delta mice treated with 5 mg/kg OTA were significantly higher than those in *p53*-proficient *gpt* delta mice. Given that p53 regulates HR repair in DSBs, these results suggest that large deletion mutations may occur during the process of HR repair for DSBs induced by OTA. Also, the suppression of karyomegaly and apoptosis by p53 suggest that these phenomena may arise from OTA-induced DNA damage.

P-53＊ Mechanisms mediating ocharatoxin A-induced deletion mutations in the kidneys of *gpt* delta rats

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We have previously reported that ochratoxin A (OTA) increases Spi$^-$ mutant frequencies when given at a carcinogenic dose, suggesting the induction of deletion mutations in the outer medulla of the kidney, a carcinogenic target site. Deletion mutations are thought to occur during DNA damage repair processes, and their patterns are likely dependent on the type of DNA damage. In the present study, to investigate the mechanisms of OTA-induced deletion mutations, spectrum analysis of deletion mutations was performed in Spi$^-$ mutants obtained following 4-week administration of OTA in *gpt* delta rats. To clarify the dose-dependent relationship between DNA damage and deletion mutations, comet assays and Spi$^-$ assays were performed in *gpt* delta rats given OTA at various doses.
<Methods> Experiment 1: Groups of 5 male *gpt* delta rats (6 weeks of –age) were fed OTA at doses of 0 or 5 ppm for 4 weeks. After Spi$^-$ assays in the kidneys (outer medulla), spectrum analysis was used to examine deletion mutations in Spi$^-$ (*red*/*gam* gene) mutants. Experiment 2: Groups of 10 male *gpt* delta rats (6 weeks of age) were treated with OTA at doses of 0, 70, 210, or 630 μg/kg by gavage for 4 weeks. Kidneys (outer medullas) were sampled at 3 h after the last dosing and used for comet assays (n = 5) and Spi$^-$ assays (n = 10).
<Results> Experiment 1: OTA significantly increased Spi$^-$ mutant frequencies. In the mutation spectrum analysis, specific mutation frequencies of large deletions (over 1,000 base pairs) and single base pair deletions were about 4 and 2 times higher in the OTA-treated group than in the control group, respectively. Experiment 2: In the comet assay, we observed a statistically significant increase in % tail DNA at doses of 70 μg/kg and higher.
<Discussion> It is highly probable that OTA is able to induce both large deletions and single-base deletions. The positive result in comet assay implied that DNA damage followed OTA treatment. Further data on the Spi$^-$ assay and spectrum analysis, along with data demonstrating the expression of DNA damage/repair-related molecules in Experiment 2 will be presented to clarify the mechanisms mediating OTA-induced deletion mutations.

P-54＊ Histopathological Examination on Hyaline droplets in the Urinary Bladder of Type 2 Diabetes Model db/db Mice

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<Background> The db/db mouse is used as a model of type 2 diabetes. Although the kidneys have been investigated in detail, there are few reports on the urinary bladder in the urinary system. In this study, we report characteristics and incidence of hyaline droplets observed in transitional epithelial cells including related lesions of the bladder in male db/db mice.
<Materials and Methods> The bladders of 9 male BKS.Cg-+ *Lepr^{db}*/+ *Lepr^{db}*/Jcl (db/db) mice (21 weeks old) were fixed in 10% phosphate buffered formalin and paraffin-embedded sections were prepared. Then, HE, PAM, PAS, AZAN, and PTAH staining were performed for histopathological examination. Also, immunohistochemical staining was performed using lysosomal membrane protein-1 (LAMP-1) antibody. Furthermore, the bladders were examined ultrastructurally with a transmission electron microscope.
<Results> Swelling or vacuolation of umbrella cells were observed in all cases and desquamation was also found in some cases. Hyaline droplets of various sizes were observed in the cytoplasm of transitional epithelial cells in 4 cases, and 3 of these cases showed hyperplasia of transitional epithelial cells and lymphocyte and plasma cell infiltration in the lamina propria and muscular layer. Hyaline droplets showed black, negative reaction, red, and yellow-brown with PAM, PAS, Azan, and PTAH stain, respectively. Immunohistochemically, peripheral of the droplets was positive for LAMP-1. Ultrastructurally, hyaline droplets were observed as electron-dense homogeneous materials surrounded by a single limiting membrane.
<Discussion> Although swelling or vacuolation of umbrella cells were observed in all cases, only the cases with hyaline droplets showed hyperplasia of transitional epithelial cells and inflammatory reactions such as lymphocyte and plasma cell infiltration in the lamina propria and muscular layer. Therefore, it was considered that hyaline droplets occur with the increase of umbrella cell damage. Also, these results suggest that hyaline droplets were associated with accumulation of unknown substances in the lysosome. Further investigation will be performed to identify the substances accumulated in the lysosome.

P-55＊ Examination of in vivo mutagenicity of rat bladder carcinogen DMA(V)

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Gpt delta rats have be widely used to determine the genotoxicity of chemicals and clarify whether its genotoxicity is involved in carcinogenicity in the target organs. However, the current standard assay protocol is only optimized for large quantity samples such as those from liver and kidney. We have successfully devolved a new technique to isolate enough genomic DNA from the bladder urothelial cells and demonstrated its packaging efficiency is sufficient for mutation assay.

This study is being undertaken to validate the usefulness of the new methodology and determine the mutagenicity of dimethyl arsenic acid (DMA^V), in which groups of gpt delta rats were treated with 92ppm DMA^V (50ppm As) or 5.0% sodium ascorbate (a nongenotoxic bladder promoter) in diet, or 0.05% BBN (a genotoxic bladder carcinogen) in the drinking water, or without any of test chemicals for 13 weeks. There was no significant difference in mutant frequency in bladder mucosa between sodium ascorbate treatment group and control group, whereas mutant frequency was significantly increased in BBN treatment group compared to control group. A reporter gene mutation assay for DMA^V is in progress.

P-56 Medium-term Urinary Bladder Carcinogenesis Bioassay of Ethyl *tertiary*-Butyl Ether (ETBE) in Rats

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<Objectives> As one goal of achieving the "Kyoto protocol", fuel derived from renewable sources (biomass or biofuel) was introduced onto the market, and there are plans to introduce ETBE, derived from biomass ethanol, in Japan. Evaluation of potential carcinogenicity of ETBE is very important in this context. Tumor promoting potential of ETBE on urinary bladder carcinogenesis was suspected from the results of a previous medium-term multi-organ carcinogenesis bioassay, since rats exposed to ETBE induced papillomatosis in the urinary bladder. Thus, it was decided to determine whether ETBE act as tumor promoter, using a medium-term urinary bladder carcinogenesis bioassay protocol.

<Method> Male F344 rats were given drinking water containing 500 ppm *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN), as an initiator, for 4 weeks. One week thereafter, the animals received ETBE by gavage at dose levels of 0 (control), 100, 300, 500 or 1000 mg/kg/day until experimental week 36. Necropsy of all rats was performed at week 37, and the urinary bladders were examined histopathologically.

<Results> No statistically significant differences in incidences of papillary or nodular (PN) hyperplasias, papillomas, and carcinomas of the urinary bladder were evident in rats treated with 100 ~ 1000 mg/kg/day ETBE as compared to control values. Furthermore, average numbers of hyperplastic or neoplastic lesions per unit length of basement membrane in rats given 100 ~ 1000 mg/kg/day ETBE were also comparable to control values. However, papillomatoses of the urinary bladder were found 4 out of 30 rats (13%) in the 1000 mg/kg/day ETBE group, and soft stones in the urinary bladder were found in 3 out of these 4 rats.

<Conclusion> The results thus demonstrated that ETBE did not exert promoting effects on urinary bladder carcinogenesis. However, papillomatosis of the urinary bladder developed in small numbers of the rats given ETBE at 1000 mg/kg/day, but not 500 mg/kg/day or lower doses.

P-57 Modifying Effects of High Fat Diet during a Juvenile Stage on DMBA-induced Mammary Carcinogenesis in Rats

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Corn oil diet (COD) and beef fat diet (BFD) fed to female F344 rats during ages of 5-6 weeks induced hyperleptinemia and hypoadiponectinemia, as compared to basal one (BD). The purpose of the present study is to clarify modifying effects of the high fat diet during a juvenile stage on DMBA-induced mammary carcinogenesis.

A total of 36 females were divided into 3 groups, and the rats were fed 1) BD, 2) COD and 3) BFD during ages of 5-9 weeks. At age of 7 week, DMBA was administered to all rats by gavage. By palpation, mammary carcinomas were initially detected at weeks 8, 8, and 4 after DMBA in groups 1-3, respectively. Histopathological carcinoma incidences were 42, 83 and 67% and multiplicities were 0.5±0.7, 1.1±0.8 and 1.0±1.0/rat in groups 1-3, respectively. Tumor volumes of group 3 rats were also larger and those of group 2 tended to be larger than those of group 1. All carcinomas in group 1 showed well differentiated phenotypes, while 8 and 25% of carcinomas in groups 2 and 3, respectively, were moderately/poorly differentiated. Western blot analysis for the moderately/poorly differentiated carcinoma samples revealed increased expression of cell cycle-related proteins, whose expressions were reported to be regulated by leptin-STAT3 signaling pathways. In conclusion, high fat feeding during a juvenile stage enhanced DMBA-induced mammary carcinogenesis and additionally influenced the phenotypes of carcinomas, and hyperleptinemia was suggested to be related especially to the latter point.

P-58 Modifying Effect of Glycidol Fatty Acid Esters on Mammary Carcinogenesis in SD Rats

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Glycidol fatty acid esters (GEs), trace contaminants in edible oils which are possibly formed during refining processes, have recently been detected in vegetable fat-containing products, including infant formulas, but not in crude and native oils and fats. The level of GEs was over ten times higher in diacylglycerol-rich oil than that in the regular cooking oil containing triacylglycerols as major components. Although there is no toxicological data available yet on the GEs, the primary toxicological concern is based on the potential release of genotoxic carcinogen, glycidol from the parent esters. In the present study, to detect the modifying effects of GEs on the mammary gland, one of the carcinogenic target organs of glycidol, we pretreated 7-week-old SD rats with *N*-methyl-*N*-nitrosourea (50 mg/kg i.p.) and then administered glycidol (800 ppm) or GEs (3600 ppm, glycidol oleate (GO) or glycidol linoleate (GL)) in the drinking water for 26 weeks. The dose levels being selected on the basis of carcinogenic dose levels in rat carcinogenicity study of glycidol (37.5 and 75 mg/kg/day) and on the equal moles of the esters. During the treatment period, general conditions were observed daily, and body weight, water intake and thoracic and abdominal mammary gland tumor appearance assessed by palpation were recorded weekly. In body weights, significant decrease was noted in the glycidol group compared to control group from week 2 through experimental period due to obvious decrease of water consumption. The calculated glycidol intake was 43 mg/kg per day and on the assumption that all treated GEs would be completely metabolized to glycidol, intake of glycidol in GO and GL groups was 93 and 74 mg/kg per day, respectively. In glycidol group, significant increase and tendency of increase in the incidences, multiplicity or volumes of palpable mammary gland tumors were observed compared to control from week 12 throughout the experimental period. The multiplicity and volume of histopathologically diagnosed mammary tumors, in particular poorly differentiated mammary carcinomas were significantly increased in the glycidol group as compared with the control. In the GO group, the multiplicity and volume of mammary tumors showed a slight tendency to increase, but no change was noted in the GL group. These results provide evidence of a mammary tumor promoting activity of glycidol, but not GEs in the present model.

P-59 Effects of Combined Treatments of DHPN and DMBA on Mammary Carcinogenesis in F344 Female Rats

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It is well known that DHPN administration in drinking water frequently induces lung carcinomas and intragastric instillation of DMBA induces mammary carcinomas in rats. We reported the relation between the expression of female hormone receptor, epidermal growth factor receptor and the size of mouse lung tumors induced by NNK. In this study, combined treatments of DHPN and DMBA on mammary carcinogenesis in F344 rats were examined.

Six weeks old female F344 rats were classified into 4 groups; (1)Control group: intragastric instillation of corn oil(1ml/rat) on day 7 (7 weeks age), (2)DHPN group: DHPN administration in drinking water for 2 weeks, (3)DMBA group: intragastric instillation of DMBA(40mg/kg bw) on day 7 (7 weeks age), (4)DHPN+DMBA group: DHPN and DMBA treatments same as group 2 and 3.On week 29, all rats were sacrificed, and the weights of liver and lungs were measured.

The incidences of mammary tumors are (1)0/10(0%), (2)0/21(0%), (3)5/21(23.8%), (4)2/21(9.5%), and all of the tumors were confirmed as adenocarcinomas histopathologicaly. The weight of body, liver and lungs were not differ among groups. In (3)DMBA group, although no significant difference about the body weight, both liver and lungs weights of 5 rats with mammary adenocarcinoma were significantly higher than those of 16 rats without mammary tumors. Similarly, in (4)DHPN+DMBA group, both liver and lungs weights of 2 rats with mammary adenocarcinoma were significantly higher than that of 19 rats without mammary tumors.

This experiment indicated that the mammary adenocarcinoma incidence of (4)DHPN+DMBA group tended to be less than that of (3)DMBA group, furthermore, both liver and lungs weights of tumor bearing rats were higher than those without tumors.

P-60* The Modifying Effects of Hyperbaric Oxygen in Mouse Skin Carcinogenesis

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Although the influence of the hyperbaric oxygen into alveolar epithelial cells is well known, the enhanced effects on chemotherapy and radiation therapy in combination with hyperbaric oxygen have been observed. On the other hand, few study of the effect for carcinogenesis under hyperbaric oxygen has been reported. In this study, we examined the effects of hyperbaric oxygen therapy (HBOT) in a two-stage chemical carcinogenesis on mouse skin.

50 mice was divided into five groups. After shaved the dorsal skin of the mice using surgical clipper, every mice groups 1-4 were applied 25 nmol 7,12-dimethylbenz[a]anthracene (DMBA) solution to the dorsal skin. Then, both groups 1 and 2 were applied 8.5 nmol 12-O-tetradecanoylphorbol-13-acetate (TPA) twice weekly. Groups 3 and 4 were applied acetone instead of TPA. Group 5 was no treatment. HBOT was started at the same time with TPA and applied for groups 2 and 3. All groups were sacrificed at 23 week from the start of the experiment. We have examined incidence, multiplicity and tumor volumes. In addition, it will be shown histopathological results.

Between the two groups treated with DMBA/TPA two-stage carcinogenesis, group 2 (HBOT) may indicate the occurrence of tumors at 8 weeks from the beginning of experiment. The group 1 (non-HBOT) may indicate the occurrence of tumors at 9 weeks. At 12 weeks, HBOT group was 38% and the non-HBOT group was 20% in the incidence of tumors. The incidence of HBOT group had almost doubled at that time. At the sacrifice on 23 weeks of TPA treatment, HBOT group was 67% and non-HBOT group was 53% in the incidence of tumors. However, there was no significant difference in the multiplicity of tumors at the sacrifice (group 1: 4.0 ±2.8, group 2: 3.9 ±2.6). Avelage volume of the tumor that occurred in HBOT group 2 (20.61 mm^3) was greater than group 1 (13.35 mm^3). Any effect of HBOT alone group (group 3) was not observed.

It might be shown that HBOT enhanced the effects of tumor promotion by TPA. And it suggested that this phenomenon was similar to the situation in which angiogenesis and epithelialization was promoted wound healing in an under hyperbaric oxygen environment.

P-61＊ Cellular Distribution of Proliferation, Apoptosis, and Cell Cycle-related Markers at the Early Stage of Tumor Promotion in Rat Two-stage Carcinogenesis Models

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We previously demonstrated that 28-day administration of carcinogens that evoked cell proliferation as determined by immunoreactivity for Ki-67 or minichromosome maintenance 3 (Mcm3), in many target organs including the kidney, liver, thyroid, urinary bladder, forestomach and glandular stomach, increased the numbers of cells immunoreactive for cell cycle-related molecules, topoisomerase (Topo) IIα, and ubiquitin D (Ubd) as well as the increase of TUNEL$^+$ apoptotic cells in rats. To clarify the role of these markers in the early carcinogenic processes to form hyperplastic or preneoplastic lesions, we examined the cellular distributions of these markers at the early stages of tumor promotion in the liver, thyroid, urinary bladder, forestomach, and glandular stomach using two-stage carcinogenesis models in rats. Promoting agents targeting the liver (piperonyl butoxide, methapyrilene), thyroid (sulfadimethoxine), urinary bladder (phenylethyl isothiocyanate), and forestomach and glandular stomach (catechol) were administered to rats after initiation treatment for each target organ. As a result, numbers of Ki-67$^+$, Mcm3$^+$, and Topo IIα$^+$ cells and apoptosis increased within preneoplastic lesions immunoreactive for glutathione *S*-transferase placental form in the liver or phospho-p44/42 mitogen-activated protein kinase in the thyroid, and hyperplastic lesions having no known preneoplastic markers in the urinary bladder, forestomach and glandular stomach. On the other hand, Ubd$^+$ cells did not increase within preneoplastic lesions in the liver and thyroid, but increased within hyperplastic lesions in the urinary bladder, forstomach and glandular stomach. These results suggest that both cell proliferation and apoptosis may be involved in the formation of preneoplastic lesions in the liver and thyroid examined here; however, spindle checkpoint disruption may not be involved in this process. Changes in hyperplastic lesions of the urinary bladder, forestomach and glandular stomach are similar to the 28-day carcinogen-treated cases, suggestive of the hyperplastic cellular character before the preneoplastic state.

P-62＊ Expression Characteristics of Cell Cycle-related Proteins at the Early Stage of Tumor Promotion in Rat Two-stage Carcinogenesis Models

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Some hepatocarcinogens have a potential to induce cytomegaly in hepatocytes after repeated exposure to experimental animals, and this cellular change reflects aberration in cell cycle. Recent studies have shown that aberrant expression of cell cycle-related proteins in the area exhibiting cellular cytomegaly may undergo a chromosomal instability linked to carcinogenicity. We previously reported that immunoreactive cells for $p21^{Cip1}$ (G_1/S checkpoint protein), Cdc2 (G_2/M checkpoint protein), and M phase-related proteins (Aurora B, p-Histone H3 and HP1α) increased in response to carcinogens evoking cell proliferation. In the present study, to examine cellular distribution of selected proteins in the early stages of carcinogenesis, two-stage carcinogenesis assays targeting liver and thyroid were performed using male F344 rats. In the liver model, rats received an intraperitoneal (i.p.) injection of 200 mg/kg body weight of *N*-diethylnitrosamine followed by a dietary exposure to piperonyl butoxide (PBO) at 20,000 ppm and methapyrilene (MP) at 1,000 ppm for 6 weeks. After one week of PBO or MP treatment, all rats were subjected to two-thirds partial hepatectomy. In the thyroid model, rats received a subcutaneous (s.c.) injection of 2,800 mg/kg body weight of *N*-bis(2-hydroxypropyl) nitrosamine followed by promotion with sulfadimethoxine through drinking water at 1,500 ppm for 4 weeks. Animals were all killed after each promotion period and immunohistochemical analysis was performed. In the liver, immunoreactive cells for Ki-67, $p21^{Cip1}$, Cdc2, Aurora B, phospho-Histone H3 and HP1α increased at the inside of the glutathione *S*-transferase placental form (GST-P)$^+$ foci as compared with the cell population at the outside of the GST-P$^+$ foci. In the thyroid, immunoreactive cells for Ki-67, Cdc2, Aurora B, phospho-Histone H3 and HP1α increased at the inside of phospho-Erk1/2$^+$ foci as compared with those at the outside of phospho-Erk1/2$^+$ foci. On the other hand, $p21^{Cip1}$ decreased at the inside of phospho-Erk1/2$^+$ foci as compared with the outside portion. These results suggest that both liver and thyroid preneoplastic lesions commonly showed high proliferation activity and increase of cell population staying at the M phase causing chromosomal instability. Difference in the expression pattern of $p21^{Cip1}$ between the liver and thyroid preneoplastic lesions may suggest difference in the G_1/S checkpoint function. The liver preneoplastic cells may maintain its function; however, thyroid cells may disrupt the function, causing progression to cancers.

P-64* Activation of the Canonical Wnt Signaling Maintains Normal and Neoplastic Gastric Epithelial Cells in Undifferentiated and Proliferative States

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The canonical Wnt signaling is frequently activated in gastric cancers but it remains poorly understood how Wnt signaling contributes to the gastric carcinogenesis. In the present study, we first investigated the effect of Wnt activation on the proliferation and differentiation of gastric epithelial cells using doxycycline-inducible β-catenin mice. When we fed adult mice doxycycline in drinking water (2.0 mg/ml) for 5 days, cytoplasmic and nuclear β-catenin accumulation were observed in both fundic and pyloric glands of the stomach, accompanied by the strong upregulation of canonical Wnt target genes such as *Myc, Ccnd1* and *Sox9*. The number of Ki-67 staining cells significantly increased in the isthmus and gastric pits of both fundic and pyloric glands in doxycycline-treated mice as compared with those in untreated mice. In addition, alcian-blue-periodic acid-Schiff (AB-PAS) staining and immunostaining for Muc5AC revealed the significantly decreased mucus production in the gastric pits of β-catenin-induced stomach, demonstrating a suppression of cellular differentiation toward surface mucous cells following β-catenin induction. Consistent with this finding, we also observed the decreased levels of *Muc5ac* mRNA in β-catenin-induced stomach by quantitative RT-PCR. In addition, qRT-PCR demonstrated that β-catenin induction led to the up-regulations of gastric epithelial stem cell markers including *Lgr5* and *Sox2*. We next examined the β-catenin expression in *N*-methyl-*N*-nitrosourea (MNU)-induced murine gastric tumors and found that cytoplasmic and nuclear β-catenin-expressing neoplastic cells formed focal areas within the tumor, which provide a good model for understanding the effect of Wnt activation on neoplastic gastric epithelial cells. Immunostaining for Ki-67 and AB-PAS staining revealed that numerous proliferating cells were randomly distributed with the absent or scant mucus production in an area of cytoplasmic and nuclear β-catenin accumulation in the tumors, consistent with the findings in β-catenin-inducible mice. Our data indicate that activation of canonical Wnt signaling maintains normal and neoplastic gastric epithelial cells in an undifferentiated and proliferative state.

P-65 Soluble VEGFR-3 Decoy but not SATB1 siRNA Suppresses Metastasis in a Highly Metastatic Mouse Mammary Cancer Model

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Overexpression of VEGF-C or VEGF-D (their receptor: VEGFR-3) has been associated with lymphangiogenesis and lymph node metastasis in a multitude of human neoplasms, including breast cancers. In addition, SATB1 has been shown to be a genome organizer of human breast cancer which enhances cell proliferation and metastasis. Soluble VEGFR-3 (sVEGFR-3) can block VEGF-C biding to the VEGFR-3. To evaluate the antimetastatic potential of sVEGFR-3 or SATB1 siRNA, gene therapy with vector expressing sVEGFR-3, SATB1 siRNA or control vector was conducted on murine metastatic mammary cancer.

<Results> Tumor volume was significantly lower in the sVEGFR-3 group as compared to the control group throughout the study. In the end of the study, bioluminescence imaging showed a tendency for a decrease in sVEGFR-3 group. Multiplicity of lymph node metastasis was significantly suppressed in sVEGFR-3 group. Moreover, total number of overall metastasis including the other organs was also decreased in this group.

<Conclusion> Our data demonstrate that sVEGFR-3 decoy but not SATB1 siRNA can inhibit mainly lymph node metastasis. The antimetastatic activity of sVEGFR-3 may be of high clinical significance.

P-66 Autophagy signalings in human breast cancer cell MCF-7 were up-regulated by allil isothiocyanate (AITC) and induced the cell death.

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In recent year, allyl isothiocyanate (AITC) was recognized as a cancer preventing agent. However, the mechanisms are not clear. Suppression of the cell cycle, induction of apoptosis is considered to be a cause for a mechanism of the cancer inhibitory effect. In this experiments, human breast cancer cells MCF analyzed contribution of autophagy in mind as the cytoplasmic activity by the AITC exposure, a mechanism at onset of cell death conduction. Also, we analyzed an expression of autophgy association protein change by Western blot. Furthermore, we examined the effect with the administration of chloroquine which was autophagy inhibitor. As a result, the AITC exposure induced inhibitory effect, cell death with the increase of the autophagy signal for breast cancer cells for a cell cycle of G2/M period, and cell death was promoted more by administration of autophagy inhibitor. As for the breast cancer cells, it was thought to be induced autophagy to resist the cell death by the AITC exposure and to survive. Therefore after culturing the cells these survived with normal culture media for 15 days, western analysis could find possible protein quantity, and the expression of the autophagy marker had the expression of the stem cells marker together when we searched it. AITC was effective in the proliferati0n suppression and cell death instruction of breast cancer cells than the above, and it was found that cancer stem cells were left. Therefore, for studies for chemoprevention and chemotherapy, concepts for autophagy and cancer stem sells may be essential.

P-67＊ Epithelial-mesenchymal Transition in Mice Bearing Human Lung Cancer Cell Lines

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Epithelial-mesenchymal transition (EMT) is a biologic process whereby epithelial cells undergo transition to a mesenchymal phenotype, which is considered to play an important role in tumor metastasis by increasing cell mobility and promoting neoplastic cell invasion. In this study, histopathological examination and immunohistochemical analysis of epithelial/mesenchymal marker expression were performed in mice bearing human lung cancer cell lines.

<Material and Methods> Male NOD-SCID mice were subcutaneously transplanted with a human non-small-cell lung cancer cell line (NCI-H2228). The subcutaneous tumor and normal adjacent tissue were collected for examination. Collected tissues were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned, and stained with hematoxylin and eosin. Additionally, immunohistochemical analysis for Ki-67, Vimetin and E-cadherin was performed.

<Results and Discussion> The mass was composed of neoplastic cells with solid and glandular areas. Neoplastic cells forming solid structure exhibited higher positive rate for Ki-67 and Vimentin compared to neoplastcic cells in the glandular area. On the other hand, the expression of E-cadherin in the neoplastic cells in the solid area was strikingly low. As the neoplastic cells forming solid structure showed up-regulation of mesenchymal marker, Vimentin, and down-regulation of epithelial differentiation marker, E-cadherin, in an epithelial-derived neoplasm, it became evident that there is a relationship between the morphological features and the expression of epithelial/mesenchymal markers. These findings are interesting to further pursue the role of EMT in epithelial-derived cancer.

P-68＊ Different Histopathological Dermal Changes Exposed Transcutaneously to Two Nano-particle Sizes of Platinum in Rats

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In our previous systematic toxicity study of two different nano-particle sizes of platinum (Pt) in rats for a 28-day transcutaneous exposure, crust accompanied by erosion appeared on the patch area from the second week of exposure. At the end of study, histopathological dermal changes in the 1 nm Pt group were more severe than those in the 20 nm Pt group.

In this study, to reveal the initial action of two different nano-particle sizes of Pt on the skin, 1 or 20 nm of Pt was exposed transcutaneously to the back skin of a rat, and morphological dermal changes was examined light and electoron microscopically on days 5, 7, and 9 of exposure in the 1 nm Pt group, and on days 7 and 14 in the 20 nm Pt group, respectively. Crust on the patch area was observed from day 5 or 6 of exposure in both groups.

In the 1 nm Pt group, eosinophil infiltration in the dermis and edema in the epidermal basal layer were observed from day 7 of exposure. By ultra-structural observation, amorphous material and dense granular material were detected in the intercellular space on the basal membrane.

In the 20 nm Pt group, vacuolar degeneration of the epidermis was observed on day 14 microscopically, and vacuoles were detected in the cytoplasm of the epidermal cell and epidermal Langerhans cell by ultra-structural observation.

From these results, the initial dermal morphological changes induced by nano-Pt were different between nano-particle sizes suggesting that induction for skin lesions can occur by different mechanisms with different nano-particle sizes.

P-69＊ A 13-Week Repeated Dose Study of Nanoclay Consisting Mainly of Montmorillonite in the Diet to F344 Rats

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<Introduction> Today, a large variety of nanomaterials are being developed, and very many industrial applications are expected. Nanoclay, consisting mainly of Montmorillonite, is being used as a component of packaging containers such as PET bottles because it has the characteristics of anti-caking and stability improvement. However, there are few reports about any possible toxicity and thus long term safety. Therefore we examined this issue with 13-week dietary administration of Nanoclay to rats in the present study.

<Materials and Methods> Two kinds of Montmorillonite, Ben-gel flake (FLAKE) and Ben-gel clea (CLEA) (HOJUN Co., Ltd., Gunma, Japan) which differ in particle size but both comply with standards and criteria for food additives, were investigated. They were mixed with powdered basal diets (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) to make pellets containing Montmorillonite at doses of 0.04, 0.2, 1.0 and 5.0%, for feeding to 6-week-old male and female F344 rats for 13 weeks. During the examination period, in-life parameters, body weights and food consumption were measured. Animals were sacrificed at 13 weeks after the start of the experiment, and blood samples were collected from the inferior vena cava under isoflurane anesthesia. Hematological, serum biochemistry and histopathological analyses were performed.

<Results and Conclusion> No obvious clinical signs and changes of food consumption, body weights or organ weights occurred in any treatment group. On hematological analysis, the 1.0% and 5.0% male FLAKE groups demonstrated increase of MCH, MCHC and PLT, and the 5.0% male FLAKE and CLEA groups featured significant increase of WBC. However, each change seemed to incidental because of the lack of any dose relationship. As for serum biochemistry analysis, in the 5.0% male CLEA group, TG was decreased significantly compared to the control group, but this was not considered to be biologically significant. In the histopathological analysis, there were no significant observations as compared to the control group. Thus, the results indicated the toxic level of Montmorillonite to be more than 5.0%, and the no-observed-adverse-effect level (NOAEL) was concluded to be 5.0% (FLAKE: 4.01 g/kg body weights/day for males, and 3.97 g/kg body weights/day for females, CLEA: 3.91 g/kg body weights/day for males, and 3.96 g/kg weights/day for females) from the present study.

P-70 Effects of Gamma-oryzanol or Glycerol on the Pulmonary Changes Due to the Intratracheally Instilled Magnetite Nanoparticles in Fischer 344 Rats

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Ferric oxide nanoparticles (magnetite) are of considerable interest for the application in the nanotechnology-related fields, including environmental catalysis, target drug delivery and hyperthermia. However, information about their potential risks is still limited. The present study, examined effects of gamma-oryzanol or glycerol, lung tumor promoters, on the pulmonary responses in rats induced by an intratracheal spray instillation.

A total of 120, 10-week-old male Fischer 344 rats were separated into 6 groups of 20 animals each, and were exposed to 4 weekly intratracheal spray instillations of 0 (control, milli-Q water; group I, III and V) or 5.0 mg/kg body weight magnetite (group II, IV and VI). From 1 week after the last instillation, groups I and II were given a basal diet and drinking water for 32 weeks. In such a period, groups III and IV were given a diet containing 1% gamma-oryzanol and drinking water, while groups V and VI were given a basal diet and 8% glycerol water. The rats were then sacrificed, and biological consequences were investigated.

Lung weights of the magnetite treated groups (II, IV and VI) were significantly higher than those of the controls. Lungs of the magnetite treated groups revealed enlargement and black patches originating from the color of magnetite. Histologically, infiltration of macrophages phagocytosing magnetite, inflammatory cell infiltration in alvelolar walls and lumens, and enlargement of alveolar type II cells were observed. In such groups, neither gamma-oryzanol nor glycerol exerted any additional effects.

P-71 Enhanced Cellular Uptake and Cytotoxicity of Curcumin-Loaded PLGA Conjugated with Anti-P-glycoprotein Ab in Drug Resistance Cancer Cells

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Curcumin (Cur) has been reported that insoluble in water is the limited efficiency factors of Cur *in vivo*. One possible way to improve the water solubility and stability of Cur is to entrap it into poly (DL-lactide-co-glycolide) (PLGA) nanoparticles in the presence of modified-pluronic® F127 stabilizer (Cur-NPs). Anti P-gp antibody at amino-terminal was conjugated to the carboxy-terminal of modified pluronic® F127 on the surface of NPs (Cur-NPs-APgp). The physical properties, cellular uptake, specificity and cytotoxicity of NPs were investigated. The particle size of Cur-NPs and Cur-NPs-APgp were 127 and 132 nm, respectively. The entrapment efficiency and actual loading of Cur-NPs-APgp were lower than Cur-NPs. Flow cytometry indicated that Cur-NPs-APgp selectively bind to KB-V1 (high expression of P-gp) than KB-3-1 cells (low expression of P-gp). Cellular uptake of Cur-NPs-APgp in KB-V1 was higher than KB-3-1 cells. Besides, cytotoxicity of Cur-NPs-APgp in KB-V1 cells was higher than in Cur and Cur-NPs. Taken together the results indicated that Cur-NPs-APgp targeted to P-gp on the cell surface membrane of KB-V1 cells could enhance cellular uptake and the cytotoxicity of Cur.

P-72＊ Effects of Grape Skin Extract on the Glandular Epithelial Cells of Parotid Glands in Rats

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In our previous study, 5.0% grape skin extract (GSE) induced hypertrophy and basophilia in the glandular epithelial cells of rat parotid glands in a 13-week feeding study. To confirm the toxicological significance of these changes, 6-week-old male F344 rats were fed a diet containing 5.0% GSE for 4 weeks. The diet was discontinued for 2 weeks to evaluate recovery. The rats were also fed a diet containing 5.0% tannic acid (TA), which is a bitter taste component to induce parotid gland hypertrophy in rats using the same protocol as the GSE feeding trial. In addition, rats were administered GSE by gavage for 4 weeks with a 2-week recovery period following treatment. The GSE dose in the gavage study corresponded to 5.0% in the feeding study. In the control groups, rats were fed a basal diet or administered sterilized distilled water by gavage. The parotid glands from the rats after the treatment and recovery periods were examined. Macroscopically, significant enlargement of the parotid glands was observed in the rats that were fed GSE and TA in the diet. Microscopically, diffuse severe hypertrophy accompanied by basophilia in the glandular epithelial cells of the parotid glands was observed in the rats that were fed GSE and TA in the diet. This pathological change was less apparent after the recovery period in the rats that were fed GSE and TA. In contrast, changes in the parotid glands were not observed in the rats administered GSE by gavage or the controls. These results indicate that parotid gland hypertrophy observed in the previous 13-week feeding study may be reversible and could be caused by direct exposure to a bitter component of GSE in the oral cavity. Based on the data from the previous study indicating that there were no toxicological changes and the data from theand present studyies, it is suggested that hypertrophy of the parotid glands is not an adverse effect of GSE.

P-73 90-Day Repeated Dose Rat Toxicity Studies of Gum Ghatti

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Gum ghatti is a polysaccharides of natural origin used in foods as thickening, gelling, emulsifying and stabilizing agent. For evaluation, groups of 10 male and female Sprague Dawley rats were exposed to 0, 0.5, 1.5, and 5% of gum ghatti in AIN-93M diet for 90 days in accordance with OECD GLP guidelines. All animals survived to terminal necropsy and there were no significant in-life, body weight, feed consumption, opthalmological or neurological findings. There were no treatment-related clinical pathology changes or gross lesions. The only tissue weight change was an increase in empty and full cecal weights in high dose groups. In H&E-stained tissues there was acute focal ulceration with inflammation in 2/10 high dose females. To confirm potential gum ghatti-related colonic lesions, a second 90-day study was conducted in groups of 20 female rats exposed to 0 or 5% gum ghatti in AIN-93M or NIH-07 diet. The only reproducible findings were increased cecal weights in rats exposed to 5% gum ghatti. There were no gum ghatti-related histopathological changes in the colon or cecum of animals fed either diet. One female control on the AIN-93M diet had an acute focal colon ulcer with inflammation. A PWG unanimously agreed that the focal colon ulcers with inflammation were sporadic changes not attributable to gum ghatti and concluded that colon changes posed a human health concern. It is concluded that the dietary NOAEL: levels for gum ghatti in both studies is 5% as follows:

<u>First Study</u>

Males – 3044 mg/kg bw/day, Females – 3309 mg/kg bw/day

<u>Second Study</u>

AIN-93M Diet – 3670 mg/kg bw/day, NIH-07 Diet – 3825 mg/kg bw/day

P-74 Historical Control Data from 104-Weeks Study in RccHan™:WIST Rats

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Non-treated RccHan™: WIST rats of both sex (53 males and 53 females) were examined to provide control data for 104-weeks study. The animals were housed individually in a wire-mesh stainless steel cage placed on a movable stainless steel rack in a semi-barrier-sustained animal room with a controlled environment. Certified MF diet (Oriental Yeast Co., Ltd., Tokyo, Japan) and local tap water were freely supplied. During the study period, bodyweight and food consumption were measured periodically. All surviving rats were sacrificed at week 104 and were subjected to macroscopic examination and tissues were examined histologically. All rats killed in extremis or found dead during the course of the study were subjected to macroscopic examination and, where possible, tissues were examined histologically. At 104 weeks on study (109 weeks of age), the survival rates were 75% in males and 66% in females, and final mean body weights were 584 g in males and 409 g in females, respectively. Major macroscopic findings were pituitary mass in both sexes, rough surface, hypertrophy and pale colored in kidney in males, and subcutaneous mass in females. In the microscopic examination, pituitary adenoma was observed in 27% males and 19% females, and mammary gland tumors were observed 66% females. Especially, thymoma was observed in 10% females.

P-75 Inhalation Carcinogenicity of 1,1,1-Trichloroethane in Rats and Mie

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1,1,1-Trichloroethane (TCE) is one of the compounds addressed by the Montreal Protocol, which stipulates that the production and consumption of these potentially ozone-depleting substances in the stratosphere are to be phased out. Under the Montreal Protocol, the final phase-out for developed countries for TCE was 1996, with selected exceptions for existing stocks and essential use; developing countries have until 2015 for their ban to take effect. Carcinogenicity of TCE by 2-year inhalation toxicity study has not yet been reported to date. International Agency for Research on Cancer (IARC) has classified as Group 3 (Not classifiable as to its carcinogenicity to human).

<Methods> Analytical-grade 1,1,1-Trichloroethane (greater than 95% pure, including 1,4-dioxane 3%) was obtained from Wako Pure Chemical Industries, Ltd. Groups of 50 male and 50 female F344 rats and BDF1 mice (starting at an age of 6 weeks) were exposed to airflow containing 1,1,1-Trichloroethane vapor at a target concentration of 200, 800 or 3200 ppm (v/v) for 6 hours/day, 5days/week and 104weeks (2-yr).

<Results> In male rats, incidences of peritoneal mesotheliomas and bronchiolar-alveolar adenomas significantly increased in exposed groups. In female rats, there was no increase in the incidences of TCE-related tumor. In male mice, incidences of malignant lymphomas of spleen and Harderian gland adenomas were significantly increased in exposed groups. In female mice, incidence of bronchiolar-alveolar adenomas and hepatocellular adenomas significantly increased in exposed groups. In male mice, survival rate was reduced due to the increased incidence of bronchiolo-alveolar carcinoma, hepatocellular carcinoma and malignant lymphoma in the 3200ppm group.

<Conclusion> This study demonstrated that 2-yr inhalation exposure to TCE vapor produced a dose-dependent increase in the incidences of benign and malignant tumors at various organs of rats and mice.

(The study was carried out on a commission from the Ministry of Health Labour and Welfare of Japan.)

P-76＊ Implantation Study of Endovascular Embolization Coil for Cerebral Aneurysm: Comparison of Histological Preparation Methods

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<Objective> Local effects after implantation of medical devices/materials are currently assessed in animal studies (ISO 10993-6 "Biolological evaluation of medical devices Part 6, Test for local effects after implantation, and the relevant MHLW guidance). Thin sectioning of tissues containing hard materials may result in tissue damage around the implantation site; therefore, the guidance on test methods suggests removing the implanted material from the tissues or polishing the material after embedding in resin. In this study, we prepared resin-embedded thin sections to assess tissue reactions caused by embolization coil for cerebral aneurysm, where an expandable polymer is packed inside the coiled metal.

<Methods> Endovascular embolization coils for cerebral aneurysm of approximately 0.3 mm in diameter (HydroSoft™) were cut into pieces of 10 mm in length and implanted in the rabbit paravertebral muscle. The muscles were excised at necropsy and fixed in 10% neutral buffered formalin solution. After excising the implantation sites, we prepared three types of histological sections: paraffin-embedded thin sections, resin-embedded polished sections (embedded in MMA resin), and resin-embedded thin sections (embedded in GMA resin). The HE-stained sections were observed under light microscopy. For paraffin-embedded thin sections, we removed the coil specimens before tissue embedding.

<Results and discussion> In the resin-embedded thin sections, the interface around the coil and polymer was maintained, which facilitated observation of the cell reactions around the implanted specimens. In the paraffin-embedded sections, no polymer was found because of removal of the coil, and damage was observed at the implant–tissue interface. In the resin-embedded polished sections, the interface around the coil and polymer was maintained. However, thick section stratified the cells and made identification of cell type difficult. Further, the cavity inside the coil had reduced transparency due to bending of the coil, which hindered observation of the interface. These results demonstrated that the resin-embedded thin section is useful method for observation of local tissue response to implants.

P-77＊ Implantation Study of Endovascular Embolization Coil for Cerebral Aneurysm: Recommendations for Successful Long-Term Implantation Test

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<Introduction> Biocompatibility and toxicopathological evaluation of implanted medical devices are critical factors because those devices are placed for long term inside a living body. Long-term implantation tests involving durations of 13 and 26 weeks have been conducted as an integral part of the safety assessment of endovascular embolization coil for cerebral aneurysm, where an expandable polymer is packed inside the coiled metal. In this report, we provided recommendations that are required for successful long-term implantation test.

<Methods> Endovascular embolization coils for cerebral aneurysm of approximately 0.3 mm in diameter (HydroSoft™) were cut into pieces of 10 mm in length ("coil specimen") and implanted in the rabbit paravertebral muscle for 13 and 26 weeks. High-density polyethylene (HDPE, diameter, 1 mm), the standard specimens, with a length of 10 mm were implanted as controls. At necropsy, the paravertebral muscles were excised including implant sites and subsequently fixed in 10% formalin neutral-buffered solution. The coil specimens were radiographed to locate and assess coil placement in the body and/or muscle. Implantation sites were embedded in resin glycol methacrylate, sectioned, and stained with hematoxylin and eosin. Cellular reactions were closely examined under light microscopy.

<Results and discussion> Of the total, 67% and 47% coil specimens and 79% and 84% HDPE specimens were collected from the rabbit paravertebral muscles at 13 and 26 weeks, respectively. Almost all other remaining implanted specimens were found from the body cavities. These results indicated that implanted specimens were eventually pushed out of the muscles along with prolonged implantation inside a living body. Tendency of such migration was more evident in coil specimens than the HDPE specimens probably because the diameter of the coil specimens was smaller than that of the HDPE specimens. Microscopic observations for implanted site collected from the muscle revealed that severity of tissue responses was reduced in a time dependent manner.

Highly accurate histological evaluation is possible by the following measures: (1) increasing the number of specimens to compensate for the eventual migration for long-term implantation, (2) tracking the position of implantation overtime, and (3) adopting appropriate methods to ensure complete recovery of implanted specimens.

P-78＊ The effect of the repeated inhalation (isoflurane) or intraperitoneal anesthesia (mixture of medetomidine, midazolam and butorphanol) for 4 weeks in rats.

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<Background and Aims> The use of anesthesia in experimental animals can be administered in single or several times during surgery or euthanasia is common. On the other hand, intratracheal and intranasal administration tests are alternative methods for inhalation exposure, because it must be administered safety and accurately test substance, need to be performed in anesthetized animals treated. These alternative methods are often not to perform a single dose as well as repeated administration, but detailed effects of repeated anesthesia are still obscure. In this study, we repeated inhalation (isoflurane: IF) and intraperitneal anesthesia (mixture of medetomidine, midazolam and butorphanol: MMB) for 4 weeks in rats, respectively, and investigated the effects of repeated anesthesia.

<Materials and Methods> Eight-week-old male Crl;CD(SD) rats were repeated inhalation exposure 3.0% IF for 10 minutes or intraperitneal administration the mixture of medetomidine, midazolam and butorphanol (0.15, 2.0, 2.5 mg/kg), once a week (IF-1, MMB-1) or 5 times a week (IF-5, MMB-5) for 4 weeks, respectively. Untreated animals were served as a control group. Observation of general condition, measurement of body weight and anesthetic time, blood biochemistry and pathological examination were carried out in all animals.

<Results and Discussion> In MMB-5 group, the body weight gain significantly decreased from 3 weeks, the anesthetic time was significantly shorter in 4 weeks, and the relative kidney weight in right side was significantly higher than that of the control group. MMB-1 group compared with the control group exhibits a significantly increase of relative kidney weight in both sides. No effects were found in blood biochemistry and pathological examination by repeated anesthesia. These data suggested that repeated MMB administration affects body weight gain, anesthetic time and relative kidney weight. On the other hand, repeated IF exposure is considered less affected, more appropriate for use in the alternative methods for inhalation exposure. Currently, in order to confirm the effects of the anesthetic in a shorter period, we have been carried out in the study for 2 weeks.

P-79＊ Deliberations on Humane Endpoints for Carcinogenicity Studies

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People engaged in animal experiments have a responsibility to observe the regulations which establish humanitarian consideration, and reduce their pain and distress as much as possible. For there is fear analgesics may have an effect on the results in toxicity studies, pain and distress is usually controlled by euthanasia. The term “humane endpoint” means the timing to apply euthanasia to release the animals from pain and distress and is used in contrast with the experiments to continue until they die (death as endpoint). It is important to assess the clinical signs accurately so that appropriate humane endpoints are applied effectively, especially in carcinogenicity studies in which animals are reared for a long term.

We marshaled the associations between the symptoms and the causes for debility and deliberated appropriate humane endpoints, targeting at the animals killed in extremis or found dead in carcinogenicity studies.

The causes of death in rats include pituitary gland tumor, mammary gland tumor, and leukemia, and those in mice include urinary disturbance and malignant lymphoma. Guidance document suggests 20% or greater body weight loss as an indicator of endpoint (OECD); however, other symptoms such as staggering gait and eye discharge should also be taken into account in pituitary gland tumor. In cases where more than 10% of body weight is a criterion in mammary gland tumor, the site of development and its surface condition should also be considered. Animals with leukemia/lymphoma showed enlargement of subcutaneous lymph nodes, abdominal distention, and anemia, and clinical exacerbation caused by anemia was considered to be a criterion.

Humane endpoints are established internationally; however, animals which satisfy one criterion don’t necessarily suffer severe pain or distress. Thus, laboratory animal caretakers, study directors, and attending veterinarians should work together to comprehensively evaluate the animals’ condition and decide when to apply euthanasia.

P-80* Investigation of Drug Induced Osteocyte Toxicity Using a Bone Organ Culture Method

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Osteocyte necrosis was observed in bone tissue when a compound X, which is currently under clinical development, was injected to a tissue adjacent to the bone. Bone organ culture and cell culture systems have been used in research for osteoblasts and osteoclasts, but generally not for osteocytes. We applied an existing bone organ culture system in a similar one using skull caps of the rabbit, and investigated possible direct effect of the compound X on the osteocyte as a help to clarify the mechanisms of the osteocyte necrosis.

Rabbits were sacrificed under anesthesia and bone fragments (4 mm in diameter x 1.5 mm in thickness) were sampled from the skull. The bone fragments were cultured in α-MEM containing 10% FBS for 24 hours, and then in media containing 2.5, 25 or 250 U/mL of the compound X, negative control (PBS) or 10 U/mL positive control (a proteolytic enzyme) for 5 days. After cultivation, the samples were fixed with 10% neutral buffered formalin, decalcified overnight, embedded in paraffin, and histology sections were stained with hematoxylin and eosin (HE). Then, photomicrographs of the four different areas near the surfaces of each bone fragment were taken under x 50 magnification. The number of the bone lacunae containing viable osteocyte was counted from a total of 200 bone lacunae, and the percentages of bone lacunae with osteocyte (osteocyte survival rate) in the test groups were calculated based on the mean value in the negative control group as 100%.

When bone fragments were cultured with the media containing the compound X for 5 days, the osteocyte survival rates were comparable with that in the negative control group at any concentration. On the other hand, the survival rate was 5.7% in the positive control group, which indicated strong osteocyte toxicity.

From these results, the compound X is not considered to possess direct cytotoxicity to the osteocyte. Since strong osteocyte toxicity was seen in the positive control group and also its reproducibility was confirmed, the test system used in this study is thought to be a possible approach to clarify mechanisms of osteocyte toxicity.

P-81* The Effect of Cathepsin K Inhibitor and Bisphosphonate on Bone Tissue in Rats

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Cathepsin K (CatK) is a proteinase that is highly expressed in osteoclasts and plays a key role in bone resorption by degradation of the organic matrix. CatK inhibitors inhibit bone resorption by mechanisms of action that are distinct from bisphosphonates, and are expected as a new treatment of osteoporosis. In this study, the effects of a CatK inhibitor and bisphosphonate (Alendronate) on bone tissue were compared histopathologically in the rats.

Female F344 rats (8-week-old, 6 animals/group) were orally administered with a CatK inhibitor at 0, 20, and 500 mg/kg or Alendronate at 5 mg/kg, once daily for 14 consecutive days. Animals were sacrificed the day after the last dose, and femurs and tibiae were sampled, decalcified with EDTA, and routinely processed paraffin sections were stained with haematoxylin–eosin (HE), tartrate-resistant acid phosphatase (TRAP), and immunostaining for CatK.

In the Alendronate-treated group, large-sized and multi-nucleated osteoclasts were frequently seen as one of the prominent findings and some osteoclasts showed apoptotsis. In addition, the area of primary spongiosa was increased in length at the metaphysis. In the CatK inhibitor-treated groups, no obvious changes were observed by HE staining including the findings seen in the Alendronate-treated group. However, stainability of osteoclasts for TRAP was strongly increased on the primary spongiosa at the metaphysis, and also that for CatK immunostaining was slightly increased at the distal part of the metaphysis. Thus Alendronate induced characteristic changes in osteoclasts and bone tissues as a bisphosphonate, however, the changes induced by the CatK inhibitor were not prominent as compared with Alendronate. Since both CatK and TRAP are localized in the intracellular vesicles in osteoclasts and they are functionally related to each other, the CatK inhibitor is thought to induce changes of TRAP intensity in osteoclasts. In addition, since the length of primary sopngiosa at the metaphysis was not changed by the CatK inhibitor even at a toxic dose, inhibited bone resoption by the compound might be compensated by some kind of factor (s) under the condition of normal bone metabolism. Further investigation, including by an electron microscopy, will be needed for the changes of fine structure of vesicles and other cytoplasmic organellas in the osteoclast.

P-82＊ Compound X-induced irreversible skeletal muscle injury

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A 28-day oral exposure study was performed in rats with a developed compound, Compound X. This study showed characteristic skeletal muscle injury that was progressive after a 28-day recovery period.

For toxicity profiling, Compound X (2000 mg/kg) was treated in 5 male F344 rats for 28 days, and the blood chemical examination (including muscle biomarker), the pathological examination (cutaneous muscle, diaphragm, esophagus, gastrocnemius, psoas, rectus femoris, soleus, and tongue), the gene expression analysis (GeneChip Rat Genome 230 2.0 array), and the measurement of the plasma and skeletal muscle concentration of the compound were conducted over time (on Day 5, 8, and 15, and on Day 29 after the recovery period).

Serum sTnI was increased after Day 8, and plasma CK-MM, serum Myl3, and serum FABP3 were increased after Day 15. In histopathology, minimal to slight degeneration of myocytes were observed in the diaphragm, gastrocnemius, psoas, rectus femoris, and tongue after Day 15. Among them, degeneration in the diaphragm was the most severe. Slight to moderate degeneration of myocytes were observed in all striated muscle of all animals on Day 29 after the recovery period. There were no clear bias in fiber type distribution of the degenerative myocytes for double immunohistochemistry with slow-myosin and fast-myosin. Even on Day 29 after the recovery period, the compound remained in the skeletal muscle in the measurement of the concentration. In gene expression analysis of rectus femoris, we suggested that Cdkn1a, Stat1, Cxcr4, Egr1, and Rac1 were the hub gene in the skeletal muscle injury, however, mechanisms underlying specific gene expressions of the toxicity were not found.

In the Compound X-induced skeletal muscle injury, an increase in serum sTnI was observed before the histological appearance, and the degeneration of myocytes was the most severe in the diaphragm. We considered that the remaining compound in the skeletal muscle after the recovery period contributed to the Compound X-induced irreversible skeletal muscle injury.

P-83＊ Immunohistochemistry with Anti-thyroid Transcriptional Factor-1 for Thyroid Proliferative Lesion in Rats; Usefulness for Tumor Diagnosis

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Thyroid transcriptional factor-1 (TTF-1) is expressed in thyroid follicular cells, alveolar type 2 epithelial cells and Clara cells in humans, Immunohistochemical analysis of TTF-1 is frequently used for diagnosis of human thyroid and lung cancer. TTF-1 mRNA expression in thyroid follicular cells, C-cells and parathyroid chief cells was reported in rats, however, it is uncertain TTF-1 could be useful for tumor diagnosis. Therefore, we investigated the usefulness of TTF-1 immunostaining in rats.

In this study, normal tissue and proliferative lesion of the thyroid and parathyroid gland (follicular cell carcinoma; 4 cases, adenoma; 1, hyperplasia; 2, C-cell adenoma; 7 cases, hyperplasia; 14, parathyroid adenoma; 2 cases, hyperplasia; 14 cases) in Crl:CD(SD)IGS BR rats were immunostained for TTF-1, thyroglobulin, calcitonin and parathyroid hormone (PTH).

In normal tissue, approximately 100% thyroid follicular cells and 70% C-cells were positive for TTF-1 and parathyroid chief cells were negative. In the proliferative lesion, all cases originated from follicular cells, 3 out of 7 cases of C-cell adenoma (42.9%) and 13 out of 14 cases of C-cell hyperplasia (92.9%) were positive for TTF-1, while all cases originated from parathyroid gland were negative. Moreover, thyroid follicular cells and C-cells in the proliferative lesion showed higher stain density than those in normal tissue.

These results suggest that immunostaining for TTF-1 could be useful for diagnosis of thyroid follicular cell derived proliferative lesions in rats.

P-84 A Spontaneous Epithelial-Myoepithelial Carcinoma of the Submandibular Gland in a Sprague-Dawley Rat

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The present report describes a rare case of spontaneous tumor of the salivary gland in a male Sprague-Dawley rat. The clinically confirmed mass rapidly developed in the cervical region between 19 and 21 weeks of age when the animal was terminally sacrificed. At necropsy, a well circumscribed approximately 7 × 6 cm-diameter nodule was found at the site of the salivary gland. The cut surface of the nodule was lobulated with a pinkish tan, soft, and fish-flesh appearance. One large cyst (approximately 3 × 2 cm in size) containing reddish fluid was also present in the nodule. Histopathologically, the tumor, with a partially lobulated structure, was surrounded by a thin fibrous capsule. The majority of tumor cells formed a diffuse solid sheet structure that mainly consisted of small ovoid or spindle-shaped cells. In the tumor periphery, some cells were arranged in nest-like structures. Small duct-like structures lined with monolayered cuboidal epithelial cells or large polygonal clear cells were also observed. Mitotic figures and necrotic foci were frequently observed in solid areas. Immunohistochemically, the tumor cells were positive for cytokeratin, epithelial membrane antigen, vimentin, p63, α-smooth muscle actin, and calponin. The cells were negative for calcitonin, synaptophysin, and chromogranin A. On the basis of these findings the tumor was diagnosed as an epithelial-myoepithelial carcinoma originating from the luminal epithelial cells and myoepithelial cells in the submandibular gland.

P-85* Ectopic Tissue of Forestomach Consisting of a mixed tissue of Glandular Stomach, Small Intestine and Exocrine Pancreas in a Rat

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Ectopic tissue in an alimentary canal has been reported in human but also in laboratory animals including rat. Most cases are well-known to consist of the highly-differentiated tissue of foregut origin which localizes in the serosa side such as muscle layer. We encountered ectopic tissue in a rat forestomach characterized by localization in lamina propria mucosa and mixed components consist of the glandular stomach, small intestine and exocrine pancreas and report here the characterization to compare with previously reported ectopic tissue in the alimentary canal.

A female Crl:CD(SD) rat from a toxicity study was sacrificed at 8 weeks of age. A solitary white nodule, 5 mm in size, was present on the luminal surface of the forestomach curvatura-ventriculi-major. Microscopically, there were a lump of ductular/glandular structures in lamina propria mucosa in the forestomach which was clearly continuous with squamous epithelium and open into forestomach lumen. Individual duct/gland was lined with tall columnar epithelial cells and branched and all of columnar epithelium was surrounded by muscularis mucosae. The branching epithelium was constituted by the cells which accumulated the mucus cells, stained a red purple in alcian blue and periodic acid-Schiff in the luminal side, and the cells which have a brush border. Those special stains showed the columnar epitheliums to differentiate into surface mucus cells of glandular stomach and absorptive epithelium of small intestine. In part of the epitheliums, pancreatic acinar cells and parietal cells were also observed. All of these cells were positive for Cytokeratin AE1/AE3. From the above findings, it was diagnosed as "Ectopic tissue consisting of a mixed tissue of glandular stomach, small intestine, and exocrine pancreas".

The etiology of this lesion is unknown, but it seems to be associated with the multilineage potential or the various tissues carried out aberrant.

P-86＊ Pathological Examination of Adenocarcinoma Originating from Pancreatic or Biliary Duct in a SD Rat

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Pancreatic ductal adenocarcinoma and biliary ductal adenocarcinoma are known as extremely rare spontaneous tumors in rats. Here we report a case of spontaneous intraperitoneal tumor suspected adenocarcinoma originating from pancreatic or biliary duct in a male SD rat at 46 week-old.

No clinical signs were observed until his sudden death. At the necropsy, two large masses were observed in the abdominal cavity. One was an intraperitoneal mass which involved pancreas and adhered with liver, stomach and colon, and another was observed in a mesentery. Organs/tissues including the masses were fixed in 10% neutral buffered formalin, embedded in paraffin wax, stained with haematoxylin and eosin and examined microscopically.

Histopathologically, the tumor invaded pancreas and intestine, and consisted of epithelial neoplastic cells showing tubular growth pattern and stromal neoplastic cells. Fibrosis and infiltration of inflammatory cells in stroma were also observed. Neoplastic cells showed marked atypia and mitotic figures.
Similar histological changes were observed in the mesenteric mass, liver, spleen, kidney and prostate glands.
Other histological lesions observed were diffuse necrosis of hepatocytes, increase of hematopoiesis in spleen, necrosis and inflammation in right testis.

The present case was considered to be an adenocarcinoma originated from pancreatic or biliary duct based on their location (invaded pancreas, adhesion with pancreas and colon) and morphological features (carcinoma with tubular formations). To our knowledge, there have been few reports on spontaneous pancreatic or biliary duct adenocarcinoma in rats, so our case was considered to be extremely rare.

Pancreatic and biliary ductal adenocarcinoma originate from same endodermal epithelial cells and are consisted of neoplastic cells with tubular growth pattern. Additional examinations are needed to distinguish between these tumors.

At the presentation, we will show the results of additional immunohistochemical examination.

P-87＊ Morphological Diversity of Endometrial Stromal Sarcoma in Rats

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<Purpose> Endometrial stromal sarcoma (ESS) is a malignant mesenchymal tumor derived from uterine endometrial stromal cells, and various mesenchymal tumors expanding into the uterine lumen have been generally diagnosed as ESS. In the present study, we investigated some tumors in rats that were previously diagnosed as ESS, and revealed their morphological and immunohistochemical characteristics.

<Materials and Methods> 11 cases of ESS (Fischer344, 7 cases; Wistar Hannover, 3 cases; SD, 1 case) were morphologically classified into the following 5 types, such as spindle cell and collagen rich type (SC, 2 cases), schwannoma Antoni A type (SA, 2 cases), schwannoma Antoni B type (SB, 3 cases), pleomorphic/spindle cell and compact type (PC, 3 cases), and decidual alteration type (DA, 1 case). They were evaluated using immunohistchemical staining of vimentin, S-100, α-smooth muscle actin (SMA) and desmin (smooth muscle markers), and CD10 (human ESS marker).

<Results and Discussion> Most of tumors showed focal or diffuse positive reactions to vimentin and S-100 and approximately half of the tumors were positive for CD10. Smooth muscle markers were shown to be specific in each type of tumors. SC and PC tended to be strongly positive for these markers with high frequency and were considered to have expressed smooth muscle characteristics. In contrast, SA and SB showed low reactions to these markers. In one case of SB showing focal reaction to SMA, the cystic and loosely arranged cell area (schwannoma like area) was negative. Moreover, the relatively closely packed cell area forming an interlacing bundle (leiomyoma like area) was positive for SMA. This result indicates that there is a cell differentiation existing within the same tumor. In conclusion, malignant mesenchymal tumors derived from uterine endometrial stromal cells showed various morphological and immunohistochemical features as schwannoma or leiomyosarcoma because of its potential for multi differentiation. If diagnosis of tumor is based on the cell origin, ESS may be applied to the tumors showing variable expressions.

P-88 A Case of Metastatic Adrenocortical Carcinoma Diagnosed by Steroidogenic Factor-1 in a Sprague-Dawley Rat

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This report describes the morphological and immunohistochemical characteristics of an adrenocortical carcinoma with distant metastasis in a female 108 week-old Sprague-Dawley rat. At necropsy, a single large mass was observed in the left adrenal gland and multiple nodules and/or enlargement were noted in the lung, liver, mediastinal lymph nodes and thyroid. Histologically, the adrenal tumor consisted of a solid growth of round cells possessing abundant eosinophilic cytoplasm with occasional vacuolation. The nuclei were round, variable in their size with distinct single nucleoli and frequent mitotic figures. Vascular invasion by the tumor cells was present and metastatic proliferative lesions were also observed in the lungs, liver, and mediastinal lymph nodes. Immunohistochemically, the nuclei of these tumor cells were positive for Steroidogenic Factor-1 (SF-1). On the other hand, thyroid C-cell carcinoma observed in this animal was immuno-negative for SF-1 but positive for calcitonin. From these results, the present case was diagnosed as adrenocortical carcinoma with distant metastases in a rat. Immunohistochemistry for SF-1 is considered to be a valuable tool for the differential diagnosis of adrenocortical tumors versus other endocrine tumors such as C-cell carcinoma.

P-89* Histopathological Changes in Fetal and Neonatal Rats Exposed to Busulfan

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<Introduction> Busulfan, an antineoplastic bifunctional-alkylating agent, is known to induce toxicity to gastrointestinal, lymphoid, gonadal, neural and ocular tissues in adult rats by long-term or high-dose treatment. We previously demonstrated busulfan-induced neurotoxicity and ocular toxicity in fetal and neonatal rats. In the present study, we demonstrated the toxicity of busulfan in the tissues other than the central nervous system and ocular tissues in fetal and neonatal rats.

<Materials and Methods>

[Fetuses] Pregnant Crl:CD(SD) rats were used on gestation day 13. Busulfan was suspended with olive oil at 30 mg/kg. The dams were sacrificed by exsanguination via the abdominal aorta under anesthesia at 12, 24, 36, 48, 72 and 96 hours after busulfan-treatment.

[Neonates] The animals were male Crl:CD(SD) rats at 6 days of age, and were treated once with busulfan at 0 or 20 mg/kg, and were euthanized at 1, 2, 4, 7 or 14 days after treatment.

[Histopathology] The organs were fixed with 10% neutral buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin and then examined microscopically.

<Results>

[Fetuses] Single cell necrosis was observed in the lungs, kidneys, liver, alimentary tract, craniofacial tissues, mandible, limb buds and tail bud. It was observed from 24 to 72 hours after treatment, but almost disappeared at 96 hours after treatment.

[Neonates] Single cell necrosis was observed in the heart, lungs, kidneys, stomach, intestines, skin, bone, thymus and lymph nodes from day 1 to day 7 after treatment, but it was no longer observed on day 14 after treatment. Single cell necrosis was also observed in the testes, and hematopoietic cells in the liver, spleen and bone marrow from day 1 to day 7 after treatment. Subsequently, depletion of germ cells in the testis was observed as well as decreased hematopoietic cells in the liver, spleen and bone marrow. These lesions were still observed on day 14 after treatment.

<Discussion and Conclusion> In this study, we clarify distribution and attribution of the busulfan-induced systemic lesions in fetal and neonatal rats. It became possible to examine the progress of the busulfan-induced toxicity associated with rat growth using the results of this study and the findings in adult rats in the literature.

P-90＊ Immunohisitochemical and comprehensive gene expression analyses of different cloned cell lines (MT-8 and MT-9) from rat malignant fibrous histiocytoma (MFH)

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【目的】MFHは成人において最も一般的な間葉系悪性腫瘍である。MFHの起源細胞は多分化能未分化間葉系細胞であるとされているが、その組織発生および細胞特性は未だ不明な点が多い。今回、ラットに自然発生したMFHから確立されたMT-8とMT-9クローン細胞の培養細胞と同系ラットでの誘発腫瘍を用いて、細胞特性の違いを免疫組織化学的及び網羅的遺伝子解析により検討した。

【方法】MT-8およびMT-9の培養細胞と、3×10^6個の細胞を同系F344雄ラットの皮下に接種し約3週間後に形成された誘発腫瘍を用いた。誘発腫瘍から切片を作製し、種々の抗体を用いて免疫組織化学的に解析した。さらに培養細胞と誘発腫瘍からmRNAを抽出し、網羅的遺伝子解析を行った。

【結果】誘発腫瘍のHE所見では、MT-8には卵円形の腫瘍細胞が充実性に増殖したのに対し、MT-9には紡錘形細胞のstoriform状の増殖様式がみられ、異なる組織像を示した。また、免疫組織化学的染色では、MT-8はvimentinおよびα-SMA等の抗体に陰性であったが、MT-9はこれら抗体に加えA3, nestin、Thy-1、CD34抗体に陽性を示した。その他種々の抗体についてもさらに検討中である。網羅的遺伝子解析の結果、MT-9での発現の2倍以上を示すMT-8での遺伝子はin vitroで2,344個、in vivoで2,871個、一方MT-8での発現の2倍以上を示すMT-9での遺伝子はin vitroで2,313個、in vivoで1,941個が検出された。間葉系細胞、脂肪細胞、筋肉細胞および上皮細胞の分化に関連する遺伝子はMT-8と比較してMT-9でより多く発現しており、さらにMT-8またはMT-9において幹細胞、間葉系細胞、骨芽細胞、軟骨細胞、筋肉細胞および上皮細胞等の分化に関連する遺伝子は培養細胞と比較して誘発腫瘍でより多く発現していた。分化に関わる遺伝子の変動についてはさらに解析中である。

【まとめ】MT-8はMT-9と比較して、より未熟な間葉系細胞の特性を有する可能性がある。これらの細胞株は同一のラットMFHに由来することから、MFHには異なる分化程度の腫瘍細胞が混在していることが示された。

P-91＊ Proposed Change to Rodent Carcinogenicity Testing of Pharmaceuticals in ICH

○Mizuho Nonaka[1], Kumiko Ogawa[2], Hiroshi Onodera[1], Dai Nakae[3], Akiyoshi Nishikawa[2]

[1]PMDA, [2]NIHS, [3]Tokyo Metropolitan Inst. Public Health

A change to the current ICH S1 guidance on rodent carcinogenicity testing is being considered. The goal of this potential change is to introduce a more comprehensive and integrated approach to address the risk of human carcinogenicity of small molecule pharmaceuticals, and to define conditions under which 2-yr rodent carcinogenicity studies add value to that assessment.

Datasets evaluated by the ICH S1 expert working group suggest that knowledge of pharmacologic targets and pathways together with toxicological and other data can, in certain cases, provide sufficient information to anticipate the outcome of 2-yr rodent studies and their potential value in predicting the risk of human carcinogenicity of a given pharmaceutical. Consideration of this information is hypothesized to provide sufficient information to conclude that a given pharmaceutical in certain cases presents a negligible risk or, conversely, a likely risk of human carcinogenicity without conducting a 2-yr rodent study.

Prospective evaluation of this proposed hypothesis is necessary to justify proceeding with revision of the ICH S1 guidance. Public comment is sought regarding the proposed change in approach to carcinogenicity assessment, on the prospective evaluation period intended to test this new approach, and on the weight-of-evidence factors proposed for predicting the risk of carcinogenicity.

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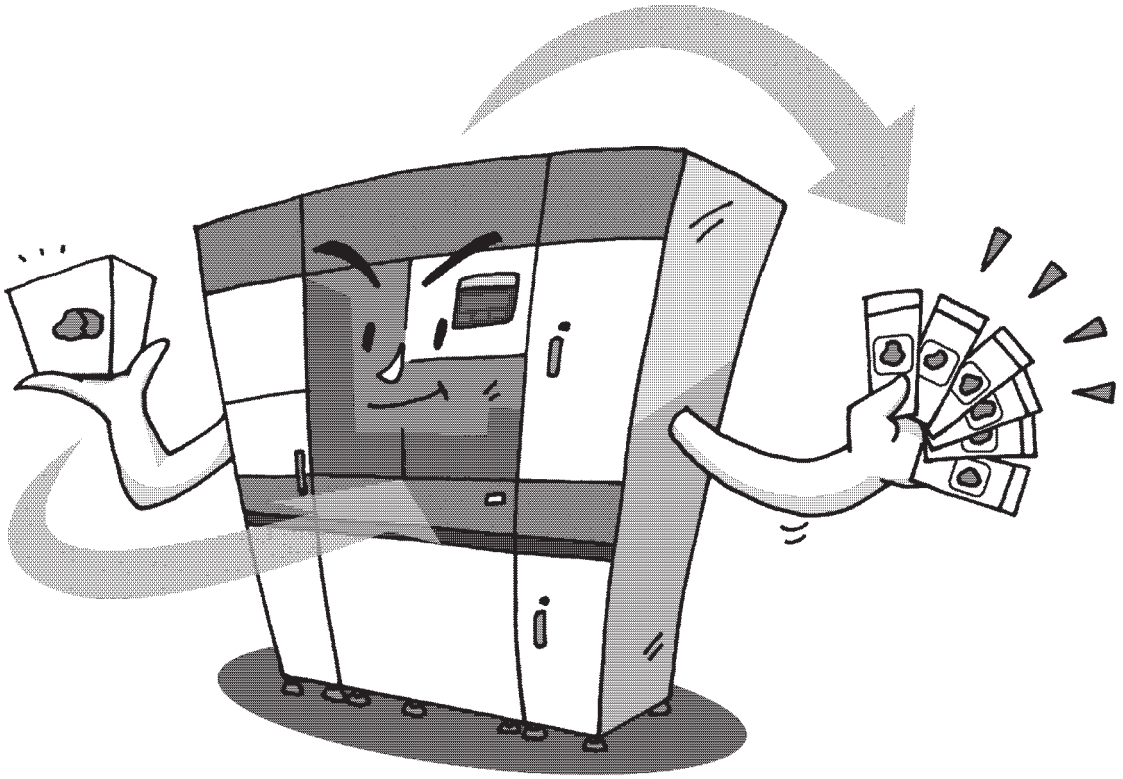

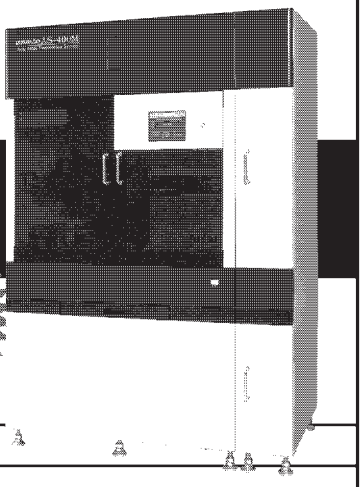

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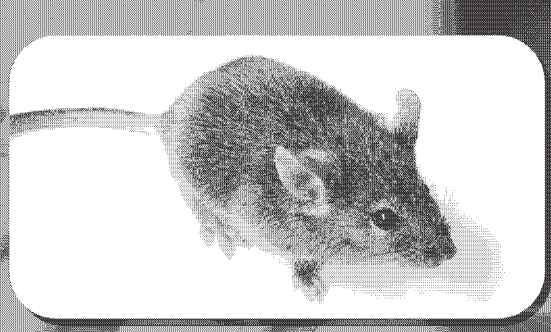

rasH2マウス

Jic:CB6F1-TgrasH2@Jcl

厚労省・米国FDA・欧州CPMPが認めたマウス

国際的な評価プロジェクトの結果から、新薬の申請用試験データとして認められています。

rasH2マウスの近況

米国研究製薬工業協会は加盟企業から提供された182化合物のデータを解析し、一定の条件を満たす医薬品については本試験法※とラット6か月慢性毒性試験で得られた成績を組み合わせることで、ラットがん原性試験を実施せずに医薬品のがん原性陰性の評価が可能であることを示し、日本製薬工業協会による追加調査でも同様の結論が得られました。これらの調査を踏まえて、2011年6月にはがん原性試験の必要性に関するICHS1Aガイドライン改訂の必要性を議論する非公式作業部会(IWG)が編成され、2012年2月28日には専門家作業部会(EWG)を結成することに日・米・EUの産官6団体が合意し、検討が進められています。

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until2003	7	1	2
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2005	4	0	2
2006	3	0	0
2007	1	3	2
2008	3	1	1
2009	1	3	0
2010	3	6	1
2011(～Aug.)	3	2	1

剤型変更、投与経路拡大等による重複は除外

※短期発がん性試験は、試験期間、費用、動物数、検体量を削減する

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  - 短期試験　1.0～1.2 万ドル
- 使用動物数 **1/2!**
  - 2年間試験　500 - 750 匹
  - 短期試験　250 - 350 匹
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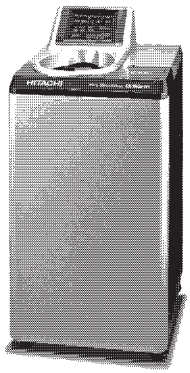

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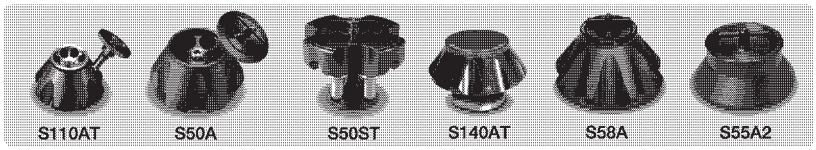
- サンプルのバランスは目分量でOK!
- ロータは全て載せるだけ！(ロータクイックセッティング方式)
- エコ対応省エネモデル

■仕様

形　名	CS150FNX	CS120FNX	CS100FNX
最高回転速度 (rpm)	150,000	120,000	100,000
最大遠心加速度 (xg)	1,050,000	771,000	571,000
サイズ (W×D×H mm)	440×520×910		
質　量 (kg)	105		
標準価格 (税別)	￥5,500,000	￥4,900,000	￥4,300,000



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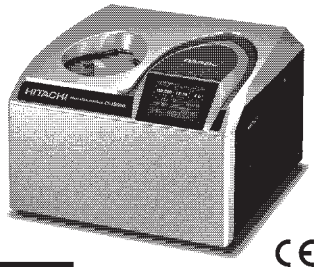

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- ロータは全て載せるだけ！

■仕様

形　名	CS150NX
最高回転速度 (rpm)	150,000
最大遠心加速度 (xg)	1,050,000
サイズ (W×D×H mm)	590×582×408
質　量 (kg)	97
標準価格 (税別)	￥6,000,000

＊(2012年3月現在当社調べによる)

■ロータ仕様

形　名	S110AT	S50A	S50ST
最高回転速度 (rpm)	110,000	50,000	50,000
容量 (ml) ×本数 (本)	5.0×8	30.0×6	7.0×4
標準価格 (税別)	￥1,100,000	￥950,000	￥1,500,000
形　名	S140AT	S58A	S55A2
最高回転速度 (rpm)	140,000	58,000	55,000
容量 (ml) ×本数 (本)	2.0×10	13.5×8	1.5×12
標準価格 (税別)	￥1,290,000	￥1,000,000	￥750,000

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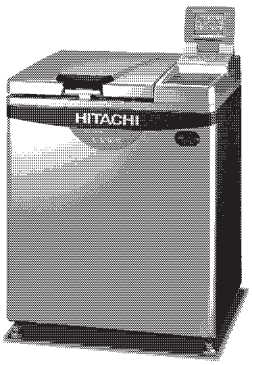
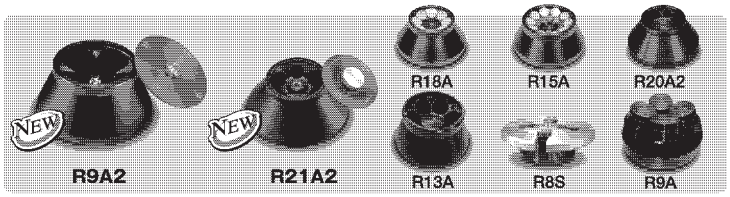


特長
- CEマーキング適合の安全性**
  (床面へのアンカーボルト固定不要)
- インバランスを正確にキャッチ
- ロータは全て載せるだけ。
  ロータ自動判別機能(特許)

＊(2012年4月現在当社調べによる高速冷却遠心機比)
**CEマーキング適合外となるロータ(旧型ロータ含む)があります。詳細はお問い合せください。

■仕様

形　名	CR22N	CR21GⅢ	CR20GⅢ
最高回転速度 (rpm)	22,000	21,000	20,000
最大遠心加速度 (xg)	55,200	50,300	48,000
サイズ (W×D×H mm)	700×810×915	752×810×1,144	
最大処理容量	1.5L×4本	1.0L×4本	
画面表示	高解像度カラー液晶	モノクロ液晶	
操作部	液晶タッチパネル	キーボード	
冷凍機	全密閉型インバータロータリー(冷媒：R410A)	1,500W全密閉型(冷媒：R404A)	
質　量 (kg)	290	300	
標準価格 (税別)	￥2,500,000	￥2,300,000	￥1,800,000



■ロータ仕様

形　名	R9A2	R21A2	R18A	R15A
最高回転速度 (rpm)	8,500	21,000	18,000	15,000
容量 (ml) ×本数 (本)	1,500×4	30×6	50×8	15／50×10
標準価格 (税別)	￥2,200,000	￥350,000	￥450,000	￥550,000
形　名	R20A2	R13A	R8S	R9A
最高回転速度 (rpm)	20,000	13,000	8,000	9,000
容量 (ml) ×本数 (本)	50×8	250×6	50×4	1,000×4
標準価格 (税別)	￥350,000	￥770,000	￥800,000	￥1,200,000

注意：R9A2ロータはCR22N専用です。CR21GⅢやCR20GⅢでは使用できません。

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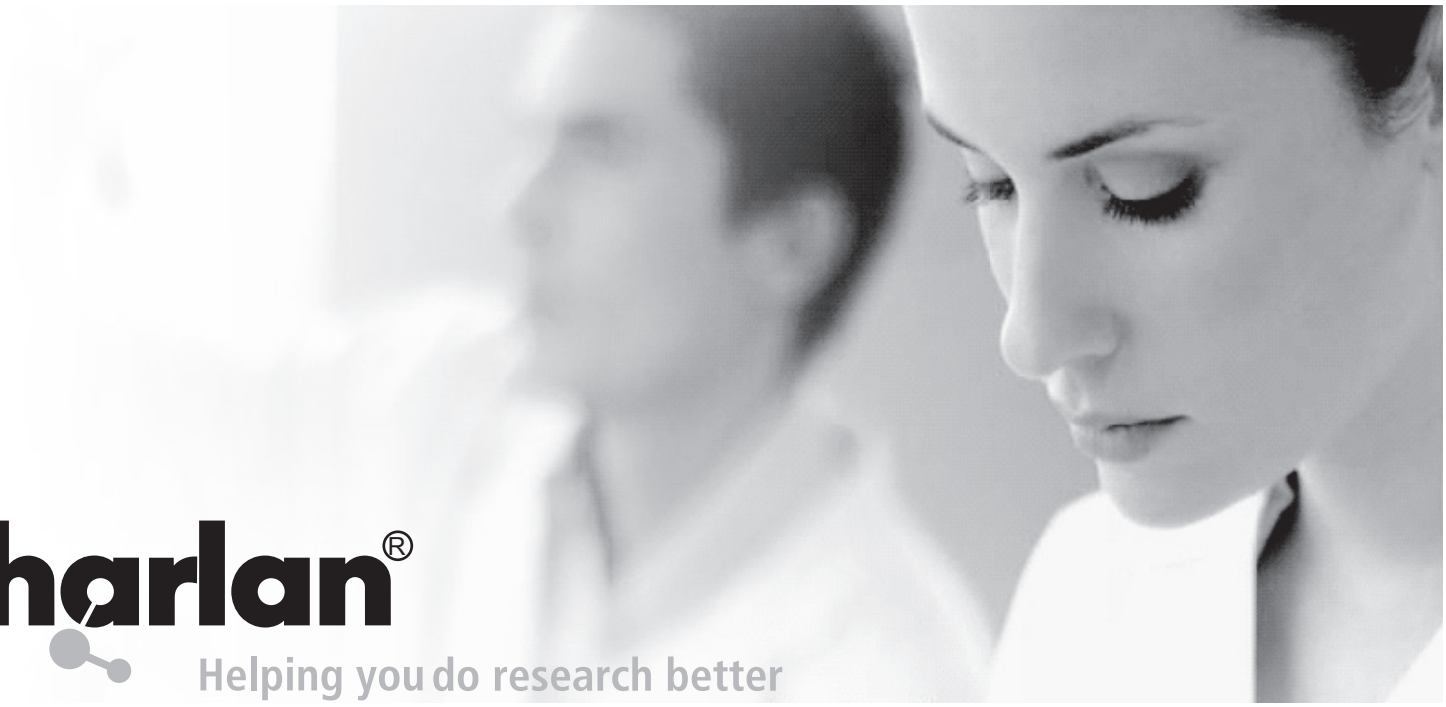

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日本毒性病理学会ランチョンセミナー

2013 年 1 月 31 日（木） 12:00 ～ 13:00

会場：オークラフロンティアホテルつくばアネックス 1 階 昴

演者： 岩田 聖（ハーランラボラトリーズ スイス）

「マウス長期発癌試験における腫瘍標的臓器を考える」

お問い合わせ

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