JSTP/IFSTP (IATP)

February 15 (Sun.) - 18 (Wed.), 2004
International Conference Center Kobe, Japan

Joint International Meeting of
The Japanese Society of Toxicologic Pathology &
The International Federation of Societies of Toxicologic Pathology;
Co-sponsored by the International Academy of Toxicologic Pathology

Program & Abstracts

The Japanese Society of Toxicologic Pathology
International Federation of Societies of Toxicologic Pathology;
Co-sponsored by the International Academy of Toxicologic Pathology

www.kitox.re.kr
AAALAC Accredited

"Korea Institute of Toxicology (KIT) Confirms Its Best Reputation for CRO in Asia"
:Specialized in Pre-clinical and Environmental Toxicology

KIT is your reliable partner in worldwide registration of your lead compounds including Asia, EU and U.S.A

"Offers exceptional combination of cutting-edge GLP systems, sound science and unmatched service to clients worldwide"

KIT Provides an Extensive Array of Tests Including: General Toxicology, Carcinogenicity Test, Non-Human Primate Toxicology, Reproductive Toxicology, Genetic Toxicology, Immunotoxicology, Pharmacology, Toxicokinetics, Toxicologic Pathology, Ecotoxicology, and Environmental Chemistry

KIT
Korea Institute of Toxigycogy
P.O.Box 123 Yuseong Daejeon 305-600 Korea Tel:+82-42-610-8080 / Fax:+82-42-610-8085

Notice

The following corrections need to be made to the Abstract Book.
Please note the revisions below when referring to the corresponding parts.

JSTP/IFSTP (IATP) 2004 Secretariat

・ Chair's Name p.29, p.95, p.286

Gerd MORAWITZ → (correct) Gerd MORAWIETZ

・ Symposium 3 - Chair p.29, p.95

Gerd MORAWIETZ → Paul-Georg GERMANN
(The Fraunhofer Institute of Toxicology and Aerosol Research, Drug Research and Clinical Inhalation) (Preclinical Safety, Novartis Pharma AG)

・ Special Presentation

Following presentation is scheduled to be held at the end of Symposium 3, February 18, 2004.

Title: Educational IT-Tools in Toxicological Pathology: The "Toxicopathology Case Collection"

Paul-Georg GERMANN	Preclinical Safety, Novartis Pharma AG
Gerd MORAWIETZ	The Fraunhofer Institute of Toxicology and Aerosol Research, Drug Research and Clinical Inhalation

The above abstract will be inserted in the abstract book.

Symposium 3 Special Presentation

Abstract

Educational IT-Tools in Toxicological Pathology: The "Toxicopathology Case Collection"

P.-G. Germann
Preclinical Safety, Novartis Pharma AG, Basel 303-0043, Switzerland
G. Morawietz
The Fraunhofer Institute of Toxicology and Aerosol Research, Drug Research and Clinical Inhalation, Hannover 30625, Germany

Background - During his illustrious career as one of the founding fathers of toxicologic pathology, **Prof. Gerhard Zbinden**, MD, F.R.C. Path., (1924 – 1993) created a unique histological slide collection (more than 1100 cases) from which about **1000 images** have been selected and stored on CD-ROM.

Scope - These images capture the essence of Prof. Zbinden's collection with emphasis on toxicopathological changes produced by xenobiotics, although many images of normal tissues and spontaneous lesions are included for reference. The collection is of considerable interest to toxicologists and other preclinical safety specialists given the variety of case material presented, but is of particular value to the learning, teaching and practicing toxicological pathologist (e.g. for the preparation of Pathology Board Examination).

What is different to other CD-ROMs - The important feature of the ESTP Toxicopathology Case Collection is the availability of the original histological slide sets to purchasers of the CD-ROM. The recipient can then study each case in more detail. Excluding mailing/insurance costs, these slide sets can be ordered on short-term loan (up to 4 weeks) free of charge. For teaching purposes, each case is available in 24 to 30 duplicate sets of histological slides.

Invited lectures - Special invited lectures from international recognized scientists are included as PDF files on this CD-ROM to cover additional important or rare aspects of toxicological pathology. One special emphasis in this invited lectures is on the **Toxicological Pathology of Male Genital System:**

Diane Creasy:	"Toxic Effects in the Testis"
Danielle Roman:	"Flow Cytometry evaluation of Testicular Toxicity"
Ulrich Hübel:	"Sperm Motility"
Jean-Loic Le Net	"Evaluation of Leydig cell toxicity"
Gabriele Pohlmeyer:	"Leydig cell tumors"

Additional special invited lectures on rare topics:

Georg Krinke:	"Toxicologic Neuropathy- the PNS"
Heinrich Ernst:	"Odontogenic Tumours in rodents"
Anne Provencher:	"Hematopoesis & Hematotoxicity in Toxicological Pathology"

Costs - All editors & authors have contributed to this first CD-ROM edition without any financial interest. Profit of the CD-ROM will be given to the ESTP, which is a non-profit organization. The CD-ROM is available (03/2004) for 70.- €, please contact: CaseCollection@Eurotoxpath.org (ISBN number: 3-9522681-1-9).

References - http://www.eurotoxpath.org/nomenclature/cd1index.htm

One Case of Ito Cell Tumor in Female F344/DuCrj Rat

Yumi UMEDA, Tetsuya TAKEUCHI, Hideki SENOH, Taku KATAGIRI, Shigetoshi AISO, Kasuke NAGANO
Japan Bioassay Research Center (JBRC)

ABSTRACT

One case of Ito cell tumor, spontaneously developing in the liver of a female rat, found from 5,000 females used for 25 studies of carcinogenicity bioassay, is presented. A mass, approximately 5 cm in size, was observed. This tumor was predominantly composed of clearly stained cells having a round, ovoid or spindle-shaped nucleus and chromophobic cytoplasm. These clearly stained cells were arranged in a cord-like structure covered with the sinusoidal endothelium. This tumor metastasized to the lung. The fluorescence microscopic examination of the liver mass revealed that fluorescence was present in the cytoplasm of the clearly stained cell, suggesting the presence of a vitamin A being characteristic of the Ito cell. Lipids were detected by Sudan-black B and Oil-red O stain. Collagenous matrix was observed among the cord-like components of the tumor. From the above-described findings, this tumor was diagnosed as a rare Ito cell tumor in the rat liver.

Table 1. Immunohistochemical characteristics of the liver tumor of BDF1 mouse

	Tumor cells	Sinusoidal endothelial cells
Factor VIII-related antigen	−	+
α-smooth muscle actin	−	−
Collagen I	+	−
Collagen III	+	−
Desmin	−	+
Laminin	+	−
Vimentin	+	+
Sudan black B	+*	−
Oil-red O	+*	−

*: Positive areas are only lipid in the cytoplasm.

CONCLUSION

Ito cells, which exist in Disse space, are the major vitamin A storage site of the body, and probably play a major role in the extracellular matrix protein synthesis (collagen I, III-VI, laminin, fibronectin, and tenascin) of the liver. The lesion with the presence of some phenotypes of the cell was observed by detection of vaitamin A with fluorescent microscope and immunohistochemical staining with a light microscope. In particular, the increased amounts of the extracellular matrix proteins, laminin and collagen III, and the detection of vitamin A suggests that the present tumor originated from the Ito cell.

Welcome

Dear Colleagues,

Welcome to Kobe. It is our great pleasure to welcome you in the Joint International Meeting of the Japanese Society of Toxicologic Pathology (JSTP) and the International Federation of Societies of Toxicologic Pathology (IFSTP), co-sponsered by the International Academy of Toxicologic Pathology (IATP); JSTP/IFSTP (IATP) 2004, entitled Investigative Toxicologic Pathology-Present and Future.

The meeting includes one special lecture, one plenary lecture, 6 symposium sessions, IATP educational session, and preferred-papers by posters. We also supply 9 luncheon seminars. In addition, we provide commercial exhibitions in the meeting break areas. We strongly hope that you will obtain important information by discussing and exchanging knowledge and experiences in the variety of fields in toxicologic pathology during the meeting.

Kobe is a highly attractive city with an international atmosphere, located on the shore of the Osaka Bay with a beautiful surrounding. Please enjoy not only the meeting, but also sight-seeing and have a safety trip back with pleasant memories.

Sincerely yours,

Shoji Fukushima, M.D.
20th JSTP Meeting Chair

Yoichi Konishi, M.D.
5th IFSTP Meeting Chair

JSTP/IFSTP(IATP)2004

Table of Contents

JSTP/IFSTP(IATP)2004

Organizing Committee

Meeting Chair

20th JSTP Meeting Chair

Shoji Fukushima (Osaka City University)

5th IFSTP Meeting Chair

Yoichi Konishi (Nara Medical University)

Secretary Generals

Dai Nakae (Sasaki Institute)
Masahiro Tsutsumi (Nara Medical University)
Hideki Wanibuchi (Osaka City University)

Program Committee

Chair

Akihiko Maekawa (Sasaki Institute)

Members

Ayumi Denda (Nara Medical University)
Kunio Doi (The University of Tokyo)
Kiyoshi Imai (Biosafety Research Center Foods, Drugs and Pesticides)
Katsumi Imaida (Kagawa University)
Satoru Mori (Shionogi & Co., Ltd.)
Keiichirou Morimura (Osaka City University)
Isao Narama (Setsunan University)
Masae Tatematsu (Aichi Cancer Center Research Institute)
Hiroyuki Tsuda (Nagoya City University)

Educational Committee

Chair

Kunitoshi Mitsumori (Tokyo University of Agriculture)

Members

Tetsuo Nunoya (Nippon Institute for Biological Science)
Yasuyoshi Okuno (Sumitomo Chemical Co., Ltd.)
Tomoyuki Shirai (Nagoya City University)
Seikou Tamano (Daiyukai Medical Research Institute)
Kazutoshi Tamura (Bozo Research Center)
Masahiro Tsutsumi (Nara Medical University)

Financial Committee

Chair

Shoji Fukushima (Osaka City University)

Members

Masao Hirose (National Institute of Health Sciences)
Yoichi Konishi (Nara Medical University)
Hiroki Kuniyasu (Nara Medical University)
Keizo Maita (The Institute of Environmental Toxicology)
Hiroaki Miyajima (Shin Nippon Biomedical Laboratories Ltd.)
Hideki Mori (Gifu University)
Takashi Nonoyama (Takeda Chemical Industries, Ltd.)
Michihito Takahashi (Pathology Peer Review Center)

International Advisory Committee

Robert A. Ettlin (IFSTP President)
Hugh E. Black (IFSTP Secretary General)

Members

Charles C. Capen (IATP Accreditation Committee, Past-Chairperson)
Patrizia Cristofori (IFSTP EC, Italian STP)
Eric Debruyne (IFSTP Treasurer)
Xavier Fouillet (IFSTP EC, French STP)
Jerry D. Frantz (IFSTP Past-President)
Paul-Georg Germann (IFSTP-councillor of the ESTP)
Chirukandath Gopinath (IFSTP Past-President)
Jerry F. Hardisty (IATP North American Director)
Michael J. Iatropoulos (IATP Secretary/Treasurer of the Board of Directors)
John M. Finch (IFSTP EC, British STP)
John Ishmael (IATP Accreditation Committee Member)
Eberhard Karbe (IATP Accreditation Committee Member)
D. Reid Patterson (IATP Board of Directors Chairperson)
Colin G. Rousseaux (IFSTP EC, North American STP)
Mikala Skydsgaard (IFSTP EC, Nordic STP)
Leander Tryphonas
(IFSTP Past-Secretary General and IATP Past-Chairman of the Board of Directors)
Joseph G. Vos (IATP Accreditation Committee Member)
Piet W. Wester (IFSTP EC, Dutch STP)
Gary M. Williams (IATP Accreditation Committee Member)
Ki-Hwa Yang (IFSTP EC, Korean STP)

Subcommittee to Nominate Foreign Chairpersons of Symposia

Paul-Georg Germann (Novartis Pharma)

Dai Nakae (Sasaki Institute)

Colin G. Rousseaux (University of Ottawa)

Honor Advisory Committee

Makoto Enomoto

Kousaku Fujiwara

Yuzo Hayashi

Eisei Ishikawa

Chitoshi Itakura

Nobuyuki Ito

Masayoshi Kanisawa

Sadashige Sakuma

General Information

JSTP/IFSTP(IATP)2004

General Information

Date

Sunday, February 15 - Wednesday, February 18, 2004

Conference Venue

International Conference Center Kobe (ICCK)
6-9-1 Minatojima-nakamachi, Chuo-ku, Kobe 650-0046, Japan
Phone: +81-78-302-5200
Fax: +81-78-302-6485

Official Language

Official language is English.

Registration

Conference participants can complete your registration during the following hours.

Sunday, February 15 ····· 11:30am-5:00pm
Monday, February 16 ····· 8:30am-6:00pm
Tuesday, February 17 ····· 8:30am-6:00pm
Wednesday, February 18 ····· 8:30am-5:00pm

1. Registration Desk is located in the Foyer (1F) at the International Conference Center Kobe (ICCK).
2. Advanced Registration: For those who have completed their registration in advance, please bring the confirmation letter with you and present it at the registration desk.
3. On-site Registration: For those who have not completed their registration, please complete registration at the on-site registration desk. Registration fee is as follows:

Registration Type ····· On-Site Registration
Member*1 ····· JPY50,000
Resident/Fellow/Student*1*2 ····· JPY20,000
Non-Member*1 ····· JPY60,000
Accompanied person*3 ····· JPY10,000

*1: The fee includes admission to all scientific sessions, exhibitions and Welcome and Gala reception.
*2: The registration form for Resident/ Fellow/ Student participants must be accompanied by a letter from the department head or a photocopy of a current registration card. Applicants should be full-time enrolled at a degree-granting university or college through the academic year of 2003 at time of registration.
*3: The fee includes admission to the meeting venue including the exhibition area, Welcome and Gala receptions but not to the inside of the rooms where scientific sessions are being held.

General Information Desk

General Information desk will be located next to the registration desk (1F). For inquiries, such as program schedule, please inquire at the desk.

Messages

A message board will be located in the registration area. Participants are encouraged to check this board regularly.

Board Meeting

Board meeting will be held with the following schedule.

JSTP Board of Directors Meeting (Rijikai)
Sunday, February 15 ····· 4:00pm-6:00pm 5F (Room 505)

JSTP Council (Hyogi Iinkai)
Tuesday, February 17 ····· 12:00pm-1:00pm 5F (Room 505)

JSTP General Meeting (Soukai)
Wednesday, February 18 ····· 11:30am-12:00pm 1F (Main Hall)

Poster Awards

For those who have presented an outstanding work will be nominated to receive a poster award. Nomination will be made by Program Committees. Recipients of the Poster Award will be announced on the last day of the conference.

Luncheon Seminar

There will be three (3) luncheon seminars held on each day, and total nine (9) seminars will be taken places through the conference. List of sponsor company can be found in the program pages, and participants are encouraged to attend. Seminars will be held at Main Hall, Room 501, and 502.

Monday, February 16 ····· 12:00pm-1:00pm
Tuesday, February 17 ····· 12:00pm-1:00pm
Wednesday, February 18 ····· 12:00pm-1:00pm

Exhibition

Exhibition will be located at the reception hall (3F). Participants are encouraged to visit the exhibition hall.

Monday, February 16 ····· 9:30am-5:30pm
Tuesday, February 17 ····· 9:30am-5:30pm
Wednesday, February 18 ····· 9:30am-5:30pm

Cloak

Cloak is located in the basement (B1F).

Sunday, February 15 ····· 11:30am-7:00pm
Monday, February 16 ····· 8:30am-7:00pm
Tuesday, February 17 ····· 8:30am-7:00pm
Wednesday, February 18 ····· 8:30am-7:00pm

JSTP/IFSTP(IATP)2004

Instructions for IATP Educational Session, Memorial Keynote/Plenary Lecture and Symposium

IATP Educational Session, Memorial Keynote Lecture, and Plenary Lecture and Symposium will be held at the Main Hall (B1F, 1F).

Chairs are asked to be prepared at the Main Hall 30 minutes before the session begins.

Speakers are expected to check the connection of their laptop at least 30 minutes prior to the start of the presentations at the preview room, Room 307 (3F).
Speakers are requested to ensure that they have a suitable connector for their laptop.
A 15 pin high density D plug connection for PCs and 15 pin D plug connection for Macs.
Please note no 35mm slides will be accepted at the conference.

JSTP/IFSTP(IATP)2004

Instructions for Poster Presentation

1. Reception desk of the poster presentation is located at the entrance hall (3F). All the poster presenters are expected to check in their names at the desk before posting the posters. This reception desk is only for the poster presentation, and all the conference participants are expected to come to the registration desk (1F) first upon your arrival to the conference venue.

2. Every poster will be on display from Monday through Wednesday.

3. Poster should be set-up and removed during the following time.

Set-up: Monday, February 16 ····· 8:30am-9:30am
Removal: Wednesday, February 18 ····· 4:00pm-5:00pm

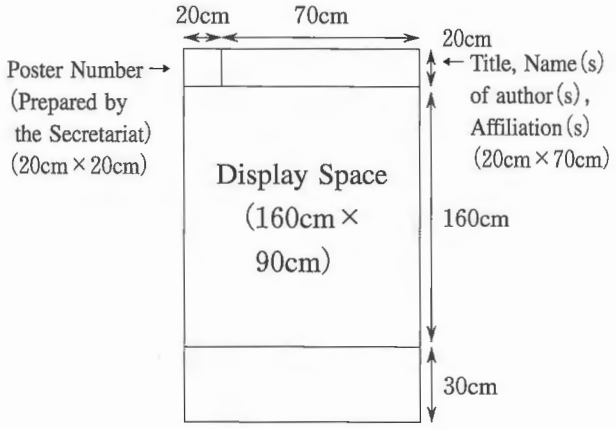
- No individual presentation will be held for the poster presentation.
- Authors will be provided, by the Secretariat, with push pins to be used in attaching material to the poster boards.
- Displays should be set up and removed strictly according to the times listed above.
- Posters not removed during the allocated times will be dismantled by the Secretariat.

4. Poster Presentation Halls will be Room 301 (3F) and Room 401, 402 (4F). Please stand next to your poster during the appointed discussion time to prepare for discussion. Core discussion times of poster session are as follow, and please refer to the program pages for your appointed date.

Monday, February 16 ····· 1:00pm-2:00pm
Tuesday, February 17 ····· 1:00pm-2:00pm
Wednesday, February 18····· 1:00pm-2:00pm

5. Language: English

Poster Board Dimension



JSTP/IFSTP(IATP)2004

Social Events/Accommodation and Tour

Welcome Reception

Sunday, February 15 ····· 7:00pm-9:00pm

Welcome Reception will be held on Sunday, February 15 from 7:00pm at PORTOPIA Hotel Main Building, Grand Banquet Room "KAIRAKU" (B1F). Participation fee is included in the registration fee.

Gala Reception

Tuesday, February 17 ····· 6:30pm-8:30pm

Gala Reception will be held on Tuesday, February 17 from 6:30pm at PORTOPIA Hotel South Wing Building, Grand Banquet Room "OWADA" (1F). Participation fee is included in the registration fee.

Official Travel Agent

JTB Corp. Event & Convention Sales Dept, Western Japan Regional Headquarters has been appointed to be the official travel agent for JSTP/IFSTP (IATP) to handle all travel arrangements to and within Japan. For those who have an inquiry about travel or sightseeing, please ask at the JTB travel desk.

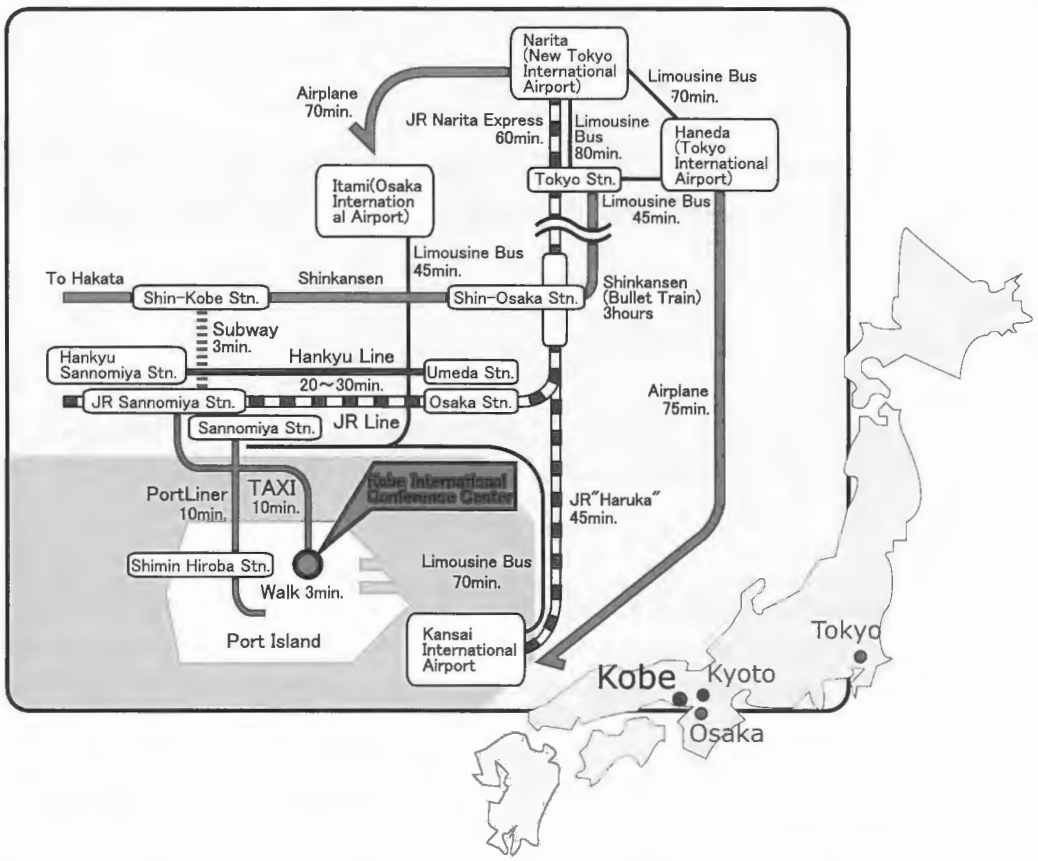
JTB Corp.,
Event & Convention Sales Dept,
Western Japan Regional Headquarters
JSTP/IFSTP (IATP) 2004 Desk
Tel:+81-6-6260-5060
Fax:+81-6-6260-5090

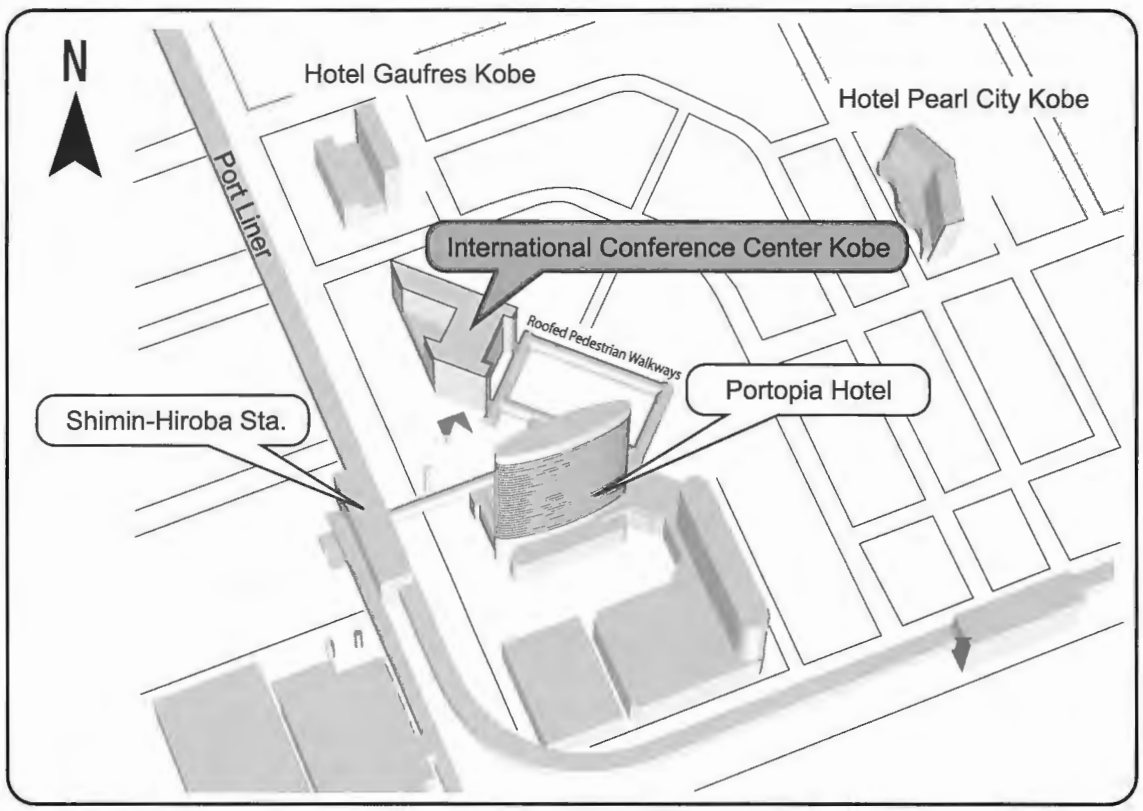
Hotels for Participants

Hotel Name	Hotel Location	Phone
Portopia Hotel	Located next to the conference venue	+81-78-302-1111
Hotel Pearl City Kobe	Walking distance from the conference venue	+81-78-303-0100
Hotel Gaufres	Walking distance from the conference venue	+81-78-303-5555

JSTP/IFSTP(IATP)2004

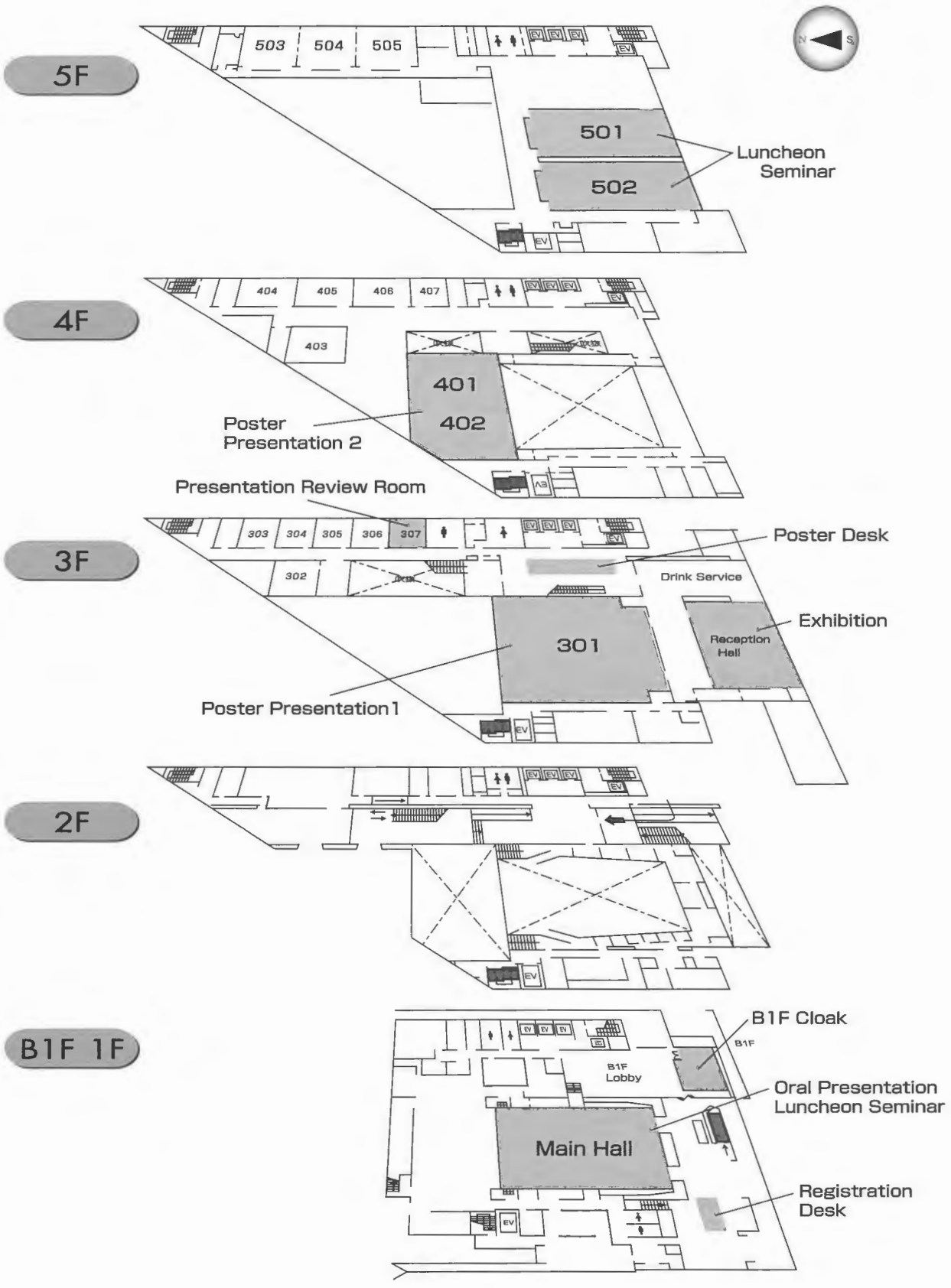
Area Map/Transportation





JSTP/IFSTP(IATP)2004

Conference Venue



Acknowledgement

Supporting Society

Asian Pacific Organization for Cancer Prevention
International Life Sciences Institute (ILSI HESI/ILSI JAPAN)
Japanese Society for Cancer Prevention
Japanese Society of Food Chemistry
The Japanese Environmental Mutagen Society
The Japanese Cancer Association
The Japanese Society of Immunotoxicology
The Japanese Society of Pathology
The Japanese Society of Toxicology
The Japanese Society of Veterinary Science

Subsidy

Kawano Masanori Memorial Foundation for Promotion of Pediatrics
The Japan Food Chemical Research Foundation
The Portopia 81 Memorial Fund
Tsutomu Nakauchi Foundation

Sponsor

Donation

Yuzo Hayashi
Adachi Co.,Ltd.
Asahi Hospital
BASF Agro, Ltd.
Chemicals Evaluation and Research Institute, Japan
Daiichi Suntory Pharma Co., Ltd.
Daiyu-kai Institute of Medical Science
JAPAN BIOASSAY RESEARCH CENTER
KANEKA CORPORATION
KIKO TECH CO., LTD.
Korean Society of Toxicologic Pathology
Mitsubishi Chemical Safety Institute Ltd.
MITSUI NORIN CO., LTD.
NARD institute, ltd.
NPO International Life Sciences Institute of Japan (ILSI Japan)
Osaka Pharmaceutical Manufacturers Association
San-Ei Gen F.F.I., Inc.
SANWARIKEN Co., Ltd.
Shin Nippon Biomedical Laboratories, Ltd.
Sumitomo Chemical Co., Ltd.
Syngenta Japan K.K.
THE INSTITUTE OF ENVIRONMENTAL TOXICOLOGY
The Pharmaceutical Manufacturers' Association of Tokyo
Wakunaga Pharmaceutical Co., Ltd.
YASHIMA PURE CHEMICALS CO., LTD.
ZERIA Pharmaceutical Co., Ltd.

Advertisement

Amersham Biosciences KK
Biopathology Institute, Co., Ltd.
CTBR (A Member of the Inveresk Research Group)
Daiyu-kai Institute of Medical Science
Dako Cytomation Co. Ltd.
Invitrogen Japan K.K.
Korea Institute of Toxicology (KIT)
KYORIN PHARMACEUTICAL CO., LTD.
KYOWA HAKKO KOGYO CO., LTD.
Nihon Bioresearch Inc.
ORIENTAL YEAST CO., LTD.
Sakura Finetek Japan Co., Ltd.
Shin Nippon Biomedical Laboratories, Ltd.
TAIHO PHARMACEUTICAL CO., LTD.
TAKARA BIO INC.

Acknowledgement

Exhibition

Affymetrix Japan K.K.
AFIP, Department of Veterinary Pathology; RTPA
ALOKA CO., LTD.
AZUMAYA CORPORATION
BIOSAFETY RESEARCH CENTER, FOODS, DRUGS AND PESTICIDES
BM EQUIPMENT CO., LTD.
Charles River Japan, Inc.
CLEA Japan, Inc.
COVANCE
CTC LABORATORY SYSTEMS Corporation
Daiyu-kai Institute of Medical Science
Dako Cytomation Co., Ltd.
Environmental Biological Life Science Research Center Inc.
Experimental Pathology Laboratories, Inc. (EPL)
GENOSTAFF CO., LTD.
H & T Corporation
Huntingdon Life Sciences Co., Ltd.
KOGAKU Co., Ltd.
Maruzen Company, Limited
MATSUNAMI GLASS IND., LTD.
MEIWA SHOJI CO., LTD.
Micro Edge Instruments Co., Ltd.
NICHIREI CORPORATION
OLYMPUS CORPORATION
ORIENTAL YEAST CO., LTD.
Shin Nippon Biomedical Laboratories, Ltd.
SUMIKA TECHNOSERVICE CORPORATION

Luncheon Seminar

Affymetrix Japan K.K.
Bozo Research Center Inc.
Dako Cytomation Co., Ltd.
Fujisawa Pharmaceutical Co., Ltd.
Japan SLC, Inc.
MEIWA SHOJI CO., LTD.
MORINAGA MILK INDUSTRY CO., LTD.
NPO International Life Sciences Institute of Japan (ILSI Japan) / ILSI Health and Environmental Sciences Institute (ILSI HESI)
OLYMPUS CORPORATION

Drink

SUNTORY LIMITED
Coca-Cola (Japan) Company, Limited
TAIHO PHARMACEUTICAL CO., LTD.
Meiji Dairies Corporation

Schedule for Conference

Sunday February 15, 2004

	Registration	Main Hall	Reception Hall	Room 301	Room 401+402	Room 501	Room 502	Other
10:00am								
11:00am								
12:00pm	11:30am							
1:00pm		JSTP Microexamination Explanation						
2:00pm								
3:00pm		Opening Remarks						
4:00pm	5:00pm	IATP Educational Session						JSTP Board of Directors Meeting (Room 505)
5:00pm								
6:00pm								
7:00pm								Welcome Reception (Portopia Hotel)
8:00pm								
9:00pm								

Monday, February 16, 2004

	Registration	Main Hall	Reception Hall	Room 301	Room 401+402	Room 501	Room 502	Other
8:00am								
9:00am	8:30am			Poster Set-up	Poster Set-up			
10:00am		Symposium 1-1						
11:00am								
12:00pm		Luncheon Seminar 1				Luncheon Seminar 2	Luncheon Seminar 3	
1:00pm			Exhibition	Poster Discussion	Poster Discussion			
2:00pm		John Faccini Memorial Keynote Lecture						
3:00pm	6:00pm							
4:00pm		Symposium 1-2						
5:00pm								
6:00pm								

Schedule for Conference

Tuesday, February 17, 2004

	Registration	Main Hall	Reception Hall	Room 301	Room 401+402	Room 501	Room 502	Other
8:00am								
9:00am	8:30am	Symposium 2-1	Exhibition					
10:00am								
11:00am								
12:00pm		Luncheon Seminar 4				Luncheon Seminar 5	Luncheon Seminar 6	JSTP Council (Room 505)
1:00pm				Poster Discussion	Poster Discussion			
2:00pm		Plenary Lecture						
3:00pm	6:00pm							
4:00pm		Symposium 2-2						
5:00pm								
6:00pm								
7:00pm								Gala Reception (Portopia Hotel)
8:00pm								

Wednesday, February 18, 2004

	Registration	Main Hall	Reception Hall	Room 301	Room 401+402	Room 501	Room 502	Other
8:00am								
9:00am	8:30am	Symposium 3	Exhibition					
10:00am								
11:00am		JSTP General Meeting						
12:00pm		Luncheon Seminar 7				Luncheon Seminar 8	Luncheon Seminar 9	
1:00pm				Poster Discussion	Poster Discussion			
2:00pm								
3:00pm	5:00pm	Symposium 4						
4:00pm				Poster Removal	Poster Removal			
5:00pm		Closing Remarks						

Program

IATP PROGRAM

Main Hall

IATP Sunday, February 15, 2004 / 3:00pm-6:30pm

CONTEMPORARY ISSUES IN TOXICOLOGIC PATHOLOGY

Chair : D. Reid PATTERSON
Reid Patterson Consulting, Inc., Illinois 60030, USA
: Charles C. CAPEN
Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Ohio 43210, USA

Enhancing the impact of toxicologic pathologists in global medical research
D. Reid PATTERSON
Reid Patterson Consulting, Inc., Illinois 60030, USA

Chemically-induced immunopathology and immune functional changes
Joseph G. VOS
National Institute for Public Health and the Environment (RIVM), Bilthoven 3720 BA, The Netherlands

Thresholds in experimental chemical hepatocarcinogenesis
Gary M. WILLIAMS, A.M. JEFFREY, J. D. DUAN and M. J. IATROPOULOS
Department of Pathology, New York Medical College, New York 10595, USA

Endocrine disruption in wildlife: The role of toxicologic pathology in fish
Pieter W. WESTER
National Institute for Public Health and the Environment (RIVM), Bilthoven 3720 BA, The Netherlands

Renal mesenchymal tumor vs. nephroblastoma: Revisited
John Curtis SEELY
Experimental Pathology Laboratories, Inc., Research Triangle Park, North Carolina 27709, USA

Demonstration of histogenesis and cytogenesis of mammary tumors from terminal endbud, mammary duct, acinus and myoepithelium cells using human c-Ha-ras proto- and mutated-oncogene transgenic rats
Hiroyuki TSUDA[1,2], Shinobu UEDA[2], Yoichiro MATSUOKA[2], Tetsuya HAMAGUCHI[2], Akihiro NAITO[2] and Nobuo TAKASUKA[2]
Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Experimental Pathology and Chemotherapy Division, National Cancer Center Research Institute[2]

The function and pathology of brown adipose tissue (BAT) in animals and humans
Michael IATROPOULOS and Gary WILLIAMS
New York Medical College, New York 10595, USA

Closing Comments
Charles C. CAPEN
Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Ohio 43210, USA

MEMORIAL/PLENARY LECTURE PROGRAM

Main Hall

Second John Faccini Memorial Keynote Lecture — **Monday, February 16, 2004 / 2:00pm-3:00pm**

Molecular aspects of toxicologic pathology

Chair : Yoichi KONISHI
Nara Medical University

Speaker : Okio HINO
Department of Experimental Pathology, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo 170-8455, Japan

Main Hall

Plenary Lecture — **Tuesday, February 17, 2004 / 2:00pm-3:00pm**

Human relevance of carcinogenesis in rodents.

Chair : Shoji FUKUSHIMA
Osaka City University Medical School

Speaker : Samuel M. COHEN
Department of Pathology and Microbiology, University of Nebraska Medical Center, Nebraska 68198, USA

SYMPOSIUM PROGRAM

Main Hall

SYMPOSIUM1-1 Monday, February 16, 2004 / 9:30am-12:00pm

Carcinogenesis-Current understanding of mechanisms and risk assessments: Non-genotoxic carcinogenesis/Low-dose carcinogenic potential of genotoxic and non-genotoxic carcinogens

Chair : Colin G. ROUSSEAUX
Department of Cellular and Molecular Medicine, Faculty of Medicine, 451 Smyth Rd, University of Ottawa, Ontario K1H 8M5, Canada
: Masao HIROSE
Division of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan

---

S1-1-1

Risk assessment of low dose carcinogenesis by genotoxic carcinogens

Hideki WANIBUCHI, Keiichirou MORIMURA, Shoji FUKUSHIMA
Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan

S1-1-2

Analysis of reporter genes for the molecular mechanisms underlying chemical carcinogenesis

Akiyoshi NISHIKAWA and Masao HIROSE
Divisions of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan

S1-1-3

Recent advances in tumor angiogenesis

Anne M. RYAN
Department of Pathology, Pfizer Global Research and Development, Connecticut 06340, USA

S1-1-4

Gene inactivation in mammalian stem cells

Michael W. McBURNEY
Ottawa Regional Cancer Center and Department of Medicine, University of Ottawa,
Ottawa K1H 1C4, Canada

Main Hall

SYMPOSIUM1-2 Monday, February 16, 2004 / 3:30pm-6:00pm

Carcinogenesis - Current understanding of mechanisms and risk assessments: Genetically modified animal models

Chair : Jerrold M. WARD
National Institute of Allergy and Infectious Diseases, NIH, Maryland 20892, USA
: Hiroyuki TSUDA
Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan,
Experimental Pathology and Chemotherapy Division, National Cancer Center Research Institute, Tokyo 104-0045, Japan

---

S1-2-1

Mouse models of human familial cancer syndromes as models for risk assessment

Jerrold M. WARD
National Institute of Allergy and Infectious Diseases, NIH, Maryland 20892, USA

S1-2-2

Mouse models of colon cancer and polyposis

Makoto Mark TAKETO
Department of Pharmacology, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan

S1-2-3

Update on use of the Trp53 +/- mouse for short-term carcinogenicity testing of pharmaceuticals

Eugenia FLOYD
Pfizer Groton Laboratories, Worldwide Safety Sciences, Connecticut 06498, USA

S1-2-4

High susceptibility of human c-Ha-ras proto-oncogene transgenic rats to various carcinogens - Possible application for a short term assay system for environmental carcinogens

Hiroyuki TSUDA[1,2], Takamasa ONISHI[2], Shinobu UEDA[2], Yoichiro MATSUOKA[2], Katsumi FUKAMACHI[2], Akihiro NAITO[2], Cheol Beom PARK[2], Beom Seok HAN[2], Chuel Kyu KIM[2] and Nobuo TAKASUKA[2]
Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Experimental Pathology and Chemotherapy Division, National Cancer Center Research Institute[2]

Main Hall

SYMPOSIUM2-1 Tuesday, February 17, 2004 / 9:00am-11:30am

Omics - A challenge for the toxicologic pathologists:Concerning general toxicity and carcinogenicity

Chair : Michael L. CUNNINGHAM
National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina27709, USA.
: Tomoyuki SHIRAI
Nagoya City University Graduate School of Medical Sciences, Aichi467-8601, Japan

---

S2-1-1

Toxicogenomic approach for detection of carcinogenic substances

Tomoyuki SHIRAI[1], Makoto ASAMOTO[1], Kazunari TSUJIMURA[1,2], Masaru SEKIJIMA[3], Nobuyoshi MIKAMI[4] and Masanori OTUSKA[2]
Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Chemical Evaluation and Research Institute[2]
Mitsubishi Chemical Safety Institute Ltd.[3]
Sumitomo Chemical Co. Ltd.[4]

S2-1-2

Proteomic expression profiling of liver toxicity in rat using 2D-DIGE technology

Hidenori YAMANAKA[1], Kazunari TSUJIMURA[1], Masanori OTSUKA[1], Yoshikuni YAKABE[1], Satsuki HOSYUYAMA[1], Koichi SAITO[2], Kayo SUMIDA[2], Masaru SEKIJIMA[3], Koji NAKAYAMA[3], Yukiko KAWANO[3], Yasuro SHINOHARA[4], Tomoyuki SHIRAI[5]
Chemical Assessment Center, Chemical Evaluation and Research Institute, Japan, Saitama 345-0043, Japan[1]
Sumitomo Chemical Co., Ltd[2]
Mitsubishi Chemical Safety Institute Ltd[3]
Hokkaido University[4]
Graduate School of Medical Sciences, Nagoya City University[5]

S2-1-3

Gene expression profiling of rat liver fibrosis, cirrhosis, and cancer induced by choline-deficient, L-amino acid-defined diet

Ivan RUSYN[1], Oksana KOSYK[1], Christine POWELL[1], Ayumi DENDA[2], Yoichi KONISHI[2], Fumiyuki UEMATSU[3] and Dai NAKAE[3]
Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, North Carolina 27599, USA[1]
Department of Oncological Pathology, Cancer Center, Nara Medical University[2]
Department of Pathology, Sasaki Institute, Sasaki Foundation[3]

S2-1-4

Gene expression changes in F344 rats following a pharmacological dose of acetaminophen

Michael L. CUNNINGHAM
National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA

Main Hall

SYMPOSIUM2-2 Tuesday, February 17, 2004 / 3:30pm-6:00pm

Omics - A challenge for the toxicologic pathologists: Concerning reproductive toxicity, immunotoxicity and toxicity of endocrine disrupting chemicals

Chair : Paul-Georg GERMANN
Preclinical Safety, Novaitis Pharma AG, Basel 303-0043, Switzerland
: Keizo MAITA
Study Planning and Consultation, The Institute of Environmental Toxicology, Ibaraki 303-0043, Japan

---

S2-2-1

Biological effects of diethylstilbestrol -Transgenerational effects of endocrine disrupting chemicals-

Tetsuji NAGAO
Department of Life Science, Kinki University, Osaka 577-8502, Japan

S2-2-2

Mechanisms of toxic effects and tumor development caused by DDT in F344 rats

Takanori HARADA, Ryoichi OHTSUKA, Makio TAKEDA, Naruto TOMIYAMA, Machiko SAKA, Tadashi KOSAKA, Junya SASAKI, Sayuri KOJIMA, Naofumi TAKAHASHI, Yukiko TAKEUCHI, Toshinori YOSHIDA, Maki KUWAHARA, Akiko ENOMOTO, Nobuaki NAKASHIMA and Keizo MAITA
The Institute of Environmental Toxicology, Mitsukaido-shi, Ibaraki 303-0043, Japan

S2-2-3

Immunotoxicology in drug safety evaluation: A medical toxicologist's view

Jacques DESCOTES
Poison Centre and Claude Bernard University, Lyon 69007, France

S2-2-4

The sensitivity and predictability of current testing strategies to evaluate immunotoxicity

Dori GERMOLEC[1], Abraham NYSKA[1], C Frieke KUPER[2], Christopher PORTIER[1], Michael KASHON[3], C KOMMINENI[3], Keith JOHNSON[4] and Michael LUSTER[3]
National Institute of Environmental Health Sciences, North Carolina 27709, USA[1]
TNO Nutrition and Food Research[2]
National Institute of Occupational Safety and Health[3]
Dow Chemical Company[4]

Main Hall

SYMPOSIUM3 **Wednesday, February 18, 2004 / 9:00am-11:30am**

Recent trend of information technology in toxicological pathology science

Chair : Gerd MORAWITZ
The Fraunhofer Institute of Toxicology and Aerosol Research, Drug Research and Clinical Inhalation, Hannover 30625, Germany
: Ikuo HORII
Pfizer Global Research & Development, Worldwide Safety Sciences - Nagoya, Aichi 470-2393, Japan

---

S3-1

Pathology information system

Nobuyuki OHASHI
Biosafety Research Center, Food, Drugs and Pesticides, Shizuoka 437-1213, Japan

S3-2

Linking worldwide pathology expertise with the telepathology system

Ingrid PRUIMBOOM-BREES, Mark TENGOWSKI and Ricardo OCHOA
Pfizer Research and Development, Department of Pathology - Groton Safety Sciences, Connecticut 06340, USA

S3-3

Image informatics: Current and future challenges

Judith A. NOLAN
Scimagix, Inc., California 94403, USA

S3-4

AFIP online veterinary pathology training programs

Mark MENSE, Terrell BLANCHARD, Thelda ATKIN, Sophie BOUCHIHA-OLSON, Sean HAHN, William INSKEEP and Dale DUNN
Department of Veterinary Pathology, Armed Forces Institute of Pathology, Washington DC 20306, USA

Main Hall

SYMPOSIUM4 Wednesday, February 18, 2004 / 2:00pm-4:30pm

Future challenges: Stem cell development, infectious agents and others

Chair : John FINCH
Inversk Research, Edinburgh EH33 2NE, UK
: Kunio DOI
Veterinary Pathology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

---

S4-1

Establishment and manipulation of monkey and human ES cell lines for biomedical research

Norio NAKATSUJI
Stem Cell Research Center, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan

S4-2

Clinical applications of stem cell research

David HARRISON
Department of Pathology, University of Edinburgh, Scotland EH8 9AG, UK

S4-3

DNA methylation pattern of CpG islands for epigenetics pathology and toxicology

Kunio SHIOTA
Cellular Biochemistry, Animal Resource Sciences / Veterinary Medical Sciences, The University of Tokyo, Tokyo 113-8657, Japan

S4-4

Emerging zoonotic diseases

Corrie BROWN
Department of Veterinary Pathology, College of Veterinary Medicine, University of Georgia, Georgia 30602, USA

POSTER PROGRAM

SESSION1 : Environmental Toxicopathology

Room 301

Endocrine Disrupters 1:00pm-2:00pm

P-1 (Mon.)

Hershberger and uterotrophic assessments to evaluate potential endocrine disrupting effects of diesel exhaust in rats

Tsuyoshi ITO[1], Hiroki OKUMURA[1], Ryo OHTA[2], Kiyoshi IMAI[3], Midori YOSHIDA[4], Dai NAKAE[4], Akihiko MAEKAWA[5]
Health Effects Research Division, Japan Automobile Research Institute, Ibaraki 305-0822, Japan[1]
Safety Testing Laboratory, Hatano Research Institute, Food and Drug Safety Center[2]
Executive Director, Biosafety Research Center, Foods, Drugs and Pesticides[3]
Department of Pathology, Sasaki Institute, Sasaki Foundation[4]
Director, Sasaki Institute, Sasaki Foundation[5]

P-2 (Tue.)

Application of hershberger assay protocol to the detection of thyroid function modulators using castrated male rats

Masakuni SAWAKI[1], Shuji NODA[1], Takako MUROI[1], Hideo MITOMA[1], Saori TAKAKURA[1], Satoko SAKAMOTO[1], Masanori OTSUKA[1], Mineo TAKATSUKI[1], Kanji YAMASAKI[1]
Chemicals Evaluation and Research Institute, Oita 877-0061, Japan[1]

P-3 (Wed.)

Lack of modifying effects of 4-tert-octylphenol and benzyl butyl phthalate on 3,2'-dimethyl-4-aminobiphenyl-induced prostate carcinogenesis in rats

Hiroyuki KOHNO[1], Rikako SUZUKI[1,2], Shigeyuki SUGIE[1], Takuji TANAKA[1]
Department of Pathology, Kanazawa Medical University, Uchinada 920-0293, Japan[1]
Research Fellow of the Japan Society for the Promotion of Science[2]

P-4 (Mon.)

Sex hormone responsiveness and ductal architecture after exposure to flutamide perinatally in the rat ventral prostate

Kaori MIYATA[1], Setsuko YABUSHITA[1], Masashi SANO[2], Yasuyoshi OKUNO[1], Masatoshi MATSUO[3]
Environmental Health Science Lab., Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan[1]
Daiyu-Kai Institute of Medical Science[2]
Cooerative Research Center for Advanced Science and Technology, Osaka University[3]

P-5 (Tue.)

Is stem cell factor related to the testicular toxicity of thiamphenycol?

Maki KUWAHARA[1], Yukiko TAKEUCHI[1], Naofumi TAKAHASHI[1], Tadashi KOSAKA[1], Nobuaki NAKASHIMA[1], Toshinori YOSHIDA[1], Akiko ENOMOTO[1], Toshiaki KITAZAWA[3], Keizo MAITA[2], Takanori HARADA[1]
Toxicology Division, The Institute of Environmental Toxicology, Ibaraki 303-0043, Japan[1]
Division of Study Plannning and Conslutation, The Institute of Environmental Toxicology[2]
Contract and Research Management Division, The Institute of Environmental Toxicology[3]

P-6 (Wed.)

No modifying effects of an estrogenic compound atrazine on 7,12-dimethylbenz(a) anthracene-induced ovarian carcinogenesis in rats

Takuji TANAKA[1], Hiroyuki KOHNO[1], Rikako SUZUKI[1,2], Shigeyuki SUGIE[1]
Department of Pathology, Kanazawa Medical University, Ishikawa 920-0293, Japan[1]
Research Fellow of the Japan Society for the Promotion of Science[2]

P-7 (Mon.)

The influence of pre- and post-natal exposure to methoxychlor on gene expression in the endometrium of rats after maturation

Ryoichi OHTSUKA[1], Makio TAKEDA[1], Satoru YAMAGUCHI[1], Koichi HAYASHI[1], Yukiko TAKEUCHI[1], Maki KUWAHARA[1], Naofumi TAKAHASHI[1], Tadashi KOSAKA[1], Keizo MAITA[1], Takanori HARADA[1]
Institute of Environmental Toxicology, Ibaraki 303-0043, Japan[1]

P-8 (Tue.)

Effects of the fetal administration of diethylstilbestrol (DES) and 4-n-octylphenol (n-OP) on 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in SD rats

Hiroaki KAWAGUCHI[1], Masakazu SOHDA[1], Shuhei TAGUCHI[1], Koichiro MIYAMOTO[1], Hiroki YOSHIDA[1]
The Department of Tumor Pathology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8544, Japan[1]

P-9 (Wed.)

The influence of pre- and post-natal exposure to methoxychlor on the rat immune system and renal function during aging process

Yukiko TAKEUCHI[1], Naofumi TAKAHASHI[1], Tadashi KOSAKA[1], Koichi HAYASHI[1], Yuko CHIBA[1], Toshinori YOSHIDA[1], Maki KUWAHARA[1], Sayuri KOJIMA[1], Ryoichi OHTSUKA[1], Keizo MAITA[2], Takanori HARADA[1]
Toxicology Division II, Institute of Environmental Toxicology, Ibaraki 303-0043, Japan[1]
Division of Study Planning and Consultation, Institute of Environmental Toxicology[2]

P-10 (Mon.)

No effects of transplacental and lactational exposure to combination of bisphenol A and 4-nonylphenol, endocrine disruptors, on morphogenesis of male reproductive organs and spermatogenesis in F344 rats.

Kyoko NABAE[1], Hiroko YOSHINO[1], Norio IMAI[1], Takeshi HIROTA[1], Mayumi KAWABE[1], Syugo SUZUKI[2], Tomoyuki SHIRAI[2]
Daiyu-kai Institute of Medical Science, Aichi 491-0113, Japan[1]
Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences[2]

P-11 (Tue.)

No effects of transplacental and lactational exposure to combination of bisphenol A and 4-nonylphenol, endocrine disruptors, on morphogenesis of male reproductive organs and spermatogenesis in ICR mice

Yuko DOI[1], Norio IMAI[1], Kyoko NABAE[1], Yousuke TODA[1], Seiko TAMANO[1], Hideki WANIBUCHI[2], Keiichirou MORIMURA[2], Shoji FUKUSHIMA[2]
Daiyu-Kai Institute of Medical Science, Aichi 491-0113, Japan[1]
Osaka City University Medical School[2]

P-12 (Wed.)

Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to 4-nonylphenol in F344 rats

Hiroko YOSHINO[1], Kyoko NABAE[1], Yuko DOI[1], Takeshi HIROTA[1], Kumiko OGAWA[2], Tomoyuki SHIRAI[2]
Daiyu-Kai Institute of Medical Science, Aichi 491-0113, Japan[1]
Deptment of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences[2]

Risk Assessment 1:00pm-2:00pm

P-13 (Mon.)

Using animal and human mode of action information in assessing human risk: a framework for analysis of various pathologies

Penelope A FENNER-CRISP[1], Dorothy E PATTON[1], The ILSI RSI WORKGROUP[1]
ILSI Risk Science Institute, Washington DC 20005, USA[1]

P-14 (Tue.)

Carcinogenicity of low-dose MeIQx, in relation to cancer risk assessment

Keiichirou MORIMURA[1], Hideki WANIBUCHI[1], Manabu HOSHI[1], Anna KINOSHITA[1], Shoji FUKUSHIMA[1]
Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

P-15 (Wed.)

Presence of the threshold for carcinogenicity and in vivo mutagenicity induced by genotoxic carcinogen, MeIQx, at low doses

Saki NAITO[1], Keiichirou MORIMURA[1], Manabu HOSHI[1], Natsuko MIYAZI[1], Shoji FUKUSHIMA[1]
Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

P-16 (Mon.)

Lack of modification of MeIQx rat liver carcinogenesis by caffeine induction of CYP1A2

Hitoshi KANDORI[1,2], Masanori KURIBAYASHI[1,3], Shingo INAGUMA[1], Makoto ASAMOTO[1],
Naomi HOKAIWADO[1], Seishiro TAKAHASHI[1], Tomoyuki SHIRAI[1]
Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Takeda Chemical Industries Ltd. Drug Safety Research Center Hikari Branch[2]
Ono Pharmaceutical Co. Ltd., Safety Research[3]

P-17 (Tue.)

Investigation of the complex carcinogenic risk by PhIP and MeIQx in the initiation stage of carcinogenesis

Akihiro HIRATA[1], Tetsuya TSUKAMOTO[1], Hiroki SAKAI[2], Masami YAMAMOTO[1], Norimitsu SHIRAI[1],
Takeshi IIDAKA[1], Tokuma YANAI[2], Toshiaki MASEGI[2], Masae TATEMATSU[1]
Division of Oncological Pathology, Aichi Cancer Center Research Institute, Aichi 464-8681, Japan[1]
Department of Veterinary Pathology, Faculty of Agriculture, Gifu University[2]

P-18 (Wed.)

Colon carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in rats: suggestion for carcinogenesis and practical threshold

Kenichiro DOI[1], Elsayed I SALIM[1], Anna KINOSHITA[1], Rawiwan PUATANACHOKCHAI[1],
Hideki WANIBUCHI[1], Shoji FUKUSHIMA[1]
Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

P-19 (Mon.)

Estimation of a no observed effect level for 2-acetylaminofluorene (2-AAF) and 2,4-diaminotoluene (2,4-DAT), genotoxic liver carcinogens, in 16-week feeding studies using male F344 rats

Akihiro HAGIWARA[1], Toshio ICHIHARA[1], Hiroko YOSHINO[1], Norio IMAI[1], Hideki WANIBUCHI[2],
Keiichirou MORIMURA[2], Seiko TAMANO[1]
Daiyu-kai Institute of Medical Science, Aichi 491-0113, Japan[1]
Department of Pathology, Osaka City University Medical School[2]

P-20 (Tue.)

Effect of phenobarbital on diethylnitrosamine-induced hepatocarcinogenesis in TGF-alpha transgenic mice-correlation with P450

Takayuki YUNOKI[1], Hideki WANIBUCHI[1], Rawiwan PUATANACHOKCHAI[1], Masakazu KAKUNI[1],
Kazuo HAKOI[1], Shoji FUKUSHIMA[1]
Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan,[1]

P-21 (Wed.)

Possible mechanism of hormetic phenomenon of alpha benzene hexachloride on diethylnitrosamine induced hepatocarcinogenesis

Rawiwan PUATANACHOKCHAI[1], Hideki WANIBUCHI[1], Mayuko OSADA[2], Keiichirou MORIMURA[1],
Yoshihiko FUNAE[2], Shoji FUKUSHIMA[1]
Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]
Department of Chemical Biology, Osaka City University Medical School[2]

P-22 (Mon.)

Effect of low-dose DDT on liver carcinogenesis in rats

Masahiko KUSHIDA[1], Tokuo SUKATA[1], Keisuke OZAKI[1], Satoshi UWAGAWA[1], Keiichirou MORIMURA[2], Hideki WANIBUCHI[2], Yasuyoshi OKUNO[1], Shoji FUKUSHIMA[2]
Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan[1]
Department of Pathology, Osaka City University Medical School[2]

P-23 (Tue.)

Activation of the ras oncogene and its relationship to aflatoxins-DNA adduct formation in rat liver treated with aflatoxins

Dae Joong KIM[1], Tae Myoung KIM[1], Jin Tae HONG[2], Hwan Soo YOO[2], Cheol Beom PARK[3], Beom Jun LEE[1], Jong-Koo KANG[1], Young Won YUN[1]
College of Veterinary Medicine, Chungbuk National University[1], Cheongju 361-763, Korea[1]
College of Pharmacy, Chungbuk National University[2]
Biotoxtech[3]

P-24 (Wed.)

Lack of effects of 1439 MHz electromagnetic near field exposure on the blood-brain barrier in immature and young rats

Masanori KURIBAYASHI[1,4], Jianqing WANG[2], Osamu FUJIWARA[2], Yuko DOI[3], Kyoko NABAE[3], Seiko TAMANO[3], Tadashi OGISO[1], Makoto ASAMOTO[1], Tomoyuki SHIRAI[1]
Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Department of Electrical and Computer Engineering, Nagoya Institute of Technology[2]
Daiyu-kai Institute of Medical Science[3]
Ono Pharmaceutical Co., Ltd., Safety Research[4]

Genotoxic and Non-genotoxic Carcinogen 1:00pm-2:00pm

P-25 (Mon.)

Specific in vivo mutational spectra of genotoxic and non-genotoxic hepatocarcinogens in gpt delta transgenic rats

Keita KANKI[1], Akiyoshi NISHIKAWA[1], Ken-ichi MASUMURA[2], Takashi UMEMURA[1], Takayoshi IMAZAWA[1], Yasuki KITAMURA[1], Takehiko NOHMI[2], Masao HIROSE[1]
Division of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan[1]
Division of Genetics and Mutagenesis, National Institute of Health Sciences[2]

P-26 (Tue.)

Mechanistic study using cDNA microarray on enhanced hepatocarcinogenesis in ICR mice fed diet containing dicyclanil

Mitsuyoshi MOTO[1], Miwa OKAMURA[1], Tomoko MUTOH[1], Takao WATANABE[1], Yoko KASHIDA[1], Noboru MACHIDA[1], Kunitoshi MITSUMORI[1]
Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan[1]

P-27 (Wed.)

Six-month carcinogenicity study of N-bis(2-hydroxypropyl)nitrosamine (DHPN) in rasH2 mice

Miwa OKAMURA[1], Mitsuyoshi MOTO[1], Yoko KASHIDA[1], Noboru MACHIDA[1], Kunitoshi MITSUMORI[1]
Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan[1]

P-28 (Mon.)

Genomic analysis of the difference between non-genotoxic carcinogen and genotocic carcinogen in the urinary bladder

Junko HATA[1], Azusa TAMURA[1], Natsuki KITAJIMA[1], Yoshinori KASAHARA[1], Hiroshi UNO[1], Keiichirou MORIMURA[2], Shoji FUKUSHIMA[2]
Pharmaceuticals Development Research Laboratories, Teijin Pharma Ltd., Tokyo 191-8512, Japan[1]
Department of Pathology, Osaka City University Medical School[2]

Infectious Agents 1:00pm-2:00pm

P-29(Tue.)

Suppurative gastritis in BALB/c mice infected with Listeria monocytogenes via the intragastric route

Jong-Hwan PARK[1], Yong-Ho PARK[2], Seung-Hyeok SEOK[1], Sun-A CHO[1], Hui-Young LEE[1], Dong-Jae KIM[1], So-Hyun KIM[2], Jae-Hak PARK[1], Young-Soon LEE[3]
Department of Laboratory Animal Medicine, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea (South)[1]
Department of Veterinary Microbiology, College of Veterinary Medicine, Seoul National University[2]
Deparment of Veterinary Public Health, College of Veterinary Medicine, Seoul National University[3]

P-30 (Wed.)

Analyses of immune response to candidate live oral Salmonella vaccine strain in mice

Sun A CHO[1], In Soo LEE[2], Jong Hwan Park PARK[1], Seung Hyeok SEOK[1], Hui Young LEE[1], Dong Jae KIM[1], Jae Hak PARK[1]
Department of Laboratory Science, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea (South)[1]
Department of Microbiology, Hannam University[2]

P-31 (Mon.)

Earlier Helicobacter pylori infection cause severer inflammation and more risk for stomach carcinogenesis in N-methyl-N-nitrosourea treated Mongolian gerbils

Tetsuya TSUKAMOTO[1], Xueyuan CAO[1], Masami YAMAMOTO[1], Harunari TANAKA[1], Masae TATEMATSU[1]
Division of Oncological Pathology, Aichi Cancer Center Research Institute, Aichi 464-8681, Japan[1]

P-32 (Tue.)

Increased oxidative stress associated with alterations in DNA damage and repair in urinary bladder carcinomas associated and non-associated with Schistosomiasis

Elsayed I. SALIM[1], Hideki WANIBUCHI[1], Amani ABDUL-HAMID[2], Keiichirou MORIMURA[1], Shoji FUKUSHIMA[1]
Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]
Department of Pathology, Tanta Cancer Institute[2]

P-33 (Wed.)

Roles of the Thai liver fluke, Opisthorchis viverrini, in the development of cholangiocellular carcinoma (CCC).

Witaya THAMAVIT[1], Tomoyuki SHIRAI[2], Malcolm A. MOORE[2], Nobuyuki ITO[2]
Department of Pathobiology, Faculty of Science, Mahidol University[1]
Department of Pathology, Nagoya City University, Medical School[2]

Heavy Metals and Natural Toxins 1:00pm-2:00pm

P-34 (Mon.)

In vitro and in vivo sensitivity of various cells to arsenic trioxide(As2O3)

Chang Suk KANG[1], Yeon Sook MOON[1], Yong Gu KIM[1], Young Jin CHOI[1], Kyung Ja HAN[1], Sang In SHIM[1]
Department of Clinical Pathology, Catholic University of Korea, Seoul 150-713, Korea (South)[1]

P-35 (Tue.)

Induction of glutathione S-transferase placental form positive foci in liver and epithelial hyperplasia in urinary bladder, but no tumor development in male Fischer 344 rats treated with monomethylarsonic acid for 104 weeks

Jun SHEN[1], Hideki WANIBUCHI[1], Elsayed SALIM[1], Kenichiro DOI[1], Min WEI[1], Shoji FUKUSHIMA[1]
Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

P-36 (Wed.)

Effects of sodium cacodylate and sodium nitrate on kidney tumor induced by dimethylnitrosamine in SD male rats.

Tsutomu HIBINO[1], Takamasa YANAGIDA[2], Junya MATUYAMA[1], Ayumi KATI[1], Naoki YAMAMOTO[2]
Department of Pathology, Fujita Health University College, Aichi 470-1192, Japan[1]
Joint Research Laboratory, Fujita Health University[2]

Food Safety 1:00pm-2:00pm

P-37 (Mon.)

Involvement of choline and amino acid metabolism in different hepatocarcinogenicity of L-amino acid-defined semisynthetic and semipurified choline-deficient diets in rats

Maki IGARASHI[1], Masakazu TAKAHASHI[2], Hitoshi ASHIDA[4], Mei-Heng MAR[5], Yutaka HATANAKA[4], Midori YOSHIDA[2], Fumiyuki UEMATSU[2], Naoto WATANABE[1], Akihiko MAEKAWA[3], Steven H ZEISEL[5], Dai NAKAE[2]
Laboratory of Protection of Body Function, Department of Food and Nutritional Science, Graduate school of Agriculture, Tokyo University of Agriculture, Tokyo 101-0062, Japan[1]
Department of Pathology, Sasaki Institute, Sasaki Foundation[2]
Director, Sasaki Institute, Sasaki Foundation[3]
Division of Life Science, Graduate School of Science and Technology, Kobe University[4]
Department of Nutrition, School of Public Health and School of Medicine, University of North Carolina at Chapel Hill[5]

P-38 (Tue.)

Induction of fatty liver by mebalonic acid in Fischer 344 rats

Naoto WATANABE[1], Midori YOSHIDA[2], Maki IGARASHI[1], Fumiyuki UEMATSU[2], Masakazu TAKAHASHI[2], Akihiko MAEKAWA[3], Dai NAKAE[2]
Department of Food and Nutritional Science, Laboratory of Protection of body function, Graduate School of Tokyo University of Agriculture, Tokyo 101-0062, Japan[1]
Department of Pathology, Sasaki Institute, Sasaki Foundation[2]
Department Director, Sasaki Institute, Sasaki Foundation[3]

P-39 (Wed.)

Induction of multiple granulation in the liver with severe hepatocyte damage by montan wax, a food additive, in a 90-day toxicity study in F344 rats

Mico IKEDA[1], Kousuke SAOO[1], Keiko YAMAKAWA[1], Hijiri TAKEUCHI[1], Masanao YOKOHIRA[1], Yoko HOSOTANI[1], Yu ZENG[1], Kyoko HOSOKAWA[1], Shigemi KINOUCHI[2], Katsumi IMAIDA[1]
Department of Onco-Pathology, Faculty of Medicine, Kagawa University, Kagawa 761-0793, Japan[1]
Diagnostic Pathology and Cytology Institute, Shikoku Cytopathologic Institutes[2]

P-40 (Mon.)

Dietary intake of various lactic acid bacteria suppresses Type 2 helper T cell production in antigen-primed mice splenocyte

Hui Young LEE[1], Jong Hwan PARK[1], Seung Hyeok SEOK[1], Sun A CHO[1], Min Won BAEK[1], Dong Jae KIM[1], Yong Soon LEE[2], Jae Hak PARK[1]
Department of Laboratory Animal Medicine, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea (South)[1]
Department of Public Health, College of Veterinary Medicine, Seoul National University, Seoul, Korea[2]

Biomakers 1:00pm-2:00pm

P-41 (Tue.)

Examination of heat shock protein 70 as an early-stage injury-biomarker in rat testes after stress-induced cell damage

Akiko IKEDA[1], Yoshihumi KANEKO[1], Makiko TAKAHASHI[1], Sumihisa SUEYOSHI[1], Yoshihiro MASUMOTO[1], Kiyoyuki TSURU[1]
Kyorin Phamaceutical Co., Ltd, Research Center, Tochigi 329-0114, Japan[1]

P-42 (Wed.)

Histopathological and endocrinological changes of cardiac natriuretic peptide in rats with drug- induced cardiotoxicity

Hiroto MIYATA[1], Rie OHNO[1], Rika SHIRANE[1], Yutaka NAKANISHI[1], Atsushi NAKAMURA[1], Masaaki KIMURA[1]
Toxicology Laboratory, Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd., Saitama 331-9530, Japan[1]

P-43 (Mon.)

Histomorphometrical parameters for evaluation of osteocompatibility of metal implants with undecalcified bone section

Miki NISHIMORI[1], Shin-ichi KATSUDA[1], Yoshimitsu OKAZAKI[2], Emiko GOTO[3], Akihito SHIMOI[1], Hidetaka SATO[1]
Japan Food Research Laboratories, Tokyo 206-0025, Japan[1]
National Institute of Advanced Industrial Science and Technology[2]
National Institute of Technology and Evaluation[3]

P-44 (Tue.)

Genetic polymorphisms of alcohol-related metabolizing enzymes and the risk of esophageal cancer in the Thai population

Pleumjit BOONYAPHIPHAT[1], Paramee THONGSUKSAI[1], Wanna SUDHIKARAN[1], Puttisak PUTTAWIBUL[2]
Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkla 90110, Thailand[1]
Department of Surgery, Faculty of Medicine, Prince of Songkla University[2]

SESSION2 : Molecular Toxicopathology or Mechanism

Room 301

Genomics 1:00pm-2:00pm

P-45 (Wed.)

Toxicogenomics using "percellome" and "mille-feuille" data system

Jun KANNO[1], Ken-ichi AISAKI[1], Atsushi ONO[1], Katsuhide IGARASHI[1]
Cellular & Molecular Toxicology Division, Biological Safety Research Center, National Institute of Health Sciences, Tokyo 158-8501, Japan[1]

P-46 (Mon.)

Microarray analysis of amplified RNA from laser- microdissected GST-P negative hepatocellular preneoplastic lesion induced by peroxisome proliferators.

Tokuo SUKATA[1], Satoshi UWAGAWA[1], Keisuke OZAKI[1], Kayo SUMIDA[1], Masahiko KUSHIDA[1], Kaoru KIKUCHI[3], Koichi SAITO[1], Kenji OEDA[1], Yasuyoshi OKUNO[1], Nobutoshi MIKAMI[1], Shoji FUKUSHIMA[2]
Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan[1]
Department of Pathology, Osaka City University Medical School[2]
Sumitomo Pharmaceuticals Co., Ltd.[3]

P-47 (Tue.)

Specific differences in gene expression profile revealed by cDNA microarray analysis of glutathione S-transferase placental form (GST-P) immunohistochemically positive rat liver foci and surrounding tissue

Shugo SUZUKI[1], Makoto ASAMOTO[1], Kazunari TSUJIMURA[1,2], Kumiko OGAWA[1], Mitsuru FUTAKUCHI[1], Tomoyuki SHIRAI[1]
Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Chemicals Evaluation and Research Institute, Chemicals Assessment Center[2]

P-48 (Wed.)

Potential for the prediction of carcinogenicity by gene expression profile in rat hepatoma cells and comparison of expression patterns between chemically treated rat and human hepatoma cells

Kazunari TSUJIMURA[1,2], Makoto ASAMOTO[1], Shugo SUZUKI[1], Shingo INAGUMA[1], Kumiko OGAWA[1], Tomoyuki SHIRAI[1]
Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Chemicals Evaluation and Research Institute, Chemicals Assessment Center[2]

P-49 (Mon.)

Gene expression profiles in F344 rat livers treated with acetaminophen or cycloheximide

Takashi YAMOTO[1], Naoki KIYOSAWA[1], Kazumi ITO[1], Takayuki SATO[1], Kyoko SAKUMA[1], Miyuki KANBORI[1], Noriyo NIINO[1], Sunao MANABE[2], Naochika MATSUNUMA[1]
Medicinal Safety Research Labs., Sankyo Co., LTD, Shizuoka 437-0065, Japan[1]
Sankyo Pharma Inc.[2]

P-50 (Tue.)

Methacarn, a versatile fixation tool for quantitative mRNA expression analysis in microdissected paraffin-embedded tissues using real-time RT-PCR and microarray systems

Makoto SHIBUTANI[1], Kyoung-Youl LEE[1], Hironori TAKAGI[1], Natsumi KATO[1], Shu TAKIGAMI[1], Masao HIROSE[1]
Division of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan[1]

P-51 (Wed.)

Transcription of rat amyloid precursor protein in rat livers is affected by the serum cholesterol concentration

Naoki KIYOSAWA[1], Kazumi ITO[1], Kayoko ISHIKAWA[1], Isao IGARASHI[1], Noriyo NIINO[1], Kyoko SAKUMA[1], Miyuki KANBORI[1], Takashi YAMOTO[1], Sunao MANABE[2], Naochika MATSUNUMA[1]
Medicinal Safety Research Labs., Sankyo Co., Ltd., Shizuoka 437-0065, Japan[1]
Sankyo Pharma Inc.[2]

P-52 (Mon.)

Gene expression profiling of terminal end buds during rat mammary carcinogenesis

Koichiro MIYAMOTO[1], Shuhei TAGUCHI[1], Hiroaki KAWAGUCHI[1], Yoshihisa UMEKITA[1], Hiroki YOSHIDA[1]
Department of Tumor Pathology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8520, Japan[1]

P-53 (Tue.)

Region-specific global gene expression analysis in the microdissected hypothalamic medial preoptic area of rat neonates exposed perinatally to di(2-ethylhexyl)phthalate

Kyoung-Youl LEE[1], Makoto SHIBUTANI[1], Hironori TAKAGI[1], Natsumi KATO[1], Shu TAKIGAMI[1], Masao HIROSE[1]
National Institute of Health Sciences, Tokyo 158-0098, Japan[1]

P-54 (Wed.)

Differential global gene expression in rat brown and white adipose tissues

Akira UNAMI[1], Yasuo SHINOHARA[2], Kazuaki KAJIMOTO[2], Kenjiro TSUBOTA[1], Yoshimasa OKAZAKI[1], Shiro FUJIHIRA[1], Masahiro MATSUMOTO[1], Yuji OISHI[1], Yoshinobu BABA[3]
Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka 532-8514, Japan[1]
Institute for Genome Research, The University of Tokushima[2]
Faculty of Pharmaceutical Sciences, The University of Tokushima[3]

P-55 (Mon.)

Microarray analysis of hepatocellular adenoma induced by di(2-ethylhexyl)phthalate in rasH 2 mice

Kaoru TOYOSAWA[1], Miwako HARADA[2], Katsuji NAKANO[2], Kohji TANAKA[1], Nobuo MATSUOKA[1]
Safety Research Laboratories, Drug Research Division, Dainippon Pharmaceutical Co., Ltd., Osaka 564-0053, Japan[1]
Pharmacology & Microbiology Research Laboratories, Drug Research Division, Dainippon Pharmaceutical Co., Ltd.[2]

P-56 (Tue.)

Mechanistic analyses using chip array analysis on inhibited uterine tumorigenesis in rasH2 mice initiated with N-ethyl-N-nitrosourea followed by dietary treatment of ethinylestradiol

Takao WATANABE[1], Kayo SUMIDA[2], Tomoko MUTOH[1], Miwa OKAMURA[1], Mitsuyoshi MOTO[1], Yoko KASHIDA[1], Kunitoshi MITSUMORI[1]
Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan[1]
Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.[2]

Carcinogenesis 1:00pm-2:00pm

P-57 (Wed.)

Tumor induction by colon carcinogen in rat gastric mucosa featuring intestinal metaplasia caused by X-irradiation

Shoji KASHIWABARA[1], Naoki KASHIMOTO[1], Toshihiro UESAKA[1], Osamu KATOH[1], Keiji WAKABAYASHI[2], Hiromitsu WATANABE[1]
Department of Cellular Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima 734-8553, Japan[1]
National Cancer Center Research Institute[2]

P-58 (Mon.)

Expression of peroxisome proliferator-activated receptor gamma in helicobacter pylori- associated mouse gastric cancer tissue and human gastric cancer cells.

Dae-Yong KIM[1], Sang-Yeon OH[1], Ki Taek NAM[2], Beom Seok HAM[2], Dong Deuk JANG[2], Ki-Hwa YANG[2], Ki-Baik HAHM[3]
Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University[1]
National Institute of Toxicological Research[2]
Genome Research Center for Gastroenterology, Ajou University School of Medicine[3]

P-59 (Tue.)

Time-course and quantitative study of the relationship between transforming growth factor-alpha and glutathione S-transferase placental form expression during rat chemical hepatocarcinogenesis

Mitsuaki KITANO[1], Jutaro WADA[1], Yutaka ARIKI[1], Masanori KATO[1], Hideki WANIBUCHI[2], Keiichirou MORIMURA[2], Kazunori HOSOE[1], Shoji FUKUSHIMA[2]
Life Science Research Laboratories, Life Science RD Center, Kaneka Corporation, Hyogo 676-8688, Japan[1]
Department of Pathology, Osaka City University Medical School[2]

P-60 (Wed.)

Enhancing effects of combined treatment with IQ and sodium nitrite on colon and liver carcinogenesis in rats

Yasuki KITAMURA[1], Kazushi OKAZAKI[1], Akiyoshi NISHIKAWA[1], Keita KANKI[1], Takashi UMEMURA[1], Takayoshi IMAZAWA[1], Masao HIROSE[1]
Division of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan[1]

P-61 (Mon.)

Involvement of the altered regulation of pre-mRNA splicing in the liver carcinogenic processes

Masakazu TAKAHASHI[1], Fumiyuki UEMATSU[1], Midori YOSHIDA[1], Maki IGARASHI[1], Naoto WATANABE[1], Akihiko MAEKAWA[2], Dai NAKAE[1]
Department of Pathology, Sasaki Institute, Sasaki Foundation, Tokyo 101-0062, Japan[1]
Director, Sasaki Institute, Sasaki Foundation, Japan[2]

P-62 (Tue.)

Genetic alterations in the Catnb gene but not the H-ras gene in hepatocellular neoplasms and hepatoblastomas of B6C3F1 mice following exposure to diethanolamine for 2 years

Shim-mo HAYASHI[1], Thai Vu TON[2], Hue-Hua L. HONG[2], Richard D. IRWIN[2], Joseph K. HASEMAN[2], Theodora R. DEVEREUX[2], Robert C. SILLS[2]
Pfizer Inc., Worldwide Safety Sciences, Taketoyo, Aichi 470-2393, Japan[1]
National Institute of Environmental Health Sciences[2]

P-63 (Wed.)

Carcinogenicity of phenobarbital in Mmh/OGG1 knockout mice

Anna KINOSHITA[1], Hideki WANIBUCHI[1], Keiichirou MORIMURA[1], Takayuki YUNOKI[1], Shoji FUKUSHIMA[1]
Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

P-64 (Mon.)

Oxidative stress and aberrant DNA methylation in cancer

Yashige KOTAKE[1], Kiyoshi ASADA[1], Hong SANG[1], Robert H. BROYLES[1], Robert A. FLOYD[1]
Oklahoma Medical Research Foundation, Oklahoma 73104, USA[1]

P-65 (Tue.)

Methylation-associated silencing of p53 responsive gene 2 and Fibrillin 2 in human pancreatic cancers

Atsushi HAGIHARA[1], Kazuaki MIYAMOTO[2], Junichi FURUTA[2], Shuichi SEKI[3], Shoji FUKUSHIMA[1], Toshikazu USHIJIMA[2]
Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]
Carcinogenesis Division, National Cancer Center Research Institute[2]
Internal Medicine, Osaka City University Medical School[3]

P-66 (Wed.)

Lack of influence of testicular castration or sialoadenectomy on sodium L-ascorbate promotion of urinary bladder carcinogenesis in male F344 rats

Satoru MORI[1], Takashi MURAI[1], Keiichirou MORIMURA[1], Hideki WANIBUCHI[1], Shoji FUKUSHIMA[1]
Deparment of Pathology, Osaka City Univercity Medical School, Osaka 545-8585, Japan[1]

P-67 (Mon.)

Effect of amiloride and ouabain on promotion by sodiium L-ascorbate in two-stage rat urinary bladder carcinogenesis

Takashi MURAI[1], Hideyuki MIYAUCHI[1], Satoshi INOUE[1], Takeki UEHARA[1], Toshiyuki MARUYAMA[1], Satoru MORI[2], Hideki WANIBUCHI[2], Shoji FUKUSHIMA[2]
Developmental Research Laboratories, Shionogi & Co., Ltd., Osaka 561-0825, Japan[1]
First Department of Pathology Osaka City University Medical School[2]

P-68 (Tue.)

Susceptibility of $p27^{kip1}$ knockout mice on N-butyl-N-(4-hydroxybutyl) nitrosamine-induced urinary bladder carcinogenesis

Toshiya MURASAKI[1], Kumiko OGAWA[1], Atsuya HIKOSAKA[1], Satoshi SUGIURA[1], Seishiro TAKAHASHI[1], Tomoyuki SHIRAI[1]
Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]

P-69 (Wed.)

Promoting effect of sodium L-ascorbate on N-butyl-N-(4-hydroxybutyl)nitrosamine-induced renal pelvic carcinogenesis in SD/cShi rats of both sexes

Takashi MURAI[1], Hideyuki MIYAUCHI[1], Satoshi INOUE[1], Takeki UEHARA[1], Toshiyuki MARUYAMA[1], Akihiro KOIDE[2], Yukio MORI[2], Satoru MORI[3], Hideki WANIBUCHI[3], Shoji FUKUSHIMA[3]
Developmental Research Laboratories, Shionogi & Co., Ltd., Osaka 561-0825, Japan[1]
Laboratory of Radiochemistry, Gifu Pharmaceutical University[2]
Department of Pathology, Osaka City University Medical School[3]

P-70 (Mon.)

Renal cell carcinoma cell lines established from Nihon rat

Izumi MATSUMOTO[1], Tadayoshi UEDA[1], Kazuyasu KIJIMA[1], Youko HIRAYAMA[2], Hiroaki MITANI[2], Kazuo OKIMOTO[1], Kohji TANAKA[1], Okio HINO[2], Nobuo MATSUOKA[1]
Drug Research Division, Dainippon Pharmaceutical Co., Ltd., Osaka 564-0053, Japan[1]
Department of Experimental Pathology, Cancer Institute[2]

P-71 (Tue.)

Strain-specific mammary proliferative lesion development following lifetime oral administration of ochratoxin A in DA and Lewis rats

Yong-Soon LEE[1], Woo-Chan SON[2], Kyung-Sun KANG[3]
Department of Public Health, Seoul National University, Seoul 151-742, Korea (South)[1]
Huntingdon Life Sciences Pathology[2]
Department of Public Health, Seoul National University[3]

P-72 (Wed.)

Strain difference in spontaneous development of uterine adenocarcinoma between donryu and Fischer344(F344)rats

Takaharu NAGAOKA[1], Hiroaki MIYAJIMA[1], Akihiko MAEKAWA[2]
Shin Nippon Biomedical Laboratories, LTD. Kagoshima 891-1394, Japan[1]
Sasaki Institute[2]

P-73 (Mon.)

Expression profile of estrogen receptor-α-related signaling pathways in the development of endometrial adenocarcinomas in Donryu rats

Midori YOSHIDA[1], Takasumi SHIMOMOTO[1], Yutaka HATANAKA[3], Takuji MIHARA[3], Akihiko MAEKAWA[2], Dai NAKAE[1]
Department of Pathology, Sasaki Institute, Sasaki Foundation, Tokyo 101-0062, Japan[1]
Director, Sasaki Institute, Sasaki Foundation[2]
BioMedical Science Department, Dako Cytomation Co., Ltd.[3]

P-74 (Tue.)

Constitutive expression of cox-2 in thyroid follicular epithelial cells and its significant reduction during DHPN-induced carcinogenesis in rats

Toshio IMAI[1], Mai HASUMURA[1], Jun-ichi ONOSE[1], Makoto UEDA[1], Tamotsu TAKIZAWA[1], Young-Man CHO[1], Masao HIROSE[1]
National Institute of Health Sciences, Tokyo 158-8501, Japan[1]

P-75 (Wed.)

PCNA labeled index of spontaneous lung proliferating lesion in rats

Masayuki MITSUI[1], Midori YOSHIDA[2], Kasuke NAGANO[3], Toshifumi TSUJIUCHI[1], Hiroshi MARUYAMA[4], Masahiro TSUTSUMI[1]
Department of Oncological Pathology, Cancer Center, Nara Medical University, Osaka 556-0005, Japan[1]
Department of Pathology, Sasaki Laboratory[2]
Division of Pathology, Japan Bioassay Research Center[3]
Department of Pathology, Hoshigaoka Koseinenkin Hospital[4]

P-76 (Mon.)

The role of polymerase beta mediated DNA repair in skin tumorigenesis: using DNA polymerase beta over-expressed transgenic mouse

Kazuo HAKOI[1], Raymond W TENNANT[2], Ronald E CANNON[2]
Drug Safety Research Lab., Taiho Pharmaceutical Co., Ltd., Tokushima 771-0194, Japan[1]
Cancer Biology Group, National Center for Toxicogenomics, National Institute of Environmental Health Sciences[2]

P-77 (Tue.)

Possible relationships between immunosuppressive effects and carcinogenicity of the synthetic gestagens with glucocorticoid-like effects in rodents

Shigeru HISADA[1], Toshi HORIUCHI[1]
Toxicology & Pharmacokinetics Research Department, Kawasaki 213-8522, Japan[1]

Cancer Prevention 1:00pm-2:00pm

P-78 (Wed.)

Chemopreventive effects of plant derived compounds in a rat medium term liver bioassay and on hepatocellular carcinoma cell lines and gene expression analysis by a microarray

Hiroyuki OHNISHI[1], Kazunari TSUJIMURA[1,2], Makoto ASAMOTO[1], Masanori KURIBAYASHI[1,3], Shugo SUZUKI[1], Tadashi OGISO[1], Tomoyuki SHIRAI[1]
Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Chemicals Evaluation and Research Institute, Chemicals Assessment Center[2]
Ono Pharmaceutical Co. Ltd., Safety Research[3]

P-79 (Mon.)

The inhibitory effects of arctiin on F344 rat multiorgan carcinogenesis

Masaharu MOKU[1], Hideki WANIBUCHI[1], Jin Seok KANG[1], Saki NAITO[1], Anna KINOSHITA[1], Shoji FUKUSHIMA[1]
Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

P-80 (Tue.)

Chemopreventive effect of PJJ-34 in rat multi-organ carcinogenesis model

Chiharu YAMAGUCHI[1], Sakai KUNIYOSI[1], Takasi YAMAGUCHI[1], Tomoko NAKATA[1], Reiko TANAKA[1], Hideki WANIBUCHI[2], Anna KINOSITA[2], Keiichirou MORIMURA[2], Shoji FUKUSIMA[2]
Department of Medicinal Chemistry, Osaka University of Parmaceutical Science, Osaka 569-1094, Japan[1]
Department of Pathology, Osaka City University Medical School[2]

P-81 (Wed.)

Dietary protocatechuic acid inhibits progression of chemically induced rat tongue carcinogenesis

Rikako SUZUKI[1,2], Hiroyuki KOHNO[1], Shigeyuki SUGIE[1], Takuji TANAKA[1]
Department of Pathology, Kanazawa Medical University, Ishikawa 920-0293, Japan[1]
Research Fellow of the Japan Society for the Promotion of Science[2]

P-82 (Mon.)

Chemopreventive effect of FBRA on N-nitrosomethylbenzylamine-induced esophageal tumorigenesis in rats

Toshiya KUNO[1], Yoshinobu HIROSE[1], Yasuhiro YAMADA[1], Keiko SAKATA[1], Nami KITAORI[1], Akira HARA[1], Hideki MORI[1]
Department of Tumor Pathology, Gifu 500-8705, Japan[1]

P-83 (Tue.)

Acyclic retinoid inhibits the aberrant crypt foci formation induced by dimethylhydrazine in the rat colon

Masumi SUZUI[1], Takahiro SHIMIZU[1], Takamitsu MORIOKA[1], Viengvansay NABANDITH[1], Morihiko INAMINE[1], Tatsuya KANESHIRO[1], Tatsuya KINJO[1], Naoki YOSHIMI[1]
Department of Pathology, University of the Ryukyus Faculty of Medicine, Okinawa 903-0215, Japan[1]

P-84 (Wed.)

Time course observation on inhibitory effects of hepato-proliferative lesions in rats induced by a liver-selective thyromimetic, KAT-681

Morimichi HAYASHI[1], Toru TAMURA[1], Yuji OKUHARA[1], Tatsuya NAGASAWA[1], Tsuyoshi KITAMURA[1], Junji KURODA[1], Nobuo SHIBATA[1], Kunitoshi MITSUMORI[2]
Kissei Pharmaceutical Co., Ltd., Nagano 399-8305, Japan[1]
Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology[2]

P-85 (Mon.)

Knockout of iNOS gene does not prevent hepatocarcinogenesis caused by a choline-deficient, l-amino acid-defined (CDAA) diet in mice

Ayumi DENDA[1], Nao MURATA[1], Toshifumi TSUJIUCHI[1], Masahiro TSUTSUMI[1], Dai NAKAE[2], Yoichi KONISHI[1], Hiroki KUNIYASU[1]
Department of Oncological Pathology, Nara Medical University, Nara 634-8521, Japan[1]
Department of Pathology, Sasaki Institute[2]

P-86 (Tue.)

Modifying effects of Terminalia catappa on azoxymethane-induced colon carcinogenesis in male F344 rats

Takamitsu MORIOKA[1], Masumi SUZUI[1], Viengvansay NABANDITH[1], Morihiko INAMINE[1], Tatsuya KANESHIRO[1], Tatsuya KINJO[1], Takahiro SHIMIZU[1], Miyuki AONAHATA[1], Naoki YOSHIMI[1]
Tumor Pathology, University of the Ryukyus Faculty of Medicine, Okinawa 903-0215, Japan[1]

P-87 (Wed.)

Lovastatin inhibits tumor growth and metastasis in a mouse mammary carcinoma model by p53-independent mitochondrial-mediated induction of apoptosis

Masa-aki SHIBATA[1], Yuko ITO[1], Junji MORIMOTO[2], Yoshinori OTSUKI[1]
Department of Anatomy & Biology, Osaka Medical College, Osaka 569-8686, Japan[1]
Laboratory Animal Center, Osaka Medical College[2]

P-88 (Mon.)

Prevention of H. pylori associated gastric carcinogenesis in N-methyl-N-nitrosourea-treated Mongolian gerbils using concentrate of Japanese apricot (Ume)

Harunari TANAKA[1], Ken-ichi INADA[2], Toru NIWA[1], Takafumi OTSUKA[1], Mitsuo GOTO[1], Hirotoshi UTSUNOMIYA[3], Tetsuya TSUKAMOTO[1], Masae TATEMATSU[1]
Division of Oncological Pathology, Aichi Cancer Center Research Institute, Aichi 464-8681, Japan[1]
1st Department of Pathology, Fujita Health University[2]
Department of Pathology, Wakayama Medical University School of Medicine[3]

P-89 (Tue.)

The effect of N-acetylecysteine on low dose 2-amino-3,8dimethylimidazo[4,5-f]quinoxaline (MeIQx)-induced rat hepatocarcinogenesis

Natsuko MIYAZI[1], Hideki WANIBUCHI[1], Masaharu MOKU[1], Keiichirou MORIMURA[1], Yoshiaki TAGAWA[1], Shoji FUKUSHIMA[1]
Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

Animal Models 1:00pm-2:00pm

P-90 (Wed.)

Glycogen granule, glycogen phosphorylase, and CYP1 after exposure to DMBA in rat liver

Shin WAKUI[1], Masakuni FURUSATO[2]
Department of Toxicologic Pathology, Azabu University School of Veterinary Medicine, Kanagawa 229-8501, Japan[1]
Department of Pathology, Kyorin University School of Medicine[2]

P-91 (Mon.)

Comprehensive gene expression profiles of hepatocellular carcinomas induced in rats fed a choline-deficient, L-amino acid-defined diet

Fumiyuki UEMATSU[1], Naoto WATANABE[2], Maki IGARASHI[2], Masakazu TAKAHASHI[1], Midori YOSHIDA[1], Akihiko MAEKAWA[3], Dai NAKAE[1]
Department of Pathology, Sasaki Institute, Sasaki Foundation, Tokyo 101-0062, Japan[1]
Department of Food and Nutritional Sciences, Graduate School of Agriculture, Tokyo University of Agriculture[2]
Director, Sasaki Institute, Sasaki Foundation[3]

P-92 (Tue.)

Enhanced preneoplastic lesion induction in the livers of gap junction disrupted transgenic rats

Naomi HOKAIWADO[1], Makoto ASAMOTO[1], Kazunari TSUJIMURA[1,2], Kumiko OGAWA[1], Tadashi OGISO[1], Tomoyuki SHIRAI[1]
Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Chemicals Evaluation and Research Institute, Chemicals Assessment center[2]

P-93 (Wed.)

In situ hybridization of interferon-gamma mRNA in concanavalin A-induced hepatitis in mice using the polymerase chain reaction-derived cRNA probes

Hideki TANAKA[1], Atsushi FUKUNARI[1], Tomomichi IWAKI[1], Shiro TAKAGI[1]
Mitsubishi Pharma Corporation, Kanagawa 227-0033, Japan[1]

P-94 (Mon.)

Rapid induction of type-1 diabetes mellitus by X-irradiation in Long-Evans Agouti (LEA) rats

Tokiko NAKAI[1], Akiko KUBO[1], Eiko TAKISHITA[1], Tamotsu TAKIZAWA[1], Takeshi TSUCHIGAUCHI[1], Keiji KODAMA[1], Keisuke IZUMI[1]
Department of Molecular and Environmental Pathology, The University of Tokushima School of Medicine, Tokushima 770-8503, Japan[1]

P-95 (Tue.)

Porcine-serum-induced hepatic fibrosis in Brown Norway and Wistar rats

Yasuko BABA[1], Koji UETSUKA[1], Hiroyuki NAKAYAMA[1], Kunio DOI[1]
Department of Veterinary Pathology, Graduate School of Agricultural and Life Science, The University of Tokyo, Tokyo 113-8657, Japan[1]

P-96 (Wed.)

Induction of mRNA related with hepatic injury response to oxidative stimuli in hepatocytes and non-parenchymal cells

Toshinobu YAMAMOTO[1], Hiroyuki UTSUMI[1], Naoko SHIMADA[1], Tetsu OGATA[1], Naohisa TSUTSUI[1]
Toxicology Laboratory, Mitsubishi Pharma Corporation, Chiba 292-0818, Japan[1]

P-97 (Mon.)

Persistence of liver cirrhosis is associated with altered location of α-smooth muscle actin (α-SMA) positive cells

Jin Seok KANG[1], Hideki WANIBUCHI[1], Keiichirou MORIMURA[1], Elsayed I. SALIM[1], Shoji FUKUSHIMA[1]
Department of Pathology, Osaka City University, Osaka 545-8585, Japan[1]

P-98 (Tue.)

Spontaneous development of maxillary incisor lesions in aged poly(ADP-ribose) polymerase-1 (Parp-1) knockout mice

Osamu KUSUOKA[1], Masahiro TSUTSUMI[2], Toshifumi TSUJIUCHI[2], Kazutoshi TAMURA[1], Kohsuke HORIGUCHI[1], Kazumi SHIRAIWA[3], Nobuo KAMADA[4], Hitoshi NAKAGAMA[5], Takashi SUGIMURA[5], Mitsuko MASUTANI[5]
Gotemba Laboratories, Bozo Research Center Inc., Shizuoka 412-0039, Japan[1]
Department of Oncological Pathology, Cancer Center, Nara Medical University[2]
Institute for Life Science Research, Asahi Kasei Corporation[3]
Chugai Research Institute for Medical Sciences, Inc.[4]
Biochemistry Division, National Cancer Center Research Institute[5]

P-99 (Wed.)

Pancreas anomaly and intestinal tumorsin the mouse small eye mutants, Pax6Sey3H and Pax6Sey4H

Yumiko NITTA[1]
Res. Inst. Radiat. Biol. Med., Hiroshima University, Hiroshima 734-8553, Japan[1]

P-100 (Mon.)

Establishment and characterization of cancer cell lines from glandular stomach carcinoma induced by MNU in p53-deficient mice

Masami YAMAMOTO[1], Hayao NAKANISHI[1], Tetsuya TSUKAMOTO[1], Akihiro HIRATA[1], Hiroki SAKAI[2], Norimitsu SHIRAI[1], Takeshi IIDAKA[1], Tokuma YANAI[2], Toshiaki MASEGI[2], Masae TATEMATSU[1]
Laboratory of Pathology, Aichi Cancer Center Research Institute, Aichi 464-8681, Japan[1]
Department of Veterinary Pathology, Gifu University[2]

P-101 (Tue.)

Influence of p53 gene deficiency on spontaneous tumor development in TCRs$^{-/-}$/p53$^{-/-}$ mice

Kana HASHIMOTO[1], Shoichi KADO[1], Kazumi UCHIDA[1], Shin IWATA[1], Yuriko NAGATA[1], Minoru ANDO[1], Hideyuki FUNABASHI[1], Masaharu ONOUE[1]
Yakult Central Institute for Microbiological Research, Tokyo 186-8650, Japan[1]

P-102 (Wed.)

Progression of glomerulonephritis in cynomolgus monkeys caused by autoimmunity to NC1 domain of type Ⅳ collagen [K35]

Masatoshi YAMAMOTO[1], Iori ITAGAKI[1], Masami HIRUMA[1], Yoshihiro TAKEI[1], Norio MUTO[1], Flordeliza P. DE VILLA[2], Tsukao YOKOYAMA[3], Syunji HATTORI[4], Hidekazu SHIGEMATSU[5]
Ina Research Inc., Ina-shi, Nagano, 399-4501 Japan, Nagano 399-4501, Japan[1]
INA RESEARCH PHILIPPINES, INC.[2]
Collagen Research Center[3]
Nippi Reseach Institute of Biomatrix[4]
Department of Pathology, Shinsyu University School of Medicine[5]

P-103 (Mon.)

Histological changes of pancreatic islets in Zucker Diabetic Fatty rats, with progress in the type 2 diabetes-like disease

Naoshi SHIMOJI[1], Wakako TOGA[1], Satoko TOMIOKA[1], Haruyo SUGIYAMA[1], Kaori SASA[1], Nobutaka DEMURA[1], Haruko KAMEDA[1], Yusuke NAGAE[1]
Preclinical Development Department, Novartis Pharma K. K., Ibaraki 300-2611, Japan[1]

P-104 (Tue.)

The mouse rasH2/BHT model as an in vivo rapid assay for lung carcinogens

Takashi UMEMURA[1], Yukio KODAMA[2], Keita KANKI[1], Yasuki KITAMURA[1], Takayoshi IMAZAWA[1], Akiyoshi NISHIKAWA[1], Masao HIROSE[1]
Division of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan[1]
Division of Toxicology, National Institute of Health Sciences[2]

P-105 (Wed.)

The utility of EHBR for toxicity study of organic anion drugs

Chitose KUWAYAMA[1], Hiroyasu NABA[1], Chihaya KAKINUMA[1], Takuo OGIHARA[1], Shuhei OHNISHI[1], Akihito SHIMOI[1]
Phamaceutical Research Center, Mochida Phamacetical Co., Ltd., Tokyo 160-8515, Japan[1]

P-106 (Mon.)

Morphological evaluation of bone dynamics using in vivo micro-CT analysis and histomorphometry in mice osteoporosis model

Anbo XIANG[1], Masahiro KANEMATSU[1], Mana MITAMURA[1], Hideo KIKKAWA[1], Kiyoshi KOBAYASHI[1], Hiroyuki HIGASHIYAMA[1], Mine KINOSHITA[1], Satoshi ASANO[1]
Pharmacology Department, GlaxoSmithKline KK., Ibaraki 300-4247, Japan[1]

P-107 (Tue.)

Expression of a system L amino acid transporter at testis of rats after prenatal exposure to di(n-butyl)phtalate

Tomoko MUTO[1], Yoshikatsu KANAI[1], Hitoshi ENDOU[1]
Department of Pharmacology and Toxicology, Kyorin University, Tokyo 181-8611, Japan[1]

P-108 (Wed.)

Infiltrating CD8+ T and NK cells, and IL-10 and TGF-β 1 cytokines expression in chemically induced neoplasias in a medium-term alternative bioassay using male Wistar rats

Ana Lucia Tozzi SPINARDI-BARBISAN[1], Luis Fernando BARBISAN[1], Joao Lauro Viana de CAMARGO[1], Maria Aparecida Marchesan RODRIGUES[1]
Dept. Pathology, UNESP Medical School, SP 18618-000, Brazil[1]

P-109 (Mon.)

Green fluorescent protein (GFP) as a marker of aryl hydrocarbon receptor (AHR) function in transgenic zebrafish (Danio rerio)

Seung H. SEOK[1], Jong-H. PARK[1], Sun A. CHO[1], Min W. BAEK[1], Hui Y. LEE[1], Dong J. KIM[1], Yong S. LEE[2], Jae H. PARK[1]
Department of Laboratory Animal Medicine, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea (South)[1]
Department of Public Health, College of Veterinary Medicine, Seoul National University[2]

SESSION3 : Organ Toxicopathology

Room 401+402

Nervous System 1:00pm-2:00pm

P-110 (Tue.)

Pathology of Minamata disease (methylmercury poisoning) - special reference to the peripheral nerves

Komyo ETO[1], Akira YASUTAKE[1], Motohiro TAKEYA[2], Michio AKIMA[3]
National Institute for Minamata Disease, Kumamoto 867-0008, Japan[1]
Graduate School for Medicial and Pharmaceutical Science, Kumamoto University[2]
Toho University, School of Medicine[3]

P-111 (Wed.)

Mechanisms and disease control of N-methyl-N-nitrosourea-induced retinal degeneration in animals

Katsuhiko YOSHIZAWA[1], Katsuji KIUCHI[3], Kaei MORIGUCHI[3], Yuji OISHI[1], Nobuaki SHIKATA[2], Airo TSUBURA[2]
Department of Toxicologic Pathology, Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka 532-8514, Japan[1]
Department of Pathology II, Kansai Medical University[2]
Department of Ophthalmology, Kansai Medical University[3]

P-112 (Mon.)

Neuronal cell death in the fetal rat spinal cord following NMDA-treatment to dams

Hirotake TAKAI[1], Tsuneo ITO[1], Kiyoka KATSUYAMA[1], Masami SUZUKI[1], Kei-ich KATAYAMA[2], Kunio DOI[2]
Safety Assessment Department, Chugai Pharmaceutical Co., Ltd., Shizuoka 412-8513, Japan[1]
Department of Veterynary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo[2]

P-113 (Tue.)

Analysis of cell cycle, migration and apoptosis of neural stem cells in the 5-azacytidine (5AzC)-treated rat fetal brain

Masaki UENO[1], Kei-ichi KATAYAMA[1], Hiroyuki NAKAYAMA[1], Kunio DOI[1]
Department of Veterinary Pathology, The University of Tokyo, Tokyo 113-8657, Japan[1]

P-114 (Wed.)

T-2 toxin-induced changes in the rat fetal brain

Shinya SEHATA[1], Naoki KIYOSAWA[2], Toshihiko MAKINO[2], Fusako ATSUMI[2], Kazumi ITO[2], Takashi YAMOTO[2], Munihiro TERANISHI[2], Sunao MANABE[2], Koji UETSUKA[1], Hiroyuki NAKAYAMA[1], Kunio DOI[1]
Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan[1]
Medicinal Safety Research Laboratories, Sankyo Co., Ltd.[2]

P-115 (Mon.)

Examination of discriminative diagnosis of brain tumors difficult to be stained with hematoxylin-eosin

Yoshihumi KANEKO[1], Akiko IKEDA[1], Makiko TAKAHASHI[1], Sumihisa SUEYOSHI[1], Yasukazu SATO[1], Yoshihiro MASUMOTO[1], Kiyoyuki TSURU[1]
Kyorin Pharmaceutical Co., Ltd. Research Center, Tochigi 329-0114, Japan[1]

P-116 (Tue.)

A spontaneous cholesterol granuloma of choroid plexus in a beagle dog

Shuji HAYASHI[1], Satoshi SUZUKI[1], Fumiko NINOMIYA[1], Kazuo HAKOI[1], Shuji YAMAGUCHI[1], Kenji IRIMURA[1], Shoji FUKUSHIMA[2]
Drug Safety Research Laboratory, Taiho Pharmaceutical Co., LTD., Tokushima 771-0194, Japan[1]
Department of Pathology, Osaka City University Medical School[2]

P-117 (Wed.)

A study on the developmental process of distal axonopathy caused by acrylamide

Naofumi TAKAHASHI[1], Maki KUWAHARA[1], Yukiko TAKEUCHI[1], Toshinori YOSHIDA[1], Akiko ENOMOTO[1], Nobuaki NAKASHIMA[1], Yasufumi SHUTOH[1], Sayaka ISHIMINE[1], Toshiaki KITAZAWA[3], Keizo MAITA[2], Takanori HARADA[1]
Toxicology Division II, The Institute of Environmental Toxicology, Ibaraki 303-0043, Japan[1]
Division of Study Planning and Consultation, The Institute of Environmental Toxicology[2]
Contract and Research Management Division, The Institute of Environmental Toxicology[3]

P-118 (Mon.)

Screening test for central neurotoxicity by a newly developed human hybrid neuron

Min-Cheol LEE[1], JK RYU[2], SU KIM[2], KH YANG[3]
Department of Pathology, Chonnam National University Medical School, Chonnam 501-190, Korea (South)[1]
Brain Disease Research Center, Ajou University[2]
Department of General Toxicology National Toxicology Institute[3]

P-119 (Tue.)

Developmental neurotoxicity of domoic acid in rat

Min-Cheol LEE[1], YS KIM[1], JY WOO[1], J KIM[1], KH YANG[2]
Department of Pathology, Chonnam National University Medical School, Chonnam 501-190, Korea (South)[1]
Department of General Toxicology National Toxicology Institute[2]

P-120 (Wed.)

Newly-established tumor lines from a spontaneous malignant schwannoma in F344 rats

Jyoji YAMATE[1], Hisae YASUI[1], Mitsuru KUWAMURA[1], Takao KOTANI[1], Sadashige SAKUMA[1], Jonathan LAMARRE[2]
Laboratory of Veterinary Pathology, Osaka Prefecture University, Osaka 599-8531, Japan[1]
Department of Biomedical Sciences, University of Guelph[2]

P-121 (Mon.)

Spontaneous malignant schwannoma in F344 rat

Bang Hyun KIM[1], Dong Deuk JANG[1], Mina CHOI[1], Beom Seok HAN[1], Ki Taek NAM[1], Chul Kyu KIM[1], Kook Kyoung LEE[2], Ki Dae PARK[1], Wan Seob CHO[1], Ki Hwa YANG[1]
Department of General Toxicology, National Institute of Toxicological Research, Seoul 122-704, Korea (South)[1]
Department of Veterinary Medicine, Cheju National University[2]

Respiratory System — 1:00pm-2:00pm

P-122 (Tue.)

Histopathological characterization of nasal airway lesions induced by intranasal challenge with ovalbumin in sensitized guinea pigs

Emiko KUWASAKI[1], Koshirou KATOKU[1], Takafumi OSHIKATA[1], Yutaka NAKAHARA[1], Hideyuki WATANABE[1], Masao HAMAMURA[1], Kosuke MORIZUMI[2]
Department of Pathology, Panapharm Laboratories Co., Ltd., Kumamoto 869-0425, Japan[1]
Department of Parmacology, Panapharm Laboratories Co., Ltd.[2]

P-123 (Wed.)

Hydroxyurea(HU)-induced apoptosis and changes in apoptosis-related genes expression in the mouse fetal lung

Gye-Hyeong WOO[1], Eun-jung BAK[2], Koji UETSUKA[1], Hiroyuki NAKAYAMA[1], Kunio DOI[1]
Department of Veterinary Pathology, The University of Tokyo, Tokyo 113-8657, Japan[1]
Department of Biomedical Science, The University of Tokyo[2]

P-124 (Mon.)

In vivo detection of lung tumors in mice by high-resolution X-ray microtomography

Horst WEILER[1], Kris MEURRENS[2], Nora M. DE CLERCK[3], Andrei A. POSTNOV[3], Piter M. TERPSTRA[2]
PHILIP MORRIS Research Laboratories, GmbH, Cologne 51149, Germany[1]
PHILIP MORRIS Research Laboratories Bvba[2]
University of Antwerp[3]

P-125 (Tue.)

Eosinophilic change of the lining cells of the nasal cavity in rasH2 mice administered di(2-ethylhexyl)phthalate

Takatoshi KOUJITANI[1], Kaoru TOYOSAWA[1], Izumi MATSUMOTO[1], Kazuo OKIMOTO[1], Mami KOUCHI[1], Koji KUROKI[1], Kohji TANAKA[1], Nobuo MATSUOKA[1]
Safety Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Osaka 564-0053, Japan[1]

P-126 (Wed.)

Biological persistence and pathological changes of potassium octatitanate of two different shapes.

Akira OGAMI[1], Takako OYABU[1], Yasuo MORIMOTO[1], Hiroshi YAMATO[1], Izumi AKIYAMA[1], Isamu TANAKA[1]
Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Fukuoka 807-8555, Japan[1]

P-127 (Mon.)

Sequential change in acute lung damage due to intratracheal instillation of Quartz in F344 male rats establishment of a biological bioassay for detection of lung toxicity by fine particles

Masanao YOKOHIRA[1], Hijiri TAKEUCHI[1], Keiko YAMAKAWA[1], Kousuke SAOO[1], Mico IKEDA[1], Yoko HOSOTANI[1], Zeng YU[1], Kyoko HOSOKAWA[1], Katsumi IMAIDA[1], Makoto SHIRAISHI[2]
Department of Onco-Pathology, Kagawa University, Kagawa 761-0793, Japan[1]
Diagnostic Pathology and Cytology Institute, Shikoku Cytopathologic Institutes[2]

P-128 (Tue.)

Immunohistochemical demonstration of IL-13 protein in rat late athmatic response model

Kiyoshi KOBAYASHI[1], Osamu KURUSU[1], Hiroyuki HIGASHIYAMA[1], Anbo XIANG[1], Hideo KIKKAWA[1], Satoshi ASANO[1], Mine KINOSHITA[1]
Pharmacology Department, High Throughput Biology, TRL, GlaxoSmithKline K.K., Tsukuba 300-4247, Japan[1]

P-129 (Wed.)

One case of pleural mesothelioma in male F344/DuCrj rat

Hideki SENOH[1], Tetsuya TAKEUCHI[1], Yumi UMEDA[1], Taku KATAGIRI[1], Shigetoshi AISO[1], Kasuke NAGANO[1]
Japan Bioassay Research Center, Kanagawa 257-0015, Japan[1]

Cardiovascular System 1:00pm-2:00pm

P-130 (Mon.)

Incidence of cardiomyopathy in rats treated with PhIP varies with the rat strain

Shoji KASHIWABARA[1], Naoki KASHIMOTO[1], Toshihiro UESAKA[1], Osamu KATOH[1], Keiji WAKABAYASHI[2], Hiromitsu WATANABE[1]
Department of Cellular Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima 734-8553, Japan[1]
National Cancer Center Research Institute[2]

P-131 (Tue.)

Histopathological effect of Enalapril maleate on fetal heart development in rat.

Arash KHAKI[1], Iraj SOHRABI HAGHDOST[1], Amir Afshin KHAKI[2], Mahnaz HAYDARI[3]
Department of Veterinary Pathology, Islamic Azad University, Tabriz/Iran, Tabriz 51388, Iran[1]
Department of Histology in Medical College of Tabriz University/Iran[2]
Department of Pharmacology, Shahid Beheshty Medical College Tehran/Iran[3]

P-132 (Wed.)

Human heart glutamate receptors (GluRs) - possible effector sites: An opportunity for drug discovery.

Olga PULIDO[1], John VEINOT[2], Ruedi MUELLER[1], Meghan KAVANAGH[1], Colin ROUSSEAUX[3], Santokh GILL[1].
Pathology Section, Toxicology Research Division, Food Directorate Bureau of Chemical Safety, HPFB, Health Canada, Ontario K1A OL[2], Canada[1]
Department of Pathology and Laboratory Medicine, University of Ottawa, Civic Hospital Campus[2]
Department of Cellular and Molecular Medicine, Faculty of medicine, University of Ottawa[3]

Digestive System 1:00pm-2:00pm

P-133 (Mon.)

Enzyme- and immuno-histochemical changes in rat salivary glands induced by theophylline

Satoru KAJIKAWA[1], Kenji NAKANO[1], Aisuke NII[1], Hiroyuki NAKAYAMA[2], Kunio DOI[2]
Safety Research Labs., Yamanouchi Pharmaceutical Co., Ltd., Tokyo 174-8511, Japan[1]
Laboratory of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo[2]

P-134 (Tue.)

Histopathological study of the time course changes in the PTHrP-induced incisor lesions of rats.

Atsuhiko KATO[1], Masami SUZUKI[1], Yayoi KARASAWA[1], Tetsuro SUGIMOTO[1], Kunio DOI[2]
Safety Assessment Dept. Chugai Pharmaceutical Co., Ltd., Shizuoka 412-8513, Japan[1]
Department of Veterinary Pathology, Faculty of Agriculture, University of Tokyo[2]

P-135 (Wed.)

Spontaneous ectopic sebaceous glands (Fordyce's granules) in the oral mucosa of Sprague-Dawley rats

Masako IMAOKA[1], Hiroshi SATOH[1], Kiyonori KAI[1], Tetsuyo KAJIMURA[2], Kazuhisa FURUHAMA[1]
Drug Safety Research Laboratory, Daiichi Pharmaceutical Co., Ltd., Tokyo 134-8630, Japan[1]
Research Planning & Administration Department, Daiichi Pharmaceutical Co., Ltd.[2]

P-136 (Mon.)

Acquisition of a gastric or duodenal phenotype on heterotropic transplantation of esophagus diaphragm, trachea and bladder tissues in F344 rats

Hiromitsu WATANABE[1], Takashi OCHIYA[2], Seiichi KAWAMATA[3], Tomoyuki KUROSE[3], Yoko KOMINAMI[1], Masayo NISHIKI[1], Atsushi SASAKI[1], Miho SHIRAISHI[1], Rina GON[1], Masaomi HAYASHI[1], Shoji KASHIWABARA[1], Naoki KASHIMOTO[1], Toshihiro UESAKA[1], Osamu KATOH[1]
Department of Cellular Biology, Research Institute for Radiation Biology & Medicine, Hiroshima University, Hiroshima 734-8553, Japan[1]
Department of Cancer Metastesis, National Institute of Cancer[2]
Institute of Health Science, Faculty of Medicine, Hiroshima University[3]

P-137 (Tue.)

Proliferative changes in upper digestive duct of rats treated with a single dose of Alloxan

Kiyokazu OZAKI[1], Yasushi KODAMA[1], Tetsuro MATSUURA[1], Isao NARAMA[1]
Research Institute of Drug Safety, Setsunan University, Osaka 573-0101, Japan[1]

P-138 (Wed.)

Gastric carcinoma in mustard gas factory workers in the Ohkuno island

Takamitsu SASAKI[1], Tomonori SASAHIRA[1], Wataru YASUI[2], Hiroki KUNIYASU[1]
Department of Oncological Pathology, Nara Medical University Cancer Center, Nara 634-8521, Japan[1]
Department of Molecular Pathology, Hiroshima University Graduate School of Biomedical Sciences[2]

P-139 (Mon.)

Gastric carcinoma in a Japanese macaque (Macaca fuscata)

Tokuma YANAI[1], Masato KOBAYASHI[1], Shunji GOTOU[2], Akino KATOU[2], Hiroki SAKAI[1], Akihiro HIRATA[1], Toshiaki MASEGI[1]
Department of Veterinary Pathology, Gifu University, Gifu 501-1193, Japan[1]
Primate Research Institute, Kyoto University[2]

P-140 (Tue.)

One case of Ito cell tumor in female F344/DuCrj rat

Yumi UMEDA[1], Tetsuya TAKEUCHI[1], Hideki SENOH[1], Taku KATAGIRI[1], Shigetoshi AISO[1], Kasuke NAGANO[1]
Japan Bioassay Research Center, Kanagawa 257-0015, Japan[1]

P-141 (Wed.)

Effects of bandage of the torso to hepatomegaly induced by phenobarbital and clofibrate in rats

Kazuhiro HAYAKAWA[1], Satoru HOSOKAWA[1], Toyohiko AOKI[1], Atsushi INAGAMI[1], Akira INOMATA[1], Jiro SONODA[1], Satoru MOTOOKA[1], George LOSOS[2], Kazuo TSUKIDATE[1]
Drug Safety Research Laboratories, Eisai Co., Ltd., Japan, Gifu 501-6195, Japan[1]
Asia Pacific Consulting Company[2]

P-142 (Mon.)

Different susceptibilities of rat liver lobes in carbon tetrachloride-induced hepatotoxicity

Takeki UEHARA[1], Takashi MURAI[1], Satoshi INOUE[1], Akira TOUCHI[1], Satoru MORI[1], Toshiyuki MARUYAMA[1]
Developmental Research Laboratories, Shionogi & Co., Ltd., Osaka 561-0825, Japan[1]

P-143 (Tue.)

Characterization of drug metabolic reaction involved in the acquired resistance to bromobenzene-induced hepatotoxicity

Kohji TANAKA[1], Satoko SATO[1], Isao IGARASHI[1], Naoki KIYOSAWA[1], Toshiyuki WATANABE[1], Munehiro TERANISHI[1], Sunao MANABE[1]
Medicinal Safety Research Laboratories, Sankyo Co., Ltd., Shizuoka 437-0065, Japan[1]

P-144 (Wed.)

Depletion of tumor-infiltrating macrophages is associated with amphoterin expression in colon cancer

Tomonori SASAHIRA[1], Takamitsu SASAKI[1], Hiroki KUNIYASU[1]
Department of Oncological Pathology, Nara Medical University Cancer Center, Nara 634-8521, Japan[1]

P-145 (Mon.)

A new medium-term bioassay system for the detection of colon carcinogenesis modifiers

Young-Man CHO[1], Jun-ichi ONOSE[1], Toshio IMAI[1], Mai HASUMURA[1], Makoto UEDA[1], Masao HIROSE[1]
Division of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan[1]

Urinary System 1:00pm-2:00pm

P-146 (Tue.)

Renal damage in newborn rats treated with p-Cumylphenol

Yuko YAMAGUCHI[1], Nobuo NISHIMURA[2], Megumi YAHATA[2], Hiroshi EDAMOTO[1], Shinichiro IKEZAKI[1], Kazutoshi TAMURA[1], Eiichi KAMATA[4], Makoto EMA[4], Ryuichi HASEGAWA[3]
Division of Pathology, Gotemba Laboratory, Bozo Research Center Inc., Shizuoka 412-0039, Japan[1]
Division of The Second Laboratory for Safety Evaluation, Gotemba Laboratory, Bozo Reseach Center Inc.[2] Division of Medicinal Safety Sciences, National Institute of Health Sciences[3]
Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences[4]

P-147 (Wed.)

A photographic spectrum of non-neoplastic renal tubule and transitional epithelial structures within selected renal neoplasm of the rat

John Curtis SEELY[1], Gordon C HARD[2]
Experimental Pathology Laboratories, Inc., Research Triangle Park, North Carolina 27709, USA[1]
Consultant in Toxicology, Pathology, Carcinogenesis.[2]

P-148 (Mon.)

Effect of water deprivation or furosemide on cefalotin and glycerol-induced proximal tubular toxicity in rats

Miyoko OKADA[1], Naoki OHYAMA[1], Shun-ichiro ISHII[1], Naoya MASUTOMI[1], Fumiko SANO[1], Jiro SUGIMOTO[1], Shiro TAKAGI[1]
Toxicology Laboratory, Mitsubishi Pharma Corporation, Chiba 292-0818, Japan[1]

P-149 (Tue.)

Pathological observation of karyomegalic cells in the kidney on male rats treated with Paecilomyces japonica

Yong-Bum KIM[1], Chang-Su HA[1], Hwa-Young SON[2], Sung-Whan CHO[2], Boo-Hyon KANG[1]
Korea Institute of Toxicology, KRICT, Yuseong, Daejeon 305-600, Korea (South)[1]
College of Veterinary Medicine, Chungnam National University[2]

P-150 (Wed.)

Long-term observation of anti-Thy-1 glomerulonephritis in uninephrectomized rats: Relationship between morphological changes and renal function

Shunji NAKATSUJI[1], Kenjiro TSUBOTA[1], Yoshimasa OKAZAKI[1], Masahiro MATSUMOTO[1], Shiro FUJIHIRA[1], Yuji OISHI[1], Masayuki TOMITA[2], Hajime SOGABE[2], Shoko NAKAZATO[2], Hiroshi KAWACHI[3], Fujio SHIMIZU[3]
Toxicology Research Laboratories, Fujisawa Pharmaceutical Co.,LTD., Osaka 532-8514, Japan[1]
Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.[2]
Institute of Nephrology, Niigata University Graduate School of Medical and Dental Sciences[3]

P-151 (Mon.)

Spontaneous glomerulonephritis in cynomolgus monkeys

Xiuying YANG[1], Shigeru SATAKE[1], Yasuhiro KAMIMURA[1], Kimiaki HIRAKAWA[1], Hiroshi MAEDA[1], Hiroaki MIYAJIMA[1]
Shin Nippon Biomedical Laboratories, Ltd., Kagoshima 891-1394, Japan[1]

P-152 (Tue.)

Spontaneous collagen glomerulopathy in a cynomolgus monkey

Kae FUJISAWA[1], Nobuo TAKASU[1], Noriko TSUCHIYA[1], Shuuichi MATSUSHIMA[1], Haruhisa INAGAKI[2], Mikinori TORII[1]
Developmental Research Laboratories, SHIONOGI & CO., LTD., Osaka 561-0825, Japan[1]
Discovery Research Laboratories, SHIONOGI & CO., LTD.[2]

Endocrine System 1:00pm-2:00pm

P-153 (Wed.)

Expression of somatostatin mRNA and peptides in C-cell tumours of the thyroid gland in Han Wistar rats

Andrew PILLING[1], Stewart JONES[2], John TURTON[3]
Huntingdon Life Sciences, Huntingdon PE28 4HS, UK[1]
GlaxoSmithKline[2]
School of Pharmacy, University of London[3]

P-154 (Mon.)

A spontaneous dwarf mutation in BrlHan:WIST@Jcl (GALAS) rat - Histopathological characteristics of the endocrine system

Takuya DOI[1], Masato NAMIKI[1], Michiko ASHINA[1], Naoto TOYOTA[1], Hiroko KOKOSHIMA[1], Yuki TOMONARI[1], Junko SATO[1], Takeshi KANNO[1], Yumi WAKO[1], Minoru TSUCHITANI[1]
Mitsubishi Chemical Safety Institute Ltd., Ibaraki 314-0255, Japan[1]

P-155 (Tue.)

Association of adrenal pheochromocytoma and lung pathology in inhalation studies with particulate compounds in the male F344 rat--the National Toxicology Program experience.

Keisuke OZAKI[1], Joseph K. HASEMAN[2], James R. HAILEY[3], Robert R. MARONPOT[3], Abraham NYSKA[3]
Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan[1]
Biostatistics Branch, National Institute of Environmental Health Sciences (NIEHS)[2]
Laboratory of Experiment Patholgy, National Institute of Environmental Health Sciences (NIEHS)[3]

Reproductive System 1:00pm-2:00pm

P-156 (Wed.)

Renal toxicity induced by folic acid is directly responsible for the enhancement of male reproductive toxicity of di (n-butyl) phthalate in male rats

Toshio ICHIHARA[1], Tomoko TSUTSUMI[1], Mayumi KAWABE[1], Hiroko YOSHINO[1], Makoto ASAMOTO[2], Syugo SUZUKI[2], Tomoyuki SHIRAI[2]
Daiyu-kai Institute of Medical Science, Aichi 491-0113, Japan[1]
Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences[2]

P-157 (Mon.)

Motional and pathological analysis for rat testicular toxicity induced by boric acid

Masakazu KAKUNI[1], Naoya KIMOTO[1], Tsuyoshi TAKEDA[1], Masato MIZUTANI[1], Kazuo SUZUKI[1], Hitoshi SATO[1], Katsumi TAKABA[1], Takuji HARA[1]
Toxicological Research Laboratories, KYOWA HAKKO KOGYO CO., LTD., Yamaguchi 755-8501, Japan[1]

P-158 (Tue.)

Early ultrastructural changes of the Sertoli cells with vacuole formations

Yoshiaki SAITO[1], Kenji USUMI[1], Noriko OSAWA[1], Tomoko NAGATA[1]
Food and Drug Safety Center, Hatano Research Institute, Kanagawa 257-8523, Japan[1]

Others 1:00pm-2:00pm

P-159 (Wed.)

International advancement in education in veterinary & comparative pathology

D. Reid PATTERSON[1]
Reid Patterson Consulting, Inc., Grayslake 60030, USA[1]

P-160 (Mon.)

Histopathology, immunocytochemistry and electron microscopy of spontaneous uveal melanoma in two HAN Wistar rats

Alexandra MORAN[1], Christopher BARTON[1]
Pathology Department, Covance Laboratories, Harrogate HG3 1PY, UK[1]

P-161 (Tue.)

Case report: naturally occuring proliferative vitreoretinopathy with multiple ocular alterations in a rabbit

Osamu KATSUTA[1], Takeshi YAMAGUCHI[1], Kenji TAKASE[1]
Drug Safety/Drug Metabolism Group, Research and Development Center, Santen Pharmaceutical Co., Ltd., Nara 630-0101, Japan[1]

P-162 (Wed.)

Histopathological findings of cleft palate in rat embryos induced by triamcinolone acetonide

Satoshi FURUKAWA[1], Koji USUDA[1], Masayoshi ABE[1], Izumi OGAWA[1]
Biological Research Laboratories, Nissan Chemical Industries, LTD., Saitama 349-0294, Japan[1]

P-163 (Mon.)

Spontaneous neural crest tumors of pinna in mice

Hiroshi EDAMOTO[1], Mariko NAGATANI[1], Rie ANDO[1], Sachiko TAMAI[1], Kenichiro KASAHARA[1], Shuzo OKAZAKI[1], Kazutoshi TAMURA[1]
Division of Pathology, Gotemba Laboratory, Bozo Research Center Inc. Shizuoka, 412-0039, Japan[1]
Division of the Second Laboratory for Safety Evaluation, Gotemba Laboratory, Bozo Research Center Inc.[2]

P-164 (Tue.)

Histological changes in the dorsal skin of YPC mice irradiated with UVB for 4weeks

Taro OKADA[1], Akira YASOSHIMA[1], Kaoru TAKANO[2], Junichiro MATSUDA[2], Koji UETSUKA[1], Hiroyuki NAKAYAMA[1], Kunio DOI[1]
Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, the University of Tokyo, Tokyo 113-8657, Japan[1]
Department of Veterinary Science, National Institute of Infectious Diseases[2]

P-165 (Wed.)

Effects of scratching on the onset and exacerbation of dermatitis in NC/Nga mice

Yutaka NAKANISHI[1], Atsushi NAKAMURA[1], Yuki HASHIMOTO[2], Iwao ARAI[2], Hiroto MIYATA[1], Masaaki KIMURA[1]
Toxicology Laboratory, Medical Research Laboratories, Taisho Pharmaceutical Co., Ltd., Saitama 331-9530, Japan[1]
Phamacology Laboratory, Medical Research Laboratories, Taisho Pharmaceutical Co., Ltd.[2]

P-166 (Mon.)

Morphological changes of auricular tissue in the mouse TNCB-repeated application model.

Chie TAKADA[1], Daisuke HARADA[1], Yukihito TSUKUMO[1], Toyoko KASHIWAGI[1], Haruhiko MANABE[1], Satoshi NISHIKAWA[1], Kazuo SUZUKI[1], Katsumi TAKABA[1]
Kyowa Hakko Kogyo Co.,Ltd., Shizuoka 411-8731, Japan[1]

P-167 (Tue.)

Application of special staining to decalcified skeletal tissues in routine toxicity studies

Aisuke NII[1], Kenji NAKANO[1], Takanori HANADA[1]
Safety Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd., Tokyo 174-8511, Japan[1]

P-168 (Wed.)

Histopathological characterization of the skeletal myopathy in rasH2 mice

Takayuki TSUCHIYA[1], Fumiko SANO[1], Kazuhiro GOTO[1], Mamoru MUTAI[1], Toshimi USUI[2], Jiro SUGIMITO[1], Shiro TAKAGI[1]
Toxicology Laboratory, Mitsubishi Pharma Corporation, Chiba 292-0818, Japan[1]
Central Institute of Experimental Animals[2]

P-169 (Mon.)

Spontaneous degenerative lesions of the femoral growth plate in rats

Shinichiro IKEZAKI[1], Osamu KUSUOKA[1], Mizuho TAKAGI[1], Katsuhiko WARITA[1], Maiko TSURUKAME[1], Kayoko KUDO[1], Sachiko TAMAI[1], Atsushi NAKAMURA[1]
Division of Pathology, Bozo Research Center Inc., Shizuoka 419-0101, Japan[1]

P-170 (Tue.)

Early pathophysiological feature of arthropathy in juvenile dogs induced by ofloxacin, a quinolone antimicrobial agent

Koichi YABE[1], Hiroshi SATOH[1], Toshimasa JINDO[1], Tadaki SUGAWARA[1], Kazuhisa FURUHAMA[1], Masanobu GORYO[2], Kosuke OKADA[2]
Drug Safety Research Lab., Daiichi Pharmaceutical Co., Ltd., Tokyo 134-8630, Japan[1]
Veterinary Pathology, Iwate University[2]

P-171 (Wed.)

Organ calcification in monkeys treated intradermally with rhPTH1-34 for 6 months followed by a 1-month recovery period

Jihong YANG[1], Peng WANG[1], Xiangting XU[1], Guoping XU[1], Ling ZHANG[1], Liuyi ZHANG[1]
Department of Pathology, Yunnan Pharmacological Laboratories of Natural Products, Kunming Medical College, Kunming City P. R. 650031, China (PRC)[1]

P-172 (Mon.)

Physiological parameters and background histopathology findings from chronic (2 year) carcinogenicity studies in the HAN wistar rat

Christopher BARTON[1]
Department of Pathology, Covance Laboratories, North Yorkshire HG31PY, UK[1]

LUNCHEON SEMINAR PROGRAM

Main Hall

LUNCHEON SEMINAR 1 — **Monday, February 16, 2004 / 12:00pm-1:00pm**

Co-sponsored by : Fujisawa Pharmaceutical Co.,Ltd.

Chair : Yoichi KONISHI
Nara Medical University

Acrylamide in foods: A global dilemma

Speaker : James R. COUGHLIN
Coughlin & Associates

Prevention of Acrylamide induced toxicity in rat

Speaker : Masao HIROSE
National Institute of Health Sciences

Room 501

LUNCHEON SEMINAR 2 — **Monday, February 16, 2004 / 12:00pm-1:00pm**

Co-sponsored by : MORINAGA MILK INDUSTRY CO., LTD.

Recent advances in lactoferrin research

Chair : Hiroyuki TSUDA
Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences

Biological and physico-chemical features of lactoferrin

Speaker : Kei-ichi SHIMAZAKI
Dairy Science Laboratory, Graduate School of Agriculture, Hokkaido University

Recent advances of clinical application of lactoferrin for the treatment of chronic hepatitis C

Speaker : Katsuaki TANAKA
Gastroenterological Center, Yokohama City University Medical Center

Room 502

LUNCHEON SEMINAR 3 — **Monday, February 16, 2004 / 12:00pm-1:00pm**

Co-sponsored by : MEIWA SHOJI CO., LTD.

Morphological Genomics - Gene analysis of the disease using Laser microdissection

Chair : Hideki WANIBUCHI
Osaka City University Medical School

Speaker : Tetsuhiko TACHIKAWA
Department of Oral Pathology, School of Dentistry, Showa University

Main Hall

LUNCHEON SEMINAR 4

Tuesday, February 17, 2004 / 12:00pm-1:00pm

Co-sponsored by : Affymetrix Japan K.K.

GeneChip® technology in the identification of mechanisms of toxicity

Chair : Sadashi SUZUKI
Affymetrix Japan K.K.

Speaker : Michael PAUMEN
Affymetrix Japan K.K.

Room 501

LUNCHEON SEMINAR 5

Tuesday, February 17, 2004 / 12:00pm-1:00pm

Co-sponsored by : OLYMPUS CORPORATION

OLYMPUS advanced technology for toxicogenomics

Chair : Kiyotsugu KOJIMA
OLYMPUS CORPORATION

Speaker : Keiichirou MORIMURA
Osaka City University Medical School

Room 502

LUNCHEON SEMINAR 6

Tuesday, February 17, 2004 / 12:00pm-1:00pm

Co-sponsored by : Bozo Research Center Inc.

Chair : Kunitoshi MITSUMORI
Tokyo University of Agriculture & Technology

In situ mRNA hybridization method with formalin-fixed and paraffin-embedded tissue section

Speaker : Yuichi SATO
Department of Molecular Diagnostics, Kitasato University School of Allied Health Sciences

Histopathology background data in laboratory beagles over ten years.

Speaker : Kazutoshi TAMURA
Division of Pathology, Gotemba Laboratories, Bozo Research Center Inc.

Inhalation toxicology -21st century equipment and techniques for precise animal treatment -

Speaker : Cristopher N. CROUCH
Division of Inhalation Toxicology, ITR Laboratories Canada

Main Hall

LUNCHEON SEMINAR 7 — **Wednesday, February 18, 2004 / 12:00pm-1:00pm**

Co-sponsored by : NPO International Life Sciences Institute of Japan (ILSI Japan)
ILSI Health and Environmental Sciences Institute (ILSI HESI)

Concordance of animal toxicity data with human clinical data

Chair : Samuel M. COHEN
University of Nebraska Medical Center

Speaker : James E. SANDERS
Aventis Pharmaceuticals

Room 501

LUNCHEON SEMINAR 8 — **Wednesday, February 18, 2004 / 12:00pm-1:00pm**

Co-sponsored by : Japan SLC, Inc.

Interbreeder's comparison of SD and F344 rats from the standpoint of pathological examinations

Chair : Kiyoshi IMAI
Biosafety Research Center Foods, Drugs and Pesticides

Speaker : Kunitoshi MITSUMORI
Tokyo University of Agriculture & Technology

Room 502

LUNCHEON SEMINAR 9 — **Wednesday, February 18, 2004 / 12:00pm-1:00pm**

Co-sponsored by : Dako Cytomation Co. Ltd.

Immunohistochemical detection of the altered expression of proteins involved in rat carcinogenesis and their digital image analysis by automated cellular scanning system (Ariol SL-50)

Chair : Katsumi IMAIDA
Faculty of Medicine, Kagawa University

Speaker : Dai NAKAE
Sasaki Institute, Sasaki Foundation

IATP

Enhancing the impact of toxicologic pathologists in global medical research

D. Reid PATTERSON

Reid Patterson Consulting, Inc., Illinois 60030, USA

In the late 1990s, the International Federation of Societies of Toxicologic Pathology, the organization bridging the numerous independent organizations of toxicologic pathologists around the world, had a vision for establishing a mechanism for recognizing or accrediting leaders and innovators in the field. These accomplished medical and veterinary pathologists would represent the field by continuing to contribute intellectually in timely scientific symposia, by challenging outdated scientific dogma, by assisting in the organization of new societies of toxicologic pathology, and by advising governmental bodies and harmonization conferees on scientific policy. The innovators envisaged this academy to establish an internationally acceptable standard of knowledge, contributions and continued competency within the field for the benefit of the global society. Thus, the International Academy of Toxicologic Pathology (IATP) was established in 1999. While still in its formative years, the Academy has attracted noted medical and veterinary pathologists from academia, government, and the pharmaceutical industry from Canada, Europe (Germany, Italy, The Netherlands), Japan, Korea, Taiwan, the United Kingdom and the United States. Additional members are needed to realize the vision of the founders of the IATP.

Chemically-induced immunopathology and immune functional changes

Joseph G. VOS

National Institute for Public Health and the Environment (RIVM), Bilthoven 3720 BA, The Netherlands

Toxicological pathology plays a most important role in the risk assessment process by identifying and defining the health effects following exposure to xenobiotics. This includes the field of immunotoxicology, as identification of chemicals that have the potential to cause injury to the immune system is of considerable public health significance in view of the role of the immune system in infectious diseases, hypersensitivity reactions and autoimmune diseases. In assessing immunotoxicity, a two-tier testing system is usually employed in rodents in which the first tier is a general screen for (immuno) toxicity including enhanced histopathology of lymphoid organs and the second tier consists of more specific immune function studies including host resistance tests or mechanistic studies. Studies with TCDD, TBTO, HCB, azathioprine and cyclosporin A will be discussed that provided data correlating histopathology with induced immune function changes. This will be followed by a discussion of the outcomes of enhanced histomorphology in the interlaboratory validation studies of azathioprine, cyclosporin A and HCB in the rat, as well as the results of a recent evaluation of extended histopathology in the mouse as an indicator of immunotoxicity. From these studies, that have been the basis for a number of regulatory activities, the following conclusions can be drawn: i) the consistency between histopathology and functional tests, ii) the complimentary information of pathology and immunology observations, and iii) the dependence on standardised protocols and trained toxicologic pathologists to accurately identify and grade microscopical changes in lymphoid organs and tissues.

Thresholds in experimental chemical hepatocarcinogenesis

Gary M. WILLIAMS, A.M. JEFFREY, J. D. DUAN and M. J. IATROPOULOS

Department of Pathology, New York Medical College, New York 10595, USA

In order to explore for potential thresholds in liver carcinogenicity, we conducted a series of studies utilizing an initiation/promotion (I/P) model in male F344 rats with the hepatocarcinogens 2-acetylaminofluorene (AAF) and diethylnitrosamine (DEN) (Toxicol. Pathol. 28, 388, 2000), which, after bioactivation, react with DNA in target tissues, particularly liver. The I/P protocol results in hepatocarcinogenicity comparable to that reported for chronic exposure to these carcinogens. In these studies, AAF or DEN was administered in exact intragastric doses during the initiation segment (IS) of up to 12 weeks followed in some experiments by phenobarbital (PB) during the promotion segment (PS) for 24 weeks to enhance manifestation of initiation. We have documented in the IS that there are no-observed-effect levels (NOELs) for formation of DNA adducts, cytotoxicity, compensatory hepatocellular proliferation, formation of preneoplastic hepatocellular altered foci (HAF), and in the PS, for tumor development. The earliest parameter to be affected with very low exposure is the formation of DNA adducts, followed at higher exposures by increased cell proliferation. The finding of NOELs for critical events of initiation of hepatocarcinogenesis supports the finding of a NOEL for PB-promotable liver tumors. We concluded that there is a practical threshold for the carcinogenicity of DNA-reactive carcinogens. Moreover, these findings provide a basis for understanding why low level environmental exposures of humans to DNA-reactive carcinogens may convey no cancer risk. Accordingly, a safe exposure level can be calculated for DNA-reactive carcinogens using DNA adducts as the most sensitive NOEL, and application of safety factors.

Endocrine disruption in wildlife: The role of toxicologic pathology in fish

Pieter W. WESTER

National Institute for Public Health and the Environment (RIVM), Bilthoven 3720 BA, The Netherlands

An emerging issue in toxicological pathology is pathology of laboratory fish. Laboratory fish are increasingly used in environmental toxicology, in particular in the context of the issue of endocrine disruption. Classical tests using fish are mainly focussed at general endpoints in ecotoxicity, i.e. with sustainability of biomass as principal goal. However, endocrine disruption as an ecotoxicological concern requires more refined endpoints, especially for shorter studies (i.e. not being multi-generation tests). Histopathology is therefore the screening tool of choice, in analogy with rodent toxicology. Efforts are currently undertaken by various organisations to develop databases, knowledge and harmonization in order to validate this emerging field where toxicologic pathologist are bound to play a principal role. We have studied some small fish species used in the laboratory (medaka, guppy and zebrafish) with emphasis on endocrine disrupting effects. The small size of these fish allows total body embedding of multiple specimen which, in step serial sectioning, enables quick screening for all relevant organs. In comparison with rodents, most histopathological reactions are rather similar, taking into account the usual typical interspecies differences. In addition, comparable data in rodents and fish enable integrated (human and environmental) risk assessment. This paper will address a series of fish- and species-specific features and effects with emphasis on endocrine disruption.

Renal mesenchymal tumor vs. nephroblastoma: Revisited

John Curtis SEELY

Experimental Pathology Laboratories, Inc., Research Triangle Park, North Carolina 27709, USA

Among the non-epithelial (tubular cell) neoplasms recognized in the rat kidney, lipoma/liposarcoma; nephroblastoma (NB); renal mesenchymal tumor (RMT); and renal sarcoma are the most commonly diagnosed. Even though excellent guideline criteria for each of these neoplasms have been published the differential diagnosis between NB and RMT may remain problematic in some cases. In the rat, the diagnosis of NB is dependent upon the identification of the highly basophilic blastemal cells which are pathognomonic for the diagnosis of NB. A biphasic cellular pattern of blastemal and epithelial elements may form clusters, nests, alveolar, papillary or tubular patterns. Organoid epithelial differentiation into glomeruli, ducts and/or tubules may also be recognized. The stromal component of the rat NB may contain stellate cells or more mature appearing cells resembling fibroblasts. On the other hand, the most important feature of RMT is the presence of a small fibroblastic-like cell which proliferates between tubules and glomeruli. These cells often form whirling patterns around encircled, pre-existing renal tubules. Occasionally, fibrous tissue and/or smooth muscle may be identified. More rarely, striated muscle, vascular, cartilaginous or osteoid tissue may be observed. RMT often contains hyperplastic tubular epithelium and urothelium. The distinction between NB and RMT may be complicated by several factors:

- some cases of induced RMT have been erroneously reported as NB;
- difficulty in comparing the rat NB and RMT to human NB;
- overlapping of similar diagnostic features;
- the presence of hyperplastic tubules and urothelium, particularly in RMT;
- the presence of neoplasms which do not easily fit into patterns typical of the rat RMT or NB.

Demonstration of histogenesis and cytogenesis of mammary tumors from terminal endbud, mammary duct, acinus and myoepithelium cells using human c-Ha-ras proto- and mutated-oncogene transgenic rats

Hiroyuki TSUDA[1,2], Shinobu UEDA[2], Yoichiro MATSUOKA[2], Tetsuya HAMAGUCHI[2], Akihiro NAITO[2] and Nobuo TAKASUKA[2]

Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Experimental Pathology and Chemotherapy Division, National Cancer Center Research Institute[2]

We have established a rat line carrying human c-Ha-ras proto-oncogene (Hras128), including its own promoter region. Both sexes were found to be highly susceptible to chemically-induced mammary, skin, bladder and esophageal carcinogenesis. Palpable multiple mammary carcinomas developed in almost all female animals in 8-12 weeks and microscopic early carcinomas in 25 days of a single i.v. dose of N-methyl -N-nitrosourea (MNU) at 50 days after birth. Preferential mutation of the transgene was found in all tumors. Furthermore, similar mutation was detected in laser captured normal looking terminal endbuds, a target tissue of MNU, at day 5 after MNU application, indicating the importance of transgene mutation.
To investigate the role of the mutated ras gene in mammary carcinogenesis, we used transgenic rats carrying a human c-Ha-ras mutated oncogene (codon 12) with Cre/loxP. construct (Hras250), thus enabling activation of the transgene by infecting the adenovirus carrying cre-recombinase (Adex/Cre). Five days after injection of the Adex/Cre into the main mammary duct, the ras oncogene activated ductal, acinar and myoepithelial cells were clearly identified by Cre-recombinase immunostaining. Myoepithelial cells were identified by positive reaction for a-smooth muscle antigen (a -SMA). Subsequently, development of atypical cell hyperplasia of the duct and acinar epithelium and myoepithelial cells was observed at day 10. Carcinoma in situ lesions of these 3 cell types developed at day 15 20 and was followed by invasive progression by week 4. Development of the myoepithelial lesions was found to take 2 patterns, one was an epithelia-like growth occurring within the ductual or acinar cell hyperplasia lesions, and the other was a sacoma-like proliferation in the stromal tissue, the former being positive for vimentin and the latter negative. Thus, we were able to characterize the histogenesis of MNU-induced mammary carcinomas from 4 different tissure/cell types by demonstration of in vivo activation of endbud tissue, mammary duct, acinus and myoepithelial cells using rats carrying ras proto- and mutated-oncogene.

The function and pathology of brown adipose tissue (BAT) in animals and humans

Michael IATROPOULOS and Gary WILLIAMS

New York Medical College, New York 10595, USA

Brown adipose tissue (BAT), an endocrine tissue, is an important regulator of nonshivering thermogenesis, energy metabolism and mitochondrial biogenesis. There is a paucity of data about BAT response in laboratory animals because BAT has not been routinely included in regulated safety assessment study protocols. We will present information on the structure, function and pathology of BAT, focusing mainly on younger and older rats. The energy supply process is stimulated in mammals mainly by hypothermia, by hypoxia and in primates by hypoglycemia. Of these, hypothermia is the main stimulus of brown adipocyte (BA) proliferation. Thermogenesis is achieved in BA in the mitochondria (mt), which are 50 to 100 times more abundant in BA than in any other cell type. The mt response involves a membranous protein in the inner membrane, uncoupling protein (UCP) or thermogenin. Triodothyronine (T3) is implicated in the regulation of the UCP gene. UCP allows mt to oxidize substrates rapidly without ADP phosphorylation, thus uncoupling oxidation from phosphorylation and promoting instead the dissipation of oxidation energy as heat. Norepinephrine (NE) binds to the s3-adrenoreceptor in BA leading to activation of lipoprotein lipase (LPL), which stimulates lipolysis and liberates FFA. Both NE and insulin (I) stimulate glucose uptake by BA, and NE increases UCP in BA. Nitric oxide (NO) stimulates the biogenesis of mt, redistribution of heat generated by the mt and inhibition of BA proliferation. NO also regulates the binding and releasing of oxygen from hemoglobin (Hgb) and the mt pathway of apoptosis. These effects of NO are mediated by peroxisome proliferator-activated receptor coactivator 1 alpha (PPARgammaCA1apha). NO is produced by endothelial NO synthase (eNOS) mainly in BA, but also in the hepatocytes and cells of the zona glomerulosa. PPARgammaCA1alpha in turn increases the expression of nuclear respiratory factor-1 (NRF-1) and mitochondrial transcription factor A (mtTFA). Thus, UCP, T3, I, NE, NO, PPARgammaCA1alpha, cGMP, eNOS, NRF-1 and mtTFA are all parts of an interactive cascade connecting the BA functions through the extracellular matrix (EMA) of all tissues to controlling the processes of EMA homeostasis and signaling, tissue normoxia and normothermia, apoptosis, proliferation, and differentiation of all cells.

Second John Faccini Memorial Keynote Lecture

Plenary Lecture

Second John Faccini Memorial Keynote Lecture

Molecular aspects of toxicologic pathology

Okio HINO

Department of Experimental Pathology, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo 170-8455, Japan

Carcinogenesis can be compared to an opened Japanese fan, because initiated cells grow in several directions and clinical tumors suggest that the edge of the fan has many gene abnormalities.
Cancer is a heritable disorder of somatic cells. Environment and heredity both operate in the origin of human cancer. The Eker (*Tsc 2* gene mutant) rat model of hereditary renal carcinoma (RC) is an example of Mendelian dominantly inherited predisposition to a specific cancer in an experimental animal. To the best of our knowledge, this was the first isolation of a Mendelian dominantly predisposing cancer gene in a naturally occurring animal model.
Recently, we discovered a new hereditary renal carcinoma in the rat in Japan, and the rat was named the "Nihon" rat and its predisposing (*Nihon*) gene could be a novel renal tumor suppressor gene.
We will present these unique models, comparing these two predisposing genes, for the study of problems in carcinogenesis ♂eg., species-specific difference in tumorigenesis, cell stage and tissue/cell-type specific tumorigenesis, multistep carcinogenesis, modifier gene(s) in renal carcinogenesis, cancer prevention and the development of the therapeutic treatments which can be translated into human patients, as well as how environmental factors interact with cancer susceptibility gene(s).

Plenary Lecture

Human relevance of carcinogenesis in rodents.

Samuel M. COHEN

Department of Pathology and Microbiology, University of Nebraska Medical Center, Nebraska 68198, USA

Over the past five decades, there has been an increasing reliance on the two-year rodent bioassay to screen chemicals for possible carcinogenic activity in humans. Considerable effort has been invested in the development of a variety of in vitro screening assays to assist in this judgment, but most of these have relied on one or more aspects of genotoxicity. In vitro cell transformation assays have also been developed, and more recently, bioassays in specialized transgenic organisms or other in vivo screening assays have been developed in attempts to shorten this process and to raise the specificity for determination of human carcinogenicity. Any time an animal model is used to evaluate any aspect of human disease, there are two fundamental assumptions: 1) observations in the species studied are pertinent to the human situation (interspecies extrapolation); and 2) effects at doses used in animal studies are relevant to the exposure levels of humans (dose extrapolation). Although these assumptions may be appropriate for many chemicals, particularly those that are DNA-reactive, increasingly, mechanisms of action have been determined in animals that either are not relevant to humans or not pertinent at the doses to which humans are exposed. Considerable insight has been gained into the various modes of action that occur in animal models, and evaluation of their relevance to humans has been ascertained. Based on this knowledge, I am proposing a considerably shortened evaluation process for screening for potential carcinogenicity in humans that eliminates the need for the two-year bioassay in rodents, both mice and rats. The process will involve screening for DNA-reactivity, in contrast to a generalized screening for genotoxicity, reliance on structure activity relationships, and an evaluation of 4-week and 13-week in vivo studies in mice and rats extensively evaluating the histopathologic effects of treatment, toxicokinetics, and cell kinetics (proliferation, necrosis, and apoptosis). Although these short-term studies require intensive investigation, the results would provide adequate information for reasonable judgments concerning potential risks to humans. Since shorter time periods are involved, a larger number of doses could be evaluated to more precisely evaluate the dose response. In addition, mechanistic evaluations could be pursued evaluating the relevance of species and dose to the human situation. The advent of newer technologies offer the possibility of further refining and shortening this process, with greater specificity regarding effects in humans.

Symposium

S1-1 Chair

Carcinogenesis-Current understanding of mechanisms and risk assessments: Non-genotoxic carcinogenesis/Low-dose carcinogenic potential of genotoxic and non-genotoxic carcinogens

Colin G. ROUSSEAUX
Department of Cellular and Molecular Medicine, Faculty of Medicine, 451 Smyth Rd, University of Ottawa, Ontario K1H 8M5, Canada

Masao HIROSE
Division of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan

Dr. Wanibuchi will talk about the risk assessment of low dose carcinogenesis in rats. It has long been believed that there is no threshold level in genotoxic carcinogens, and thus they are not allowed to include as food additives and pesticides. He will present the existence of threshold levels for several endpoints such as tumor incidence, DNA damage and mutation even in genotoxic carcinogens. The results may give us important information for the future risk assessment of environmental genotoxic carcinogens.

Dr. Nishikawa will present the recent results of reporter gene analysis of several genotoxic and non-genenotoxic carcinogens using transgenic animal models such as *lacI* and *gpt* delta. In this assay, carcinogenicity, and mutation frequency and spectra of target organs can be evaluate in a same animal. Therefore risk assessment of some possible genotoxic carcinogens have recently been conducted using this assay. However, there may be problems as well as merits in this assay. He also will discuss the problems as a tool for assessing the risk of unknown environmental carcinogens.

In the second part of the session,two speakers will address conference attendees regarding the latest developments in gene inactivation and its role in carcinogenicity and current developments in angiogenesis as it relates to the development and progression of neoplasia.

Dr. Ryan will discuss the critical role that angiogenesis plays early in the multistage process of carcinogenesis. Following an overview of angiogenesis as a normal process embryogenesis (implantation, organ development and tissue differentiation) and also a key role in postnatal physiologic processes such as wound healing, longitudinal bone growth and reproduction, Dr. Ryan will brief outline the role of angiogenesis in pathologic processes, including rheumatoid arthritis, psoriasis, and intraocular neovascularization. The majority of the presentation will focus on the positive and, particularly, negative regulators of angiogenesis, which many of these negative factors are currently being evaluated in clinical trials as potential anti-angiogenic therapeutics for oncology indications. Dr. Ryan's presentation will highlight the various experimental methods and models used in elucidating the mechanisms of induction and inhibition of angioneogensis.

Gene silencing is necessary for growth, differentiation, repair and maintenance of healthy tissue by limiting the expression of some genes while permitting the expression of others. Invasion, a characteristic of many malignancies, may seem to contradict this concept and appear to be a "new event." However, the recognition that none of us would be here today if, as a trophoblast, we had no method of invading our mothers endometrium through the expression of certain genes. These genes then normally become and remain silent. The converse also holds true. Human cancers arise from cells that have inactivated tumor suppressor genes either by mutation or transcriptional inactivation. The means by which genes become transcriptionally silenced is poorly understood in mammalian cells. Dr. McBurney will present his resent research in murine embryonal carcinoma stem cells growing in cell culture. His research suggests that the silencing of transgenes in stem cells is triggered by chromatin assembly or reassembly following DNA recombination and that the active splicing of transcripts maintains chromatin in the open configuration that supports continued transcription.

S1-1-1

Risk assessment of low dose carcinogenesis by genotoxic carcinogens

Hideki WANIBUCHI, Keiichirou MORIMURA, Shoji FUKUSHIMA

Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan

For a long period, it has been generally considered that genotoxic carcinogens have no threshould in exerting carcinogenic potential. This is because genotoxic carcinogens are mutagenic, and seem to act through interaction with DNA to produce irreversible genetic changes in target organ cells. This theory is based on acceptance of a linear relationship which approaches zero at low doses for risk assessment of exposure to man with chemicals found to be carcinogenic in animal studies. However, there are limited data available for estimation of cancer risk assessment in man exposed to genotoxic carcinogens, and the validity of the no-threshold theory can be challenged in this case.

In these situation, low dose hepatocarcinogenicity of genotoxic, environmental carcinogens were examined as an aid to cancer risk assessment in humans. Male F344 rats, 21-day-old at the start of the experiment, were administered 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), a food-derived hepatocarcinogen, in the diet at various doses for a maximum of 32 weeks. Quantitative values of glutathione *S*-transferase placental form (GST-P) positive foci, a preneoplastic marker for tumors in the liver, were similar among the 0 to 1 ppm MeIQx groups, while MeIQx at a dose of 10 ppm showed a tendency for increase and 100 ppm significantly elevated their numbers. MeIQx-DNA adduct formation in the liver demonstated a linear relationship with all the doses tested. Levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the liver were linearly elevated from 1 ppm MeIQx at week 4, and from 0.01 ppm MeIQx at week 16. Interestingly, there were no differences in H-ras mutation frequencies with 0.01 ppm MeIQx or less, while they were significantly increased at 0.1 ppm MeIQx and above. In a Big Blue transgenic rat mutagenesis assay, MeIQx at low doses was found not to induce *lacI* gene mutations in the liver, this tending to correlate with non-induction of GST-P positive foci. However, the dose of MeIQx at which in vivo mutagenicity was significantly increased was lower than that for GST-P positive foci. Data for GST-P positive focus development with MeIQx followed by phenobarbital treatment were similar to those with MeIQx-alone. Co-administration of carbon tetrachloride or ethanol enhanced the induction of liver GST-P positive foci by MeIQx in each group, numbers being significantly increased with 10 ppm and higher doses of MeIQx. Thus, these results indicate that MeIQx have no-observed effect levels for induction of carcinogenesis-relating changes in the rat lives, implying a threshold for carcinogenicity of genotoxic carcinogens.

S1-1-2

Analysis of reporter genes for the molecular mechanisms underlying chemical carcinogenesis

Akiyoshi NISHIKAWA and Masao HIROSE

Divisions of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan

Recent progress in transgenic animal models with reporter genes such as *lacI*, *lacZ* and *gpt* has enabled us to assess *in vivo* genotoxicity and carcinogenicity simultaneously. In order to analyze the molecular mechanisms underlying chemical carcinogenesis, we have investigated genotoxic as well as non-genotoxic carcinogens using *lacI* transgenic and *gpt* delta rodents. As genotoxic carcinogens, the heterocyclic amines MelQx and IQ, the nitroso compounds dimethylnitrosamine (DMN) and *N*-nitrosopyrrolidine (NPYR), and the dinking water contaminants potassium bromate (PB) and MX were given to these mice or rats. Dietary 12- or 13-week MelQx or IQ treatment consistently increased mutant frequencies (MFs) of reporter genes in the liver of mice or rats, of which predominant type of mutations was GC to TA transversion. Interestingly, although the MFs were significantly increased even at low doses such as 30 ppm, 78-week feeding of 300 ppm MeIQx failed to induce murine liver tumors. It was thus suggested that post-mutational epigenetic processes might also play a key role in chemical carcinogenesis. Intraperitoneal DMN treatment significantly increased MFs in the liver, kidneys and lungs, of which predominant type of mutations was deletions as well as GC to AT transition, but not in other non-target organs. In fact, the mice receiving DMN for 78 weeks proved to have liver cell adenomas. It was also suggested that both induced and spontaneous MFs are accumulated with aging. Although no significant increase in the MFs was detected in the kidney of mice given PB their drinking water at 800 pm for 12 weeks, a significant increase in the MFs was detected in rats given at 500 ppm, in line with 8-oxoG formation. However, molecular analysis of mutations indicated base substitutions other than GC to TA transversion in addition to short deletions. Surprisingly, a strong *in vitro* mutagen MX did not increase the MFs in mice at all. Meanwhile, as a non-genotoxic carcinogen, di(2-ethylhexyl)phthalate (DEHP) was given to *gpt* delta rats for 13 weeks. However, no increase in *gpt* MFs was found in rats given DEHP. In addition, DEHP failed to increase deletions detected by Spi- assay. Our results thus strongly suggest that these reporter gene transgenic models could offer powerful tools for analyzing molecular mechanisms underlying chemical carcinogenesis and for assessing the risk of unknown environmental carcinogens.

S1-1-3

Recent advances in tumor angiogenesis

Anne M. RYAN

Department of Pathology, Pfizer Global Research and Development, Connecticut 06340, USA

Angiogenesis plays a critical role in normal embryogenesis (implantation, organ development and tissue differentiation) and also a key role in postnatal physiologic processes such as wound healing, longitudinal bone growth and reproduction. Angiogenesis has also been implicated in a variety of pathologic processes, including rheumatoid arthritis, psoriasis, intraocular neovascularization and carcinogenesis. Several positive (aFGF, bFGF, TGF-α, TGF-β, HGF, IL-8, VEGF, angiopoietin and αvβ3) and negative (TSP, SPARC, angiostatin and endostatin) regulators of angiogenesis have recently been identified. Many of the latter are currently being evaluated in clinical trials as potential anti-angiogenic therapeutics for oncology indications. Recent data from several rodent models implicates angiogenesis as a key early event in the multistage process of carcinogenesis. VEGF up-regulation (and subsequent increases in microvessel density) has been reported in the early stages of BBN-induced bladder cancer and MNU-induced prostate cancer in rats. COX-2, which may play a role in carcinogenesis by stimulating tumor angiogenesis via VEGF, is upregulated in 3-methylcholanthrene + diethylnitrosamine-induced lung tumors in rats and correlated with tumor progression. COX-2 inhibitors have been shown to decrease tumor progression and microvessel density and to enhance the anti-cancer effects of radiotherapy and chemotherapy in experimental animals. In addition to experimental rat models of carcinogenesis, several genetically modified mouse models (H-ras Tg, TSP-1 Tg, K14-HPV16 Tg, RIP1-TAG2, APC min/+) have also recently demonstrated the pivotal role of angiogenic factors in the pathogenesis of skin, intestine and pancreatic islet neoplasia. Finally, several new knockout mouse models (TSP-1, TSP-2, IFNR) have been reported with increased susceptibility to carcinogenesis due to alterations in endogenous angiogenic signaling pathways.

S1-1-4

Gene inactivation in mammalian stem cells

Michael W. McBURNEY

Ottawa Regional Cancer Center and Department of Medicine, University of Ottawa,
Ottawa K1H 1C4, Canada

Human cancers arise from cells that have inactivated tumor suppressor genes either by mutation or transcriptional inactivation. The means by which genes become transcriptionally silenced is poorly understood in mammalian cells. We have studied this process in murine embryonal carcinoma stem cells growing in cell culture. Following their stable integration into the genome, transfected reporter genes spontaneously inactivate at high frequency. This gene silencing can be forestalled by treating cells with inhibitors of histone deacetylases such as trichostatin A but once inactivation has occurred, trichostatin A does not reactive expression. DNA methylation of transfected genes occurs but does not seem involved in the initiation of gene silencing although DNA methylation may be important for maintaining the silent state and for suppressing recombination between repeated sequences in the transgene. Direct repeats of identical gene sequences do predispose to more efficient silencing of the repeated genes suggesting that mammalian cells carry out repeat-induced gene silencing (RIGS), a phenomenon well described in simpler eukaryotic systems. The promoters driving expression of our reporter genes appear to promote transcription in both directions creating the potential for repeated genes to give rise to double stranded RNA that could trigger gene inactivation by a process similar to that described in yeast that is dependent on the enzymology of the RNA interference pathway. Finally, we find that inactivation of transgenes can be forestalled by creating transgenes comprised of introns and exons. Our evidence is that the active splicing of primary transcripts feeds back on the local chromatin structure to prevent the invasion of heterochromatinization. Our data suggests that the silencing of transgenes in stem cells is triggered by chromatin assembly or reassembly following DNA recombination and that the active splicing of transcripts maintains chromatin in the open configuration that supports continued transcription.

S1-2 Chair

Carcinogenesis - Current understanding of mechanisms and risk assessments: Genetically modified animal models

Jerrold M. WARD
National Institute of Allergy and Infectious Diseases, NIH, Maryland 20892, USA

Hiroyuki TSUDA
Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan,
Experimental Pathology and Chemotherapy Division, National Cancer Center Research Institute, Tokyo 104-0045, Japan

Over the past 30 years, carcinogenesis assessment of chemicals for human risk assessment has utilized the 2 year rat and mouse biossay as a standard test. Recently, the costs of these tests has risen to 1-2 million dollars per GLP study. Also, modern day human risk assessment requires considerations of possible mechanisms of toxicity and carcinogenesis in rodents and humans. In the search for a more cost effective and perhaps more relevant assays, genetically engineered mice (GEM) and rats (GER) have been developed. These new rodent models have genetic changes in genes for which similar changes have been found in human tumors or in human familial cancer syndromes. Use of these new rodent assays require less animals, time and costs. In addition, relevance to the human situation may be more applicable. Studies to date have shown that genotoxic and nongenotoxic carcinogens usually induce tumors in these rodents faster and with earlier and more multiple preneoplastic and neoplastic lesions than in conventional rodents. Some exceptions to these usual findings have also been seen. The new rodent GEM and GER models may offer faster, more efficient and more relevant models for carcinogenicity assays and human risk assessment. Although further work is needed to develop these models for routine use, they presently have offered initial benefit for drug and chemical screening. The presentations will review concepts and models with the eye to the future.

S1-2-1

Mouse models of human familial cancer syndromes as models for risk assessment

Jerrold M. WARD

National Institute of Allergy and Infectious Diseases, NIH, Maryland 20892, USA

Of the more than 50 characterized familial cancer syndromes, most involve diseases affecting multiple organs and many can be traced to one or more abnormalities in specific genes. These syndromes may be responsible for up to 5% of all human cancers. The genes are often tumor suppressor genes and the syndromes are inherited as a dominant trait. Mutations in the same genes are also often found in sporadic cancers. Genetically engineered mouse models of these syndromes provide a direct application of a mouse model to a human disease including human risk of exposure to carcinogenic substances. Genetically engineered mice (null, heterozygous, conditional knockout) as models for human familial cancer syndromes include those with mutations in *Apc, Atm, Blm, Brca1, Brca2, Lkb1, Men1, Mlh, Msh, Nf1, Tp53, Pten, Rb1, Tsc1, Tsc2, Vhl*, and *Xpa*. These mouse lines provide models not only for clinical disease and pathology, but also provide avenues to investigate carcinogenesis of chemicals and other substances, chemoprevention, molecular pathology, gene and protein functions and therapeutic intervention. This presentation reviews these mouse models and examines their usefulness in toxicology assessment and other aspects of medical research. The mice are available in mouse repositories (http://emice.nci.nih.gov/emice/resources/repositories).

S1-2-2

Mouse models of colon cancer and polyposis

Makoto Mark TAKETO

Department of Pharmacology, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan

As a model for human familial adenomatous polyposis (FAP), we earlier constructed a knockout mouse strain $Apc^{\Delta 716}$. Using this mouse model, we found that cyclooxygenase 2 (COX-2) is markedly induced in the polyp stromal cells, and that inhibition of COX-2 can suppress intestinal polyposis, either by introduction of a COX-2 gene knockout mutation, or by dosing COX-2 inhibitors to the $Apc^{\Delta 716}$ mice (Cell 87:803-809, 1996). Although the direct metabolite of arachidonic acid by COX-2 is PGH_2, it is then converted to varisous PGs. Among them, PGE_2 appears to be the major player in the polyposis and carcinogenesis, and PGE_2 signals through four different cell surface receptors, EP1, EP2, EP3 and EP4. We recently found that the mRNA for the EP2 receptor is strongly induced in the polyps. We then crossed the $Apc^{\Delta 716}$ knockout mice with knockout mice for the EP1, EP2, and EP3 genes, respectively. Only in the EP2 compound mutants, the polyp number and size were reduced dramatically, showing very similar phenotypes to those in the COX-2 gene compound mutants. These results indicate that the PGE_2 secreted from the stromal cells activates cell surface receptor EP2 in an autocrine manner, and increases the cellular cAMP level, which in turn induces more COX-2; a positive feedback mechanism. At the same time, the increased cAMP level induces biosynthesis of angiogenic factors such as VEGF and Ang2 (Nat. Med. 7:1048-1051, 2001). In fact, the microvessel densities (MVD) increased when polyps reached beyond 1 mm in diameter, and this increase was inhibited in the compound $Apc^{\Delta 716}$ mutants with the COX-2 or EP2 gene mutation, indicating that tumorigenic PGE2 signal is mediated through EP2 to cause angiogenesis (Cancer Res. 62:506-511, 2002). While COX-2 plays a key role in tumorigenesis, our recent data suggest that COX-1 is equally important (Cancer Res. 62:in press, 2003). These results will be presented and discussed.

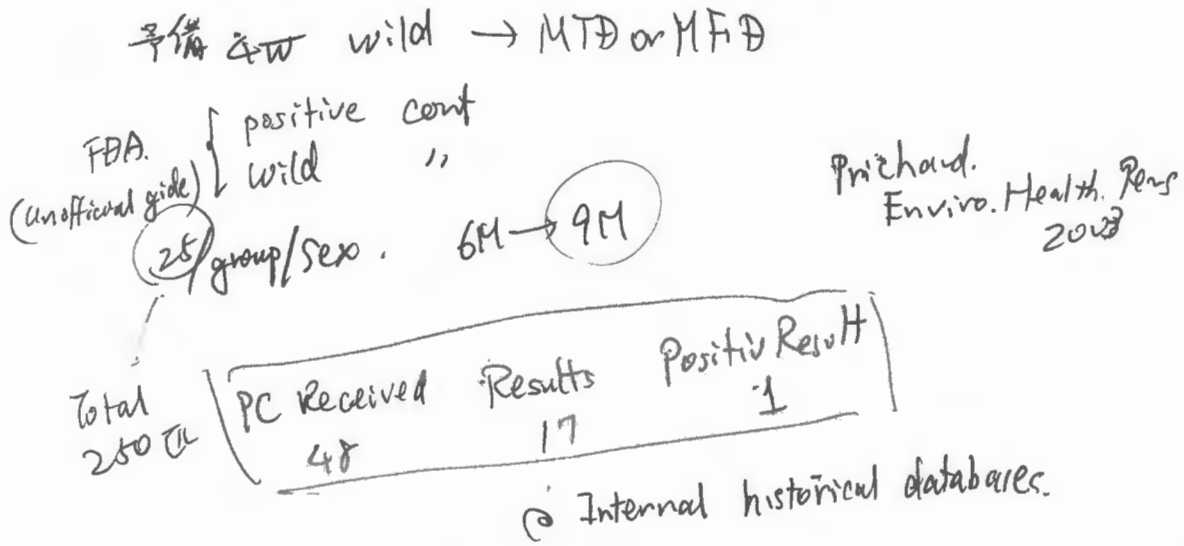
S1-2-3

Update on use of the Trp53 +/- mouse for short-term carcinogenicity testing of pharmaceuticals

Eugenia FLOYD

Pfizer Groton Laboratories, Worldwide Safety Sciences, Connecticut 06498, USA

Over two years have passed since publication of the results from the ILSI-HESI international consortium for the evaluation of alternative models for short-term carcinogenicity testing of pharmaceuticals. The *Trp53* +/- mouse is now generally accepted to be the alternative model of choice for compounds, or compounds with metabolites, that are clearly or equivocally positive in one or more tests of the standard genetic toxicology battery. Regulatory agencies have used negative *Trp53* +/- assay results in weight-of-evidence arguments to allow the continued development of a number of compounds having positive Ames and/or in vitro chromosomal aberration assays. As their experience with the *Trp53* +/- assay has grown, regulators have updated their informal guidance on protocol design and study interpretation. In conjunction, they have identified areas of regulatory concern regarding assays with the *Trp53* +/- mouse. The major recommended protocol change is the increase in group size from 15 to 25 mice per sex per dose to increase the statistical power. A positive control group remains an essential element of current protocol design, although discussions continue about the possibility of substituting genotype confirmation and periodic colony testing for use of concurrent positive controls. Regulatory agencies have expressed concern about several documented failures of response to positive control compounds, the most commonly used being p-cresidine. In response, some laboratories are evaluating methyl nitrosourea (MNU) for use as a standard positive control. Routine inclusion of a wild-type high-dose arm also remains highly recommended by regulatory agencies. Two strains of the TSG-p53® mouse of are now commercially available, the N5 and N12 backcrosses, creating a new challenge for sponsors performing short-term bioassays. The N12 is more closely congenic to the C57 BL/6 (B6) background strain, making it scientifically preferable to the N5. However, the ILSI studies used the N5 strain, and use of the N12 has lagged because of concerns about the extended applicability of the ILSI historical database. Use of *Trp53* +/- mice in dose range finding studies is the safest way to select doses for a bioassay, but wild-type mice (N5 or B6) still can be substituted for genetically altered strains (N5 or N12, respectively). Of most importance, some regulators' concerns about the sensitivity of the current *Trp 53* +/- bioassay have lead to discussions about increasing its duration from 6 to 9 months, a protocol change with potential to greatly impact future use of this model by pharmaceutical companies.



Symposium

S1-2-4

High susceptibility of human c-Ha-ras proto-oncogene transgenic rats to various carcinogens - Possible application for a short term assay system for environmental carcinogens

Hiroyuki TSUDA[1,2], Takamasa ONISHI[2], Shinobu UEDA[2], Yoichiro MATSUOKA[2], Katsumi FUKAMACHI[2], Akihiro NAITO[2], Cheol Beom PARK[2], Beom Seok HAN[2], Chuel Kyu KIM[2] and Nobuo TAKASUKA[2]

Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Experimental Pathology and Chemotherapy Division, National Cancer Center Research Institute[2]

A rat line carrying three copies of the human c-Ha-ras proto-oncogene, including its own promoter region was established (Hras128) and both sexes were found to be highly susceptible to chemically-induced mammary, skin, bladder and esophageal carcinogenesis. Multiple mammary carcinomas developed in almost all animals in as short as 8-12 weeks after oral doses of MNU, DMBA or PhIP on day 49 after birth. Furthermore, skin tumors were induced in males with a DMPA-TPA protocol. Preferential mutation of the transgene but not the endogenous c-Ha-ras gene and relative increase in the activated form of ras protein in these tumors indicated an importance of transgene mutation. An obvious increase in the number of endbuds, the targets of carcinogens, and enhanced proliferation of the component epithelial cells, associated with an increase in the pMAPK/MAPK ratio and c-myc expression, might explain the elevated susceptibility to carcinogens. Furthermore, the observation that mammary carcinomas can be induced by various carcinogens including mammary and various other non-mammary carcinogens in 12 weeks and induction of early stage carcinomas in the abdominal-inguinal mammary glands in all rats within as short as 20 days after MNU application may indicate possible utilization for medium to short term screening of environmental carcinogens irrespective of their organotropism.

S2-1 Chair

Omics - A challenge for the toxicologic pathologists:concerning general toxicity and carcinogenicity

Michael L. CUNNINGHAM
National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina27709, USA.

Tomoyuki SHIRAI
Nagoya City University Graduate School of Medical Sciences, Aichi467-8601, Japan

Toxicogenomics is a new scientific field that studies how the genome is involved in responses to environmental stressors or toxicants. It includes studies of genetics, genomic-scale mRNA expression cell and tissue-wide protein expression(poteomics),metabolite profiling(metabonomics)and bioinformatics for understanding the role of gene-environment interactions in disease. New technologies, such as DNA microarray analysis, can measure the expression of thousands of genes at a time providing the potential to accelerate discovery of toxicant pathways and specific gene targets. Studies of gene and protein expression can provide mechanistic insight into toxic responses and disease pathologies. It is expected that the power and potential of these new toxicogenomics methods are capable of revolutionizing the field of toxicology. Changes in gene expression as a result of chemical exposure can indicate activation of a signal transduction pathway that is responsible for disorder of cell function. Such information can be combined with other biological and chemical information to explain the mechanism(s)underlying a toxic response. Global gene expression profiles for chemicals from different mode-of-action classes can provide gene expression" signatures"of chemical. Gene expression profiling may be consistently and mechanistically linked to specific toxic effects and disease. In other words, if gene profiling specific to certain class or category of chemicals was established, these new technologies can be applied to predict outcome of a certain chemical (predictive toxicology). Here we have4speakers focusing liver disorders such as liver cell degeneration/necrosis and liver tumors. Two of them will present current data on gene expression profilings or protein profilings as predictive tool for hepatocarcinogenic potential of chemicals, and the other two will talk about gene expression profiles in the liver of rats exposed to a hepatotoxin or stress induced by a specific diet.

S2-1-1

Toxicogenomic approach for detection of carcinogenic substances

Tomoyuki SHIRAI[1], Makoto ASAMOTO[1], Kazunari TSUJIMURA[1,2], Masaru SEKIJIMA[3], Nobuyoshi MIKAMI[4] and Masanori OTSUKA[2]

Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Chemical Evaluation and Research Institute[2]
Mitsubishi Chemical Safety Institute Ltd.[3]
Sumitomo Chemical Co. Ltd.[4]

A long-term rodent bioassay for carcinogenicity is a standard method to predict carcinogenic hazard of chemicals to human beings. However, this assay needs a long term and a lot of costs. To overtake these issues, short or medium-term bioassays have been developed, but there are still limitations in these assays especially for detection of non-genotoxic carcinogens. To address these, the Ministry of Economy, Trade and Industry, Japan has started a toxicogenomics project in 2001 to develop a novel bioassay based on toxicology studies and gene expression profiles.
Five-week old male Fisher F344 rats were treated by daily oral gavages with known carcinogenic and non-carcinogenic compounds at two doses for up to 28 days. Reference compounds are classified to categorizations; mutagenic hepatocarcinogen, non-mutagenic hepatocarcinogen, mutagenic non-hepatocarcinogen, non-mutagenic non-hepatocarcinogen and non-carcinogen. The liver and colon were sampled at 1, 3, 7, 14 and 28 days. Besides of classical toxicological studies, gene expression profiles were obtained using in-house cDNA microarrays containing approximately 9,000 genes. Genes for prediction of carcinogenicity were selected by statistical methods and analyzed by cross validation probability tests, clustering analysis and principal components analysis. Correct predictive ratio for carcinogenicity was over 95% when rats were treated with chemicals over 14 days.
These results indicate short-term bioassay systems of rats for carcinogenicity using gene expression profiles are promising.

S2-1-2

Proteomic expression profiling of liver toxicity in rat using 2D-DIGE technology

Hidenori YAMANAKA[1], Kazunari TSUJIMURA[1], Masanori OTSUKA[1], Yoshikuni YAKABE[1], Satsuki HOSYUYAMA[1], Koichi SAITO[2], Kayo SUMIDA[2], Masaru SEKIJIMA[3], Koji NAKAYAMA[3], Yukiko KAWANO[3], Yasuro SHINOHARA[4], Tomoyuki SHIRAI[5]

Chemical Assessment Center, Chemical Evaluation and Research Institute, Japan, Saitama 345-0043, Japan[1]
Sumitomo Chemical Co., Ltd[2]
Mitsubishi Chemical Safety Institute Ltd[3]
Hokkaido University[4]
Graduate School of Medical Sciences, Nagoya City University[5]

Hepatic responses to 28-day repeated dose of 24 chemicals were monitored using two-dimensional differential gel electrophoresis (2D-DIGE) technology (Amersham Biosciences, UK Limited). The method, which has ability to measure expression change of hundreds of proteins at once, provided new insights into the effects of chemicals on biological systems. For example, the genotoxic hepatocarcinogen diethylnitrosoamine (DEN), peroxisome proliferator Clofibrate and non-carcinogen 4-nitro-o-phenylenediamine gave rise to greater than 1.5-fold quantitative changes compared to control in 236 and 108 and 60 liver proteins after 28 days of 20, 250 and 250 mg/kg/day repeated dose, respectively. Especially, glutathione S-transferase, pi, that known to express in a wide variety of tumors, showed 95-fold increase by administration of DEN. The proteomic expression signatures of 24 chemicals including genotoxic carcinogen, non-genotoxic carcinogen and non-carcinogen were compared and hierarchical clustering was applied to them to find the characteristic pattern of liver protein to the type of toxicity of chemicals.

S2-1-3

Gene expression profiling of rat liver fibrosis, cirrhosis, and cancer induced by choline-deficient, L-amino acid-defined diet

Ivan RUSYN[1], Oksana KOSYK[1], Christine POWELL[1], Ayumi DENDA[2], Yoichi KONISHI[2], Fumiyuki UEMATSU[3] and Dai NAKAE[3]

Department of Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, North Carolina 27599, USA[1]
Department of Oncological Pathology, Cancer Center, Nara Medical University[2]
Department of Pathology, Sasaki Institute, Sasaki Foundation[3]

The consecutive emergence of the fatty liver, necrosis, compensatory proliferation, accumulation of preneoplastic foci, development of fibrosis, cirrhosis, adenomas and carcinomas are the hallmark pathological changes induced in rat liver by choline-deficient, L-amino acid-defined (CDAA) diet. This model of hepatocarcinogenesis has been extensively studied and the mechanisms that are thought to underline the rapid progression of the disease include, among other things, altered fatty acid metabolism, hypomethylation of various genes, changes in gene expression, activation of hepatic stellate cells, oxidative stress to DNA, and accumulation of mutations. The goals of this study were to investigate the mechanisms of oxidative DNA damage and repair induced by CDAA diet, and to determine what genes are regulated at different stages of liver injury in this model. Male Fisher 344 rats were given CDAA diet, or matching choline-sufficient (CSAA) control diet for up to 80 weeks and liver samples were collected. A significant increase in oxidized purines in genomic DNA was detected as early as 4 weeks of CDAA treatment. The number of mutagenic apurinic/apyrimidinic sites was increased at 12 and 30 weeks on a choline-deficient diet. Furthermore, the expression of base excision DNA repair genes for 8-OH-dG DNA glycosylase, N-methylpurine DNA glycosylase, apurinic/apyrimidinic endonuclease, polymerase beta, methylguanine methyltransferase and proliferating cell nuclear antigen was significantly elevated in CDAA-treated animals beginning at 4 weeks. Gene expression profiling was conducted on liver samples from 4, 12 and 80-week groups, as well as on liver tumors from the 80 week CDAA-fed animals. The selection of tissue samples was performed to reflect various stages of liver pathology in this model. In general, three biological replicates were analyzed in each group. Affymetrix® microarrays (RAE230A, ~16,000 genes) were used. Several normalization and image intensity calculation procedures were used to analyze the results. Clustering analysis of the individual samples on all of the genes represented on the array revealed a strong segregation of CSAA and CDAA groups with a further separation of replicate samples into individual treatment groups. The analysis of individual clusters of genes specific for each time point and treatment showed that CDAA-induced liver pathology could be correlated to specific changes in gene expression. Collectively, this study demonstrated that global changes in gene expression can be phenotypicaly anchored to liver pathology, and this new information may be used to uncover the complexity of the molecular changes associated with various stages of liver disease.

S2-1-4

Gene expression changes in F344 rats following a pharmacological dose of acetaminophen

Michael L. CUNNINGHAM

National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA

Acetaminophen (APAP) is a commonly used over-the-counter analgesic and antipyretic that is hepatotoxic in high doses. Centrilobular necrosis occurs in the liver following APAP exposure after glutathione stores are depleted, allowing its toxic metabolite N-acetyl-p-benzoquinone imine to react with cellular macromolecules causing mitochondrial dysfunction and hepatotoxicity. We treated ad libitum fed male Fisher 344 rats orally with APAP at doses that may be experienced during normal therapeutic regimes, 50 and 150 mg/Kg and examined gene expression changes in the liver after 6 hours. Although no clinical signs of toxicity were observed by histopathologic examination or clinical chemistry measurements, gene expression changes were observed consistent with energy metabolism disruption. The adaptive response by the liver included down regulation of fatty acid synthesis and cholesterol metabolism, pathways that are energy intensive. Upregulation of stress related genes such as heme oxygenase and metallothionein 1 were also observed. Several of the changes were consistent with previous reports but additional novel gene changes were observed. By 48 hours after dosing only a small number of genes are differentially expressed and these appear to be associated with a return to energy homeostasis. These early changes in gene expression indicate a disruption of energy metabolism and of normal metabolic processes at pharmacologic doses of APAP. These data suggest that differential gene expression analysis may provide a more sensitive indicator of toxicity than routine histology and clinical chemistry evaluations.

S2-2 Chair

Omics - A challenge for the toxicologic pathologists: Concerning reproductive toxicity, immunotoxicity and toxicity of endocrine disrupting chemicals

Paul-Georg GERMANN
Preclinical Safety, Novaitis Pharma AG, Basel 303-0043, Switzerland

Keizo MAITA
Study Planning and Consultation, The Institute of Environmental Toxicology, Ibaraki 303-0043, Japan

There has been an increasing concern among people if their substantial system maintaining healthy life and reproductive activity might be affected by environmental toxicants while they are not aware of it. To develop proper methods evaluating the toxicity and to study the mechanism of the mode of action with those toxicants should be an urgent task for the toxicologic pathologists.

This first part of this session will present sophisticated interactions between environmental endocrine active contaminants and sensitive tissues in the talks of Drs. Nagao and Harada. Dr. Nagao will give his presentation on "Biological effects of diethylstilbestrol in rats and mice" and Dr. Harada on "Mechanism of toxic changes and tumor development by DDT in F344 rats."

In the second part of this session the current understanding of immunological responses against chemical exposure will be presented by Drs. Descotes and Germolec. During the last years, accumulating evidence occurred that potential immunotoxicity might be associated with significant increase in incidence and severity of clinical side effects. The scope of the second part of this IFSTP/JSTP workshop is dedicated to immunotoxicology & immunotoxicopathology. There we would like to present different aspects, views and issues of the current state of the art of this important field, also by giving specific examples. The awareness of immunotoxicology has led to an intensified and controversial discussion within the different Guideline Committees to address this important field adequately in preclinical studies. Specialised animal models and modified study designs in preclinical immunotoxicology studies are of increasing importance. Their difficulties especially their limitations in interpretation are the topic of Dr. Descotes presentation: "Importance of Immunotoxicology on Safety Assessment: A Toxicologists View." Dr. Germolec is contributing the toxicological pathologists' view in her talk about "Improving the Sensitivity and Predictability of Current Testing Strategies for Immunotoxicology." Pathologists are playing an increasing role with their interpretation of immunotoxic effects. An international pathology working group report with its results is pointing to the usefulness of extended pathological contributions. Finally, in the discussion we will try to figure out future development trends in the assessment of new drugs as well as open and emerging issues in immunotoxicology.

The result of the discussions will be also included in the full summary of the workshop.

S2-2-1

Biological effects of diethylstilbestrol -Transgenerational effects of endocrine disrupting chemicals-

Tetsuji NAGAO

Department of Life Science, Kinki University, Osaka 577-8502, Japan

In parallel with increasing concern about the reproductive effects of endocrine disrupting chemicals in the environment, evidence has been accumulating that developmental exposure to estrogenic hormones can cause a variety of abnormalities in the male reproductive tract (reviewed by Nagao *et al*., 1998, 1999, 2003). However, little is known about transgenerational toxicity of estrogens, except for the potential of a transgenerational carcinogenic effect of diethylstilbestrol (DES) (Newbold *et al*., 1998, 2000). We carried out the present study as an attempt to fill this gap in our knowledge. Male mice prenatally treated with DES were mated with untreated females, and the resulting fetal offspring were inspected for external malformations. A significant increase in the incidence of malformed fetuses over the control level was noticed in the offspring whose sires were treated with a high DES dose, but it was not seen in the offspring of males treated with a low dose. The high dosing, but not the low dosing, caused partial atrophy and feminization in the genital tracts. Ethinyl estradiol and estradiol benzoate also showed transgenerational teratogenicity when applied prenatally at a dose which caused histopathological changes in the testes. Germ-cell series in normal testis have mechanisms to select against spontaneously arising mutations. However, these selection mechanisms may not function efficiently in chemically damaged testis. Based on these results and considerations, I propose as a working hypothesis that male-mediated developmental toxicity (teratogenicity) may occur as a consequence of testicular toxicity.

(supported by a grant from The Science and Technology Agency: Special Coordination Funds for Promoting Science and Technology)

Nagao T., Fujikawa K., 1998, *Cong Anom* **38**:1-8.
Nagao T., 1999, *Environ Mutagen Res* **21**:267-72
Nagao T., 2003, *Advances in Male Mediated Developmental Toxicity.,* Kluwer Academic/Plenum Publishers
Newbold RR., Hanson RB., Jefferson WJ. *et al*., 1998, *Carcinogenesis* **19**:1655-63.
Newbold RR., Hanson RB., Jefferson WJ. *et al*., 2000, *Carcinogenesis* **21**:1355-63.

S2-2-2

Mechanisms of toxic effects and tumor development caused by DDT in F344 rats

Takanori HARADA, Ryoichi OHTSUKA, Makio TAKEDA, Naruto TOMIYAMA, Machiko SAKA, Tadashi KOSAKA, Junya SASAKI, Sayuri KOJIMA, Naofumi TAKAHASHI, Yukiko TAKEUCHI, Toshinori YOSHIDA, Maki KUWAHARA, Akiko ENOMOTO, Nobuaki NAKASHIMA and Keizo MAITA

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

In order to clarify the mechanisms of toxic effects and tumor induction by DDT, we conducted a 2-year feeding study of *p,p'*-DDT in F344 rats of both sexes at dose levels of 0, 5, 50, and 500 ppm. These animals were sacrificed after 26, 52, 78, and 104 weeks of treatment and subjected to toxicokinetics, hematology, biochemistry, necropsy, and histopathology. In addition, potential factors involved in hepatocarcinogenesis including microsomal enzymes, oxidative stress, cell proliferation, gap junctional intercellular communication (GJIC), and gene expression in target tissues were examined. Hormone assay was also performed on control and high-dose animals at termination of treatment.

Males and females in the high-dose group showed tremor and depression of body weight gain which were more evident in females. A marked hair loss was also noted in females. The concentrations of DDT and its metabolites in the plasma and brain tended to be higher in females, while those in the liver were higher in males. This difference might be one of the factors contributing to sex differences in toxic effects and tumor induction. Analysis of hepatic microsomal enzymes demonstrated dose-dependent increases in P450 isozyme contents of CYP2B1 and CYP3A2 which were more evident in females. Since CYP3A2 is androgen-dependent, the induction of this isozyme in females indicates that DDT is able to modulate sexual metabolic dimorphism. As to serum hormone levels in the high-dose group, a decrease in testosterone and an elevation in progesterone were noted in males and a decline in estrogen in females. Histopathologically, centrilobular hepatocellular hypertrophy was observed in all dose groups. Eosinophilic hepatocellular foci increased in number and size with time in a dose-dependent manner and the incidence of hepatocellular tumors increased in males in the mid-dose group and both sexes in the high-dose group. These groups exhibited increases in hepatic lipid peroxide and 8-hydroxydeoxyguanosine and an inhibition of GJIC. The oxidative stress may play an important role in the hepatocarcinogenesis by DDT. In other organs/tissues, the occurrence of chronic nephropathy was accelerated in the mid- and high-dose groups of both sexes. In the high-dose group, the incidences of pituitary adenomas and testicular Leydig cell tumors increased in males and hypertrophy/hyperplasia of ovarian interstitial gland were frequently observed in females. These findings may reflect an endocrine disrupting effect of DDT. Microarray analysis of gene expression in target tissues is now underway.

S2-2-3

Immunotoxicology in drug safety evaluation: A medical toxicologist's view

Jacques DESCOTES

Poison Centre and Claude Bernard University, Lyon 69007, France

Over the past decades, the clinical experience unambiguously demonstrated that drug-induced immunotoxic effects can result in significant morbidity and even mortality. Immunotoxic effects are divided into four broad categories: immunosuppression, immunostimulation, hypersensitivity and autoimmunity. Each category is associated with a specific pattern of possible adverse health consequences, the nonclinical prediction of which should follow different strategies. Concern was initially on immunosuppression, but hypersensitivity and immunostimulation are increasingly key issues. Although absolutely essential, the histological examination of the lymphoid organs is not considered sufficient to assure the reliable detection of unexpectedly immunosuppressive drugs. At least one immune function assay, primarily a T-dependent antibody response assay, is recommended. Additional testing is decided case by case depending on early toxicity findings, possible histology changes, and the structure and pharmacological properties of the drug. It is not known whether function assays typically used to predict immunosuppression are also applicable to detect unexpectedly immunostimulatory drugs. Specific assays, e.g. cytokine release assays, can be helpful in some circumstances. For the purpose of immunotoxicity risk assessment, host resistance assays, e.g. experimental infections (immunosuppression) or autoimmunity models (immunostimulation) may be needed. Current animal models and assays are seldom valid to predict the risk of drug-induced hypersensitivity. Guinea pig models, but not the LLNA can be used for the prediction of contact sensitization induced by drugs. Immunogenicity of therapeutic proteins is sometimes evaluated in monkeys. Finally, drug-induced autoimmune reactions cannot be predicted in most instances. The timing and design of nonclinical immunotoxicity studies are critical issues. Although no guidelines require the evaluation of drug immunotoxicity prior to clinical trials, it would be logical to perform some immunotoxicity studies early in nonclinical safety evaluation. Inclusion of a T-dependent antibody response assay in safety pharmacology is an interesting alternative. Other critical factors to be considered include particular dose-response relationships, the redundancy of immune responses, the role of genetic factors and inter-species differences. Finally, as more and more studies will be performed routinely to meet recent regulatory requirements, a wealth of unexpected immune changes is likely to be seen in nonclinical studies. It is therefore essential that selected endpoints of nonclinical studies can also be measured in clinical trials.

S2-2-4

The sensitivity and predictability of current testing strategies to evaluate immunotoxicity

Dori GERMOLEC[1], Abraham NYSKA[1], C Frieke KUPER[2], Christopher PORTIER[1], Michael KASHON[3], C KOMMINENI[3], Keith JOHNSON[4] and Michael LUSTER[3]

National Institute of Environmental Health Sciences, North Carolina 27709, USA[1]
TNO Nutrition and Food Research[2]
National Institute of Occupational Safety and Health[3]
Dow Chemical Company[4]

The identification of chemicals that have the potential to cause injury to the immune system is of considerable public health significance, as alterations in immune function can lead to increased incidence of hypersensitivity and autoimmune disorders, infectious diseases or neoplasias. Experimental animal data collected using standardized testing panels have provided a database from which the sensitivity and predictability of a variety of tests commonly used for the screening of chemicals for immunotoxicity has been evaluated. A working group was established to design and implement a study to evaluate the sensitivity and predictability of extended histopathology as an indicator of immunotoxicity, as compared with the National Toxicology Program's functional testing battery. Standardized slide sets from thymus; spleen and mesenteric lymph nodes were generated for 10 chemicals, which had previously been evaluated for their immunotoxicity using functional tests. A semiquantitative assessment was adopted in order to estimate the histopathological changes within different anatomical compartments of the lymphoid organs using the diagnostic terms for identifying and semiquantitating the histopathologic changes recommended by Kuper *et al*. (2000). Findings from each pathologist were entered into a common database and evaluated for agreement between pathologists, sensitivity of individual parameters and correlation with the "immunotoxic" call. A direct comparison of the ratings for each pathologist indicated that certain individuals tended to be more conservative while others were more able to discern subtle changes. Additional analyses examined the consistency of a pathologist's ratings in a single tissue by investigating the correlation among all the measures in the same tissue type. When data were examined for each pathologist independently, it was noted that specific histopathological changes such as cortical cellularity in the thymus were more easily evaluated and demonstrated the best correlation. For several spleen and lymph node measures, the outcomes for each pathologist were less well correlated. When comparing histopathology to functional tests, the antibody forming cell (AFC) assay detected immune suppression in two instances where no changes in pathology were indicated. In contrast, the AFC assay failed to detect oxymetholone as immunotoxicant, although other immune parameters, as well as extended histopathology indicated immunologic changes. These studies suggest that there is not a single most sensitive parameter for assessing damage to the immune system, but rather that one or two functional tests combined with pathology examinations would be needed to accurately flag a compound as immunotoxic and provide data into its mechanism of action.

S3 Chair

Recent trend of information technology in toxicological pathology science

Gerd MORAWITZ
The Fraunhofer Institute of Toxicology and Aerosol Research, Drug Research and Clinical Inhalation, Hannover 30625, Germany

Ikuo HORII
Pfizer Global Research & Development, Worldwide Safety Sciences - Nagoya, Aichi 470-2393, Japan

Recently, introduction and integration of novel information technologies (IT) has become the dominant trend in various scientific domains, and yielded considerable results in data management with synergistic effects on scientific data evaluation. Application of IT has been spreading deeply and widely also into the field of toxicological pathology (TP) science.

This symposium has been organized to study the necessity of the effective use of IT and its applicability in the process of data gathering, data retrieval, report preparation and evaluation relevant to TP. It is essential to smartly introduce / develop the IT system to establish a significant database for effective use of data obtained, and also indispensable in terms of TP safety evaluation to develop a synergistic workstation towards the goal of establishing a mutually accessible database between TP science and the other fields of science. In other words, sufficient database establishment using a warehousing approach is expected in order to improve the difficulties in data management due to the diversity and complexity in TP safety evaluations. Related to these, we will touch upon the valid database management, telepathology system, imaging informatics system and pathology training program.

Presentations and discussions will be held mainly on the following themes.
(1) Pathological data gathering / retrieval / evaluation and its usability as a database
(2) Strategy and prospect of telepathology system
(3) TP imaging technology as a new approach
(4) Pathological evaluation / training program under the information technology

We hope that information / outcome at this symposium, basically on IT, will offer insights that will lead to future development in the field of TP science.

S3-1

Pathology information system

Nobuyuki OHASHI

Biosafety Research Center, Food, Drugs and Pesticides, Shizuoka 437-1213, Japan

An-Pyo Center has been using the PATHOTOX, an Integrated Pathology and Toxicology System Network, since 1991 with the successional improvement by user's requirement and for the increased reliability. This system has been used to evaluate many toxicological examinations including over one hundred carcinogenetic studies up to now. This computer system offers high user interface with a network multi-windows system (X-windows) and prompt response by using UNIX server (Solaris 8) and windows 2000 client. Simultaneous recording of microscopic findings can be done on a parallel with evaluating observation of histopathological slides. Entry of histopathological findings is done by selecting words or phrases from multiple windows comprising organ and tissue specific finding list, a general histological dictionary, an organ dictionary, a comment list, and cause of death section. The management of these dictionaries is important to use this system more efficiently because pathologists need to confirm every word in the dictionary for every histopathological lesion according to the universally accepted criteria. Currently, the combined image system with histopathological findings is available to share criteria for each pathological finding by pathologists. Pathologists are able to discuss on the histopathological features with dictionary images on the computer screen. Furthermore, necropsy findings and clinical data, including hematology, clinical chemistry, and urinalysis data of individual animals can be displayed in screens. The system produces a variety of reporting and retrieval functions combined with statistical analyses. Peto's trend test can be executed for some of tumor findings to reveal a significant occurrence of tumors. For the authorized user, senior pathologist, this system has a peer review function, being divided into the macro findings input, the micro findings input, the findings data reference, the master management, etc. For security, each user has the account and must change the password every three months. Network security system is equipped with number of firewalls and operating system functions. Users living in the remote area from the An-Pyo Center can safely use this system from another site (The Saitama branch) through the VPN (Virtual Private Network).

S3-2

Linking worldwide pathology expertise with the telepathology system

Ingrid PRUIMBOOM-BREES, Mark TENGOWSKI and Ricardo OCHOA

Pfizer Research and Development, Department of Pathology - Groton Safety Sciences, Connecticut 06340, USA

The telepathology system consists of a suite of integrated internet-enabled, computer-driven microscopes installed at each user site. Each system is equipped with a standard computer and internet server, a robotic optical microscope; a video or digital camera and an automatic multi slide holder. By automating the slide loading and unloading process, up to 50 slides can be scanned and digitized unattended, and overnight. Because the computer connects the microscopes to the Internet, the users can view and navigate the microscopic slides from across the globe or just down the hall using their standard PC and internet connection. The microscopic images appear on the users computer screen in real time, and the software allows selection and full navigation of the slide, including control of the objective (up o 100X dry objective), focus, illumination and digital photography. Thanks to the automatic loading and the remote internet access, pathologists can collaborate asynchronously or concurrently with colleagues across the globe, and this make the telepathology system ideal for applications such as consultation around individual microscopic findings or to peer review an entire toxicology study. In conclusion, the telepathology system allows industries to breakdown distance barriers and to leverage worldwide pathology expertise while reducing travel expenses and the costs of duplicating areas of expertise at multiple sites.

S3-3

Image informatics: Current and future challenges

Judith A. NOLAN

Scimagix, Inc., California 94403, USA

Digital imaging techniques have provided researchers with new capabilities but with an equal number of difficulties. With the growing acceptance of digital imaging technology in medical research, there is a rapidly expanding need for systems to aid in the management and mining of this type of data. One of the main challenges is to develop a system that can read and display a large number of different image formats, including proprietary formats from instrument manufacturers. These images may be created with a variety of techniques, including histochemical staining of tissues, confocal and video output and multi-channel fluorescent detection. Users require the ability to search both the images and their associated metadata, and to be able to easily compare them on any workstation. Centralized management enables these capabilities, along with providing a mechanism to consistently annotate and share the data among colleagues. Once such a system is put in place, users will be able to access images readily and may concentrate on gaining more quantitative data from the images using image analysis techniques. Most imaging scientists will combine data obtained from commercial and their own proprietary algorithms. The main difficulty with the analyses is comparing results from different analyses when data is in very different formats. Technology producing large volumes of images, such as High Content Screening, further magnifies the problem. Researchers in pre-clinical and clinical environments have additional validation considerations for images and associated data, since these electronic records may invoke the 21 CFR Part 11 ruling. This presentation will discuss these issues and review methodologies that will assist imaging scientists to best control and extract valuable information from their data.

S3-4

AFIP online veterinary pathology training programs

Mark MENSE, Terrell BLANCHARD, Thelda ATKIN, Sophie BOUCHIHA-OLSON, Sean HAHN, William INSKEEP and Dale DUNN

Department of Veterinary Pathology, Armed Forces Institute of Pathology, Washington DC 20306, USA

The Department of Veterinary Pathology, Armed Forces Institute of Pathology, has implemented three online resources in veterinary pathology. 1) The Veterinary Systemic Pathology Resource, funded from a Department of Education, Fund for the Improvement of Post-secondary Education grant, utilizes the same eleven-organ system training archive as the AFIP residency program. Each organ system is divided into bacterial, fungal, metabolic/miscellaneous, neoplastic, parasitic, toxic, and viral entities. The database can be viewed as unknowns or sorted by organ system, animal species, and disease category for over 675 entities in numerous species. Linked sequential images with legends are provided to simulate examination of the tissue at the microscope. Lower magnification images contain hyperlinked "hotspots" which delineate the viewable area contained in the next higher magnification. Each case manuscript is reviewed and updated every three years. Future projections include the addition of virtual slides, gross pathology images, cytology/clinical pathology data, electron micrographs, immunohistochemistry micrographs, links from references to online publications, and development of online testing modules. 2) The Registry of Toxicologic Pathology web conference provides toxicologic pathologists with an anonymous forum for the exchange of ideas and information concerning toxicologic pathology research and related issues. There are nine conferences annually, with four cases per conference. Registered participants in the conference include more than 45 international corporate and government agencies, greater than 675 individual users, and nine US veterinary schools. Four hours of continuing education credit is offered per conference under the AAVSB of RACE approval program. 3) The Department of Veterinary Pathology provides the Wednesday Slide Conference on the World-Wide-Web to veterinary pathologists worldwide in an effort to enable everyone to participate in this valuable forum. The Wednesday Slide Conference is in its 51st year, has 135 international participants and is considered an integral part of many international veterinary training programs. This yearly conference is held on each Wednesday for 25 weeks from September through May for residents and board-certified veterinary pathologists. Each conference presents four unknown cases of classic or newly discovered disease entities, encompassing modern concepts and the most recent literature references. The conference focuses on morphologic and etiologic diagnoses, board quality descriptions, differential diagnoses, and classic gross and histologic lesions, with comparative aspects among the common species affected.

S4 Chair

Future challenges: Stem cell development, infectious agents and others -Introduction

Kunio DOI

Veterinary Pathology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

The last symposium of JSTP/IFSTP (IATP) 2004 deals with some possibilities of future challenges in toxicologic pathology. Recently stem cells have been attracting a great attention in the field of biomedical science. During the last decade, a good deal of information about stem cells has been newly added every year, and it is no doubt that such information will be necessary for the development of toxicologic pathology in near future. In this symposium, at first, three researchers show us the current of stem cell research from various viewpoints. Namely, Dr. Nakatsuji gives lecture on establishment and manipulation of monkey and human ES cell lines as well as reprogrammming of somatic cells into pluripotent stem cells, and further mentions ethical problems of usage of human ES cells. Dr. Shiota emphasizes an importance of DNA methylation pattern of CpG islands, and proposes a need of evaluation of mutagens as well as epimutagens in pathological and toxicological researches. Dr. Harrison introduces many examples of clinical applications of stem cell research. Next, Dr. Brown gives us a general view of emerging zoonotic diseases such as bovine spongiform encephalopathy, and refers to how emerging zoonotic diseases reflect upon toxicologic pathology.

S4-1

Establishment and manipulation of monkey and human ES cell lines for biomedical research

Norio NAKATSUJI

Stem Cell Research Center, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan

We have established several ES cell lines from blastocysts of the cynomolgus monkey. After various improvements of culture methods, they can be maintained in culture stably for long periods. They maintain the normal karyotype and produce several differentiated cell types in culture. When such ES cells were transplanted into SCID mice, they produced teratomas containing many differentiated tissues. Unlimited proliferation capacity of ES cells in culture and subsequent differentiation into various types of functional cells could be used for cell transplantation therapy. For example, we have succeeded to produce dopaminergic and other neurons and pigmented epithelia from our cynomolgus ES cells.

For further progress in basic research and the medical application of primate and human ES cells, we need to improve many aspects of the manipulation of stem cells. For example, we like to produce genetically modified monkey and human ES cells for reduction of antigenicity in cell therapy or efficient selection of particularly useful cell types. After improvement of the transfection and selection methods, we can produce ES cell clones with integrated transgenes at efficient and reliable rates.

Nonhuman primate ES cell lines provide important research tools for basic and applicative research. In most countries, usage of human ES cells is strictly regulated because of ethical problems. In such situations, monkey ES cells provide invaluable materials for research to advance various aspects of regenerative medicine. Also, they are indispensable for pre-clinical research using primate models of allogenic cell transplantation therapy to evaluate effectiveness, safety and immunological reaction in physiological conditions similar to the treatment of human diseases. Our cynomolgus ES cells have been already distributed and utilized by many laboratories.

We are now carrying out a project to establish human ES cell lines from frozen surplus human embryos. The Japanese government guidelines, issued in September 2001, require strict procedures for establishment and research usage of human ES cells. We have obtained the approval and national grants to produce human ES cell lines in April 2002. So far, we have established and characterized one human ES cell lines. We expect to produce additional few lines. We have obligation to distribute ES cells to other institutes in Japan with approved research plans, and we plan to start the distribution by the end of this year.

We are also investigating reprogramming of somatic cells into pluripotent stem cells by cell fusion with ES cells. Such reprogramming could be used to produce pluripotent stem cells that have the same genome with a patient to avoid immunological rejection without production of the nuclear-transfer-cloned embryos, which would raise various ethical problems in many countries including Japan.

S4-2

Clinical applications of stem cell research

David HARRISON

Department of Pathology, University of Edinburgh, Scotland EH8 9AG, UK

One of the challenges is knowing which stem cells are going to be useful for therapeutic purposes. Should we be relying on embryonal stem cells or can adult derived stem cells, haematopoietic or otherwise, be used. In this overview we will explore possible uses as well as discussing, from a pathology perspective, some of the key issues still to be resolved.

The first key issue is plasticity: the ability of a stem cell to adopt the characteristics of the differentiated cell intended. Whilst it is clear that embryonal cells are totipotent there is considerable debate about the plasticity of other stem cells, for example bone marrow derived.

There are a number of overlapping possible uses for stem cell therapeutics some of which, for example bone marrow transplantation, are already widely used. They are (i) replacement (ii) substitution (iii) regeneration (iv) repair of gene defects (v) reproductive cloning.

Replacement therapy may offer hope in Parkinson's disease or Alzheimer's by directly adding back cells that have been damaged or destroyed. The combination of gene therapy with stem cell therapy offers the possibility of treating old diseases in new ways, for example glucagons-like peptide 1 in treatment of type 1 diabetes. Regeneration is widely thought to offer possibilities in diseases like liver failure, renal disease and emphysema. Yet, as pathologists we are aware that chronic disease is not always a deficit of cells but rather a problem of order, architecture and remodelling. Specific repair of gene defect, for example sickle cell anemia, is an attractive target with many millions of potential patients. Reproductive cloning and the associated technology of nuclear transfer raise moral and ethical problems, as well as considerable technical problems.

Stem cell research is expanding rapidly. It is swallowing up gene therapy and there is a need to pause and reflect what we are trying to achieve, and to place therapy in the context of an understanding of the disease we are trying to treat.

S4-3

DNA methylation pattern of CpG islands for epigenetics pathology and toxicology

Kunio SHIOTA

Cellular Biochemistry, Animal Resource Sciences / Veterinary Medical Sciences, The University of Tokyo, Tokyo 113-8657, Japan

"Epigenetics" means the study of heritable changes in gene-activity without changes in DNA sequences. In vertebrates, methylation of DNA mainly occurs at the 5'-position of cytosine in a CpG dinucleotide forming 5-methylcytosine. Methylation of DNA plays a profound role in transcriptional repression of gene expression through several mechanisms. Sequences of CpGs are not evenly distributed in the mammalian genome. They appear at a 10 to 20 times higher density in selected regions than in other regions, and regions enriched with CpGs are known as CpG islands. These CpG islands are used as landmarks to find genomic regions in bulk DNA sequences, because CpG islands are generally found in transcription units. Generally, it has been recognized that CpG islands are unmethylated in normal tissues, except the CpG islands involved in X inactivation and genomic imprinting. However, most data on DNA methylation mediated gene repression concerns TATA-less and CG-rich promoters that are associated with CpG islands. Scanning of a few thousands of islands from various cells and tissues of mouse, including ES cells and embryo revealed that CpG islands having tissue-dependently and differentially methylated region (T-DMR) were numerous and widespread in the genome. The T-DMR panel clearly indicates that DNA methylation is cell type specific. The human genome project identified 30,000-40,000 protein coding genes, and there are approximately 29,000 CpG islands. There are 30,000 genes and 15,000 CpG islands in the mouse genome. Tissue-specific promoters revealed that 50% of CpG islands are linked to tissue-specific genes. The remaining tissue-specific promoters do not associate with CpG islands. A single fertilized egg gives rise to a complex multi-cellular organism consisting of at least 200 differentiated cell types. Most cells differentiate without changes in DNA sequence through activation of a particular set of genes and inactivation of others. The molecular basis for the memory for activated or inactivated gene sets, which is inherited to the cells' next generations, is critical for differentiation and development of multicellular organisms. Thus, formation of DNA methylation pattern underlies mammalian development, and epigenetic errors cause other diseases. Changes in heritable DNA methylation which alter phenotype are referred to as epimutagen. Now, evaluation of mutagens as well as epimutagens are needed in the pathology and toxicology research.

Ref

Shiota K. et al., *Genes Cells* 7:961-969 (2002).
Shiota K, Yanagimachi R: *Differentiation* 69:162-166 (2002).

S4-4

Emerging zoonotic diseases

Corrie BROWN

Department of Veterinary Pathology, College of Veterinary Medicine, University of Georgia, Georgia 30602, USA

The interface between animal and human health is becoming increasingly blurred as new diseases cross continents and oceans and from one species to another. The last few years has witnessed a dismaying array of novel human/animal disease events. Bovine spongiform encephalopathy traveled to many new parts of the world, with each appearance creating considerable consternation about health of both agriculture and the public. Avian influenza continues to emerge and re-emerge, often with movement into human populations. Particularly disquieting is the possibility of a reassortment event occurring between human and avian influenza viruses within a human host. A newly recognized human disease, severe acute respiratory syndrome, galvanized the international public health community into concerted efforts to contain the spread of a newly recognized coronavirus, which presumably moved into the human population from an animal reservoir. An outbreak of monkeypox in prairie dogs and humans in the Midwestern United States underscores the potential for any disease to show up anywhere and unexpectedly affect new species. Basically, given the interconnectedness of today's world, trying to separate diseases into the categories of "human" and "non-human" has become increasingly artificial. In fact, as we continue to change the environment, modify habitats, move species around, and alter the way we live and raise animals, pathogens from the animal and human worlds will not only continue to proliferate and mutate but also collide in a multitude of unpredictable ways. It is imperative that medical and veterinary fields interact and communicate in a fluid manner, so that we can respond effectively to each new disease as it arises, and continue to protect our global populations of all species.

S4 Chair

Future challenges: Stem cell development, infectious agents and others -Summary

John FINCH
Inveresk Research, Edinburgh EH33 2NE, UK

As Dr Doi said in his introduction to our symposium, we have been trying this afternoon to describe future challenges to toxicological pathology. Whilst some of what we have heard today might seem remote from the day to day concerns of the toxicological pathologist the principles that are applied to these new challenges are those that we are already applying in the more traditional aspects of our profession. Dr Nakatsuji described the establishment of embryonal stem cell lines, and how monkey ES cells provide a valuable alternative to human cells in research. The establishment of human ES cell lines was also described. Dr Harrison told us about the uses such cells can have in the armamentarium of the clinician. Exciting possibilities include treatments for Parkinson's disease, Alzheimer's disease, diabetes, liver failure and many others. Here the challenge is one of recognising opportunity and ensuring that as guardians of public safety we share the enthusiasm of our colleagues in drug discovery and pure research.

Other challenges involve the recognition of new threats to our well being. Genotoxicity is well known to us, but in Dr Shiota's talk were introduced to epigenetics as a real possibility of extending our safety assessments of medicines and environmental chemicals. Changes in heritable DNA methylation can affect the phenotype and evaluation of the epimutagenic of substances may one day be as routinely performed as current genotoxicity tests. A central theme of our symposium has been the introduction of foreign cells as therapeutic agents. These do of course carry a risk of carrying unwanted pathogens and it is there fore appropriate that finally we turned our attention to the world of infectious disease to get Dr Brown's excellent update on what's new in human and animal disease from SARS to BSE.

I hope that this glimpse of what the future may hold has been as stimulating for you as it has for me. In any event our goal remains what it has always been - better and safer medicines for mankind.

Posters

P-1

Hershberger and uterotrophic assessments to evaluate potential endocrine disrupting effects of diesel exhaust in rats

Tsuyoshi ITO[1], Hiroki OKUMURA[1], Ryo OHTA[2], Kiyoshi IMAI[3], Midori YOSHIDA[4], Dai NAKAE[4], Akihiko MAEKAWA[5]

Health Effects Research Division, Japan Automobile Research Institute, Ibaraki 305-0822, Japan[1]
Safety Testing Laboratory, Hatano Research Institute, Food and Drug Safety Center[2]
Executive Director, Biosafety Research Center, Foods, Drugs and Pesticides[3]
Department of Pathology, Sasaki Institute, Sasaki Foundation[4]
Director, Sasaki Institute, Sasaki Foundation[5]

In recent years, potential endocrine disrupting effects of diesel exhaust, in particular those on reproductive organs have received much attention. Diesel exhaust is demonstrated to inhibit the spermatogenesis in rats and mice. It is also indicated that diesel exhaust particle extracts exert an antagonistic effect on androgen receptors and an agonistic effect on estrogen receptors *in vitro*. Such effects is attributed mainly to polycyclic aromatic hydrocarbons represented by benzo[*a*]pyrene (B[*a*]P), major component of diesel exhaust particles, because B[*a*]P is antiandrogenic, its hydroxylated metabolites being estrogenic in culture cells. In this context, the present study was conducted to clarify whether diesel exhaust modulates overall androgen- and estrogen-mediated biological mechanisms *in vivo* by using Hershberger and uterotrophic assays in Fischer 344 rats. In the Hershberger assay, castrated male rats were inhaled diluted diesel exhaust at the concentrations of particles of 0, 0.1, 0.5 or 2.0 mg/m^3 or filtered exhaust with particles of 2.0 mg/m^3 for 16 hours/day for 10 days. To examine the antiandrogenic activity, subcutaneous doses of 0.2 mg/kg of teststerone propionate was additionally administrated to half of the animals in every group on each day. After the final diesel exhaust exposure, weights of the seminal vesicle and coagulating gland, ventral prostate, levator ani, bulvocavernous muscle and cowper's glands were measured. In the uterotrophic assay, ovariectomized female rats were inhaled diluted diesel exhaust with conditions identical to those used in the Hershberger assay for 16 hours/day for 14 days. To examine the antiestorogenic activity, subcutaneous doses of 0.1 μg/kg of ethynylestradiol was additionally administered to half of the animals in every group on each day. After the final diesel exhaust exposure, weight of the uterus was measured. As a result, diesel exhaust did not change any organ weights in either assay. These results indicate that diesel exhaust does not modulate the overall androgen- and estrogen-mediated biological mechanisms *in vivo*.

P-2

Application of hershberger assay protocol to the detection of thyroid function modulators using castrated male rats

Masakuni SAWAKI[1], Shuji NODA[1], Takako MUROI[1], Hideo MITOMA[1], Saori TAKAKURA[1], Satoko SAKAMOTO[1], Masanori OTSUKA[1], Mineo TAKATSUKI[1], Kanji YAMASAKI[1]

Chemicals Evaluation and Research Institute, Oita 877-0061, Japan[1]

In vivo screening methods for detection of thyroid function modulators are now under development in many research laboratories. We examined the applicability of Hershberger assay protocol to the screening of thyroid function modulators. Hershberger assay is a screening method for (anti-) androgenic compounds using castrated male rats. Castrated male BrlHan WIST@Jcl (GALAS) rats at 8 weeks of age were administered by gavage with a potent thyroid peroxidase inhibitor 3-amino-1,2,4-triazole (AT) 0, 40, and 200 mg/kg/day for 10 consecutive days (n = 5 or 6/group). Castration was performed at the age of 6 weeks. To examine the effect of castration, intact male rats were also subjected to the same protocol. All animals were sacrificed approximately 24 h after the final dosing. The thyroid glands and hypophysis of each rat were collected and weighed approximately 24 h after fixation with 10% neutral-buffered formalin to avoid crushing during manipulation. In addition, the thyroid glands of all animals were prepared for hematoxylin and eosin staining for histopathological examination and morphometry of the follicular epithelial height. As a result, significant increase of the thyroid weight was observed at the 200 mg/kg group in the castrated and intact rats. The hypophysis weight was not changed by AT treatment, however, comparing the vehicle-treated groups, the hypophysis weight of the castrated rats was higher than that of the intact rats. Enlargement of the thyroid glands in the AT-treated rats was observed at necropsy. Histopathlogically, the thyroid glands of all the AT-treated animals showed hypertrophy and hyperplasia of the follicular epithelial cells and the height of follicular epithelium of the thyroid glands increased in a dose-dependent manner in both of the castrated and intact rats. These results suggest that the effect of AT could be detected using Hershberger assay protocol regardless of castration and this protocol may be useful for screening of thyroid function modulators, although other compounds with mechanisms different from AT, such as deionase inhibitors and thyroid hormone metabolism enhancers should be tested.

P-3

Lack of modifying effects of 4-tert-octylphenol and benzyl butyl phthalate on 3,2'-dimethyl-4-aminobiphenyl-induced prostate carcinogenesis in rats

Hiroyuki KOHNO[1], Rikako SUZUKI[1,2], Shigeyuki SUGIE[1], Takuji TANAKA[1]

Department of Pathology, Kanazawa Medical University, Uchinada 920-0293, Japan[1]
Research Fellow of the Japan Society for the Promotion of Science[2]

Certain types of cancer development is related to environmental factors including diet and environmental chemicals. In the current study, the effects of dietary feeding of estrogenic compounds 4-*tert*-octylphenol (4-*t*-OP) and benzyl butyl phthalate (BBP) on 3,2'-dimethyl-4-aminobiphenl (DMAB)-induced prostatic carcinogenesis were investigated in male F344 rats to know whether these compounds exert modifying effects on prostatic carcinogenesis. We also assessed the effects of test compound on proliferating cell nuclear antigen (PCNA)-index in induced neoplasms, hyperplastic lesions, and non-lesional glands in the prostate. Rats were given intraperitoneal injections of DMAB (25 mg/kg body weight) every other week for 10 times to induce prostatic neoplasms. They also received the experimental diet containing 10 or 100 ppm OP and BBP for 40 weeks, starting one week after the last dosing of DMAB. DMAB exposure produced prostatic adenocarcinoma with an incidence of 41% (7/17 rats) at the end of the study (week 60). Dietary administration of 4-*t*-OP and BBP did not affect the incidence of prostatic adenocarcinoma: 44% (7/16 rats) in the DMAB → 10 ppm 4-*t*-OP group; 25% (4/16 rats) in the DMAB → 100 ppm 4-*t*-OP group; 44% (7/16 rats) in the DMAB → 10 ppm BBP group; and 44% (7/16 rats) in the DMAB → 100 ppm BBP group. The incidences of prostatic adenocarcinoma in other groups were 0% (0/8 rats) in the 100 ppm 4-*t*-OP alone group, 13% (1/8 rats) in the 100 ppm BBP alone group, and 0% (0/8 rats) in the untreated group. The PCNA-indices in adenocarcinomas, hyperplastic lesions, and non-lesional glands in rats treated with DMAB and 4-*t*-OP or BBP were slightly lower than that of the DMAB alone group, and the differences among the groups were not statistically significant. These results might suggest dietary feeding of two estrogenic compounds 4-*t*-OP and BBP did not have modulating effects on DMAB-induced rat prostatic carcinogenesis.

P-4

Sex hormone responsiveness and ductal architecture after exposure to flutamide perinatally in the rat ventral prostate

Kaori MIYATA[1], Setsuko YABUSHITA[1], Masashi SANO[2], Yasuyoshi OKUNO[1], Masatoshi MATSUO[3]

Environmental Health Science Lab., Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan[1]
Daiyu-Kai Institute of Medical Science[2]
Cooerative Research Center for Advanced Science and Technology, Osaka University[3]

Effects on ventral prostate in male rats exposed to flutamide perinatally were examined to clarify the hormone responsiveness and the main factor with respect to the irreversible effects.
Material & Methods: [Study 1] Pregnant rats were administered flutamide at a daily dose of 10mg/kg from gestation day 14 (GD14) to post-parturition day 3. Male pups were castrated on post-natal day 36 (PND36) and treated with vehicle, testosterone propionate (TP; 2 mg/kg/day, s.c.) or dihydrotestosterone (DHT; 1.25 mg/kg/day, s.c.) for 10 days, beginning at PND46. On PND56, serum testosterone, LH and FSH levels, ventral prostate weight were measured, and RT-PCR (for AR, C3, VEGF, TGF-beta1, beta2, KGF and CK8 mRNAs) was carried out. **[Study 2]** Pregnant rats were administered flutamide at doses of 10mg/kg from GD12 to GD21, or 30mg/kg on 2 successive days in the period from GD14 to 21 (GD14-15, GD16-17, GD 18-19, and GD20-21). RT-PCR at PND1 for the 10mg/kg group, and morphological analysis at PND7, 14 and 21, and organ weight measurement and receptor binding assay at PND76 or 78 for all groups were conducted.
Results: [Study 1] The degree of increase in the weight after TP or DHT treatment was smaller in the 10mg /kg group. Serum hormone levels and the results of RT-PCR revealed no significant differences. **[Study 2]** RT-PCR at PND1 demonstrated increase in the expression of AR and KGF mRNAs. Morphological analysis showed a consistent reduction in the number of main ducts and ductal branchpoints, as well as the complexity of the terminal ductal network in the 10mg/kg group, and the 30mg/kg GD16-17 and GD18-19 groups. The organ weights at PND76 or 78 were reduced in all treated groups, and the severity of effect was matched with those on ductal architecture. Receptor binding assays exhibited no significantly difference regarding maximum binding capacity and dissociation constant.
Conclusion: In flutamide exposure group, the increased degree of organ weight after androgen treatment was small, and an irreversible effect on the prostatic ductal architecture caused. The morphological effect was severest when the prostatic buds are developing, and accompanied an increase in KGF mRNA expression just after exposure. The effect on organ weight after androgens treatment was likely related to the ductal architecture, there were not any effects on serum hormone levels, expression of mRNAs, nor androgen receptor function later in life.

P-5

Is stem cell factor related to the testicular toxicity of thiamphenycol?

Maki KUWAHARA[1], Yukiko TAKEUCHI[1], Naofumi TAKAHASHI[1], Tadashi KOSAKA[1], Nobuaki NAKASHIMA[1], Toshinori YOSHIDA[1], Akiko ENOMOTO[1], Toshiaki KITAZAWA[3], Keizo MAITA[2], Takanori HARADA[1]

Toxicology Division, The Institute of Environmental Toxicology, Ibaraki 303-0043, Japan[1]
Division of Study Plannning and Conslutation, The Institute of Environmental Toxicology[2]
Contract and Research Management Division, The Institute of Environmental Toxicology[3]

Previously, we demonstrated the testicular toxicity of thiamphenicol (TAP), derivate of chroramphenicol, in Spraque-Dawley rats. In the testis of rats treated with 200 mg/kg/day TAP, spermatogonia in the stage V and preleptoten spermatocyte in the stage VII were significantly decreased from week 2. At week 4, degeneration and apoptosis of germ cells were frequently seen in most of the seminiferous tubules together with retention of sperm, dissociation of germ cell arrangement, and frequent presence of giant cells. There were no obvious changes in the Leidig cells. These changes incompletely disappeared after a week-10 of withdrawal period following 4 weeks of treatment, in spite of the presence of BrdU positive spermatogonia in seminiferous tubules which appeared to be "Sertoli only syndrome". These data have suggested that Sertoli cells are most likely to be the primary target in TAP testicular toxicity. As it was recently reported that irreversible testicular toxicity of hexandione was promoted by deficient of stem cell factor (SCF), which is secreted by Sertoli cells and plays key roles in the development of the testis in the neonatal stage, we examined the relationship between the SCF and thiamphenicol-induced testicular toxicity. Testes of rats orally treated with 200 mg/kg/day TAP for 7, 14, and 28days were fixed with FSA fixatives, routinely sectioned and immunostained with anti-SCF by ABC method. In the control testis, a strong positive reaction was observed in elongated spermatid in stage XI to XIII and spermatogonia in stage I to II. These types of germ cells are reported to express c-kit, the receptor protein of the SCF, and SCF binding to c-kit is necessary to differentiate these cells. No Sertoli cells showed positive reaction against the SCF antibody. Testes treated with 200 mg/kg/day TAP for 7 and 14 days showed almost same staining manner with the controls. After 28 days of treatment, although positive reaction observed in elongated spermatid and spermatogonia were maintained, some sprematocytes in disarranged seminiferous tubules exhibited a positive reaction. It is concluded that Sertoli cells still produce SCF in the TAP-treated testis, however, some functional alteration might occur.

P-6

No modifying effects of an estrogenic compound atrazine on 7,12-dimethylbenz(a)anthracene-induced ovarian carcinogenesis in rats

Takuji TANAKA[1], Hiroyuki KOHNO[1], Rikako SUZUKI[1,2], Shigeyuki SUGIE[1]

Department of Pathology, Kanazawa Medical University, Ishikawa 920-0293, Japan[1]
Research Fellow of the Japan Society for the Promotion of Science[2]

Atrazine with an estrogenic action is one of the widely used pre-emergence and post-emergence triazine herbicides that have made their way into the potable water supply of many agricultural communities. Atrazine was reported to induce mammary tumors in experimental animals. In the current study, the modifying effect of dietary feeding of a synthetic estrogenic compound atrazine, on 7,12-dimethylbenz(*a*) anthracene (DMBA)-induced ovarian carcinogenesis was studied in female Sprague-Dawley rats. We also assessed the effect of atrazine on the growth of ovarian adenocarcinoma by measuring proliferating cell nuclear antigen (PCNA)-index and by assessing the immunohistochemical expression of estrogen receptor (ER)-αand -βand androgen receptor (AR) in induced neoplasms. Rats were given a single injection of DMBA (0.01 ml of 0.5% DMBA suspended in olive oil) into their left ovary to induce ovarian neoplasms. They also received the experimental diet containing 5, 50 or 500 ppm atrazine for 50 weeks, starting one week after the dosing of DMBA. DMBA exposure produced ovarian adenocarcinoma with an incidence of 45% (9/20 rats) at the end of the study (week 51). Dietary feeding with atrazine reduced the incidence of ovarian adenocarcinoma without statistically significance: 22% (4/18 rats), 28% (5/28 rats), and 26% (5/19 rats) incidences in rats fed 5 ppm, 50 ppm, and 500 ppm atrazine containing diets after DMBA exposure, respectively. The PCNA-index in adenocarcinomas was greater than that of surface ovarian epithelium. ER-α, βand AR were expressed in a variable percentage of moderately and poorly differentiated adenocarcinoma cell nuclei, but their reactivity was extremely weak or negative in well differentiated adenocarcinoma cells. In additionm atrazine treatment did not affect tumor development in other organs (mammary gland and subcutaneous tissue). These results might suggest dietary feeding of a synthetic estrogenic compound atrazine did not affect DMBA-induced rat ovarian carcinogenesis.

P-7

The influence of pre- and post-natal exposure to methoxychlor on gene expression in the endometrium of rats after maturation

Ryoichi OHTSUKA[1], Makio TAKEDA[1], Satoru YAMAGUCHI[1], Koichi HAYASHI[1], Yukiko TAKEUCHI[1], Maki KUWAHARA[1], Naofumi TAKAHASHI[1], Tadashi KOSAKA[1], Keizo MAITA[1], Takanori HARADA[1]

Institute of Environmental Toxicology, Ibaraki 303-0043, Japan[1]

In order to determine whether pre- and post-natal exposure to methoxychlor (MXC), an endocrine disrupting chemical, influences the gene expression in the rat uterus after maturation, we conducted microarray analysis for mRNA expression in endometrial cells captured by Laser Microdissection (LCM) system.
For the present study, 32 female pups were obtained from SD rat dams receiving MXC at a dietary level of 0 or 1000 ppm during the gestation/lactation period. The pups were fed a normal diet after weaning and kept up to 52 weeks of age. At 10 and 52 weeks of age, 8 animals from each group were euthanized and their uteri were sampled immediately after necropsy. The uterine tissue samples were embedded in tissue compound for microarray analysis and also fixed in 10% neutral buffered formalin for conventional histopathology.
For microarray analysis, endometrial cells were captured from the frozen uterine sections stained with HistoGene LCM Frozen Section Staining Kit using LCM system, and the total RNA samples were extracted by Absolutely RNA Microprep Kit. The antisense RNA (aRNA, reflecting mRNA expression) samples were synthesized from extracted total RNA samples by MessageAmp aRNA Kit for 2 rounds according to the manufacture's protocol. Before starting microarray analysis, a pooled control aRNA sample was prepared from 8 control samples and was used as reference of each sample by labeling with Cy3. The samples from each animal (including controls) were labeled with Cy5, and incubated with Cy3-labeled pooled control on Microarrays (24 arrays were used) and scanned with array scanner.
In MXC-treated rats at 10 weeks of age, about 300 genes expressed higher (>150%) and 5 genes expressed lower (<66.7%) signals than those of Cy3-labeled pooled control. In control rats, only 6 genes expressed higher signals than that of pooled control. These results may indicate the possibility of the fetal imprinting effect of MXC on the rat endometrial cells. Analysis for animals at 52 weeks of age is now underway.

P-8

Effects of the fetal administration of diethylstilbestrol (DES) and 4-n-octylphenol (n-OP) on 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in SD rats

Hiroaki KAWAGUCHI[1], Masakazu SOHDA[1], Shuhei TAGUCHI[1], Koichiro MIYAMOTO[1], Hiroki YOSHIDA[1]

The Department of Tumor Pathology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8544, Japan[1]

Introduction Recently, breast cancer in humans has been increasing both in Japan and the rest of the world. Endocrine disrupting chemicals are thought to be one of the factors involved in this phenomenon. We have, therefore, studied the effects of the fetal administration of diethylstilbestrol (DES) and 4-n-octylphenol (n-OP) on 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in SD rats.
Materials and Methods Pregnant rats were divided into 3 groups. The rats in group I were intact during the gestation period. The other rats were given DES (0.1, 1, 10, 100 ppm) and/or n-OP (10, 100, 1000 ppm) in their feed on gestation days 0-21 (group II) and on gestation days 13-21 (group III). All offspring were given 10 mg DMBA by gastric intubation at the age of 50 days, and palpated for mammary masses.
Result Early abortion (Group II) and abnormal delivery (Group III) were seen in pregnant rats given DES, and growth disorders were seen in their offspring. However, no abnormal changes in pregnancy or delivery were seen in pregnant rats given n-OP or their offspring. The induction of mammary tumors induced by DMBA was suppressed in the female fetal rats given DES in Group II, but was promoted in the female fetuses given DES in Group III and n-OP in Groups II and III.
Discussion We suggest that the fetal administration of DES (strong estrogen) induced disorders of the endocrine mechanism and induction of mammary tumors, whereas, the fetal administration of n-OP (very weak estrogen) promoted the induction of mammary tumors.

P-9

The influence of pre- and post-natal exposure to methoxychlor on the rat immune system and renal function during aging process

Yukiko TAKEUCHI[1], Naofumi TAKAHASHI[1], Tadashi KOSAKA[1], Koichi HAYASHI[1], Yuko CHIBA[1], Toshinori YOSHIDA[1], Maki KUWAHARA[1], Sayuri KOJIMA[1], Ryoichi OHTSUKA[1], Keizo MAITA[2], Takanori HARADA[1]

Toxicology Division II, Institute of Environmental Toxicology, Ibaraki 303-0043, Japan[1]
Division of Study Planning and Consultation, Institute of Environmental Toxicology[2]

We previously demonstrated that apoptotic cell death occurred in the thymus of rat pups of both sexes exposed to a high dose of methoxychlor (MXC) during the pre- and post-natal period. Based on the result, we have undertaken a further investigation to determine whether the pre- and post-natal exposure to MXC persistently influence the rat immune system until a mature age. For the present study, male and female rat pups were obtained from dams receiving MXC at dietary levels of 0, 30, 100, 300, and 1000 ppm during the gestation and lactation period. They were fed a normal diet after weaning and kept up to 52 weeks of age. These animals were examined for immune response at 10 and 52 weeks and also for renal function at 52 weeks. At 10 weeks of age, there were no abnormalities in the thymus of either sex, but splenic T-cell subsets were significantly decreased in females at 300 and 1000 ppm. At 52 weeks of age, significant increases in the relative weights (ratio to body weight) of the spleen and kidneys were observed in females at 1000 ppm although there were no significant changes in the thymus and splenic lymphocyte subsets in either sex. These female rats showed an abnormal excretion of urinary protein and increases in plasma creatinine and chloride which indicate renal dysfunction. However, such changes were not observed in male rats. Histopathologically, the incidence of nephropathy increased in females at 1000 ppm and their kidneys had significantly large-sized glomeruli. In addition, increases in glomerular IgG and IgM deposits and plasma IgM levels were observed in these female rats. These results indicate that the immune system damage caused by the pre- and post-natal exposure to MXC could be repaired during aging process but not completely in females even at a mature age. The increased incidence of nephropathy characterized by enlarged glomeruli associated with increased IgG and IgM deposits may reflect an imbalance of population among lymphocyte subsets which persistently remains after exposure to MXC.

P-10

No effects of transplacental and lactational exposure to combination of bisphenol A and 4-nonylphenol, endocrine disruptors, on morphogenesis of male reproductive organs and spermatogenesis in F344 rats.

Kyoko NABAE[1], Hiroko YOSHINO[1], Norio IMAI[1], Takeshi HIROTA[1], Mayumi KAWABE[1], Syugo SUZUKI[2], Tomoyuki SHIRAI[2]

Daiyu-kai Institute of Medical Science, Aichi 491-0113, Japan[1]
Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences[2]

Bisphenol A (4,4'-isopropylidene-2-diphenol, BPA) is widely used in the polycarbonate plastic industry. BPA has been reported to be weakly estrogenic, both in vitro and in vivo. BPA has been found in umbilical cord blood and mother's milk, and both fetus and newborn are suspected to be much more sensitive to estrogenic compounds than adult. 4-Nonylphenol (4-NP), a surfactant used in the production of laboratory plasticware, and was also reported to exert estrogenic activity. Previously, we also reported that transplacental and lactational exposure to BPA and 4-NP did not each exert any adverse effects on morphogenesis of accessory sex organs or spermatogenesis in F344 male rats. This study was conducted to evaluate the combination risk of endocrine disruptors on the accessory sex organs and spermatogenesis in F344 male rats offspring exposed them preneonatally and neonatally. Non-treated female and male F344 rats were mated at 11 weeks of age, and the pregnant rats were administered by gavage 0 (control), BPA 0.05 mg/kg, 4-NP 0.1 mg/kg and mixture of BPA 0.05 mg/kg and 4-NP 0.1 mg/kg during gestation and lactation period. When male F1 rats reached 13 weeks old, they were euthanized, and final body and organs weights were recorded. And spermatogenesis (account of sperm, sperm mobility and morphological abnormality) and distribution of stage of seminiferous epithelium were investigated, and serum levels of testosterone were measured. No effects on the body weights of treated dams, the gestational period and the numbers of stillborn and newborn were apparent. No differences in the sex ratios of live newborn at birth was observed between the treated and control groups. Furthermore, no effects on male F1 rats in body weights and food consumption were apparent. No differences in organ to final body weight ratios of testes, prostate, epididymides, seminal vesicles and LA-BC muscle were noted between the treated and control groups. No differences in spermatogenesis in the testis and epididymis were noted between the treated and control groups. No effects on distributions of stage of seminiferous epithelium were also observed. And no observable histopathological abnormalities were present in the accessory sex organs. We concluded that transplacental and lactational exposure to combination of BPA and 4-NP did not exert any adverse effects on the spermatogenesis and the morphogenesis of rat accessory sex organs.

P-11

No effects of transplacental and lactational exposure to combination of bisphenol A and 4-nonylphenol, endocrine disruptors, on morphogenesis of male reproductive organs and spermatogenesis in ICR mice

Yuko DOI[1], Norio IMAI[1], Kyoko NABAE[1], Yousuke TODA[1], Seiko TAMANO[1], Hideki WANIBUCHI[2], Keiichirou MORIMURA[2], Shoji FUKUSHIMA[2]

Daiyu-Kai Institute of Medical Science, Aichi 491-0113, Japan[1]
Osaka City University Medical School[2]

Recently, a number of chemicals, which disrupt normal endocrine functions and misgivings in worldwide have been identified. Bisphenol A (BPA) and 4-nonylphenol (4-NP) are widely used and both compounds were reported to have weak estrogenic effects. The important endocrine-disrupting effects might be modification of development in hormone-sensitive organs such as the prostate, uterus, breast and testis. Previously, we exposed transplacentally and lactationally to female mice BPA or 4-NP alone at 0.05 mg/kg/day by gavage and concluded that transplacental and lactational exposure to BPA or 4-NP was no effect in male offspring mice. Since we are exposed by many chemicals, we investigated the combination effect of two chemicals, BPA and 4-NP. We designed to examine the effect of transplacental and lactational exposure of BPA and 4-NP in combination on morphogenesis of male reproductive organs and spermatogenesis. Female and male ICR mice were mated, and the pregnant female mice were given vehicle (corn oil), BPA at 0.05 mg/kg/day, 4-NP at 0.05 mg/kg/day or mixture of BPA and 4-NP intragastrically during the gestation and lactation period. Male F1 body weights and food consumptions were measured once a week until 13 weeks old. At week 13, all F1 male mice were killed and reproductive organ weights were recorded. Thereafter, they were observed histopathologically. The numbers of sperm in the testis and epididymis were counted, and the motility and malformation of sperm were also observed. In maternal mice, implantation marks were decreased in combination group. No. of newborn and live newborn, and sex ratio of newborn were also decreased. Body weights and food consumptions were no different during experimental period. Relative testis, prostate (ventral lobe) and epididymis weights were not affected and no histopathological abnormality was observed compared with control group. In each group treated with chemicals, there were no changes on morphological abnormalities in the reproductive organs of male offsprings. For F1 male mice, number of sperms in testis and epididymis were not affected in combination group. Furthermore, data for other parameters (movement and morphological abnormalities) also did not significantly differ between control and treatment group. These data indicate that transplacental and lactational exposure to combination of BPA and 4-NP does not affect on morphogenesis and spermatogenesis in male offspring mice.

P-12

Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to 4-nonylphenol in F344 rats

Hiroko YOSHINO[1], Kyoko NABAE[1], Yuko DOI[1], Takeshi HIROTA[1], Kumiko OGAWA[2], Tomoyuki SHIRAI[2]

Daiyu-Kai Institute of Medical Science, Aichi 491-0113, Japan[1]
Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences[2]

4-Nonylphenol (4-NP), a surfactant used in the production of laboratory plasticware, and has been reported to have estrogenic activity. The compound has been concerned as an estrogenic xenobiotic. One of the endocrine-disrupting effects might be modification of carcinogenicity in hormone-sensitive organs such as the prostate, uterus, breast and testis. We previously reported that estrogen may play an important role in prostate carcinogenesis. Therefore, the current study was designed to examine the modulating effects of 4-NP on prostate cancer risk in male offspring exposed transplacentally and lactationally. Non-treated female and male F344 rats were mated at about 11 weeks of age, and females which were confirmed to be pregnant was given intragastrically by gavage 0 (control), 0.1, 10 or 100 mg/kg/day of 4-NP during the gestation and lactation periods. The body weight of each dam was monitored during gestation and lactation period. When F_1 males reached 5 weeks old, all groups were given 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine intragastrically by gavage at a dose of 100 mg/kg b.w. weekly for 19 weeks. All surviving rats were killed at experimental week 60 and subjected to complete autopsy. The body weight of F_1 male rats was monitored weekly for experimental period, at autopsy, F_1 rats were euthanized, and final body and organ weights recorded. The prostates, seminal vesicles and all other organs were examined for gross abnormalities and fixed in 10% buffered formalin, processed routinely, and examined histopathologically. The body weights of dams were significantly suppressed by 4-NP 100mg/kg/day administration during gestation and lactation period. And gestation period of rats treated 100 mg/kg/day were elongated compared to control group. However, the numbers of stiilborn and newborn were not affected. There were no differences in the sex ratios at PND 4 between the treated and control groups. There were no observable external anormalies at birth and no significant differences in offspring body weights at any point before weaning.The final body and organ weights were not significantly affected. The preneoplastic and neoplastic lesions of the accessory sex organs were histopathologically classified into prostatic intraepithelial neoplasia (PIN) and carcinomas of prostate, as well as atypical hyperplasia of the seminal vesicle. None of the incidences in the experimental groups were significantly different from those in the control group. Our data suggest that maternal exposure to 4-NP during gestation and lactation periods does not affect the risk of prostate carcinogenesis in male offspring.

P-13

Using animal and human mode of action information in assessing human risk: a framework for analysis of various pathologies

Penelope A FENNER-CRISP[1], Dorothy E PATTON[1], The ILSI RSI WORKGROUP[1]

ILSI Risk Science Institute, Washington DC 20005, USA[1]

In late 2003, the International Life Sciences Institute Risk Science Institute (ILSI RSI) published the product of a two-year effort on using animal and human mode of action (MOA) information to evaluate the human relevance of tumor induction by non-genotoxic carcinogens. This report featured case study analyses of six carcinogens, which then led to the development of a four-part Human Relevance Framework (HRF) for systematic and transparent analysis of MOA data and related information. With such information increasingly available for various pathologies in addition to neoplasia, the sponsors of this project, the U.S. Environmental Protection Agency and Health Canada, requested ILSI RSI to evaluate the utility and applicability of this new HRF for other endpoints. The current RSI Workgroup, including some participants from the original project, has applied the HRF to case studies involving developmental effects such as skeletal and other abnormalities, male reproductive effects such as the androgen insufficiency syndrome (AIS), neurotoxic effects as a result of premature onset of cell replication in the developing brain and renal tubular damage. The Workgroup also examined the value of application of the Framework to genotoxic carcinogens, including those with and without mutagenic properties. This poster will exhibit the Framework and several of the cases used to examine its utility for analysis of a broad variety of pathologies.

P-14

Carcinogenicity of low-dose MeIQx, in relation to cancer risk assessment

Keiichirou MORIMURA[1], Hideki WANIBUCHI[1], Manabu HOSHI[1], Anna KINOSHITA[1], Shoji FUKUSHIMA[1]

Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

More than 80% of human cancers may be related to carcinogenic environmental chemicals, indicating an importance for managing the hazards of environmental carcinogens. For managing these hazards, an assessment based on some scientific data should be necessary. As for the genotoxic carcinogens, it has been generally accepted that there have no threshold in exerting carcinogenic potential, because genotoxic carcinogens are mutagenic. In the present study, for clarification of human risk assessment of genotoxic carcinogens, we examined the low-dose carcinogenicity of 2-amino-3,8-dimethylimidazo[4,5-f]quinoline (MeIQx) which is one of food-derived heterocyclic amine mutagens. A total of 885 male 21-day-old F344 rats were received MeIQx at doses of 0, 0.001 (equivalent to human daily intake), 0.01, 0.1, 1, 10, 100 ppm in powered diet for 4 or 16 weeks. After 16 weeks treatment, the numbers of GST-P positive foci of rat liver in groups receiving 0.001 to 1 ppm of the carcinogen did not differ from the control value, in contrast to the increase observed with 10 ppm and the clear, significant elevation with 100 ppm. We also examined the formation of MeIQx-DNA adducts and 8-Hydroxy-2'-deoxyguanosine (8-OHdG) levels in the liver. There was linear relationship between the various doses of MeIQx and the levels of MeIQx-DNA adducts. Concerning the 8-OHdG levels, no significant differences among groups receiving MeIQx from 0.001 ppm to 0.1 ppm and the control group were apparent, although values were linearly elevated from 1 ppm and above, with statistical significance. cDNA microarray using 3D array system was applied for liver tissues of 16 weeks treatment groups. No over-expression was detected in metabolizing enzymes for 0 to 1 ppm but slight increase was seen in high dose treatment. Some genes related with signaling pathways were down-regulated. Moreover, the in vivo mutagenicity assay using Big Blue rats treated for 16 weeks with same low doses of MeIQx was examined. The mutation frequency from 0.001 ppm to 1 ppm were same level at control group and the values for 10 ppm and 100 ppm showed significant increase compared with the control group. The present results indicate that the dose-response curve of genotoxic carcinogen, MeIQx, at low-dose is different from that of high-dose. Threshold may exist for the hepatocarcinogenic potential of MeIQx. These results are important regarding how we should view the impact of genotoxic carcinogens, in the human environment in relation to cancer risk assessment and management.

P-15

Presence of the threshold for carcinogenicity and in vivo mutagenicity induced by genotoxic carcinogen, MeIQx, at low doses

Saki NAITO[1], Keiichirou MORIMURA[1], Manabu HOSHI[1], Natsuko MIYAZI[1], Shoji FUKUSHIMA[1]

Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

More than 80% of human cancers may be related to carcinogenic environmental chemicals. Carcinogenicity is generally detected by long-term carcinogenicity testing in rodents using doses considerably in excess of human exposure levels. The situation is complicated by the fact that carcinogens can be classified as either genotoxic or non-genotoxic according to results of *in vivo* genotoxity tests. The currently accepted view is that no threshold exists for the cartcinogenic potential of genotoxic carcinogens, with the response curve approaching zero in a linear fashion with extrapolation from the doses used in carcinogenicity testing. Actual human cancer risk of genotoxic carcinogens is very difficult to assess, because few directly obtained data are available from the carcinogenecity testing at low doses such as human daily exposure levels. Therefore, practical information concerning cancer risk remains inadequate, and the need for investigation in this field is urgent.To elucidate the relationship between *in vivo* carcinogenic and mutagenic potentials of genotoxic carcinogens, low doses were tested in the Big Blue transgenic rats with 2-amino-3,8-dimethylimidazo[4,5-*f*] quinoxaline (MeIQx). In disagreement with a general belief that genotoxic carcinogens show no threshold for carcinogenic potential, a threshold was demonstrated for both carcinogenicity in terms of preneoplastic lesion induction and *in vivo* mutagenicity of MeIQx.

P-16

Lack of modification of MeIQx rat liver carcinogenesis by caffeine induction of CYP1A2

Hitoshi KANDORI[1,2], Masanori KURIBAYASHI[1,3], Shingo INAGUMA[1], Makoto ASAMOTO[1], Naomi HOKAIWADO[1], Seishiro TAKAHASHI[1], Tomoyuki SHIRAI[1]

Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Takeda Chemical Industries Ltd. Drug Safety Research Center Hikari Branch[2]
Ono Pharmaceutical Co. Ltd., Safety Research[3]

2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), one of the most abundant carcinogenic heterocyclic amine in cooked meat and fish, is speculated to be associated with human carcinogenesis. MeIQx is known to be metabolically activated by several enzymes, especially CYP1A2, to form DNA adducts. Therefore analysis of interactions between MeIQx with various chemicals which affect MeIQx metabolism is important for risk assessment. In order to evaluate modifying effects of CYP1A2 inducers on MeIQx hepatocarcinogenesis, caffeine or diethylnitrosamine (DEN) was given together with MeIQx to rats. Modification was assessed using a medium-term liver bioassay system. Six-week-old male F344 rats were given a single dose of DEN, 200 mg/kg b.w., i.p. as an initiator. Starting 2 weeks later, MeIQx was given together with or without 0.1% caffeine or 0.01% DEN in the diet or drinking water, respectively for 6 weeks. At week 3, all rats were subjected to a two-thirds partial hepatectomy. At week 8, the experiment was terminated and the livers were taken for analysis. First, we analyzed the numbers and areas of glutathione S-transferase (GST-P) positive liver foci, putative preneoplastic lesions, after immunohistochemical staining. Then, mRNA expression levels of several metabolic enzymes were compared between GST-P positive foci and the surrounding liver tissue. As a result, DEN given together with MeIQx showed a synergistic promoting effect on development of GST-P positive foci. However, no modification was found with caffeine, although it up-regulated CYP1A2 mRNA expression. DEN is reported to also be a CYP1A2 inducer but we were not able to confirm this. In the GST-P positive foci, phase II detoxifying enzymes including GSTs and UDPGTs were up-regulated. In the background tissue of rat livers after exposure to DEN as a CYP inducer, specific expression of extracellular matrix-related genes including sparc and laminin receptor 1 was detected. These results suggest that modifying effects of caffeine on MeIQx through CYP1A2 are limited because caffeine also up-regulates various phase II detoxifying enzymes. Moreover, our findings indicate that changes in extracellular matrix-related genes in the surrounding tissue are important for progression of GST-P positive foci.

P-17

Investigation of the complex carcinogenic risk by PhIP and MeIQx in the initiation stage of carcinogenesis

Akihiro HIRATA[1], Tetsuya TSUKAMOTO[1], Hiroki SAKAI[2], Masami YAMAMOTO[1], Norimitsu SHIRAI[1], Takeshi IIDAKA[1], Tokuma YANAI[2], Toshiaki MASEGI[2], Masae TATEMATSU[1]

Division of Oncological Pathology, Aichi Cancer Center Research Institute, Aichi 464-8681, Japan[1]
Department of Veterinary Pathology, Faculty of Agriculture, Gifu University[2]

[Purpose] In order to examine the complex carcinogenic risk by different heterocyclic amines (HCAs), the initiation activities of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-b]quinoxaline (MeIQx) and their combination were investigated with an in vivo 5-week initiation assay model.
[Method] Seven-week-old male F344 rats were undergone two-thirds partial hepatectomy (PH) and the compounds were administered intragastrically at 12 and 30 hours after PH. Group 1 animals received simultaneous administration of PhIP (200 mg/kg body weight (b.w.)) and MeIQx (25 mg/kg b.w.) at 12 hours and then vehicle (corn oil) alone at 30 hours. Rats in groups 2 were given both compounds separately (PhIP at 12 hours and then MeIQx at 30 hours). Group 3 received both of them in a reverse order (MeIQx -> PhIP). Single compounds were given at 12 or 30 hours to group 4 (PhIP -> vehicle), group 5 (vehicle -> PhIP), group 6 (MeIQx -> vehicle), and group 7 (vehicle -> MeIQx). Control group rats were given only vehicle (group 8). Subsequently, the rats were fed on basal diet for 2 weeks, followed by diet containing 0.015% 2-acetylaminofluorene for the next 2 weeks. Three weeks after PH, all rats received carbon tetrachloride as a stimulus for proliferation. At the end of week 5, all the survivors were sacrificed. Initiation activities of each group were assessed with the induction of glutathione S-transferase placental form (GST-P) positive foci in the liver.
[Results] The value of GST-P positive foci in the group taking MeIQx prior to PhIP (group 3) was significantly higher than those in the groups having PhIP or MeIQx alone at the comparable time points ($P<0.05$, vs. group 5 and 6), indicating the summation effect of the two chemicals. Nonetheless, there was no significant difference in the number of GST-P positive foci between the groups with serial treatment of PhIP then MeIQx (group 2) and with PhIP (group 4) or MeIQx (group 7) alone. Furthermore, the number of GST-P positive foci induced with MeIQx alone (group 6) was significantly suppressed with the simultaneous administration of PhIP (group 1) ($P<0.05$, vs. group 6).
[Conclusion] PhIP, when administered beforehand or simultaneously, could inhibit the carcinogenic potential of MeIQx in the initiation stage of carcinogenesis.

P-18

Colon carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in rats: suggestion for carcinogenesis and practical threshold

Kenichiro DOI[1], Elsayed I SALIM[1], Anna KINOSHITA[1], Rawiwan PUATANACHOKCHAI[1], Hideki WANIBUCHI[1], Shoji FUKUSHIMA[1]

Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

Colon carcinogenesis is thought to be a multi-step process caused by plural genetic alterations. Genotoxic carcinogens, such as food-derived heterocyclic amines (HCAs), have been implicated as initiating agents to exert its mutagenicity through binding DNA and forming DNA adduct. Most abundant HCA, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), is revealed to be rodent's colon carcinogen and constantly taken from our usual environment, resulted in suspicion of an etiology for human colon cancer. However, PhIP could induce quite low levels of tumor yield when administered to rats. Therefore, we presently hoped to examine an actual potential of PhIP on rat colon carcinogenesis at lower dose levels that mimic to human practical exposure. A total of 192, 6-week-old, male F344 rats were divided into 8 groups (24 rats in each) and all animals were subcutaneously injected two times of 15 mg/kg body weight, azoxymethane (AOM), followed by continuous feeding of PhIP in basal diet. At weeks 16 and 36, animals were sacrificed and histopathologically examined. As a result, PhIP exerted so strong enhancing effects on colon tumorigenesis at more than 50 ppm, oppositely which clearly revealed no-observed effect levels (0.001-10 ppm) including human daily exposure. Furthermore, several modifying effects on tumor distribution or histological types were observed at higher-doses. These results may postulate the strong promotion effect of PhIP in the latter carcinogenic process, also may suggest a 'practical threshold' for rats, providing further possibility for human risk assessment.

P-19

Estimation of a no observed effect level for 2-acetylaminofluorene (2-AAF) and 2,4-diaminotoluene (2,4-DAT), genotoxic liver carcinogens, in 16-week feeding studies using male F344 rats

Akihiro HAGIWARA[1], Toshio ICHIHARA[1], Hiroko YOSHINO[1], Norio IMAI[1], Hideki WANIBUCHI[2], Keiichirou MORIMURA[2], Seiko TAMANO[1]

Daiyu-kai Institute of Medical Science, Aichi 491-0113, Japan[1]
Department of Pathology, Osaka City University Medical School[2]

The carcinogenic potential at very low doses of 2-AAF and 2,4-DAT was evaluated in 16-week feeding studies, which applied quantitative analysis of glutathione S-transferase placental form (GST-P) positive hepatocytic foci, using male F344 rats. In experiment 1, groups of 100 or 50 animals (21-day old at commencement) were given 0 (as control), 0.001, 0.01, 0.1, 1, 10 and 100 ppm 2-AAF in their diet for 16 weeks. Marked body weight retardation was found in 100 ppm group, but no influence was evident in groups fed a diet containing less than 10 ppm. Administration of a dietary concentration of 100 ppm 2-AAF to rats caused prominent elevation of liver weight, serum g-glutamyltranspeptidase (GGT), bromodeoxyuridine (BrdU) labeling indices (LI) in the hepatocyte, and quantitative values for small sizes of GST-P positive foci (comprised from 2-4 cells, 5-10 cells, and 11 cells). Significant increase in liver weight was also noted in rats fed 10 ppm 2-AAF, but not in rats receiving less than 1 ppm 2-AAF. Quantitative values for GST-P positive foci were statistically increased in the 1 and 10 ppm groups, but not at 0.1 ppm or lower. No significant elevation of the 8-hydroxyguanine (8-OHdG) level in the liver DNA was found at the highest dietary level. In experiment 2, groups of 90 or 30 animals were also given dietary levels of 0, 0.001, 0.01, 0.1, 1, 10, 100 and 300 ppm 2,4-DAT for same period. Marked retardation of body weight increase was found in 300 ppm groups, but not in animals fed a diet containing less than 100 ppm. Administration of a dietary concentration of 300 ppm 2,4-DAT to rats caused prominent elevation of liver weight, and quantitative values for small GST-P positive foci. Significant increased liver weight was also noted in rats fed 100 ppm 2,4-DAT, but not with doses of 10 ppm 2,4-DAT or less. Quantitative values for GST-P positive foci were significantly increased in 100 ppm groups, but at 10 ppm and lower there were no differences from controls. BrdU LI in the hepatocyte and 8-OHdG level in the liver DNA were not affected at 300 ppm 2,4-DAT group. Thus, practical threshold may exist for carcinogenicity of 2-AAF and 2,4-DAT, and a no-observed effect level of 2-AAF and 2,4-DAT, as assessed with reference to GST-P positive foci, was estimated to be 0.1 ppm (6.81 mg/kg/day) and 10 ppm (0.654 mg/kg/day), respectively, under the present experimental conditions.

P-20

Effect of phenobarbital on diethylnitrosamine-induced hepatocarcinogenesis in TGF-alpha transgenic mice-correlation with P450

Takayuki YUNOKI[1], Hideki WANIBUCHI[1], Rawiwan PUATANACHOKCHAI[1], Masakazu KAKUNI[1], Kazuo HAKOI[1], Shoji FUKUSHIMA[1]

Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan,[1]

[Objective] To study the effect of various concentrations of phenobarbital on DEN induced Hepatocarcinogenesis with TGF-alpha transgenic mice.
[Methods] All mice was administerd DEN intraperitoneal dose of 5mg/kg body weight at 15 days old,and given PB at dietary concentration of 0,2 15,500ppm from the end of week2.
[Result] All mice were sacrificed at the of 22 weeks .The incidence of hepatocellular carcinoma was 80% at dietary concentration of 500ppm.The incidence was 20% and 24 % at DEN alone group and DEN+PB2% grup,respectively.But hepatocellular carcinoma was not obserbed at DEN+PB15%grup.8-OHdG in liver slightly decreased at PB 2ppm group,but there was no significant difference among all groups.The lever of The level of TGF-alpha slightly decreased at PB 2ppm group and 15ppm group.
[Conclusion] Phenobarbital enhanced DEN-induced liver hepatocarcinogenesis at 500 ppm, on the other hand, tend to suppressed hepatocarcinogenesis at 15 ppm in TGF-alpha transgenic mice. One possible mechanism of the suppression of liver carcinogenicity by medium dose of phenobarbital might be influence on TGF-a expression.The result suggest that the mechanism correlate with p-450.

P-21

Possible mechanism of hormetic phenomenon of alpha benzene hexachloride on diethylnitrosamine induced hepatocarcinogenesis

Rawiwan PUATANACHOKCHAI[1], Hideki WANIBUCHI[1], Mayuko OSADA[2], Keiichirou MORIMURA[1], Yoshihiko FUNAE[2], Shoji FUKUSHIMA[1]

Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]
Department of Chemical Biology, Osaka City University Medical School[2]

Alpha benzene hexachloride (alpha-BHC), an organochlorine insecticide, was banned about 25 years ago from Japan but some countries still employed. Male F344 rats were injected by 100 mg/kg body weight of diethylnitrosamine (DEN) once a week for 3 times via intraperitoneum. After the last injection 1 week, diet containing various concentrations of alpha-BHC, range from 0.01 to 500 ppm, was fed for 10 weeks. It was found that high concentration of alpha -BHC, 500 ppm, treated rats significantly increased the number and area of glutathione S-transferase placental form-positive foci (GST-P), a marker of preneoplastic lesion of the liver. On the other hand, low doses of alpha-BHC, 0.05 ppm, significantly reduced both number and area of GST-P positive foci. Thus, in plotting concentration of alpha-BHC versus number and area of GST-P positive foci results in a U-shape dose-response curve. Furthermore, biphasic effect of alpha-BHC on cytochrome P 450 contents was revealed that significantly reduced at low dose, 0.05 ppm, but significantly raised at high dose. 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, production was significantly increased in high dose treated groups but significantly decreased in low dose treated groups. In addition, the levels of CYP2B1, a source of free radicals production, mRNA and protein using competitive RT-PCR and western blotting, respectively, were significantly induced in 50 and 500 ppm of alpha-BHC treated group. There, however, is no significantly difference among low dose treated group. From these results suggested that hormetic phenomenon of alpha-BHC might be involved in xenobiotic metabolism, cytochrome P450 oxidoreductase system, that produce free radicals followed by oxidative DNA damage and consequently pathological change in the liver.

P-22

Effect of low-dose DDT on liver carcinogenesis in rats

Masahiko KUSHIDA[1], Tokuo SUKATA[1], Keisuke OZAKI[1], Satoshi UWAGAWA[1], Keiichirou MORIMURA[2], Hideki WANIBUCHI[2], Yasuyoshi OKUNO[1], Shoji FUKUSHIMA[2]

Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan[1]
Department of Pathology, Osaka City University Medical School[2]

Inhibitory effects of DDT on rat hepatocarcinogenesis have been reported, when given to rodents at a dose of 0.005, 0.01 ppm, while higher doses exhibited promoting activity. The mechanism of DDT promotion as well as its inhibitory activity remain unclear and raise the question of whether the treatment with low doses of DDT will reduce cancer risk. We investigated its effect in male 6-week-old F344 rats injected by N-diethylmitrosoamine (DEN) once a week for 2 times, ip. After 1 week of the last injection, diet containing various concentrations of DDT, 0.005, 0.5, 500 ppm, was fed for 11 and 43 weeks. The inhibitory effect of DDT treatment at a dose of 0.005 ppm on formation of GST-P positive foci and tumors has been observed after 11 and 43 weeks of treatment, respectively. CYP3A2 and 8-hydroxy-2'-deoxyguanosin (8-OHdG), which is the marker of oxidative DNA damage, levels in rat livers were correlated to induction of GST-P positive foci. On the contrary, when DDT was applied at a high dose, a formation of GST-P positive foci and tumors, CYP3A2 protein expression and 8-OHdG levels in rat livers highly elevated in the rat liver at weeks 11 and 43, respectively. Thus these results suggested that hormetic phenomenon of DDT might be involved in CYP450 system that produce free radicals followed by oxidative DNA damage and resulting to pathological change in the liver.

P-23

Activation of the ras oncogene and its relationship to aflatoxins-DNA adduct formation in rat liver treated with aflatoxins

Dae Joong KIM[1], Tae Myoung KIM[1], Jin Tae HONG[2], Hwan Soo YOO[2], Cheol Beom PARK[3], Beom Jun LEE[1], Jong-Koo KANG[1], Young Won YUN[1]

College of Veterinary Medicine, Chungbuk National University[1], Cheongju 361-763, Korea[1]
College of Pharmacy, Chungbuk National University[2]
Biotoxtech[3]

Aflatoxins are produced by Aspergillus flavus, parasiticus and their related fungi that grow in improperly stored foods such as corn, rice, peanuts and other cereals. In addition to its high mutagenicity and cytotoxicity, aflatoxin B_1(AFB_1) is a potent hepatocarcinogen in experimental animals and an important factor for the human liver cancer. In spite of a high attention to the hepatocarcinogenicity of aflatoxins, the relative toxicity, for the risk assessment, of other types (AFB_2, AFG_1 and AFG_2) of the toxin was not fully studied. In the present study, the relative potency for the hepatotoxicity, mutagenicity, and DNA-adduct formation by aflatoxins B_1, B_2, G_1 and G_2 were investigated. Sprague-Dawley male rats were orally administered with AFB_1, AFB_2, AFG_1 or AFG_2 at doses of 0.25, 1.25 or 2.5 mg/kg. Animals were killed at 12, 24 or 48 hrs following alflatoxin exposure, the histopathological examination, expression of ras oncogene and 8-OxodG production as the biomarkers of hepatotoxicity, mutagenicity and DNA-adduct formation, respectively, were examined and analyzed for the relative toxicity of the types of aflatoxins.

P-24

Lack of effects of 1439 MHz electromagnetic near field exposure on the blood-brain barrier in immature and young rats

Masanori KURIBAYASHI[1,4], Jianqing WANG[2], Osamu FUJIWARA[2], Yuko DOI[3], Kyoko NABAE[3], Seiko TAMANO[3], Tadashi OGISO[1], Makoto ASAMOTO[1], Tomoyuki SHIRAI[1]

Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Department of Electrical and Computer Engineering, Nagoya Institute of Technology[2]
Daiyu-kai Institute of Medical Science[3]
Ono Pharmaceutical Co., Ltd., Safety Research[4]

The use of cellular phones has been spreading rapidly all over the world, especially in the developed countries. They are being used not only by adults but also by children and the potential risk of electromagnetic near-fields (EMFs) from their antennae to human health, especially with respect to brain tumor development or disturbance of the blood-brain barrier (BBB), is a source of concern. In the present study, we performed experiments to explore whether EMF exposure causes any damage to the BBB in rats with ages equivalent to the time of BBB genesis (4-weeks-old) and the teenage (10-weeks-old) period. For this purpose, alteration of BBB-related genes, such as those encoding p-glycoprotein, aquaporin-4 and claudin-5, was assessed by immunohistochemistry and quantitative RT-PCR in the brain. First, we verified that expression of the three BBB-related genes is influenced by 1,3-dinitrobenzene (DNB), a chemical reported to destroy the BBB, and then we performed an EMF exposure experiment with 0, 2.0 and 6.0 W/kg brain-average specific absorption rates (SARs) at 1.5 GHz for 90 min/day for 1 or 2 weeks. Vascular permeability was also investigated using dye-transfer technique by FITC-dextran and albumin immunohistochemistry. As a result, expression of the three BBB-related genes was found to be clearly decreased after administration of DNB as a positive control, when compared with the control values. However, there were no biologically meaningful differences with the EMF at any exposure level at either age. Vascular permeability, monitored with reference to transfer of FITC-dextran, was not affected by EMF exposure. Furthermore, albumin immunohistochemistry clearly demonstrated albumin leakage from capillary blood vessels to brain parenchymal tissue in the brains of rats receiving DNB, although there was no change with EMF exposure. Thus, these findings suggest that local exposure to 1.5 GHz EMF exerts no adverse effects on the BBB in immature and young rats.

P-25

Specific in vivo mutational spectra of genotoxic and non-genotoxic hepatocarcinogens in gpt delta transgenic rats

Keita KANKI[1], Akiyoshi NISHIKAWA[1], Ken-ichi MASUMURA[2], Takashi UMEMURA[1], Takayoshi IMAZAWA[1], Yasuki KITAMURA[1], Takehiko NOHMI[2], Masao HIROSE[1]

Division of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan[1]
Division of Genetics and Mutagenesis, National Institute of Health Sciences[2]

Environmental carcinogens are classified into genotoxic and non-genotoxic carcinogens based on *in vitro* bacterial mutagenicity. However, data from transgenic rodents with reporter genes including *lacI* and *lacZ* have suggested some discrepancy between *in vitro* and *in vivo* mutagenicity of carcinogens. *gpt* delta Transgenic rodents have advantages to efficiently detect different types of mutations. For example, point mutations and deletions can be detected by 6-thioguanine selection using the *gpt* gene of *E. coli* and Spi$^-$ selection using the *red/gam* genes of lambda phage, respectively. In the present experiment, the *in vivo* mutant frequency (MF) and mutation spectrum of the known genotoxic rodent hepatocarcinogens 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and *N*-nitrosopyrrolidine (NPYR), and the non-genotoxic hepatocarcinogen di(2-ethylhexyl)phthalate (DEHP) were investigated in *gpt* delta transgenic rats. Although there was no growth inhibition in any group after the 13-week treatment, liver/body weight ratio was significantly increased in all groups. GST-P positive liver cell foci were significantly increased in the 300 ppm IQ and 200 ppm NPYR groups, but little was induced in 12,000 ppm DEHP group. 6-Thioguanine selection revealed significant increases of *gpt* MF in the liver of IQ group ($18.8X10^{-5}$) and NPYR group ($5.7X10^{-5}$), being 35- and 10-fold higher than that of control ($0.55X10^{-5}$), respectively. Predominant type of base substitutions observed in *gpt* genes were G:C to T:A transversion in IQ group, and A:T to G:C transition in NPYR group. There was no difference of *gpt* MF and mutation spectrum between the control and DEHP groups. Similarly, there was no difference of Spi$^-$ MF and mutation spectrum in DEHP groups. Thus, IQ and NPYR showed potent mutagenicity with specific mutation spectrum in the liver of *gpt* delta rats. G:C to T:A base substitution induced by IQ has been also reported in other transgenic rodents such as MutaMouse and BigBlue. Although NPYR has been reported to form deoxyguanosine adducts by simple alkylation, adduction to deoxyadenosine by its metabolites such as crotonaldehyde may be responsible to *in vivo* mutation of NPYR because A:T to G:C transition was a major type of mutation in the present study. Since no mutagenicity was detected with DEHP, hepatocarcinogenicity of DEHP in rodents was confirmed to be non-genotoxic pathways. In conclusion, our data indicates that analysis for specific *in vivo* mutational spectrum can reversely provide useful information on the mechanism underlying chemical carcinogenesis.

P-26

Mechanistic study using cDNA microarray on enhanced hepatocarcinogenesis in ICR mice fed diet containing dicyclanil

Mitsuyoshi MOTO[1], Miwa OKAMURA[1], Tomoko MUTOH[1], Takao WATANABE[1], Yoko KASHIDA[1], Noboru MACHIDA[1], Kunitoshi MITSUMORI[1]

Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan[1]

Dicyclanil, a pyrimidiamine-derived insect growth regulator used as a veterinary medicine, is known as a hepatocarcinogen in mice. In our previous study, negative results were obtained in an *in vivo* comet assay in mice and a liver initiation assay in rats using dicyclanil. These results suggested that dicyclanil was a non-genotoxic carcinogen having a potent tumor promoting effect. However, detailed mechanism of the promoting effects for hepatocarcinogenesis of dicyclanil is still unclear. In the present study, to investigate the mechanism of hepatocarcinogenesis in mice induced by dicyclanil, cDNA microarray and RT-PCR were performed in mice fed diet containing dicyclanil. At first, to obtain the gene expression profile by the administration of dicyclanil, male ICR mice were given 0 or 1500 ppm dicyclanil for 2 weeks. Although an increase in relative liver weights and slight swelling of hepatocytes were observed in the mice treated with dicyclanil, no other histopathological changes were observed in the liver. In microarray analyses, upregulations of gene expression of oxidation and/or redox-related metabolic enzyme, such as alcohol dehydrogenase, cholesterol 24-hydroxylase, coproporphyrinogen oxidase, CYP 1A1, CYP 1A2, Glu-6 phosphate dehydrogenase, glutathione peroxidase and thioredoxin reductase 1 (TrxR 1), were observed in the liver of dicyclanil-treated mice. Especially, the mRNA expressions of CYP 1A1, CYP 1A2 and TrxR 1 were remarkably increased. The gene expression of thioredoxin, known as an oxidative stress marker, was also increased in the liver of mice given dicyclanil. Based on these results, using the two stage hepatocarcinogenesis model, male ICR mice received an i.p. injection of 5 mg/kg of *N*-nitrosodimethylamine and were then given diet containing 0 or 1500 ppm dicyclanil for 7 weeks with two thirds partial hepatectomy, to examine liver promoting effects and alteration of gene expressions induced by dicyclanil. In histopathological investigations, the centrilobular swelling of hepatocytes were observed in the dicyclanil group, and the numbers of gamma glutamyltransferase positive micro foci were increased in the liver of this group, indicating enhanced tumor promoting effects. In RT-PCR and real-time PCR analyses, upregulations of mRNAs that were similar to those in 2-week study were also observed in the liver of the dicyclanil group. These results suggest that oxidative stress partly plays a role in the enhancement of liver tumor promoting effects by dicyclanil. Additional molecular pathological investigations are now in progress in our laboratory.

P-27

Six-month carcinogenicity study of N-bis(2-hydroxypropyl)nitrosamine (DHPN) in rasH2 mice

Miwa OKAMURA[1], Mitsuyoshi MOTO[1], Yoko KASHIDA[1], Noboru MACHIDA[1], Kunitoshi MITSUMORI[1]

Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan[1]

[Purpose] The rasH2 mice carrying the human prototype c-Ha-*ras* gene have been used in many 6-month short-term carcinogenicity tests. In the present study, we evaluated the carcinogenic susceptibility of rasH2 mice to *N*-bis(2-hydroxypropyl)nitrosamine (DHPN).
[Methods] Total of 73 male rasH2 mice and 35 their wild-type littermates (non-Tg mice), 6-week old, were given DHPN in drinking water at 0, 20 or 200 ppm, and 0 or 200 ppm, respectively, for 26 weeks. All animals were subjected to a complete necropsy for histopathological examinations. The unilateral lobes of the lung, including normal lung tissue (0 ppm) and the whole lung tissue containing a neoplastic mass induced by DHPN (200 ppm), were frozen for RT-PCR analyses of transgene and endogenous mouse *ras* gene.
[Results and Discussion] The experiment of rasH2 and non-Tg mice given 200 ppm DHPN was completed at 20 weeks, since the survival rates of these animals were remarkably decreased as compared with that in the control group. Histopathologically, adenomas and adenocarcinomas of the lung and squamous cell hyperplasias and papillomas of the trachea were frequently observed in rasH2 and non-Tg mice given 20 or 200 ppm DHPN. Hemangiosarcomas of the liver were induced with a high incidence in both mice of the 200 ppm group, but there was no induction of hemangiosarcomas of the liver in rasH2 mice of the 20 ppm group. In addition, squamous cell hyperplasias and papillomas of the forestomach, transitional cell hyperplasias and papillomas of the urethra, squamous cell papillomas and carcinomas of the excretory duct of salivary glands were induced in rasH2 mice in DHPN treated group. No induction of neoplastic lesions of the thyroids and kidneys was found in rasH2 and non-Tg mice. RT-PCR analysis showed that there was no marked difference in the expression of mRNA of the transgene and endogenous mouse *ras* gene between the whole lung tissue of rasH2 mice with a neoplasm and normal lung tissue in the control rasH2 mice. These results suggest that rasH2 mice are highly susceptible to DHPN and have many tumor target organs such as the forestomach, salivary gland and urethra, which have not been found in long-term carcinogenicity studies of rats and mice.

P-28

Genomic analysis of the difference between non-genotoxic carcinogen and genotocic carcinogen in the urinary bladder

Junko HATA[1], Azusa TAMURA[1], Natsuki KITAJIMA[1], Yoshinori KASAHARA[1], Hiroshi UNO[1], Keiichirou MORIMURA[2], Shoji FUKUSHIMA[2]

Pharmaceuticals Development Research Laboratories, Teijin Pharma Ltd., Tokyo 191-8512, Japan[1]
Department of Pathology, Osaka City University Medical School[2]

Uracil is known to be non-genotoxic carcinogen, calculus is induced by the uracil-administration and long-term stimulation of the calculus produces the neoplastic lesion in the urinary bladder. However, N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) and p-Cresidine is known to be genotoxic carcinogen. We examined the difference of the expression levels of various mRNA in the urinary bladder treated with these substances. For the purpose of this study, we used the glass array system (Atlas Glass Array, Rat Toxicology 1.0) and real time PCR system method. The urinary bladder was prepared from twelve F344 rats 4-week treated with 3% uracil in the feeding, 0.05% BBN in the drinking water or 0.5% p-Cresidine in the feeding, respectively. Histopathologicaly, the papillomatosis was observed in all Uracil-treated urinary bladder, and simple hyperplasia was observed in all of the BBN-treated. But no change was observed in the control and p-Cresidine-treated. The mRNA levels were compared and the differences in mRNA expressions between these three compounds were detected by glass array. Although extreme differences were not detected, the increasing more than two-times or decreaasing more than half were detected. We selected several genes, and reconfirmed the expression level by real time PCR. The number of the gene detected the difference from control was the largest in the BBN treated group and the smallest in the p-Cresidine treated group. Some selected data of mRNA level was reconfirmed by real time PCR, and we confirmed 70% data of the Glass Array was the same data of the real time PCR. Moreover, by using the in situ RT-PCR method, we examined the localization of the mRNA expression of the Glutation S-transferase (GST), which detected down regulation in the Uracil-treated urinary bladder only in the glass array analysis. The expression was observed in the cytoplasm of the transitional cell. Especially, the signal of the normal transitional cell in control and p-Cresidine-treated was stronger than that of the papillomatosis (Uracil) and simple hyperplasia (BBN). In conclusion, we detected the difference of the mRNA level of three compounds by using glass array and real time PCR for the trigger of this study, and we aimed at the GST mRNA expression. Though the difference of the gene expression between the carcinogen and non-carcinogen was not clarified regarding the GST mRNA expression, we detected the GST mRNA in the normal transitional cell stronger than that of papillomatosis treated with Urasil and simple hyperplasia treated with BBN.

P-29

Suppurative gastritis in BALB/c mice infected with Listeria monocytogenes via the intragastric route

Jong-Hwan PARK[1], Yong-Ho PARK[2], Seung-Hyeok SEOK[1], Sun-A CHO[1], Hui-Young LEE[1], Dong-Jae KIM[1], So-Hyun KIM[2], Jae-Hak PARK[1], Young-Soon LEE[3]

Department of Laboratory Animal Medicine, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea (South)[1]
Department of Veterinary Microbiology, College of Veterinary Medicine, Seoul National University[2]
Deparment of Veterinary Public Health, College of Veterinary Medicine, Seoul National University[3]

Suppurative gastritis was demonstrated in BALB/c mice 3 days after intragastric inoculation with 10^9 organisms of *Listeria monocytogenes* strain ATCC19113 (serotype 3). Also tested were four other strains of mice (C3H, C57BL/6, FVB and ICR). After inoculation with ATCC19113 the numbers of bacteria found in the stomach wall were greater in C57BL/6 and ICR mice than in C3H and FVB mice; moreover, the gastritis produced in BALB/c and C57BL/6 mice was more severe than that produced in the other mouse strains The inflammatory response occurred in the lamina muscularis and mucosa of the fundus. Massive necrosis of the gastric epithelium was observed, and oedema in a large part of the mucosal layer of the fundus. In addition, the submucosal layer was apparently expanded due to oedema, and in the cardia, the mucosal layer had become thin and flattened. Immunohistochemically, a polyclonal antibody against *Listeria* spp. produced labelling was observed in areas of the gastric mucosa in which there was an inflammatory response and gastric epithelial necrosis.

P-30

Analyses of immune response to candidate live oral Salmonella vaccine strain in mice

Sun A CHO[1], In Soo LEE[2], Jong Hwan Park PARK[1], Seung Hyeok SEOK[1], Hui Young LEE[1], Dong Jae KIM[1], Jae Hak PARK[1]

Department of Laboratory Science, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea (South)[1]
Department of Microbiology, Hannam University[2]

This study was performed to provide the guideline for evaluating the efficacy of a recombinant *Salmonella* live vaccine. We examined the immunologic efficacy of a candidate typhoid vaccine strain (recombinant *salmonella typhimurium* LF 878). Ten each mouse was orally administered with 10^5 and 10^6 CFU of LF878, $10^7$and $10^9$of *S. typhi* Ty2, respectively, and control with sterile PBS. Five mice of each group were necropsied at 5 and 12 days after infection. On ELISA and RT-PCR using serum and spleen tissue, expression of immunoglobulins and cytokines was higher in mice immunized with LF878 and *S. typhi* Ty2 than in control. When treated with T- and B-cell mitogens, spleen cells of mice immunized with LF878 and S. typhi Ty2 was more proliferative than those of control. And expression of CD4+ T lymphocyte in spleens of mice with LF878 and *S. typhi* Ty2 also increased more than control.

P-31

Earlier Helicobacter pylori infection cause severer inflammation and more risk for stomach carcinogenesis in N-methyl-N-nitrosourea treated Mongolian gerbils

Tetsuya TSUKAMOTO[1], Xueyuan CAO[1], Masami YAMAMOTO[1], Harunari TANAKA[1], Masae TATEMATSU[1]

Division of Oncological Pathology, Aichi Cancer Center Research Institute, Aichi 464-8681, Japan[1]

Purpose: Helicobacter pylori (H. pylori) is now well known to be associated with stomach carcinogenesis. Infection during childhood rather than in adults is considered to be more important.
Methods: [Experiment 1] To evaluate the difference in susceptibility to stomach carcinogenesis among various ages to acquire H. pylori infection, we designed an experiment with inoculation of H. pylori (ATCC 43504) followed by N-methyl-N-nitrosourea (MNU) treatment at different ages. Four-week-old male Mongolian gerbils were divided into twelve groups. H. pylori were inoculated at 4, 18 and 32 weeks old, as representative of early, middle and late infection, respectively. Two weeks later, the animals were treated with MNU. Groups without H. pylori and/or MNU were included as controls. [Experiment 2] To compare the severity of inflammation in association with stomach carcinogenesis, duration of H. pylori infection was set to long (20 weeks), intermediate (8 weeks), and short (2 weeks) terms as a model of severe, moderate, and mild inflammation before the administration of MNU at 27 weeks old. Gerbils were sacrificed at 77 weeks old. Controls were designed as above.
Results: [Experiment 1] The incidences of adenocarcinomas at 52 weeks after the inoculation in the early (H. pylori +MNU), middle (H. pylori +MNU), and late (H. pylori +MNU) groups were 60% (12/20), 18.4% (2/11), and 10% (2/20), respectively. The corresponding figures were 14.8% (4/27), 0% (0/11), and 0% (0/21) in the MNU-alone groups. A higher titer of serum IgG for H. pylori and gastrin level was seen in the early-infected compared to the middle and the late groups ($P<0.01$). [Experiment 2] Anti-H. pylori antibody and serum gastrin level were the highest in the long-term group, indicating the severest inflammation. Incidence of adenocarcinomas were 45% (9/20), 20% (2/10), and 23.1% (3/13) in the long, intermediate, and short Hp+MNU groups, respectively. The tumor incidence in the long-term group was significantly higher ($P<0.05$) than that in the corresponding MNU alone group (0%=0/16).
Conclusion: The results clearly demonstrated that early acquisition of H. pylori with MNU significantly increases gastric chemical carcinogenesis, as compared to the case with later infection, possibly because of differences in host gastric mucosal factors, immunologic responses, and subsequent inflammation.

P-32

Increased oxidative stress associated with alterations in DNA damage and repair in urinary bladder carcinomas associated and non-associated with Schistosomiasis

Elsayed I. SALIM[1], Hideki WANIBUCHI[1], Amani ABDUL-HAMID[2], Keiichirou MORIMURA[1], Shoji FUKUSHIMA[1]

Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]
Department of Pathology, Tanta Cancer Institute[2]

To cast light on the underlying mechanisms predisposing Egyptian urinary bladder carcinomas associated and non-associated with Schistosomiasis to high grades of aggressiveness, we analyzed immuno-histochemically the relationship between oxidative stress markers, DNA single strand breaks (ssDNA) and DNA repair genes in function with nitric oxide synthases expression levels in bladder cancers of 36 Egyptian cases with or without Schistosoma infection. Marked elevated levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were found in SCCs and TCCs associated with Schistosomiasis as compared with non-Schistosomal TCCs. This was accompanied by strong overexpression of inducible nitric oxide synthase (iNOS), ssDNA marker, and DNA damage repair genes; the 8-oxoguanine-DNA-glycosylase (OGG1) and apirinic/apirimidinic endonuclease (APE-1) in Schistosomal carcinomas. However, expression of the endothelial nitric oxide synthase (eNOS) was slightly stronger in non-Schistosoma-associated than the Schistosoma-associated cancers. In conclusion, these findings suggest a strong correlation between Schistosoma haematobium infection in Egyptian patients, and explains mechanisms of induction by increased levels of oxidative stress accompanied by a continuous DNA damage in urothelium followed by a subsequent overexpression of DNA repair genes, all together may function in a possible correlation with increased levels of iNOS in Schistosomal bladder cancers.

P-33

Roles of the Thai liver fluke, Opisthorchis viverrini, in the development of cholangiocellular carcinoma (CCC).

Witaya THAMAVIT[1], Tomoyuki SHIRAI[2], Malcolm A. MOORE[2], Nobuyuki ITO[2]

Department of Pathobiology, Faculty of Science, Mahidol University[1]
Department of Pathology, Nagoya City University, Medical School[2]

Association of CCC development and O.viverrini infection has been well known in the Thai Northeast. In experimental animals that received combination of the liver fluke and dimethylnitrosamine (DMN) developed 100% CCC. Animals with single DMN-initiated dose and subsequent infection by the liver fluke also developed CCC 44% of animals with other bile duct precancerous lesions ie. cholangiofibrosis, mucinous cystadenoma etc. in almost all the animals. Whereas, no CCC development were detected in animals monthly treated for 10 times with 50, 25 and 12 metacercariae in both sexes and left for one year. The results indicates promoting roles of the parasite in causing chronic cell death and cell profiferation of bile duct epithelium which persist as long as the presence of the parasite of which its lifespan is not less than 25 years. The histopathological lesions that imply cell death and cell proliferation of bile ducts are: egg and parasite granulomas, hyperplasia of main ducts, cholangiectatic cyst of bile duct resulting in partial obstruction of larger duct and bile ductule proliferation as a consequence, and mechanical damage of bile duct due to movement of the parasite etc. In the Thai Northeastern people, children received infection via mother feeding of infected raw fishes at the age of two month old. Continuous induction of bile duct cell death and cell proliferation creates fertile soil for initiation and subsequent promotion which perpetuates for life reflecting coinitiator and promoter roles of the parasite. In Thailand, initiators of carcinogens are believed to contaminate widely in foods and foodstuffs and everywhere. These reasons might explain world highest incidence of CCC in that region".

P-34

In vitro and in vivo sensitivity of various cells to arsenic trioxide(As_2O_3)

Chang Suk KANG[1], Yeon Sook MOON[1], Yong Gu KIM[1], Young Jin CHOI[1], Kyung Ja HAN[1], Sang In SHIM[1]

Department of Clinical Pathology, Catholic University of Korea, Seoul 150-713, Korea (South)[1]

Recently, inorganic arsenic trioxide (As2O3) was reported to induce complete remission in many patients with refractory acute promyelocytic leukemia (APL) and its growth inhibitory effects on several lymphoid neoplasms and solid tumors, in vitro, has been studied. However, the effect of As2O3 on cancer cells from malignant patients is not established. especially, in vivo. We assayed arsenic sensitivities on LoVo, OVCAR-3 and PA-1 cell lines and cancer cells obtained from patients with peritoneal carcinomatosis. And we evaluated the therapeutic effects of As2O3 on mice implanted cancer cell line. We cultured the LoVo, OVCAR-3 and PA-1 cell lines in media with variable As2O3 concentration for 24 and 48 hours. The detection methods for apoptotic or damaged cells were flow cytometry using annexin V-FITC/PI, terminal deoxynucleotide transferase (Tdt) -mediated dUTP- digoxigenin nick-end labeling (TUNEL), trypan blue dye exclusion test, acridine orange/ethidium bromide (AO/EtBr) uptake test, morphologic examination after Wright staining, and electrophoresis using 1% agarose gel for DNA ladder pattern identification. The cancer cells from patients are incubated by same process for 24 hours and 48 hours and measured percentage of damaged cells by trypan blue dye exclusion method. To find out in vivo therapeutic effect of As2O3, we introduced the PA-1 cells into the peritoneal cavity of mice and injected As2O3 into peritoneal cavity of the tumor implanted mice. Then, we weighed the mice (group I; peritoneal carcinomatosis group without As2O3 injection, group II; As2O3 injected group after peritoneal carcinomatosis developed, group III; PA-1 cells and As2O3 injected group at first time) and analyzed macro- and microscopic findings of tumor masses and survival rate between group II and group III. Among the above mentioned methods, Trypan blue dye exclusion was the most useful and reproducible method for As2O3 susceptibility test, and PA-1 cell line was most susceptible. Ladder pattern was not observed in DNA electrophoresis of As2O3 exposed cell lineds. Of eight peritoneal carcinomatosis cases, two in 5μmol/L, four in 10μmol/L, seven in 50μmol/L were susceptible. In vivo study, group III developed peritoneal carcinomatosis more lately, and lived longer than group I and II. Conclusively, the As2O3 sensitivity was variable in each cancer cell lines and cancer cells from patients. The As2O3 injection could delay development of peritoneal carcinomatosis in the early stage of peritoneal seeding. The cell death effect of As2O3 exposure might be result from not apoptosis induction but other mechanism.

P-35

Induction of glutathione S-transferase placental form positive foci in liver and epithelial hyperplasia in urinary bladder, but no tumor development in male Fischer 344 rats treated with monomethylarsonic acid for 104 weeks

Jun SHEN[1], Hideki WANIBUCHI[1], Elsayed SALIM[1], Kenichiro DOI[1], Min WEI[1], Shoji FUKUSHIMA[1]

Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

The carcinogenicity of monomethylarsonic acid (MMA(V)), a major metabolite of inorganic arsenics in human and experimental animals, was investigated in male Fischer 344 rats. A total of 129 rats at 10 weeks of age were randomly divided into three groups and received drinking water containing MMA(V) at doses of 0 (Control), 50 and 200 ppm ad libitum for 104 weeks. No significant differences were found between the control and MMA(V)-treated groups regarding clinical signs, mortality, hematological and serum biochemistry findings. Quantitative analysis of glutathione S-transferase placental form (GST-P) positive foci in liver revealed a significant increase of numbers and areas in the 200 ppm MMA(V)-treated group. In the urinary bladder MMA(V) induced simple hyperplasia, and significantly elevated the proliferating cell nuclear antigen (PCNA) positive index in the urothelium. A variety of tumors developed in rats of all groups, including the controls, but all were histologically similar to those known to occur spontaneously in F344 rats and there were no significant differences among the groups. Thus, it could be concluded that, under the present experimental conditions, MMA(V) induced lesions in the liver and urinary bladder, but did not cause tumor development in male F344 rats even after two years exposure.

P-36

Effects of sodium cacodylate and sodium nitrate on kidney tumor induced by dimethylnitrosamine in SD male rats.

Tsutomu HIBINO[1], Takamasa YANAGIDA[2], Junya MATUYAMA[1], Ayumi KATI[1], Naoki YAMAMOTO[2]

Department of Pathology, Fujita Health University College, Aichi 470-1192, Japan[1]
Joint Research Laboratory, Fujita Health University[2]

This study examined the effects of sodium cacodylate as an arsenic compound and sodium nitrate as a food additative on kidney tuomorigenesis induced by dimethylnitrosamine (DMN) in rats. Male Sprague-Dawley rats, 6 weeks olds at the start of the experiment, were divided into experiment 1 and 2. A single dose of 40 mg/kg DMN were dissolved in 0.5 ml of saline, were intragastrically intubated. One gram/liter Sodium cacodylate (SC) and ten grams/liter sodium Nitrate (SN) were mixed in drinking water respectively and were administered ad libitum. Experiment 1: Group 1 (16 rats) was gavaged DMN and 1 week later, was administrated SC. Group 2 (10 rats) was gavaged DMN alone. Group 3 (14 rats) was gavaged saline and SC. Group 4 (10 rats) was control. At 40 weeks, the incidence of renal cell carcinoma (RCC) was in 13 of 16 rats (81.3%) of group 1, and in 5 of 9 rats (55.6%) of group 2. Experiment 2: Group 1 (15 rats) was treated with DMN and SN. Group 2 (15 rats) was gavaged DMN alone. Group 3 (10 rats) was administered SN alone. Group 4 (10 rats) was controls. At 40 weeks, the incidence of RCC was in 15 of 15 rats (100%) of group 1, and 14 of 15 rats (93.3%) in Group 2. We concluded that sodium cacodylate were promoted the incidence of renal cell carcinoma induced by a single dose of DMN in male Sprague-Dawley rats, but sodium nitrate was not promoted.

P-37

Involvement of choline and amino acid metabolism in different hepatocarcinogenicity of L-amino acid-defined semisynthetic and semipurified choline-deficient diets in rats

Maki IGARASHI[1], Masakazu TAKAHASHI[2], Hitoshi ASHIDA[4], Mei-Heng MAR[5], Yutaka HATANAKA[4], Midori YOSHIDA[2], Fumiyuki UEMATSU[2], Naoto WATANABE[1], Akihiko MAEKAWA[3], Steven H ZEISEL[5], Dai NAKAE[2]

Laboratory of Protection of Body Function, Department of Food and Nutritional Science, Graduate school of Agriculture, Tokyo University of Agriculture, Tokyo 101-0062, Japan[1]
Department of Pathology, Sasaki Institute, Sasaki Foundation[2]
Director, Sasaki Institute, Sasaki Foundation[3]
Division of Life Science, Graduate School of Science and Technology, Kobe University[4]
Department of Nutrition, School of Public Health and School of Medicine, University of North Carolina at Chapel Hill[5]

Introduction : Choline is a vitamin that is involved in a wide variety of physiological regulations. Altered bioavailability of choline thus causes various pathological conditions. For instance, its dietary deficiency is hepatocarcinogenic in rats. In order to clarify its underlying mechanisms, we developed a L-amino acid-defined semisynthetic choline-deficient (CDAA) diet and found its much stronger carcinogenicity than a conventional, semipurified choline-deficient (CD) diet containing proteins with an equivalent amino acid profile to the CDAA diet. To assess factors involved in this phenomenon, the present study compared plasma and hepatic levels of choline derivatives and amino acids in male Fisher 344 rats given a short-term feeding of the CDAA or CD diets.

Methods : A total of 30 male Fisher 344 rats were used and equally divided into 5 groups at their age of 7 weeks old. Group 1 received a basal laboratory diet. Groups 2 and 3 received a semipurified, choline-supplemented (CS) diet and the CD diet, respectively. Groups 4 and 5 received a L-amino acid-defined semisynthetic, choline-supplemented (CSAA) diet and the CDAA diet, respectively. At the end of a 4-week feeding, all rat were killed, and the and heparinized plasma and liver were collected. Plasma and liver levels of choline derivatives; the choline, phosphatidylcholine, betaine, sphingomyeline, glycerophophocholine and phosphocholine; were analyzed by a liquid chromatography-mass spectrometry. Plasma and liver levels of amino acids were measured by a liquid chromatography.

Results and discussion : Both the CDAA and CD diets decreased levels of all determined choline derivatives, reflecting insufficient choline availability, and increased levels of almost all amino acids, suggesting enhanced protein degradation and diminished amino acid utility, either in plasma or livers. In particular, the increase of an essential amino acid cysteine, urea cycle members urea and ornithine, and fibrosis-related proline may correspond to hepatotoxicity induced by the diets. While the changes of choline derivative levels occurred in a similar extent for the by CDAA and CD diets, the changes of amino acid levels were substantially more prominent in the CDAA case than in the CD case . These results suggest that the different amino acid metabolism, but not the similar choline metabolism, may be involved in the different hepatocarcinogenicity in between rats fed the CDAA diet and these feed the CD diets.

P-38

Induction of fatty liver by mebalonic acid in Fischer 344 rats

Naoto WATANABE[1], Midori YOSHIDA[2], Maki IGARASHI[1], Fumiyuki UEMATSU[2], Masakazu TAKAHASHI[2], Akihiko MAEKAWA[3], Dai NAKAE[2]

Department of Food and Nutritional Science, Laboratory of Protection of body function, Graduate School of Tokyo University of Agriculture, Tokyo 101-0062, Japan[1]
Department of Pathology, Sasaki Institute, Sasaki Foundation[2]
Department Director, Sasaki Institute, Sasaki Foundation[3]

Purpose Mebalonic acid (MA) is used as a food additive or an integrant of cosmetics, but its safety evaluation including a repeated toxicity remains undetermined. In the present study, therefore, we conducted a 13-week oral toxicity study to evaluate subchronic oral toxicity and its reversibility for MA in rats.
Materials and methods Groups each consisting of 15 male or female Fisher 344 rats were administered MA at concentrations of 0 (a basal diet only), 400, 2,000 and 10,000 ppm in diet from their 6 weeks of age for 13 weeks. At this time-point, ten each of animals were sacrificed, and the remaining 5 each of animals were switched to the basal diet for additional 4 weeks. After terminations of the treatment or recovery periods, the blood was collected from all animals for the hematological and blood biochemical analyses. After a complete necropsy, all organs and/or tissues were fixed in a 10% neutrally buffered formaldehyde solution and routinely processed for the histopathological examination.
Results No clinical abnormalities or no death attributed to the MA treatment were observed in any animals. The body weight gain of the highest dose groups in either sex was slightly but significantly depressed at the last half of the MA-treated period, whereas the depression was abolished during the recovery period. Food consumptions in all treated groups were comparable to those in the control groups. At the necropsy, the livers of the highest dose group in either sex showed fatty-liver appearance, and the changes histopathologically correlated to fatty changes of the hepatocytes in the centrilobular zone with a slight aggregation of inflammatory cells. Also in the highest dose groups, the ALT activity was increased with slight anemia for both sexes, while the total cholesterol and triglyceride levels were increased for only females. After the recovery period, all of such histopathological, hematological and blood biochemical changes were almost reversed. There were no treatment-related changes observed in the other dose groups of either sex.
Discussion and conclusion These results indicate that a 13-week treatment of 10,000 ppm of MA induces fatty change of the liver with its related blood biochemical and hematological changes in male and female rats. The depression of the body weight at the highest dose is also considered treatment-related. All these changes are, however, reversible after a 4-week recovery period.

P-39

Induction of multiple granulation in the liver with severe hepatocyte damage by montan wax, a food additive, in a 90-day toxicity study in F344 rats

Mico IKEDA[1], Kousuke SAOO[1], Keiko YAMAKAWA[1], Hijiri TAKEUCHI[1], Masanao YOKOHIRA[1], Yoko HOSOTANI[1], Yu ZENG[1], Kyoko HOSOKAWA[1], Shigemi KINOUCHI[2], Katsumi IMAIDA[1]

Department of Onco-Pathology, Faculty of Medicine, Kagawa University, Kagawa 761-0793, Japan[1]
Diagnostic Pathology and Cytology Institute, Shikoku Cytopathologic Institutes[2]

Montan wax is a mineral wax which is extracted from lignite formed by the decomposition of vegetable substances. As it is hard and resistant to decomposition, when used as an ingredient in polishes and waxes, it imparts scuff resistance, water repellency and a high gloss. It has many other applications, including its use in emulsions, as an asphalt additive, in casting and leather finishing and as an additive in paper making and lubricant manufacture. Montan wax has also found employment as a food additive. As a part of the safety assessment of montan wax, we have performed a 90-day toxicity study in Fischer 344 rats.
Groups of 10 males and 10 females were given the material at dose levels of 0, 0.56, 1.67 or 5 % in the diet for 90 days. Clinical signs were noted once a day and body weights and food intakes were measured once a week. During the experiment, there were no remarkable changes in general appearance and no deaths occurred in any group. All animals were sacrificed after 90 days treatment. Hematological and serum biochemical examinations as well as necropsies were performed at the end of the experiment, when blood samples were collected after 16 hours of starvation. On hematological examination, Hb, Ht, MCV and MCH were significantly decreased and WBC was significantly increased in all treated rats. On serum biochemical examination, AST and ALT were found to be elevated more than four fold in all treated groups as compared to the control group in both sexes. Furthermore, significant increases of total cholesterol, platelets and potassium were also observed in all treated rats in all treated rats. Relative organ weights of liver, spleen, lung and kidneys in all treated groups of both sexes were increased. Histopathological observation revealed diffuse multiple microgranulation in the liver with severe hepatocyte damage and lymphocytic infiltration. Granulomatous lesion were also apparent in the mesenteric lymph nodes in all treated males and females. These findings clearly demonstrate that montan wax, at doses of more than 0.56 % in the diet can induce severe granulomatous inflammation in the liver. Because of pathological, hematological and serum biochemical changes were observed in the lowest dose group, a no-observed-adverse-effect level (NOAEL) could not be determined in a present study.

P-40

Dietary intake of various lactic acid bacteria suppresses Type 2 helper T cell production in antigen-primed mice splenocyte

Hui Young LEE[1], Jong Hwan PARK[1], Seung Hyeok SEOK[1], Sun A CHO[1], Min Won BAEK[1], Dong Jae KIM[1], Yong Soon LEE[2], Jae Hak PARK[1]

Department of Laboratory Animal Medicine, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea (South)[1]
Department of Public Health, College of Veterinary Medicine, Seoul National University, Seoul, Korea[2]

Lactic acid bacteria (LABs) have been proposed as a potential oral allergy-therapeutic mean of modulating immune phenotype expression in vivo, via promoting or reducing cytokine production. This study investigated the ability of LABs to suppress allergic response via modulating cytokine production in mice splenocytes. BALB/c mice were intraperitoneally primed to ovalbumin in alum adjuvant to invoke antigen-specific Th1/Th2 cytokine-secreting cell populations in splenocytes. Spleen cells from mice fed with Lactobacillus confusus PL9001(KCCM-10245), L. fermentum PL9005(KCCM-10250), L. plantarum PL9011 (KCCM-10358) and Bifidobacterium infantis PL9506(KCCM-10406) during antigen sensitization suppressed the levels of Th2 cell cytokines such as IL-4 and IL-5. And all mice fed with LABs induced Th1 cell cytokines such as IL-2 secretion by splenocyte. Results suggested that these LABs are anti-allergic agents, in the view of the Th1/anti-Th2 immunoregulation.

P-41

Examination of heat shock protein 70 as an early-stage injury-biomarker in rat testes after stress-induced cell damage

Akiko IKEDA[1], Yoshihumi KANEKO[1], Makiko TAKAHASHI[1], Sumihisa SUEYOSHI[1], Yoshihiro MASUMOTO[1], Kiyoyuki TSURU[1]

Kyorin Phamaceutical Co., Ltd, Research Center, Tochigi 329-0114, Japan[1]

Heat shock proteins (HSPs) are increased at an early stage of injury associated with stress conditions such as hyperthermia, ischemia, UV radiation, oxidative stress, and chemicals. Heat shock protein 70 (HSP70), one of such proteins, may serve as a sensitive biomarker for cell damage. Thus, this study was aimed to examine whether HSP70 would be used as an injury-biomarker for testes by determining the time-courses for HSP70 and apoptosis after cell damaging. Cell damages were induced by surgical crytorchidism or administration of human chorionic gonadtrophin (hCG). Surgical cryptorchidism has been frequently used to study germ cell apoptosis due to heat stress. Administration of hCG is known to induce precapillary vasoconstriction resulting in cell damage.

In cryptorchidism with Wistar rats, the day of surgery was designed as day 0, and the testes were removed 4 h, 8 h, 24 h, and 3 days, 5 days, 7 days and 14 days after surgery. In administration of hCG, Wistar rats were injected s.c. with 100 i.u. hCG, and then sacrificed to remove the testes 4 h, 8 h, 24 h and 2 days after injection. The levels of HSP70 expression and apoptosis in spermatocytes were detected by immunoperoxidase staining and by TUNEL, respectively.

In cryptorchidism, HSP70 was rarely observed in pachytene spermatocytes and round spermatids at 0 h after surgery. HSP70 increased in pachytene spermatocytes from 4 h after surgery, and peaked 8 h after surgery. The increase in HSP70 preceded germ cell apoptosis, which tended to increase from 24 h after surgery. Germ cell apoptosis was noted in the majority of tubules from 3 days after surgery, and the apoptotic cells peaked 5 days after surgery. Staining with periodic acid shiff showed the presence of degenerating germ cells in almost all tubules from 3 days after surgery. Similarly, administration of hCG increased the HSP70 expression, which preceded germ cell apoptosis in pachytene spermatocytes. HSP70 increased at 8 h, and peaked at 24 h after administration. In the both types of cell damage induction, HSP70 was temporally correlated with germ cell apopstosis despite some time lag each other. In conclusion, HSP70 increased earlier than germ cell apoptosis in pachytene spermatocytes under stress conditions both in surgical cryptorchidism and administration of hCG. These results suggest that HSP70 may serve as an early injury-biomarker for rat testes.

P-42

Histopathological and endocrinological changes of cardiac natriuretic peptide in rats with drug- induced cardiotoxicity

Hiroto MIYATA[1], Rie OHNO[1], Rika SHIRANE[1], Yutaka NAKANISHI[1], Atsushi NAKAMURA[1], Masaaki KIMURA[1]

Toxicology Laboratory, Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd., Saitama 331-9530, Japan[1]

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are circulating hormones secreted from heart predominantly in cases of hypertension or congestive heart failure. We have previously reported on the relationship between these hormones in plasma and cardiac injury in rats. In the present study, in order to confirm whether ANP and BNP levels can be appropriate indicators in evaluation of cardiac injury, we investigated the relationship between the plasma or heart levels of these hormones and heart injury in rats given isoproterenol (ISO) to induce cardiac injury. Eighty eight male Crj; CD (SD) IGS rats (10 weeks old) were given a single intravenous injection of ISO. Blood and heart samples were collected 0.5, 2, 4, 8, 48 hrs, 7 and 14 days after the dosing. Six hearts per group were used for histopathological investigations. Each specimen was fixed in 10% neutral phosphate-buffered formalin, dehydrated in absolute alcohol, and embedded in paraffin wax. 4μm-thick sections processed from these paraffin blocks, were stained with hematoxylin and eosin (H&E) or used for immunohistochemical studies using anti-ANP or anti-BNP antibody. Measurements of plasma and heart concentration were perfomed by radioimmunoassay. Myocardial degeneration or necrosis with inflammatory cell infiltration was observed in the cardiac ventricle 4 hrs after the dosing. At 48 hrs there was myocarditis with neutrophil and mononuclear cell infiltration in the ventricle, more extensively than at earlier points. In some specimens, fibrosis had formed in the myocarditis 4 days after the dosing. BNP positive cells were recognized in myocardial degenaration part or its circumference 4 days after the dosing. ANP levels in plasma and heart concentration did not change after ISO dosing. However, BNP levels in plasma significantly increased 0.5-48 hrs after the dosing, peaking 4 hrs after the dosing. BNP levels in cardiac atrial measurement increased 7 days after the dosing while those in the cardiac ventricle significantly increased 2-8 hrs after the dosing. These data indicate that peak BNP levels in plasma parallel those of the cardiac ventricle, revealing that they reflect histopathological alterations of the heart. These results suggest that plasma ANP and BNP levels can be useful indicators of the location of injuries in the heart.

P-43

Histomorphometrical parameters for evaluation of osteocompatibility of metal implants with undecalcified bone section

Miki NISHIMORI[1], Shin-ichi KATSUDA[1], Yoshimitsu OKAZAKI[2], Emiko GOTO[3], Akihito SHIMOI[1], Hidetaka SATO[1]

Japan Food Research Laboratories, Tokyo 206-0025, Japan[1]
National Institute of Advanced Industrial Science and Technology[2]
National Institute of Technology and Evaluation[3]

We evaluated histocompatibility of alloys such as Co-CrF-75, Ti6-4ELI and Ti-15Zr by comparing osteogenesis histomorphometrically on undecalcified bone section. Alloys were implanted into femoral and tibial bones of male Wistar/ST rats for 1, 3, 6, and 12 months. At the end of each implantation period, bones including implants were excised and polyester-resin-sections were ground to approximately 70 to 80 micrometers thick. They were stained with Villanueva bone stain, Toluijine blue stain or Giemsa stain, and observed with light and/or fluorescent microscopes. For histomorphometry, (1) thickness of new bone surrounding the surface of the implant, (2) rate of bone contact with the implant, (3) rate of perimeter of new bone, (4) rate of osteoid and calcified bone in new bone were measured with a quantitative television microscope. (1) Thickness, (2) rate of bone contact, and (3) rate of perimeter were increased from 1 to 6 months in all alloys. However, these factors were constant after 6 months. The calcified new bone surrounded all alloys, (4) the rate of calcified bone being more than 80 % of the new bone in 1 month. At Ti -15Zr implanted sites, rates of osteoid were approximately 10, 6, 6, 3 % in 1, 3, 6 and 12 months, respectively. At Co-CrF-75 and Ti6-4ELI implants, rates of osteoid were approximately 5 % in 3 months. However, the rates of osteoid were increased up to approximately 10 % after 6 months. From these finding, Ti-15Zr appeared to show good osteocompatibility and osteoconductivity, and histomorphometrical measurements of the relation between new bones and implants were suggested to be good parameters for osteogenesis with undecalcified bone section.

P-44

Genetic polymorphisms of alcohol-related metabolizing enzymes and the risk of esophageal cancer in the Thai population

Pleumjit BOONYAPHIPHAT[1], Paramee THONGSUKSAI[1], Wanna SUDHIKARAN[1], Puttisak PUTTAWIBUL[2]

Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkla 90110, Thailand[1]
Department of Surgery, Faculty of Medicine, Prince of Songkla University[2]

The association of life style habits and polymorphism of *ADH2*, *ALDH2* and *CYP2E1* genes with the risk of esophageal squamous cell carcinoma in the Thai population was investigated in a hospital-base case-control study:163cases and221controls. The results of multivariate logistic analysis revealed that alcohol consumption>40g/day;tobacco smoking>10cigarettes/d and betel chewing>5quids/day increased the risk significantly(odds ratio(OR)7.75,95%confidence interval(CI)3.65-16.44;2.72,95%CI1.10-6.68and2.3695%CI 1.25-4.44respectively).The associations between individual gene and the interaction of genes and environment on the risk of cancer were not found. Interestingly, a significant gene-gene interaction was observed. Individual with *CYP* 2*E* 1(*c* 1/*c* 2)and *ALDH* 2(2*/2*)had the risk6.27folds(95%CI1.96-20.01).In conclusion, these results demonstrate that alcohol is a strong risk factor for esophageal cancer. Furthermore, the combined genotype of mutant *CYP* 2*E* 1and *ALDH* 2is also a significant risk factor for esophageal cancer in our population.

This work was supported by a grant from Prince of Songkla University

P-45

Toxicogenomics using "percellome" and "mille-feuille" data system

Jun KANNO[1], Ken-ichi AISAKI[1], Atsushi ONO[1], Katsuhide IGARASHI[1]

Cellular & Molecular Toxicology Division, Biological Safety Research Center, National Institute of Health Sciences, Tokyo 158-8501, Japan[1]

The whole genome information for humans and rodents, and technology for monitoring whole genome expression will enable us to perform the comprehensive profiling of transcriptome. The major characteristics of this profiling are that the overt phenotypes are not the primary factors for the construction of toxicology database/informatics. This "gene first, phenotype second" approach named "Phenotype-Independent Toxicology" will compile the future predictive toxicogenomics database in combination with the current toxicology based on "phenotype first, gene second" strategy.
To build up such database, multiple experiments must be conducted and data collected over a certain period of time. Commonly, DNA microarray data are normalized relatively against mRNA concentration of certain housekeeping gene(s) or total mRNA quantity, or other empirical baselines that are considered to be universal among samples. Although such relative measurements are satisfactory for smaller numbers of genes that are changing drastically, smaller changes in many genes are not well analyzed. To overcome these problems, here we introduce a system that will absolutize the gene expression values in a "per one cell" basis ("percellome") . Once absolutized, the data from each samples and studies can be compared without further normalization. This system is made primarily for Affymetrix GeneChips, but can be expanded to other platforms and RT-PCR, as long as they fulfill the demands of this system. This "percellome" absolutization will enable us to express data in a linear scale from zero, thus generating "mille-feuille" data using virtually all gene data generated by the microarray.
The "percellome" will facilitate the analysis of low expression genes including those of knocked out genes in gene knock out mice. Unsupervised clustering is much easier for this type of data than commonly used ratio data against concurrent controls. As a whole, this system will contribute to the predictive toxicology by the development of a high-precision large-scale database/informatics.

P-46

Microarray analysis of amplified RNA from laser- microdissected GST-P negative hepatocellular preneoplastic lesion induced by peroxisome proliferators.

Tokuo SUKATA[1], Satoshi UWAGAWA[1], Keisuke OZAKI[1], Kayo SUMIDA[1], Masahiko KUSHIDA[1], Kaoru KIKUCHI[3], Koichi SAITO[1], Kenji OEDA[1], Yasuyoshi OKUNO[1], Nobutoshi MIKAMI[1], Shoji FUKUSHIMA[2]

Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan[1]
Department of Pathology, Osaka City University Medical School[2]
Sumitomo Pharmaceuticals Co., Ltd.[3]

In the present study, we tried to obtain a gene expression profile, which is specific for glutathione-S-transferase placenatal form (GST-P) negative foci induced by peroxisome proliferator (PP), by microarray analysis of micro-lesions. GST-P negative foci were induced using the initiation-promotion model. Study 1: 6-week-old, F344 male rats were given a single i.p. injection of diethylnitrosamine (DEN, 200mg/kg) at the start of the experiment and subjected to two-thirds partial hepatectomy at week 3. Two weeks after the initiation by DEN, Wy-14,643 and clofibrate were fed to rats for 10 weeks at doses of 0, 1000ppm (Wy-14,643) and 3000ppm (clofibrate), respectively. Study 2: one week after the i.p. injection of DEN (100mg/kg) once a week for 2 weeks, clofibrate was fed to 6-week-old, F344 male rats for 26 weeks at dose of 3000 ppm. GST-P positive (induced by DEN alone and DEN+clofibrate in Study 1) and GST-P negative foci (induced by DEN+ Wy-14,643 in Study 1 and DEN+clofibrate in Study 2), and the adjacent normal tissues were microdissected using laser microdissection system. Subsequently, a slight amount of total RNA was isolated, linear- amplified, and analyzed using gene tips. The genes were filtered with a minimum standard of at least a 1.5-fold change in expression level compared to the adjacent normal tissue. As the results, the numbers of up-regulated genes were 175 in Wy-14,643 and 260 in clofibrate, and the numbers of down-regulated genes were 30 in Wy-14,643 and 190 in clofibrate. In the up- regulated genes, 18 genes were common in the GST-P negative foci induced by the PPs (EST, 7; cell growth, 2; protease, 2; metabolism, 1; signal transduction, 1; receptor, 1; membrane protein, 1; the others, 3), and only one gene was specific for GST-P negative foci (not expressed in GST-P positive foci) among them. On the other hand, in the down-regulated genes, 8 genes were common in the GST-P negative foci induced by the PPs (metabolism, 4; genetic stability, 1; steroid hormone synthesis, 1; EST, 1; the other, 1), and none of them were specific for GST-P negative foci. Furthermore, fluctuation of these GST-P negative foci susceptibility genes generally got to be more distinct in hepatocellular adenoma. These lesion-specific analysis will be useful to elucidate the relation between morphological change and fluctuation of gene expressions.

P-47

Specific differences in gene expression profile revealed by cDNA microarray analysis of glutathione S-transferase placental form (GST-P) immunohistochemically positive rat liver foci and surrounding tissue

Shugo SUZUKI[1], Makoto ASAMOTO[1], Kazunari TSUJIMURA[1,2], Kumiko OGAWA[1], Mitsuru FUTAKUCHI[1], Tomoyuki SHIRAI[1]

Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Chemicals Evaluation and Research Institute, Chemicals Assessment Center[2]

The glutathione S-transferase placental form (GST-P), one of the glutathione S-transferase family of detoxification enzyme, is a very useful marker of rat liver preneoplastic lesions. We here investigated the gene expression profile in GST-P positive foci as compared with surrounding GST-P negative areas in the same livers of rats. For induction of the GST-P positive foci, seven-week-old male rats received an intraperitoneal injection of diethylnitrosamine at a dose of 200 mg/kg b.w. for initiation, and two weeks later, they were administered basal diet containing 100 ppm 2-acetylaminofluorene for 4 weeks. The rats were subjected to two-thirds partial hepatectomy at the end of experimental week 3. All surviving animals were killed under ether anesthesia at week 6. For the purpose of extracting RNA from prepared GST-P immunostained tissue, liver frozen sections were fixed in ice-cold acetone, and treated with rabbit anti-rat GST-P antibody and secondary antibody at 4℃ with RNase inhibitor. The sites of peroxidase binding were demonstrated with diaminobenzidine at room temperature. After the immunostaining of frozen tissue, microdissection was performed using the AS LMD system. Total RNAs were extracted to allow gene expression profiles to be assessed by cDNA microarray technology. As a result we found 37 genes to be up-regulated and 10 genes to be down-regulated in the GST-P positive foci. All these genes were found to be associated with metabolism in the biological process category, and catalytic activity in the molecular function category. The results provide for an important role of metabolism in carcinogenesis included GSTs, GCS, aldo-keto reductases, glutathione synthetase and P450s. Transaldolase, rat aflatoxin B1 aldehyde reductase (AFAR) and gamma-glutamylcysteine synthetase (GCS) were found as up-regulated genes and regucalcin as a down-regulated gene, in line with findings for hepatocellular carcinomas. The results indicate that the approach adopted is useful for understanding mechanisms of hepatocarcinogenesis and identification of new markers for rat liver preneoplastic foci.

P-48

Potential for the prediction of carcinogenicity by gene expression profile in rat hepatoma cells and comparison of expression patterns between chemically treated rat and human hepatoma cells

Kazunari TSUJIMURA[1,2], Makoto ASAMOTO[1], Shugo SUZUKI[1], Shingo INAGUMA[1], Kumiko OGAWA[1], Tomoyuki SHIRAI[1]

Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Chemicals Evaluation and Research Institute, Chemicals Assessment Center[2]

[Object] The long-term rodent bioassay is the standard method to predict carcinogenic hazard of chemicals for human beings. However, this assay takes a long time and high costs. Recently, gene expression profiling, with the use of microarray technology, has be increasingly contributing to an improved understanding of global, coordinated cellular events under a variety of conditions. In the field of toxicology, this approach has been also applied to assess the toxicity of unknown compounds, in the field of "Toxicogenomics". Assessment using cell lines is simple and reproducible and our aim has been to develop a rapid and reliable prediction method for carcinogenicity based on microarray technology in cultured cells.
[Materials and Methods] We selected 39 chemicals that have been well characterized for carcinogenicity. Rat hepatoma cells (MH1C1) and human hepatoma cells (HepG2) were treated with the chemicals for three days at a non-toxic dose. Gene expression changes were analyzed using our in-house microarray for MH1C1 cells and the AceGene microarray (Hitachi soft ltd.) for HepG2. Genes for prediction of carcinogenicity were selected with statistical methods and analyzed by clustering analysis, with a support vector machine (SVM), that is supervised machine learning classifiers. As a test set, additional 6 chemicals underwent prediction of carcinogenicity based on the results of the SVM analysis and comparison with actual findings. Twenty-four of 39 chemicals were compared for expression responses in MH1C1 and HepG2 regarding ortholog significant genes using the Homologene database.
[Result] A significant gene set for the prediction of carcinogenicity was selected. As the result of cross-validation, the predictive ratio for carcinogenicity was approximately 80%. With the six test set chemicals, the results predicted by SVM were reasonable. In contrast to MH1C1, when human HepG2 cells were used, significant genes could not be selected for the prediction of carcinogenicity, and the pattern of gene expression induced by chemicals was very different from the human (HepG2) case.
[Discussion] These results indicate that short-term bioassay systems of hepatoma cells for carcinogenicity using gene expression are promising. With interspecies analysis, careful consideration is needed for the application of rat data to the human situation.
[Acknowledgement] This study was financially supported by the New Energy and Industrial Technology Development Organization (NEDO) for basic research on "Development of a High Precision Summary Toxicity (Hazard) Assessment System".

P-49

Gene expression profiles in F344 rat livers treated with acetaminophen or cycloheximide

Takashi YAMOTO[1], Naoki KIYOSAWA[1], Kazumi ITO[1], Takayuki SATO[1], Kyoko SAKUMA[1], Miyuki KANBORI[1], Noriyo NIINO[1], Sunao MANABE[2], Naochika MATSUNUMA[1]

Medicinal Safety Research Labs., Sankyo Co., LTD, Shizuoka 437-0065, Japan[1]
Sankyo Pharma Inc.[2]

Acetaminophen (APAP) and cycloheximide (CHX) are known as chemicals that induce the cell death in hepatocytes by different mechanisms. The aim of this study was to evaluate the feasibility of gene expression data for the analysis of the cytotoxic mechanisms of APAP and CHX. F344/DuCrj male rats (9 weeks of age) were administrated 1000 mg/kg of APAP or 6 mg/kg of CHX. The liver of treated animals were collected at 1, 2, 6 and 24 hours after dosing of APAP, or at 1, 2 and 6 hours after dosing of CHX. Rat Genome U34A Array (Affymetrix) was used for the collection and analysis of comprehensive gene expression data in rats liver. Among the 8799 probes in the Rat Genome U34 Array, 1049 and 1315 probes were selected as the probes that represent the remarkable gene expression changes according to the APAP and CHX treatment, respectively. The expression of the genes associated with inflammation detected relatively soon after APAP treatment and the gene expression profile was changed in the course of time. On the other hand, specific changes were observed at first and then the inflammation related gene expression was detected in the CHX toxicity study. The gene expression changes in rat livers caused by the treatments with APAP or CHX could be classified as different clusters. In conclusion, the difference between the cytotoxic mechanisms of APAP (necrosis) and CHX (apoptosis) in rat livers was distinguished by the difference in their gene expression profiles. The accumulation of data that associates between pathological observations and the changes in gene expression profiles will provide vast useful information for the prediction of the toxicity of chemicals.

P-50

Methacarn, a versatile fixation tool for quantitative mRNA expression analysis in microdissected paraffin-embedded tissues using real-time RT-PCR and microarray systems

Makoto SHIBUTANI[1], Kyoung-Youl LEE[1], Hironori TAKAGI[1], Natsumi KATO[1], Shu TAKIGAMI[1], Masao HIROSE[1]

Division of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan[1]

We have previously demonstrated methacarn to be a versatile fixation tool for molecular analysis of proteins, DNAs and RNAs in paraffin-embedded tissues (PETs). This time, we wish to clarify the performance of methacarn for quantitative measurement of mRNA expression in microdissected PET specimens by real-time RT-PCR and microarray systems. RNA-yield from methacarn-fixed PET section was equivalent to the unfixed cryosection and hematoxylin staining also did not affect the yield. By real-time RT-PCR, relative abundance of measurable mRNAs in methacarn-fixed PET section was found to be 80-100 % of unfixed cryosection. Hematoxylin staining resulted in only 0-20 % reduction of amplifiable mRNAs from the unstained case. Correlation between the expression levels of target genes and input amount of extracted RNA in the range of 1-1000 pg was very high (correlation coefficient:>0.98) with regression curves equivalent to the unfixed cryosection case. Although the cell number within the dynamic range of PCR amplification for cytochrome P450 2B1 and glyceraldehyde 3-phosphate dehydrogenase was found to be 52 and 13, respectively in the 10 μm-thick section of the phenobarbital (PB)-treated rat liver, measured values were varied among samples when the size of microdissected areas was decreased, and ≥ 300 cells would be required for accurate measurement if normalization of expression values with the input amount of total RNA was intended. As for microarray analysis, up to 0.5 million-fold of antisense RNAs (aRNAs) could be amplified by two-round (2x) *in vitro* transcription with 50 ng of total RNA from PB-treated rat liver PET section. With the amplified aRNAs, expression profile was compared with those in 1x or 2x-amplified aRNAs from unfixed tissue, and high fidelity was confirmed by a correlation efficient of 0.91 with 1x-amplified aRNAs from unfixed tissue. For practical measurement, region-specific gene expression profiles in the microdissected medial preoptic area (MPOA) were analyzed in rat neonates injected s.c. with estradiol benzoate (EB; 10 μg/pup) or flutamide (FA; 250 μg/pup), to identify genes linked to disruption of brain sexual differentiation. MPOA-specific gene expression profile by each treatment was examined 24 hour later, and genes showing altered expression by EB or FA with sex-dependent manner could be identified as candidates to play a role in central endocrine disruption. These results demonstrate the high performance of methacarn close to that of unfixed tissues in the quantitative expression analysis of mRNAs in microdissected PET-specimens by both real-time RT-PCR and microarray systems.

P-51

Transcription of rat amyloid precursor protein in rat livers is affected by the serum cholesterol concentration

Naoki KIYOSAWA[1], Kazumi ITO[1], Kayoko ISHIKAWA[1], Isao IGARASHI[1], Noriyo NIINO[1], Kyoko SAKUMA[1], Miyuki KANBORI[1], Takashi YAMOTO[1], Sunao MANABE[2], Naochika MATSUNUMA[1]

Medicinal Safety Research Labs., Sankyo Co., Ltd., Shizuoka 437-0065, Japan[1]
Sankyo Pharma Inc.[2]

Microarray analysis is a useful tool for detecting and investigating unknown behaviors of gene expression. Analyzing a gene expression database constructed with Rat Genome U34 Array (Affymetrix Inc.), we found that transcription of amyloid precursor protein (APP) in male F344 rat liver is progressively increased by the repetitive phenobarbital (PB) treatments. APP transcriptional induction by the PB treatment did not show dose dependency, suggesting it is not an extension of direct pharmacological effect of PB, but caused from secondary effect by repetitive PB treatments. On the other hand, APP transcription decreased in the clofibrate (CPIB)-treated rat livers. The objective of this study is to investigate the molecular mechanism of hepatic APP induction and repression in rat livers after repetitive PB or CPIB treatments.
The hepatic APP transcription profiles correlated with serum total cholesterol (T.CHO) concentration profiles in both chemical-treated rats. Therefore we explored the genes whose time-course transcription profiles are correlated highly with serum T.CHO concentration profile, using microarray database on rat livers treated with PB or CPIB. Of the 8799 probes in the Rat Genome U34 Array, transcription of GST alpha, UDPGT-1, Apolipoprotein A-I or cMOAT, all of which are involved in bilirubin or bile acid metabolism and efflux, were highly affected by the serum T.CHO concentration, and APP transcription exhibited the 6th most correlated transcription profiles to that of serum T.CHO concentration. These data suggest that hepatic APP transcription is strongly affected by the serum T.CHO concentration, supposedly involved in cholesterol and bile acid metabolism. To confirm the notion, we fed a high-cholesterol (1%) diet for 33 days, and both serum T.CHO and hepatic APP transcription were measured. Serum T.CHO concentration and hepatic APP transcription in treated rat livers increased by 4.6-fold and1.9-fold compared to those in the control, respectively. These data suggest that the APP transcription in rat liver is positively regulated by the serum T. CHO, and that increase in APP transcription in the PB-treated rat liver was brought about via an elevation of serum T.CHO concentrations.

P-52

Gene expression profiling of terminal end buds during rat mammary carcinogenesis

Koichiro MIYAMOTO[1], Shuhei TAGUCHI[1], Hiroaki KAWAGUCHI[1], Yoshihisa UMEKITA[1], Hiroki YOSHIDA[1]

Department of Tumor Pathology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8520, Japan[1]

We have reported that a rat mammary carcinoma is induced in rats by the administration of 7,12-dimethylbenz[a]anthracene (DMBA). In the present study, we tried to clarify molecular paths involved in progression of normal tissue to malignancy using cDNA microarray and laser microdissection methodology. Inbred female Sprague-Dawley rats were divided into two groups. At the age of 50 days, rats of Group I and II were given 20 mg of 7, 12-dimethylbenz[a]anthracene (DMBA) and 1 ml of sesame oil, respectively. Two weeks after the administration of DMBA and sesame oil, rats of both groups were killed for removal of mammary glands. Removed mammary glands were frozen on dry ice and embedded in OCT. Frozen sections were stained with HistoGene (Arcturus Engineering). Terminal end buds were transferred onto caps using the PixCell IIe LCM System (Arcturus Engineering). Total RNA was extracted from caps using PicoPure (Arcturus Engineering). cDNA was synthesized from total RNA and amplified by Atlas SMART Probe Amplification Kit (CLONTECH). 32P-labeled cDNA probes were hybridized to Atlas Rat Toxicology 1.2 Array membranes. The hybridization pattern was analyzed by autoradiography and quantified by phosphorimaging. Results were normalized by global normalization and compared between two groups using AtlasImage (CLONTECH). Fifteen genes that demonstrated more than 1.69-fold change values between Group I and Group II were evaluated as significant. Especially transcripts for ribosomal proteins, receptors of the extracellular matrix and folding-related proteins were up-regulated in DMBA-treated rats. Study for abnormal gene expression at the early stage of carcinogenesis is important and we are going forward with further investigations by changing conditions and using real-time PCR.

P-53

Region-specific global gene expression analysis in the microdissected hypothalamic medial preoptic area of rat neonates exposed perinatally to di(2-ethylhexyl)phthalate

Kyoung-Youl LEE[1], Makoto SHIBUTANI[1], Hironori TAKAGI[1], Natsumi KATO[1], Shu TAKIGAMI[1], Masao HIROSE[1]

National Institute of Health Sciences, Tokyo 158-0098, Japan[1]

Phthalate esters affect male sexual differentiation in rats exposed prenatally or perinatally by affecting perinatal testosterone surge due to testicular toxicity. To identify genes linked to disruption of brain sexual differentiation by phthalate esters, a region-specific global gene expression analysis was performed in the medial preoptic area (MPOA) microdissected from hypothalamus of rat neonates at postnatal day (PND) 2 following maternal exposure to di(2-ethylhexyl)phthalate (DEHP; 6000 ppm) in diet from gestational day 15. For comparison, a group of neonates were exposed to flutamide (FA; 250 μg/pup, s.c.) at birth. At PND 2, brains of animals (3 males and 3 females per group) were fixed with methacarn and paraffin-embedded. Total RNA (50 ng) extracted from microdissected MPOA was subjected to two-round *in vitro* transcription, hybridized with Affymetrix GeneChip Rat Genome U34A Array containing 8000 probes, and analyzed the expression data by GeneSpring software ver. 5 (Silicon Genetics). In the MPOA, about 3600 genes showed presence call in both sexes. Among these genes, a total of 5% showed sex difference in the expression (>2-fold) in untreated controls, with 62 and 124 genes showing higher expression in males and females, respectively. By DEHP-treatment, up- or down-regulation (>2-fold) was observed with 24 and 21 genes in males, and 31 and 418 genes in females, respectively. Among them, only one and 2 genes showed up- or down-regulation commonly to both sexes, respectively. On the other hand, 2 of up-regulated genes by DEHP in males showed down-regulation in females and 2 other genes showed opposite expression pattern. Among genes showing a sex difference with higher expression in males, 7 genes showed down-regulation in males and 5 genes showed up-regulation in females by DEHP. Similarly, among genes showing sex difference with higher expression in females, 22 genes showed down-regulation in females and 10 genes showed up-regulation in males by DEHP. By single injection of FA, up- or down-regulated genes were found to be 6 each in males, and 9 and 29 in females, respectively. Only cyclin G1 showed up-regulation commonly to FA and DEHP in males, and apolipoprotein E and death-associated like kinase showed down-regulation commonly to both chemicals in females. In summary, we identified genes showing altered expression by perinatal DEHP exposure with apparent sex difference or common to the neonatal FA-treatment to be candidates playing role(s) for central endocrine disruption by DEHP.

P-54

Differential global gene expression in rat brown and white adipose tissues

Akira UNAMI[1], Yasuo SHINOHARA[2], Kazuaki KAJIMOTO[2], Kenjiro TSUBOTA[1], Yoshimasa OKAZAKI[1], Shiro FUJIHIRA[1], Masahiro MATSUMOTO[1], Yuji OISHI[1], Yoshinobu BABA[3]

Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka 532-8514, Japan[1]
Institute for Genome Research, The University of Tokushima[2]
Faculty of Pharmaceutical Sciences, The University of Tokushima[3]

It is well known that white and brown adipocytes are present in mammals and that those function as counter actors in energy metabolism. The physiological role of white adipocytes is to accumulate excess energy as fat, and that of brown adipocytes is its expenditure as heat. Increasing in adipose cell number and size results in obesity, which is one of risk factors for hypertension, hyperlipidemia and diabetes. Recently, some proteins that participate in the physiological function of these adipocytes have been noticed as new drug targets. Therefore, to clarify the molecular mechanisms of energy metabolism in adipocytes is considered to be very useful to develop the therapy for obesity. In the present study, we analyzed gene expression profiles in brown (BAT) and white (WAT) adipose tissues of rat using a high-density cDNA microarray to characterize unknown gene associating with energy metabolism in those tissues.
Microarray analysis showed that 499 cDNA/ESTs were expressed at least 5-fold higher or lower in BAT than in WAT. Genes encoding uncoupling protein 1 (UCP1), muscle-type carnitine palmitoyltransferase (M-CPTI), carnitine carrier (CC) and some other proteins involved in energy metabolism were highly expressed in BAT. Most of the genes encoding mitochondrial proteins, such as subunits of ATP synthase, cytochrome c oxidase and NADH dehydrogenase, were higher expressed in BAT than WAT, which might be dependent on the cellular content of mitochondria in BAT and WAT. However, several genes encoding mitochondrial protein, such as liver mitochondrial aldehyde dehydrogenase and dicarboxylate carrier, were remarkably lower expressed in BAT than in WAT. Furthermore, we examined the expression levels of proteins which showed higher transcript levels in BAT by Western blotting. UCP1, M-CPTI and CC were higher expressed in BAT than in WAT even in protein levels, whereas expression levels of two protein subunits of redox complex and ATP complex were not markedly different between two adipose tissues. Taken together, it suggested that expression levels of these subunits would be regulated in dependence on expression levels of the other subunits of the complexes. These results may provide further insight for clarifying the unique energy metabolism in BAT.

P-55

Microarray analysis of hepatocellular adenoma induced by di(2-ethylhexyl) phthalate in rasH2 mice

Kaoru TOYOSAWA[1], Miwako HARADA[2], Katsuji NAKANO[2], Kohji TANAKA[1], Nobuo MATSUOKA[1]

Safety Research Laboratories, Drug Research Division, Dainippon Pharmaceutical Co., Ltd., Osaka 564-0053, Japan[1]
Pharmacology & Microbiology Research Laboratories, Drug Research Division, Dainippon Pharmaceutical Co., Ltd.[2]

We have reported that Di(2-ethylhexyl)phthalate (DEHP), a peroxisome proliferator, induces hepatocellular adenomas in transgenic mice carrying a human prototype c-Ha-*ras* gene (rasH2 mice), there are no mutations of the transgene found in the hepatocellular adenoma induced by DEHP, and the mRNA levels of the transgene and peroxisome proliferator activated receptor (PPAR) α are essentially equal between the hepatocellular adenoma and adjacent liver tissue (1,2).
In the present study, comprehensive analyses were performed using microarrays to find genes related to tumorigenesis. Gene expressions related to fatty acid metabolism and peroxisome increased in the DEHP-treated liver in comparison with the control liver, whereas they decreased in the hepatocellular adenoma in comparison with the adjacent liver tissue, and those related to cell cycle increased unequivocally in the hepatocellular adenoma in comparison with the adjacent liver tissue. Phenotypic expressions reflecting these gene expressions will be reported together on a poster presentation.

1) K.Toyosawa et al. Toxicologic Pathology 29:458-466, 2001.
2) K.Toyosawa et al. Cancer Letter 192:199-203, 2003.

P-56

Mechanistic analyses using chip array analysis on inhibited uterine tumorigenesis in rasH2 mice initiated with N-ethyl-N-nitrosourea followed by dietary treatment of ethinylestradiol

Takao WATANABE[1], Kayo SUMIDA[2], Tomoko MUTOH[1], Miwa OKAMURA[1], Mitsuyoshi MOTO[1], Yoko KASHIDA[1], Kunitoshi MITSUMORI[1]

Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan[1]
Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.[2]

Estrogen has a potent tumor promoting effect in the uterus, liver and kidney of experimental animals. In the uteri, estrogen stimulates DNA synthesis and cell proliferation in the luminal and glandular epithelia of rodent uteri *in vitro*, and induces the expression of genes such as c-*fos*, c-*jun*, c-*myc*, c-Ha-*ras*, epidermal growth factor (EGF), EGF receptor, and estrogen receptor in mammalian uteri. Although, to date, several experiments on the uterine carcinogenesis have been introduced in rats and mice, links between hormonal carcinogenesis promoted by estrogens and overexpression of such oncogenes have yet to be proven. In our previous studies in which *N*-ethyl-*N*-nitrosourea (ENU) was intraperitoneally given to transgenic mice carrying a human prototype c-Ha-*ras* gene (rasH2 mice), the incidence of uterine adenocarcinomas, atypical hyperplasias and endometrial hyperplasias were significantly increased in rasH2 mice, as compared to those in non-transgenic mice with initiation of ENU. On the other hand, the incidences of uterine tumors such as adenocarcinomas, atypical hyperplasias and endometrial hyperplasias in ENU-initiated rasH2 mice followed by dietary treatment of 2.5 ppm ethinylestradiol (EE) for 26 weeks were completely inhibited, whereas significantly high incidences of those in ENU-initiated ICR mice followed by dietary treatment of 2.5 ppm EE for 26 weeks were observed. In the present study, in order to clarify this paradoxical inhibitory mechanism of uterine tumors in rasH2 mice by 2.5 ppm EE treatment, the gene expression patterns from uterine tissues in rasH2 mice given EE for 6 weeks after ENU initiation were examined using high density DNA microarray (Affymetrix, Santa Clara, CA) in comparison with those in ICR mice subjected to the same treatment. As a result, the decreases of m-RNA expression for ER alpha, CYP1B1and EGF receptor, and the increase of 17-beta hydroxysteroid dehydrogenase, CYP3A16 were detected. These results, thus, suggest that the alterations of these m-RNAs may be related to the inhibitory mechanism for uterine tumorigenesis in ENU-initiated rasH2 mice followed by 2.5 ppm EE treatment. Additional molecular pathological investigations are now in progress in our laboratory.

P-57

Tumor induction by colon carcinogen in rat gastric mucosa featuring intestinal metaplasia caused by X-irradiation

Shoji KASHIWABARA[1], Naoki KASHIMOTO[1], Toshihiro UESAKA[1], Osamu KATOH[1], Keiji WAKABAYASHI[2], Hiromitsu WATANABE[1]

Department of Cellular Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima 734-8553, Japan[1]
National Cancer Center Research Institute[2]

Based on investigations in humans, intestinal metaplastic changes in the stomach have been considered to be precancerous lesions or a predisposing condition for differentiated gastric carcinoma development. However, we have experimentally reported an inverse relationship between intestinal metaplasia, with or without Paneth cells, and gastric tumor development, and we have established that the presence of intestinal metaplasia does not promote induction of gastric neoplasia by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) or N-methylnitrosourea (MNU) in rats. Nakagawa et al. have reported that colorectal mucosa implanted into the glandular stomach is like the intrinsic large intestine sensitive to tumorigenesis caused by 1,2-dimethylhydrazine (DMH), in contrast to the normal gastric mucosa. The present study was designed to examine whether intestinal metaplasia might be a target for colon carcinogen-induction of malignant tumors in the glandular stomach. Male 5-week-old Crj:CD, Crj:Wistar and F344/DuCrj rats were X-irradiated with a total of 20 Gy in two equal fractions with a 3-day interval. Beginning 16 weeks after the first irradiation, DMH was injected i.m. at a dose of 20 mg/kg body weight weekly for 10 times to CD and Wistar rats. Azoxymethane (AOM) was injected i.m. at a dose of 15 mg/kg body weight weekly for 3 weeks and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) was given every 2 days, 3 times per week, for a total 10 doses of 75 mg/kg body weight by intragastric intubation to F344 rats. Twelve months after the initial carcinogen treatment, tumors in the pylorus of the glandular stomach were observed in 2 (3 lesions) of 30 CD rats, 5 of 23 Wistar rats and 4 of 29 animals in the X-rays+AOM groups and 4 of 25 F344 rats receiving X-rays+PhIP. No such lesions were found in the DMH, AOM, PhIP or X-ray alone or nontreated groups. Large intestinal tumors developed in the colon carcinogen treated groups. Skin, pancreatic and kidney tumors developed in the X-irradiated groups. While we must consider the alternative possibility that the effects of irradiation and DMH and other colon carcinogens on glandular stomach epithelial cells are additive or synergistic, it appears likely that intestinal mucosal stem cell(s) are susceptible to colon carcinogenesis, independently of the administration route or their location. In summary, the presence of intestinal metaplasia, with or without Paneth cells, may increase the sensitivity of the stomach to the induction of tumors by carcinogens like DMH, AOM or PhIP, but not MNNG or MNU.

P-58

Expression of peroxisome proliferator-activated receptor gamma in helicobacter pylori- associated mouse gastric cancer tissue and human gastric cancer cells.

Dae-Yong KIM[1], Sang-Yeon OH[1], Ki Taek NAM[2], Beom Seok HAM[2], Dong Deuk JANG[2], Ki-Hwa YANG[2], Ki-Baik HAHM[3]

Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University[1]
National Institute of Toxicological Research[2]
Genome Research Center for Gastroenterology, Ajou University School of Medicine[3]

Peroxisome proliferator-activated receptor (PPAR) is nuclear hormone receptors that can be activated by a variety of compounds. Two PPAR gamma isoforms are expressed at the protein level in mouse, gamma 1 and gamma 2. And PPAR gamma is intimately associated with cell differentiation and proliferation. Based on the recent study established mice model of H. pylori-associated gastric cancer, the aim of this study is to investigate the expression pattern of PPAR gamma in mouse gastric cancer tissues and human gastric cancer cell lines. To generate stomach cancer, female C57BL/6 mice were treated with N-methyl-N-nitrosourea (MNU) and H. pylori. All mice were sacrificed at the 50th week after carcinogen treatment, and histopathology, immunohistochemistry, and western blotting for PPAR gamma was performed. In vitro experiment was performed using gastric cancer cells and administration of PPAR gamma ligands and H. pylori. Cell viability and death after PPAR gamma ligand treatment was assessed. PPAR gamma protein was expressed normal gastric epithelial cells, and more highly expressed in gastric adenoma and adenocarcinoma. PPAR gamma protein expression in gastric adenocarcinoma was higher than adenoma. In gastric cancer cells, PPAR gamma protein expression decreased in treatment of PPAR gamma agonists. However, when we treated PPAR gamma antagonist, PPAR gamma protein was more highly expressed. The expression of RXR alpha heterodimerized with PPAR gamma was decreased in treatment of PPAR gamma antagonist. PPAR gamma agonists induced apoptosis in gastric cancer cells, but not PPAR gamma antagonist.

P-59

Time-course and quantitative study of the relationship between transforming growth factor-alpha and glutathione S-transferase placental form expression during rat chemical hepatocarcinogenesis

Mitsuaki KITANO[1], Jutaro WADA[1], Yutaka ARIKI[1], Masanori KATO[1], Hideki WANIBUCHI[2], Keiichirou MORIMURA[2], Kazunori HOSOE[1], Shoji FUKUSHIMA[2]

Life Science Research Laboratories, Life Science RD Center, Kaneka Corporation, Hyogo 676-8688, Japan[1]
Department of Pathology, Osaka City University Medical School[2]

The expression of transforming growth factor-alpha (TGF- alpha) during progression from glutathione S-transferase placental form (GST-P) positive foci to neoplasms in rat chemical hepatocarcinogenesis was investigated. One hundred F344 rats were given a single intraperitoneal injection of diethylnitrosamine (DEN) (200 mg/kg body wt.) and subjected to two-thirds partial hepatectomy at week 3. Commencing 2 weeks from the start, sodium phenobarbital (PB) at doses of 0 or 500 ppm was fed to the rats for 48 weeks. Groups of ten rats given DEN only and ten rats given DEN and PB were killed at weeks 4, 8, 16, 32, 48 and their livers were immunohistochemically examined for expression of GST-P, TGF- alpha, and epidermal growth factor receptor (EGF-R). TGF- alpha positive foci were observed from week 4. They were seen within GST-P positive foci and were much fewer than these latter. The numbers of TGF- alpha positive foci did not increase from weeks 4 to 48, but their areas did show increment at weeks 32 and 48. Almost all of the tumors observed at week 32 and 48 were positive for TGF- alpha (98%). PB administration enhanced the areas of TGF- alpha positive foci. EGF-R overexpression was observed in TGF- alpha positive lesions. Our data suggest that TGF- alpha positive foci observed in the early stage of rat hepatocarcinogenesis might develop into tumors in the progression stage.

P-60

Enhancing effects of combined treatment with IQ and sodium nitrite on colon and liver carcinogenesis in rats

Yasuki KITAMURA[1], Kazushi OKAZAKI[1], Akiyoshi NISHIKAWA[1], Keita KANKI[1], Takashi UMEMURA[1], Takayoshi IMAZAWA[1], Masao HIROSE[1]

Division of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan[1]

[Objectives] Combined effects of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), a heterocyclic amine, and sodium nitrite ($NaNO_2$) were examined in a rat two-stage carcinogenesis model targeting the liver and colon.
[Methods] Experiment 1: For the initiation treatment, male 6-week-old F344 rats were given a single intraperitoneal injection of 200 mg/kg body weight of diethylnitrosamine (DEN) and subcutaneous injections (four times) of 40 mg/kg body weight of 1,2-dimethylhydrazine (DMH) during initial 2 weeks. Then, animals were administrated 0 or 300 ppm IQ in diet and/or 0, 0.1 or 0.2% $NaNO_2$ in drinking water for 27 weeks. All surviving animals were killed at week 29 for the histopathological examination. Experiment 2: Male 6-week-old F344 rats were given IQ and/or $NaNO_2$ under the same conditions as Experiment 1, for one week. All animals were sacrificed and their liver, lung and colon (mucosa only) were sampled for analyzing 8-oxodeoxyguanosine (8-OHdG) levels by HPLC with an electrochemical detection system and bromodeoxyuridine (BrdU)-labeling index (LI) by immunohistochemical procedure.
[Results] Experiment 1: In both liver and colon, incidences and multiplicities of benign and malignant tumors in the groups given IQ were significantly higher than those in the groups receiving the initiation treatment alone. The combined treatment with IQ and $NaNO_2$ synergistically enhanced colon carcinogenesis and also tended to enhance hepatocarcinogenesis. Interestingly, incidences of lung tumors in the group given IQ (73%) were significantly increased as compared with the initiation alone group (5%) irrespective of combined treatment with $NaNO_2$. Experiment 2: In both liver and colon, the 8-OHdG levels were increased by the co-treatment with IQ and $NaNO_2$. In colon, particularly, the 8-OHdG levels and BrdU-LI in the group given 0.2% $NaNO_2$ concomitantly with IQ were significantly higher as compared with the group given IQ alone.
[Conclusion] These results indicated that $NaNO_2$ enhanced carcinogenic potential of IQ in the liver and colon and oxidative DNA damage might be involved in the observed enhancement of carcinogenesis. In addition, it is suggested that IQ is a potent lung carcinogen in rats.

P-61

Involvement of the altered regulation of pre-mRNA splicing in the liver carcinogenic processes

Masakazu TAKAHASHI[1], Fumiyuki UEMATSU[1], Midori YOSHIDA[1], Maki IGARASHI[1], Naoto WATANABE[1], Akihiko MAEKAWA[2], Dai NAKAE[1]

Department of Pathology, Sasaki Institute, Sasaki Foundation, Tokyo 101-0062, Japan[1]
Director, Sasaki Institute, Sasaki Foundation, Japan[2]

Introduction: Altered expression of genes and their products is crucially involved in carcinogenesis. Such events are partly ascribed to the aberrant pre-mRNA splicing, and aberrant spliced mRNAs of specific genes and their product proteins are frequently detected in a variety of malignancy cases. Its rationale remains largely obscure. The present study was thus conducted to examine the involvement of the altered regulation of pre-mRNA splicing in hepatocarcinogenesis. Nagase analbuminemic rat (NAR) have a 7-base-pair deletion at the 5' splice site of the HI intron of the albumin gene, and the normal albumin generation is abolished. In this rat, however, the aberrantly spliced mRNA and protein of albumin are generated in the liver by aging or the administration of hepatocarcinogen, 3'-meyhyl-4-dimethylaminoazobenzene (3'-Me-DAB). There were increased amount of exon H-skipped (delta H), exons G and H-skipped (delta GH), and exons H and I-skipped (delta HI) mRNAs and aberrant 60-kDa of rat albumin. NAR can thus serve as an useful tool in the present study.
Methods: Seven weeks old male NAR were divided into four groups. Groups 1 and 2 received the control CE2 diet alone and containing 0.06% of 3'-Me-DAB, respectively, while groups 3 and 4 received choline-supplemented (CSAA) and choline-deficient, L-amino acid-defined (CDAA) diets, respectively, for 18 weeks. Serum albumin concentration was measured by single radial immunodiffusion. In the liver, numbers and sizes of glutathione S-transferase placental form (GST-P)-positive, preneoplastic lesions and numbers of albumin-positive hepatocytes were determined by immunohistochemicaly, and mRNA expression of albumin, alternative splicing factor (ASF), 30kDa arginine-serine-rich protein (SRp30) and 55kDa arginine-serine-rich protein (SRp55) was examined by RT-PCR.
Results and Discussion: Numbers and sizes of GST-P-positive lesions were significantly higher in groups 2 and 4 than in 1 and 3, respectively. Serum albumin content, number of albumin-positive hepatocytes, and mRNA expression of aberrantly spliced albumin, ASF and SRp55, but not SRp30, significantly increased only in group 2. These results indicate that, 3'-Me-DAB causes aberrant pre-mRNA splicing by virtue of its altered regulation, and thus suggest that the altered regulation of pre-mRNA splicing may be involved in the liver carcinogenic processes.

P-62

Genetic alterations in the Catnb gene but not the H-ras gene in hepatocellular neoplasms and hepatoblastomas of B6C3F1 mice following exposure to diethanolamine for 2 years

Shim-mo HAYASHI[1], Thai Vu TON[2], Hue-Hua L. HONG[2], Richard D. IRWIN[2], Joseph K. HASEMAN[2], Theodora R. DEVEREUX[2], Robert C. SILLS[2]

Pfizer Inc., Worldwide Safety Sciences, Taketoyo, Aichi 470-2393, Japan[1]
National Institute of Environmental Health Sciences[2]

The present study characterized the immunohistochemical localization of β-catenin protein in hepatocellular neoplasms and hepatoblastomas in B6C3F1 mice exposed to diethanolamine (DEA) for 2 years and evaluated genetic alterations in the Catnb and H-ras genes which are known to play important roles in the pathogenesis of liver malignancies. Genomic DNA was isolated from paraffin sections of each liver tumor. Catnb exon 2 (corresponds to exon 3 in human) genetic alterations were identified in 18/18 (100%) hepatoblastomas from DEA exposed mice. Deletion mutations (15/18, 83%) were identified more frequently than point mutations (6/18, 33%) in hepatoblastomas. Eleven of 34 (32%) hepatocellular adenomas and carcinomas from DEA treated mice had mutations in exon 2 of the β-catenin gene, while only 1 of 10 spontaneous neoplasms had a deletion mutation of codon 5-6. Common to all liver neoplasms (hepatocellular adenomas, carcinomas and hepatoblastomas) was membrane staining for the β-catenin protein, while cytoplasmic and nuclear staining was observed only in hepatoblastomas. The lack of H-ras mutations in hepatocellular neoplasms and hepatoblastomas suggests that the ras signal transduction pathway is not involved in the development of liver tumors following DEA exposure which is different from that of spontaneous liver tumors that often contain H-ras mutations.

P-63

Carcinogenicity of phenobarbital in Mmh/OGG1 knockout mice

Anna KINOSHITA[1], Hideki WANIBUCHI[1], Keiichirou MORIMURA[1], Takayuki YUNOKI[1], Shoji FUKUSHIMA[1]

Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

The carcinogenic potential of phenobarbital (PB), was assessed in a mouse line carrying a mutant *Mmh* allele of the *Mmh/OGG1* gene. *Mmh* homozygous and wild type male and female 10-week-old mice were treated with PB in diet at a dose of 500 ppm for 70 weeks. Non-treated knockout mice developed only lung tumors at a low rate. An increase of total tumor incidence and multiplicity in mutant, but not in the wild type, male (25%) and predominantly female (60%) mice was induced by PB treatment as compared to control (15%). Hepatocellular carcinomas were found only in PB-treated mutant mice, while wild type animals developed only liver adenomas. In PB-treated but not in control *Mmh/OGG1*$^{-/-}$ females, lymphomas were observed (35%). The 8-hydroxydeoxyguanosine (8-OHdG) level was significantly elevated in the liver of all mutants as compared to wild type mice. Moreover, 8-OHdG levels and cell proliferation was significantly increased by PB in the liver of *Mmh/OGG1*$^{-/-}$. Microarray analysis of the livers of control *Mmh/OGG1*$^{-/-}$ mice revealed a significant underexpression of several oncogenes, genes involved in xenobiotic metabolism, intracellular kinase network, DNA damage and repair, and encoded extracellular matrix and cytoskeleton proteins. PB treatment of *Mmh/OGG1*$^{-/-}$ mice resulted in significant overexpression of genes involved in xenobiotic metabolism, DNA damage and repair (females), encoded extracellular matrix proteins and ornithine decarboxylase as compared to both *Mmh/OGG1*$^{-/-}$ and PB-treated *Mmh/OGG1*$^{+/+}$ controls. These results indicate that PB is carcinogenic in *Mmh/OGG1* mutant mice, which might be related to the increased level of non-repaired DNA oxidative base modifications.

P-64

Oxidative stress and aberrant DNA methylation in cancer

Yashige KOTAKE[1], Kiyoshi ASADA[1], Hong SANG[1], Robert H. BROYLES[1], Robert A. FLOYD[1]

Oklahoma Medical Research Foundation, Oklahoma 73104, USA[1]

The relationship between cytosine methylation and 8-hydroxyguanine (8-OHdG) formation in DNA is not well understood. In vitro, the presence of 8-OHdG in the CG sequence in synthetic oligonucleotides has been shown to interfere with maintenance DNA methyltransferase activity which results in aberrant methylation in replicated strands. We hypothesize that 8-OHdG-mediated hyper- or hypo-methylation occurs in carcinogenesis in vivo in CG islands within the promoter region of crucial genes. Therefore, the objective of this study was to show that the 8-OHdG level in DNA correlates with aberrant methylation in vivo in a hepatocarcinogenesis model, and that decrease in 8-OHdG by antioxidant treatment leads to normalization of methylation status. In a choline-deficient (CD) diet induced hepatocarcinogenesis model in rats, we have shown that: 1) there is an increase in 8-OHdG level in liver DNA; 2) the 8-OHdG increase was prevented by co-feeding with the synthetic antioxidant, free radical trap phenyl N-t-butylnitrone (PBN); and 3) PBN inhibits formation of frank hepatocellular carcinoma (75-week feeding) as well as preneoplastic foci (16-week feeding). In addition, previous studies in this model demonstrated that aberrant DNA methylation occurred in global DNA and in specific genes. We will determine the methylation status of global DNA and of the promotor of four specific genes (c-myc, p16, GST-P, and SOD2) by using 5-methylcytidine HPLC, ELISA and bisulfite sequencing. Liver genomic DNA was obtained from non-tumor liver tissues in four groups of rats (5 animals each group): group 1) fed choline deficient L-amino acid defined (CDAA) diet (Dyets Inc); group 2) fed CDAA diet plus 0.03% PBN in food; group 3) fed choline sufficient L-amino acid diet (CSAA); and group 4) fed CSAA diet plus 0.03% PBN. The feeding period was 30 weeks. Preliminary results in p16 and SOD2 show the tendency that methylation status of PBN treated group is closer to control groups than to CDAA group. We anticipate the results which indicate that PBN treatment prevent aberrant DNA methylation and experiments are in progress. Supported in part by NIH CA-082506.

P-65

Methylation-associated silencing of p53 responsive gene 2 and Fibrillin 2 in human pancreatic cancers

Atsushi HAGIHARA[1], Kazuaki MIYAMOTO[2], Junichi FURUTA[2], Shuichi SEKI[3], Shoji FUKUSHIMA[1], Toshikazu USHIJIMA[2]

Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]
Carcinogenesis Division, National Cancer Center Research Institute[2]
Internal Medicine, Osaka City University Medical School[3]

Pancreatic cancer is one of the most refractory neoplasms, with the average 5-year survival being less than 20 %. In addition to the anatomical location of the pancreas that makes early detection of pancreatic cancers very difficult, several genetic and biological characteristics, such as the presence of severe chromosomal instability and the strong involvement of stromal tissues, are considered to underlie their poor prognosis. In this study, aberrant methylation in human pancreatic cancers was searched for, using the genome scanning technique, methylation-sensitive-representational difference analysis (MS-RDA). CpG islands (CGIs) in the 5' regions of 30 genes were found to be methylated in at least one of seven pancreatic cancer cell lines, while unmethylated in two human pancreatic ductal epithelial cell lines. Among these, four genes, *p53 responsive gene 2 (PRG2/MG50)*, *Fibrillin 2 (FBN2)*, *ras protein-specific guanine nucleotide-releasing factor 2 (RASGRF2)*, and *Thrombomodulin (THBD)*, were found to be abundantly expressed in the ductal cell lines, while the others were expressed in small amounts. Expression of the first four genes was lost in cancer cell lines with methylation, and their expression was restored by 5-aza-2'-deoxycytidine, showing their silencing. In seven and nine of 12 primary pancreatic adenocarcinomas, respectively, the CGIs of the *PRG2* and *FBN2* were methylated. These data suggested that *PRG2* and *FBN2* could be involved in pancreatic cancer development and progression.

P-66

Lack of influence of testicular castration or sialoadenectomy on sodium L-ascorbate promotion of urinary bladder carcinogenesis in male F344 rats

Satoru MORI[1], Takashi MURAI[1], Keiichirou MORIMURA[1], Hideki WANIBUCHI[1], Shoji FUKUSHIMA[1]

Deparment of Pathology, Osaka City Univercity Medical School, Osaka 545-8585, Japan[1]

The study was designed to investigate whether testicular castration (TC) or sialoadenectomy (SE) can influence sodium L-ascorbate (Na-AsA)-promoting effects on urinary bladder carcinogenesis in male F344/DuCrj rats. The animals, 6-weeks-old at the commencement, were given 0.05% N-butyl-N- (4-hydroxybutyl) nitrosamine (BBN) in their drinking water for 4 weeks and then underwent TC or SE. Thereafter they received basal diet with or without Na-AsA supplement for 32 weeks. Na-AsA increased significantly urinary pH and concentrations of sodium ion and total ascorbic acid. TC significantly decreased the relative organ weights of accessory sexual glands, kidneys and livers whereas SE was without effect on these organs. Both decreased very slightly BBN-induced carcinogenesis, but did not influence the Na-AsA -promoting effects. The results indicate that Na-AsA -promotion of two-stage urinary bladder carcinogenesis does not depend on the presence of testes or salivary glands.

P-67

Effect of amiloride and ouabain on promotion by sodiium L-ascorbate in two-stage rat urinary bladder carcinogenesis

Takashi MURAI[1], Hideyuki MIYAUCHI[1], Satoshi INOUE[1], Takeki UEHARA[1], Toshiyuki MARUYAMA[1], Satoru MORI[2], Hideki WANIBUCHI[2], Shoji FUKUSHIMA[2]

Developmental Research Laboratories, Shionogi & Co., Ltd., Osaka 561-0825, Japan[1]
First Department of Pathology Osaka City University Medical School[2]

The effects of amiloride (AM), which is a potent inhibitor of the Na^+/H^+exchanger, and ouabain (OU), which is a potent inhibitor of the Na^+/K^+-ATPase on the promotion by sodium L-ascorbate (Na-AsA) in two-stage rat urinary bladder carcinogenesis were examined to evaluate the potency of promotion mechanism of Na-AsA. In Experiment 1, F344/DuCrj rats were initiated with 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) in drinking water for 4 weeks or left intact and subsequently given basal MF diet with or without a 5% Na-AsA for 24 weeks. During the administration period of Na-AsA, the initiated or intact rats were given 6.6 mg/kg AM subcutaneously three times a week. In the rats treated with both BBN and Na-AsA, AM treatment delayed the occurrence of the occult blood in urine and hematuria and significantly decreased the incidence of them. Although the AM treatment did not influence urinary pH, the concentration of sodium ion in the urine decreased in the rats given AM. The increase in urinary bladder weight due to the neoplasia and the number of carcinoma per rat were significantly inhibited by AM treatment. In Experiment 2, the similar two-stage bladder carcinogenesis protocol as in Experiment 1 was carried out to evaluate the effects of OU and to confirm the effect of AM on the second stage of urinary bladder carcinogenesis. The OU treatment showed significant decrease of body weight, but did not affect any urinalysis data. Histopathological evaluation of urinary bladder revealed that the number of urinary bladder carcinoma per rat was significantly inhibited by AM. OU showed slightly increase in size of carcinoma. The effect of AM on the initiation by BBN alone in Experiment 3 or the effects of AM and OU on the promotion by Na-AsA alone in Experiments 4 and 5 was examined in the shorter observation period than Experiments 1 and 2. As the results, only AM inhibited cell proliferation by Na-AsA, although AM did not affect mitogenesis by BBN. These results indicate that the oscillation of intracellular Na^+ which induce cell proliferation is involved in the promotion stage by Na-AsA in two-stage rat urinary bladder carcinogenesis.

P-68

Susceptibility of p27^{kip1} knockout mice on N-butyl-N-(4-hydroxybutyl) nitrosamine-induced urinary bladder carcinogenesis

Toshiya MURASAKI[1], Kumiko OGAWA[1], Atsuya HIKOSAKA[1], Satoshi SUGIURA[1], Seishiro TAKAHASHI[1], Tomoyuki SHIRAI[1]

Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]

p27^{kip1} is a cyclin dependent kinase (CDK) inhibitor regulating the cell cycle. It is present abundantly and ubiquitously in quiescent cells but declines in proliferating cells in response to mitogenic stimulation by growth factors and cytokines. Although mutations of the *p27^{kip1}* gene are rare in human tumors, decrease in p27^{kip1} protein correlates with a poor prognosis in breast, colon, stomach, prostate and bladder cancers.
Our previous study on chemical-induced rat urinary bladder carcinogenesis revealed that cell cycle regulation of p27^{kip1} is maintained in hyperplastic lesions but lost in carcinomas, presumably contributing to their disordered proliferation.
In the present study for further elucidation the role of p27^{kip1} in urinary bladder carcinogenesis, alteration of susceptibility to N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN), a rodent urinary bladder carcinogen, was assessed in p27^{kip1} knockout mice.
Male and female mice of three p27 genotypes, i.e. nullizygous (p27 $^{-/-}$), heterozygous (p27$^{+/-}$) and wild (p 27$^{+/+}$), originating from the C57BL/6 strain were at the age of 6 weeks divided into one group of 18-20 mice given drinking water containing 0.05% BBN for 10 weeks and the other group of 11-15 mice receiving plain drinking water. At the 20th experimental week, all mice were killed under ether anesthesia and their urinary bladders, kidneys, livers and spleens were excised. They were weighed and bladders and kidneys were processed for histological examination.
Simple hyperplasia or papillary or nodular (PN) hyperplasia, putative premalignant lesions of murine urothelia, occurred in all mice receiving the BBN treatment. Carcinomas were observed in 100, 85 and 80% of male p27 $^{-/-}$, p27$^{+/-}$ and p27$^{+/+}$ mice, respectively. The incidences of carcinomas in female groups were much lower at 39, 15 and 0% respectively, demonstrating a significant difference among the three p27 genotypes. In both male and female groups, quantitative assessment with an image analyzer, revealed larger tumors in p27 $^{-/-}$ than p27$^{+/+}$ mice, while p27$^{+/-}$ mice had intermediate-sized lesions although no significant difference were seen.
In conclusion, loss of p27 clearly increases the susceptibility to BBN-induced mice urinary bladder carcinogenesis.

P-69

Promoting effect of sodium L-ascorbate on N-butyl-N-(4-hydroxybutyl) nitrosamine-induced renal pelvic carcinogenesis in SD/cShi rats of both sexes

Takashi MURAI[1], Hideyuki MIYAUCHI[1], Satoshi INOUE[1], Takeki UEHARA[1], Toshiyuki MARUYAMA[1], Akihiro KOIDE[2], Yukio MORI[2], Satoru MORI[3], Hideki WANIBUCHI[3], Shoji FUKUSHIMA[3]

Developmental Research Laboratories, Shionogi & Co., Ltd., Osaka 561-0825, Japan[1]
Laboratory of Radiochemistry, Gifu Pharmaceutical University[2]
Department of Pathology, Osaka City University Medical School[3]

Susceptibility to the promoting effects of sodium L-ascorbate (Na-AsA) on the development of pelvis and urinary bladder tumors in male and female SD/cShi rats, featuring spontaneous hydronephrosis, was investigated. Rats received 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) in their drinking water for 4 weeks and subsequently given basal diet with or without a 5% Na-AsA supplement for 32 weeks. Histopathological examination revealed the promoting effect of Na-AsA on not only the development of urinary bladder tumors but also renal pelvic tumors in the animals of both sexes in this two-stage carcinogenesis experiment, the effect being more prominent in males. Administration of either BBN or Na-AsA alone also induced papillomas and papillary or nodular hyperplasia of renal pelvis or urinary bladder, respectively, in male but not female rats. However, the 5-bromo-2'-deoxyuridine-labeling index of urothelium in the pelvis and bladder increased slightly in male rats and significantly in female rats given Na-AsA alone for 8 weeks. N-butyl-N-(3-carboxypropyl)nitrosamine, which is a metabolite of BBN and proximate carcinogen, was found more in the urine of urinary bladder than that of renal pelvis. These results indicate that the urothelium of the renal pelvis and urinary bladder in SD/cShi rats is susceptible to promoting effects of Na-AsA in the present two stage model urinary tract carcinogenesis, with the urinary bladder of male rats as the most sensitive organ.

P-70

Renal cell carcinoma cell lines established from Nihon rat

Izumi MATSUMOTO[1], Tadayoshi UEDA[1], Kazuyasu KIJIMA[1], Youko HIRAYAMA[2], Hiroaki MITANI[2], Kazuo OKIMOTO[1], Kohji TANAKA[1], Okio HINO[2], Nobuo MATSUOKA[1]

Drug Research Division, Dainippon Pharmaceutical Co., Ltd., Osaka 564-0053, Japan[1]
Department of Experimental Pathology, Cancer Institute[2]

A novel rat model of hereditary renal cell carcinoma (RC) was discovered in a rat colony of the Sprague-Dawley strain in Japan, and thus was named "Nihon rat". The phenotype of RCs in Nihon rats is clear cell type predominant, the phenotype being common in human. The mutation gene is located on the chromosome 10, and finally predisposing gene was identified. LOH was detected, fitting Knudson's "two hit" model and proving us with insights into a novel tumor suppressor gene. To further investigate the RCs in Nihon rats, cell lines were isolated and established.

A RC was aseptically removed from a 10-month-old male rat, finely minced and incubated in Dulbeco's modified Eagle media supplemented with 10% fetal calf serum. The outgrowth of cells was selected for epithelial cells that were harvested and passaged to establish cell colonies showing a strong ability of proliferation. Seven cell lines were finally isolated from these colonies using the limited dilution method. Characterization of the cell lines included growth curves, morphological examination with a light and an electron microscope, immunohistochemical staining for renal epithelial cells, chromosome analysis, gene analysis and tumorigenicity in nude mice. These cell lines will provide insights into a novel tumor suppressor gene involved renal carcinogenesis.

P-71

Strain-specific mammary proliferative lesion development following lifetime oral administration of ochratoxin A in DA and Lewis rats

Yong-Soon LEE[1], Woo-Chan SON[2], Kyung-Sun KANG[3]

Department of Public Health, Seoul National University, Seoul 151-742, Korea (South)[1]
Huntingdon Life Sciences Pathology[2]
Department of Public Health, Seoul National University[3]

OTA, a potent nephrotoxin in several species, is a renal carcinogen in animals and is implicated in the etiology of BEN. The NTP classified OTA as having clear evidence of carcinogenic activity, based on uncommon tubular adenomas and tubular cell carcinomas of the kidney and multiple fibroadenomas of the mammary gland, seen in the rat. As shown previously (Castegnaro et al., Int J Cancer 1998;77:70.5), induction of renal tumors by OTA is sex- and strain-specific in DA and Lewis rats, with DA males being most responsive and DA females being resistant; however, that report was confined to the kidney and urinary tract. To obtain OTA induced tumorigenic information in rats, we administered OTA (0.4 mg/kg) by oral gavage to both DA and Lewis rats for their lifetimes and extended the investigation to complete histopathology of all tissues and organs. We also observed the characteristic renal tumor that is highly strain- and sex-specific, and there were increased incidences of proliferative mammary lesions in Lewis rats but not in DA rats, indicating that these were also strain-specific. In view of the NTP report of OTA treatment-related mammary fibroadenoma in F344 rats, we observed increased mammary proliferative lesions in Lewis rats but not in DA rats. Our results suggest that OTA may play some role in mammary tumor development in some rat strains. Key words : ochratoxin A; renal tumor; mammary gland; fibroadenoma; DA rat; Lewis rat

P-72

Strain difference in spontaneous development of uterine adenocarcinoma between donryu and Fischer344(F344)rats

Takaharu NAGAOKA[1], Hiroaki MIYAJIMA[1], Akihiko MAEKAWA[2]

Shin Nippon Biomedical Laboratories, LTD. Kagoshima 891-1394, Japan[1]
Sasaki Institute[2]

Strain difference in spontaneous development of uterine adenocarcinoma was investigated between Donryu and F344 rats. 1. Incidence of uterine endometrial proliferative lesions. In Donryu rats, the incidence of endometrial adenocarcinoma, the most commonly occurring condition, was 35.1%. Endometrial hyperplasia also developed at a high incidence. Conversely, in F344 rats, endometrial stromal polyp was the most frequently observed lesion, at an incidence density of 21.2 %. The incidence of endometrial adnocarcinoma was less than 1 %. 2. Characteristics in histology of endometrial adenocarcinoma. In Donryu rats, endometrial adenocarcinomas were well differentiated at 51.3%, moderately differentiated at 38.5%, and poorly differentiated at 10.3 %. In F344 rats, 60% were moderately differentiated and 40% were poorly differentiated. 3. Expression of p53 positive phenotype in endometrial adenocarcinoma. "P53 Positive phenotype" was only observed in the well-differentiated adenocarcinomas in Donryu rats. Conversely, in F 344 rats, the majority of adenocarcinomas and hyperplasias were positive, regardless of the level of differentiation. 4. Changes in sequential hormone production. Sequential analyses of plasma gonad steroid levels determined that there was a significant link between the increase in the ratio of estrogen/progesterone and the higher incidence in the development of endometrial adenocarcinomas in Donryu rats. However, changes in hormone production were not evident in F344 rats. 5. Incidence of endometrial proliferative lesions in androgenized animals. Incidences of 15.7% for endometrial adenocarcinomas and of 51.1% for endometrial hyperplasias were detected in Donryu rats. Conversely, in F344 rats, only endometrial hyperplasia was observed, at an incidence of 45.7%. The findings in the two rat strains stated above, indicate a clear strain difference in the spontaneous development of uterine proliferative lesions. The incidences of adenocarcinomas in Donryu and F344 rats were high and low, respectively. In addition, the imbalance of hormone production and the aberrant expression of p53 phenotype may be related to the development of endometrial carcinogenesis.

P-73

Expression profile of estrogen receptor-α-related signaling pathways in the development of endometrial adenocarcinomas in Donryu rats

Midori YOSHIDA[1], Takasumi SHIMOMOTO[1], Yutaka HATANAKA[3], Takuji MIHARA[3], Akihiko MAEKAWA[2], Dai NAKAE[1]

Department of Pathology, Sasaki Institute, Sasaki Foundation, Tokyo 101-0062, Japan[1]
Director, Sasaki Institute, Sasaki Foundation[2]
BioMedical Science Department, Dako Cytomation Co., Ltd.[3]

Endometrial adenocarcinoma is a common malignant tumor in women. For its improvement of diagnosis, management and prevention, molecular profiling is essential but not as yet sufficiently elucidated. In Donryu rats, endometrial adenocarcinomas are spontaneously developed and have various similarities regarding morphological and biological properties to the human counterparts, and are also inducible by intrauterine treatment of *N*-ethyl-*N*'-nitro-*N*-nistroguanidine (ENNG). The underlying molecular pathway in rats, however, has been largely obscure. In this context, we investigated gene expressions of estrogen receptor (ER)-α-related signaling pathways and their product proteins in endometrial proliferating lesions in rats. Assessed materials were histopathologically confirmed normal epithelia, hyperplasias and well- and poorly-differentiated adenocarcinomas in Donryu rats induced by the ENNG. Immunohistochemical expressions for proteins were examined and image-analyzed using the automated cellular scanning system (Ariol SL-50, USA). The assessed proteins were ER-α, proliferating cell nuclear antigen (PCNA), Bcl-2, epidermal growth factor receptor (EGFR), insulin-like growth factor binding protein 5 (IGF-BP5), transforming growth factor (TGF)- β, PTEN/MMAC1 (PTEN), p53 (both wild and mutated), β-catenin and phosphorylated p44/42 MAP kinase (MAPK/Erk1/2). RT-PCR was performed to assess mRNA expressions of the IGF-BP5, EGFR, Bcl-2 and PTEN genes in normal epithelium and hyperplasia tissues obtained by a laser capture microdissection after the confirmation of sufficient mRNA expression of GAPDH gene.
ER-α was expressed in the normal epithelia, hyperplasias and well-differentiated adenocarcinomas with a similar intensity but not in poorly-differentiated ones. PCNA-positive cells increased with the progression of proliferating lesions. p53 was only detected in poorly-differentiated adenocarcinomas. Nuclear MAPK/Erk1/2 was expressed in poorly-differentiated adenocarcinomas and slightly scattered at periphery of hyperplasias. PTEN was similarly expressed among all categories. Bcl-2, EGFR, IGF-BP5, TGF-β or nuclear β-catenin was not detected in any cases. No mRNA expressions of assessed genes were detected with the exception of PTEN expressed in both normal epithelia and hyperplasias.
These results indicate the close correlation between expressions of mRNA and proteins and suggest that: 1) Expression of ERα might be related to endometrial carcinogenesis in rats and the malignant progression of endometrial adenocarcinomas is related to the loss of hormone dependency; 2) the alteration on p53 is a late event during endometrial carcinogenesis, and 3) the alteration on the mitogen-activated protein kinase cascade plays some roles in both of its early and late stages. These findings are relevant to the human endometriod adenocarcinomas.

P-74

Constitutive expression of cox-2 in thyroid follicular epithelial cells and its significant reduction during DHPN-induced carcinogenesis in rats

Toshio IMAI[1], Mai HASUMURA[1], Jun-ichi ONOSE[1], Makoto UEDA[1], Tamotsu TAKIZAWA[1], Young-Man CHO[1], Masao HIROSE[1]

National Institute of Health Sciences, Tokyo 158-8501, Japan[1]

In human thyroid follicular epithelial cells, constitutive expression of cyclooxygenase-2 (COX-2) has been demonstrated (Smith et al., 1999). To assess the role of COX-2 during rat thyroid carcinogenesis, we evaluated its expression in normal rat thyroid tissue and the proliferative lesions induced by goitrogens with or without *N*-bis(2-hydroxypropyl)nitrosamine (DHPN)-initiation. DHPN (2800 mg/kg body weight) was subcutaneously injected once to 40 male 6-week-old F344 rats. One week after the initiation, goitrogens such as propylthiouracil (PTU, 0.003%) and sulfadimethoxine (SDM, 0.1%) were administered in drinking water for 4 or 10 weeks, and then thyroid samples were collected for histopathology, Western blot analysis and immunohistochemistry for COX-2. Goitrogen alone and basal diet alone groups were also placed. At week 4, multiple focal follicular cell hyperplasias and adenomas were frequently observed in DHPN + PTU and DHPN + SDM groups. In DHPN + SDM and SDM alone groups, severe inflammation with fibrosis in the thyroid capsule with migrated follicular epithelial cells into the capsule were also observed. At week 10, increased incidences and multiplicities of focal hyperplasias and adenomas in DHPN + PTU and DHPN + SDM groups were observed. In addition, invasive adenocarcinomas to capsule were also detected in 3 of 10 rats of the DHPN + SDM group. These carcinomas were suggested to originate in the focal hyperplasias or adenomas adjacent to the capsule. Western blot analysis and immunohistochemistry for COX-2 revealed the COX-2 expression in the normal follicular epithelial cells. In the proliferative lesions observed in DHPN + PTU and DHPN + SDM groups as well as the migrated follicular epithelial cells into the capsule in DHPN + SDM and SDM alone groups, COX-2 reactivity was frequently reduced or negative. These results suggested that capsular inflammation and reduction of COX-2 expression in the preneoplastic and neoplastic lesions play roles in the development of invasive adenocarcinomas and promotion/progression of rat thyroid carcinogenesis, respectively.

P-75

PCNA labeled index of spontaneous lung proliferating lesion in rats

Masayuki MITSUI[1], Midori YOSHIDA[2], Kasuke NAGANO[3], Toshifumi TSUJIUCHI[1], Hiroshi MARUYAMA[4], Masahiro TSUTSUMI[1]

Department of Oncological Pathology, Cancer Center, Nara Medical University, Osaka 556-0005, Japan[1]
Department of Pathology, Sasaki Laboratory[2]
Division of Pathology, Japan Bioassay Research Center[3]
Department of Pathology, Hoshigaoka Koseinenkin Hospital[4]

Some endpoint markers such as gamma glutamyl-transferase (GGT)-positive phenotype has been reported to examine lung carcinogenicity in the lung proliferating lesions of rats, however the GGT histochemical staining can not react to formaldehyde-fixed lung tissues. Therefore, it is searching for more conventional markers available to routine formaldehyde fixative. Proliferating cell nuclear antigen (PCNA;36 kDa protein acting as a subunit of DNA polymerase delta) is one of the most common indicator to evaluate cell proliferating activity or tumor malignancy in many tissue and/or organs. In the present study, we investigated usefulness of PCNA labeling index to evaluate relationship between malignancy and morphological variations in spontaneous lung proliferating lesions of rats.

Material and Method: Samples were formaldehyde-fixed and paraffin-embedded sections, and supplied from various types of lung proliferating lesions in F344 rats of control groups of carcinogenicity studies. The lesions classified into hyperplasia, adenoma and adenocarcinoma were sub-divided by proliferating pattern (tubular, papillary and solid), nuclear atypism and existence of active stroma using HE staining. The nuclear atypism was characterized by enlarged atypical nucleus with marked nucleolus and rough and coarse chromatin granules, and active stroma was consisted with fibroblast, inflammatory cells and collagen fiber. Serial sections of each sample were made and incubated with PCNA antibody (DAKO Epos-anti-PCNA antigen) after heating at 100° C for 5 minutes in distilled water to retrieve antigen. PCNA labeled index(LI) in the lung proliferating lesions were countered after visualization by DAB and shown as percents.

Result and discussion: The LI in lung proliferating lesions were sequentially increased with malignancy from hyperplasia to adenocarcinoma via adenoma. Based on the sub-division of lesions, nuclear atypism was highly correlated with PCNA labeled cells. In addition, there was a tendency that LI was increased with activity stroma. On the other hand, there was no correlation between the LI and all proliferating pattern (tubular, papillary and solid).

Conclusion: These results demonstrate that evaluation of PCNA labeled cells is a useful and convenient marker to estimate malignancy of spontaneous lung proliferating lesions in rats.

P-76

The role of polymerase beta mediated DNA repair in skin tumorigenesis: using DNA polymerase beta over-expressed transgenic mouse

Kazuo HAKOI[1], Raymond W TENNANT[2], Ronald E CANNON[2]

Drug Safety Research Lab., Taiho Pharmaceutical Co., Ltd., Tokushima 771-0194, Japan[1]
Cancer Biology Group, National Center for Toxicogenomics, National Institute of Environmental Health Sciences[2]

Transgenic mice engineered to over express DNA polymerase beta were initiated with a single topical dose of 7,12-dimethylbenz [*a*] anthracene (DMBA) followed by 25 weeks of promotion with 12-O-tetradecanoylphorbol-13-acetate (TPA) two times per week to investigate the effect of DNA polymerase beta on skin tumorigenesis. Non-transgenic isogenic mice were used as a genotypic control. Induced skin tumors were observed for 52 weeks post initiation to investigate benign and malignant tumor formation.
The incidence of keratoacanthoma and papilloma with codon 61 H- *ras* mutation was determined to be significantly higher in male mice that over expressed polymerase beta as compared to all other genotypes and genders. The incidence of squamous cell carcinoma, BrdU labeling index, expression of COX-2 and P53 were no different across genotypes or genders. These data indicate that the over expression of DNA polymerase beta does not effect the malignant conversion under this experimental paradigm. These data also indicate that type of tumors induced is not dependant on genotype or gender.
The conclusion from these results that a male associated effect enhances the incidence of skin tumors induced by a two-stage protocol in transgenic mice that over express DNA polymerase beta.

P-77

Possible relationships between immunosuppressive effects and carcinogenicity of the synthetic gestagens with glucocorticoid-like effects in rodents

Shigeru HISADA[1], Toshi HORIUCHI[1]

Toxicology & Pharmacokinetics Research Department, Kawasaki 213-8522, Japan[1]

The ultimate end point of immunotoxicy is increased susceptibilities to infection and tumorigenseis. In the present study we discus the relationship between weak immunosuppression and tumorigenesis observed in the compounds with glucocorticoid-like effect.
Medroxyprogesterone acetate (MPA), a synthetic gestagen, has a glucocorticoid-like effect and the female Crj:CD(SD) rats receiving MPA at the dose levels of 1 and 10 mg/kg for 4 weeks showed atrophy of the adrenal (decreased weight and cortical atrophy), atrophy of the thymus (decreased weight and cortical atrophy), atrophy of the spleen (decreased weight and atrophy of periarteriolar lymphoid sheath), slightly decreased numbers of CD5 and CD8-possitive cells in the peripheral blood but no changes in anti-ovoalubumin(OVA) antibody production. On the other hand, another synthetic gestagen osateron acetate (TZP-4238) has a rather week glucocorticoid-like effect and the female Crj:CD(SD) rats receiving TZP-4238 at the dose levels of 0.1, 1 and 10 mg/kg showed a slightly decreased weight of the adrenal but no immunosuppressive changes were observed including organ weights and histopathology of the thymus, spleen and lymph nodes, flowcytometry of peripheral blood and anti-OVA antibody production.
The results of the carcinogenicity testing of MPA and TZP-4238 were as follows.
1) Female F344 rats fed MPA in the diet at 300 ppm for 104 weeks showed a statistically significant increase in lethality of mononuclear cell leukemia (MCL) accompanying decreased weight of the adrenal with cortical atrophy and decreased weight of the spleen with atrophy of periarteriolar lymphoid sheath.
2) Males and females of F344 rats fed TZP-4238 in the diet at 6, 30 and 150 ppm for 104 weeks showed a statistically significant increase in lethality of MCL accompanying decreased weight of the adrenal with cortical atrophy and decreased weight of the spleen with atrophy of periarteriolar lymphoid sheath at 150 ppm.
3) An additional study in which female F344 rats were fed TZP-4238 in the diet at 150 ppm for 104 weeks revealed no changes in lethality of MCL and weight of the adrenal.
4) Female Crj:CD(SD) rats and female ACI/N rats fed TZP-4238 in the diet at 150 ppm for 104 weeks showed no increases in incidence of tumors although the weight of the adrenal was decreased.
Based on these results, it seemed likely that synthetic gestagens with glucocorticoid-like effects induced MCL in the MCL-prone F344 rats when adrenocortical atrophy was apparent. These steroids showed little effects on antibody production but might affect NK cell activity.

P-78

Chemopreventive effects of plant derived compounds in a rat medium term liver bioassay and on hepatocellular carcinoma cell lines and gene expression analysis by a microarray

Hiroyuki OHNISHI[1], Kazunari TSUJIMURA[1,2], Makoto ASAMOTO[1], Masanori KURIBAYASHI[1,3], Shugo SUZUKI[1], Tadashi OGISO[1], Tomoyuki SHIRAI[1]

Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Chemicals Evaluation and Research Institute, Chemicals Assessment Center[2]
Ono Pharmaceutical Co. Ltd., Safety Research[3]

Recently, it has repeatedly been shown that compounds derived from several plants of various origins have chemopreventive effects on carcinogenesis. In the present study we investigated the effects of chemicals in this category, such as nobiletin, garcinol, auraptene, β-cryptoxanthin hesperidin rich pulp (CHRP), and 1'-acetoxychavicol acetate (ACA), expected to have inhibitory effects on liver carcinogenesis, using a rat medium term liver bioassay system and hepatocellular carcinoma (HCC) cell lines. Six week-old male F344 rats were given a single dose of diethylnitrosamine (DEN; 200 mg/kg b.w., i.p.), then during experimental weeks 2 to 8, test chemicals were given in the diet (0.05%). At week3, all rats were subjected to a two-thirds partial hepatictomy. At week 8, the experiment was terminated and the livers were taken for analysis of development of glutathione S-transferase (GST-P) positive liver foci, putative preneoplastic lesions, with the aid of an image analyzer after immunohistochemical staining. There were no significant differences in the numbers and areas of GST-P positive foci between the controls and any of the chemical-treated groups. Then, we evaluated the effect of chemicals on the growth of HCC cell lines, HepG2 and MH1C1, to see whether they might suppress the progression stage of hepatocarcinogenesis. Nobiletin had an inhibitory effect on the growth of HepG2 and MH1C1 cells and promoted apoptosis. Furthermore, we also performed microarray analysis to detect alteration of gene expression in HCC cells and found a group of genes whose expression pattern was specifically altered with the nobiletin treatment. These genes might point to mechanisms of chemoprevention by nobiletin. Our results suggest that nobiletin might have chemopreventive potential regarding progression of hepatocellular carcinoma. In conclusion, while the chemicals did not suppress the development of GST-P foci, nobiletin might have a suppressive action on the progression stage of hepatocarcinogenesis.

P-79

The inhibitory effects of arctiin on F344 rat multiorgan carcinogenesis

Masaharu MOKU[1], Hideki WANIBUCHI[1], Jin Seok KANG[1], Saki NAITO[1], Anna KINOSHITA[1], Shoji FUKUSHIMA[1]

Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

Arctiin is a naturally occurring lignan which is extracted from the seeds of burdock (*Arctium Lappa*). Some kinds of lignans have been shown to have phytoestrogenic characteristics and chemopreventive effects on chemically induced tumors of different organs such as liver, skin and lung in the rat. In the present experiment, we evaluated inhibitory effects of arctiin on rat multiorgan carcinogenesis model. Eighty four, 6 week-old, male, F344 rats were divided into 4 groups (groups 1-4) and given initiation treatment with diethylnitrosamine (DEN)(100mg/kg body weight, i.p., once at the start), n-methylnitrosourea (MNU)(20mg/kg body weight, i.p., 4times for first 2weeks), 1,2-dimethylhydrazine (DMH)(40mg/kg body weight, s.c.,4 times for following2weeks), N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN)(0.05%in drinking water, during weeks1and 2) and 2,2-dihydroxy-di-n-propylnitrosamine (DHPN)(0.1%in drinking water, during weeks 3 and 4) (DMBDD treatment). Another18rats, divided into 2 non-initiated groups (groups 5 and 6), were given the vehicle alone. After DMBDD treatment, rats in groups 1 to 4 received 0, 0.004, 0.02 and 0.1% arctiin in NIH-07 (soy bean free) diet respectively. Groups 5 and 6 received 0 and 0.1% arctiin in their diet. All surviving rats were killed under ether anesthesia at week33and all organs were excised and subjected to pathological examination. Arctiin has no toxity in all experimental dose. In high dose (0.1%) group, both incidence and multiplicity of bladder carcinoma were significantly decreased as compared with control group. No any significant effect was observed in other organs. Our results indicate that arctiin exerts chemopreventive effects in the bladder of the F344 rats in multiorgan carcinogenesis model.

P-80

Chemopreventive effect of PJJ-34 in rat multi-organ carcinogenesis model

Chiharu YAMAGUCHI[1], Sakai KUNIYOSI[1], Takasi YAMAGUCHI[1], Tomoko NAKATA[1], Reiko TANAKA[1], Hideki WANIBUCHI[2], Anna KINOSITA[2], Keiichirou MORIMURA[2], Shoji FUKUSIMA[2]

Department of Medicinal Chemistry, Osaka University of Parmaceutical Science, Osaka 569-1094, Japan[1]
Department of Pathology, Osaka City University Medical School[2]

Our efforts to search for tumor-chemopreventive agents have been focused in constituents from coniferous trees plants. An extract of picea jezonesis, 13a,14a-epocy-3b-methoxyserratan-21b(PJJ-34), is a serratane-type triterpenoid. PJJ-34 is reported to show inhibitory effects on skin carcinogenesis with the Epstein-Barr virus early antigen activation test. In this experiment, we examined the modifying effect of PJJ-34 on rat multi-organ bioassay. A total of 70 male 6 weeks old F344/DuCrj rats were divided randomly into 5 groups. (20 rats each for groups 1-3: 5rats each for groups 4-5). Animals in groups 1-3 were treated sequentially with N-Nitrosodiethylamine (100mg/kg.b.w. i.p., single dose at the commencement), N-Methyl-N-nitrosourea (20mg/kg.b.w. i.p., 4 times, until 2weeks), 1,2-DimethylhydrazineDihydrochloride (40mg/kg.b.w. s.c, 6 times, during weeks 2 and 6), N-n-Butyl-N-butan-4-ol-nitrosamine (0.05% in the drinking water, until 2weeks) and Diisopropunolnitrosamine (0.1% in the drinking water, during weeks 2 and 6). Groups 4 and 5 were given vehicle as control. After the completion of 5 carcinogens treatment (DMBDD treatment) groups1,4 and group2 were administered PJJ-34 at the concentration of 10 and 5mg/kg.b.w. i.g., up to the end of the experiment, respectively. All rats were killed under the ether anesthesia at weeks 30 from the start of the experiment, all major organs were excised, and subjected to histological and immunohistcemical analysis. Relative weights of liver, kidney and spleen did not demonstrate any significant differences among the groups. The incidence of lung tumor was 72, 55 and 84% for group1, 2 and 3, respectively. Statistical significance was observed between group2 and 3. The incidence of colon tumor was 22, 45 and 58% for group1, 2 and 3, respectively and statistical significance was detected between group1 and 3. Moreover, the incidence of thyroid tumor of group2 (10%) was significantly decreased, compared with that of group3 (53%). PJJ-34 showed inhibitory effect of tumor formation in several organs on this rat carcinogenesis model. Immunohistcemical analysis for PCNA and cyclinD1 were performed with lung tissue. The percentage value of positive stain on normal appearance mucosa was reduced in groups1 and 2, compared with group3. These results might suggest that PJJ-34 has an inhibitory effect, based on the cell cycle regulation. To further investigate the mechanism of inhibitory effect of PJJ-34, cDNA microarray analysis on short-term carcinogenesis model is now in progress.

P-81

Dietary protocatechuic acid inhibits progression of chemically induced rat tongue carcinogenesis

Rikako SUZUKI[1,2], Hiroyuki KOHNO[1], Shigeyuki SUGIE[1], Takuji TANAKA[1]

Department of Pathology, Kanazawa Medical University, Ishikawa 920-0293, Japan[1]
Research Fellow of the Japan Society for the Promotion of Science[2]

The frequent consumption of edible plants is associated with a low incidence of a variety of cancer. Protocatechuic acid (PCA), a simple phenolic acid, is a constituent of many edible plants. Our previous experiment demonstrated that PCA inhibits the development of 4-nitroquinoline 1-oxide (4-NQO)-induced tongue carcinogenesis when fed during the initiation and promotion phases. The compound can also inhibit chemical carcinogenesis in other organs. In the present study, we investigated the modifying effects of dietary administration of PCA during the progression phase of tongue carcinogenesis initiated with 4-NQO in male F344 rats. For tumor progression, we developed a new animal model, where rats initiated by 4-week treatment of 20 ppm 4-NQO in drinking water and thereafter received four cycles of 20 ppm 4-NQO (one cycle: 2 weeks of 4-NQO followed by 2 weeks of tap water, starting at 14 weeks after the initiation) to induce advanced tongue cancer. The treatment enabled to induce high incidence of tongue cancer with or without metastases of tongue cancer in lungs, when compared with rats given the initiation treatment alone, suggesting that this model can be used for tongue tumor progression study. Starting two weeks before the cycle treatment with 4-NQO, animals were fed the 2000 ppm PCA containing diet and continued on this diet until the end of the study. At the termination of the experiment (week 32), the incidences of tongue neoplasms and preneoplastic lesions, and total polyamine levels in the tongue tissue, and cell proliferation activity estimated by morphometric analysis of silver-stained nucleolar organizer regions' protein were compared among the groups. Feeding with PCA containing diet during the progression phase significantly reduced the incidence of tongue squamous cell carcinoma (31% inhibitory by PCA, $P<0.05$) and preneoplasia (hyperplasia and dysplasia) (29% inhibitory by PCA, $P<0.001$). Rats given PCA-containing diet have no metastasis. In addition, PCA feeding significantly lowered polyamine levels of the tongue at the end of the experiment (week 32): 3.80 ± 0.44 in the group initiated with 4-NQO + cycle treatment with 4-NQO; and 3.23 ± 0.34 in the group initiated with 4-NQO + cycle treatment with 4-NQO + PCA ($P<0.001$). Our results suggest that a simple phenolic acid PCA has inhibitory effect on 4-NQO-induced rat tongue carcinogenesis when given during the progression phase of tumorigenesis, and such inhibition might be related to suppression of cell proliferation by PCA.

P-82

Chemopreventive effect of FBRA on N-nitrosomethylbenzylamine-induced esophageal tumorigenesis in rats

Toshiya KUNO[1], Yoshinobu HIROSE[1], Yasuhiro YAMADA[1], Keiko SAKATA[1], Nami KITAORI[1], Akira HARA[1], Hideki MORI[1]

Department of Tumor Pathology, Gifu 500-8705, Japan[1]

FBRA (Fermented brown rice by Aspergillus Orizae) is a natural product occurring in rice germ with properties associated with reduced liver and colon cancer risk in rat. Modifying effects of FBRA during the initiation or post-initiation phase of *N*-nitrosomethylbenzylamine (NMBA)- induced esophageal tumorigenesis were investigated in male F344 rats. A total of 150 rats were used. Five-week-old rats were divided into 7 groups, and at 7 weeks of age, rats in groups 1 through 5 received 15 s.c. injections of NMBA over the course of 5 weeks (0.5mg/kg /dose, three dose /week). Rats in groups 2 and 4 were given the diet containing 5 and 10% FBRA, respectively, for 7 weeks (starting at 6 weeks of age until 1week after the last dosing of the carcinogen). They were switched to the basal diet and maintained on that until the termination. Starting 1 week after the final injection of NMBA, rats in groups 3 and 5 were fed the diet mixed with 5 and 10% FBRA, respectively, until the termination. Group 6 was given the diet containing 10% FBRA throughout the experiment, and group 7 was kept on basal diet alone and served as an untreated control. All rats sacrificed at 32 weeks were examined for tumor formation, and esophageal tumor were processed for histopathological evaluation. In group 1, the incidence of total neoplasmas was 88%. The incidences of esophageal papilloma in groups 2-5 were 69, 76, 69 and 62%, respectively. The incidence of total esophageal neoplasms in group 5 was significantly less than that in group 1 ($P<0.05$). Multiplicity of total neoplasms in group 3 and 5 (1.16+/-0.99, 0.81+/-0.85, respectively) were significantly smaller than that in group 1 (1.77+/-1.07) ($P<0.05$). These findings indicate that FBRA inhibits NMBA-induced esophageal tumorigenesis when given the post initiation phase rather than initiation phase. Incidence and multiplicity of preneoplastic lesions and proliferative activity of each lesion are just exploring. These data will be demonstrated at the meeting.

P-83

Acyclic retinoid inhibits the aberrant crypt foci formation induced by dimethylhydrazine in the rat colon

Masumi SUZUI[1], Takahiro SHIMIZU[1], Takamitsu MORIOKA[1], Viengvansay NABANDITH[1], Morihiko INAMINE[1], Tatsuya KANESHIRO[1], Tatsuya KINJO[1], Naoki YOSHIMI[1]

Department of Pathology, University of the Ryukyus Faculty of Medicine, Okinawa 903-0215, Japan[1]

Acyclic retinoid (ACR), a novel synthetic retinoid, has recently been demonstrated by us to inhibit the growth of human hepatoma cells by inhibiting in the levels of expression of the cell cycle related molecules thus this agent may be useful in the chemoprenevtion and therapy of hepatoma and possibly other human malignancies (Suzui, M. et al. Cancer Res. 62: 3997-4006, 2002). Therefore, in the present studies we investigated the possible chemopreventive effect of dietary administration of ACR on rat colon carcinogenesis. Aberrant crypt foci (ACF) are putative preneoplestic lesions of colon carcinogenesis thus these lesions are widely utilized as useful proliferating biomarkers. The number of ACF in rat colonic epithelium was examined. A total of 35 male F344 rats were randomly divided into the following 5 experimental groups. At 5 weeks of age, rats in groups 1, 2, and 3 were weekly given s.c. injections of a carcinogen dimethylhydrazine (DMH, 40 mg/kg body weight) for 2 weeks to induce ACF, rats in groups 2 and 3 were fed a diet containing ACR at concentrations of 50 and 150 ppm, respectively, throughout the experiment. Rats in group 4 were served as untreated controls. Rats in group 5 were fed a diet containing 150 ppm ACR. All animals were sacrificed at 5 weeks after the start of the experiment. Dietary administration of ACR caused a significant decrease in number of ACF at both doses ($P<0.003$), when compared with the group treated with DMH alone (group 1). No toxic effect caused by ACR was observed in all organs examined. These findings suggest that ACR may be a promising chemopreventive agent for human colon carcinogenesis. These novel effects of ACR provide further evidence that ACR may be a valuable compound in the chemoprevention and therapy of various malignancies.

P-84

Time course observation on inhibitory effects of hepato-proliferative lesions in rats induced by a liver-selective thyromimetic, KAT-681

Morimichi HAYASHI[1], Toru TAMURA[1], Yuji OKUHARA[1], Tatsuya NAGASAWA[1], Tsuyoshi KITAMURA[1], Junji KURODA[1], Nobuo SHIBATA[1], Kunitoshi MITSUMORI[2]

Kissei Pharmaceutical Co., Ltd., Nagano 399-8305, Japan[1]
Laboratory of Veterinary Pathology, Tokyo University of Agriculture and Technology[2]

Our previous study showed that a liver-selective thyromimetic, KAT-681 (KAT), inhibited the development of hepato-proliferative lesions positive for glutathione S-transferase placental form (GST-P) in rats that were induced by diethylnitrosamine (DEN)-initiation treatment followed by promotion treatment with 2-acetylaminofluorene (2-AAF) and partial hepatectomy. To clarify the inhibitory effect, a time course observation on the hepato-proliferative lesions in rats treated with KAT was performed in the present study. For the induction of hepato-proliferative lesions, male F344 rats were treated with 2-AAF (7.5 mg/kg/day) by gavage twice a day from Weeks 2 to 4 and a partial hepatectomy at Week 3 after single intraperitoneal injection of DEN (150 mg/kg). From 5 weeks after completion of 2-AAF administration, rats were treated orally with KAT at the dose of 0.25 mg/kg/day for up to 3 weeks. These animals were sacrificed on Days 0, 1, 2, 4, 7, 14 or 21 of KAT treatment, and a quantitative morphometric analysis using a computer-assisted image processor was performed on the GST-P-positive lesions such as altered hepatic foci (AHF) and hepatocellular adenomas (HCAs). In addition, the labeling indices of proliferating cell nuclear antigen (PCNA) and single-strand DNA (ssDNA) were measured as a proliferative index (PI) and an apoptotic index (AI) in the GST-P-positive lesions, respectively. The total area and number, except for mean size, of AHF were significantly decreased from Days 4 or 7 to 21 of KAT treatment. Those of HCAs did not show any significant changes, except for the reduction of mean size on Day 14. The PI was significantly increased in AHF, but not in HCAs, with a peak on Day 2. The AI showed no significant changes in both AHF and HCAs. These results suggest that the inhibitory effect of KAT on hepatic proliferative lesions is attributable to the reduction in the total area and number of AHF.

P-85

Knockout of iNOS gene does not prevent hepatocarcinogenesis caused by a choline-deficient, l-amino acid-defined (CDAA) diet in mice

Ayumi DENDA[1], Nao MURATA[1], Toshifumi TSUJIUCHI[1], Masahiro TSUTSUMI[1], Dai NAKAE[2], Yoichi KONISHI[1], Hiroki KUNIYASU[1]

Department of Oncological Pathology, Nara Medical University, Nara 634-8521, Japan[1]
Department of Pathology, Sasaki Institute[2]

Inflammation has been postulated to involve in human carcinogenesis, one of key molecules in which being a frequently and highly expressed inducible NO synthase (iNOS) during inflammation. NO produced by iNOS, can cause DNA damages and thus gene alterations, and can promote growth of tumors through inhibition of apoptosis and enhancement of angiogenesis and invasion. Moreover, tumors of several organs including colon in humans and animals have been reported to express iNOS, in fact, with iNOS gene knockout preventing colon carcinogenesis in min mice. In human livers, especially of patients infected with HCV, iNOS is reportedly highly expressed with the development of cirrhosis and hepatocellular carcinomas (HCC). Nevertheless, roles of iNOS in hepatocarcinogenesis remain to be elucidated. A CDAA diet causes hepatocarcinogenesis associated with development of fatty liver, fibrosis and cirrhosis in rats and mice, which is the only animal model possessing similar histological sequences to those of human hepatocarcinogenesis, especially of that caused by HCV. Recently, in this rat model, we have found expression of iNOS protein in the livers during hepatocarcinogenesis. In the present study, in order to seek into roles of iNOS in the hepatocarcinogenesis, hepatocarcinogenicity of a CDAA diet in iNOS -/- mice was examined.
[Methods] Male 7-8 weeks old wild C57BL/6J (Japan Clea) and iNOS -/- (NOS2 tm/Lau, Jackson) mice, 60 each, were fed a CDAA or the control choline-supplemented (CSAA) diet for 21, 44 and 80 weeks. Development of prenoplstic and neoplastic liver lesions was histologically examined, and expression of iNOS by RT-PCR and immunohistiochemistry. Serum NOx levels assessed by the method of Miranda.
[Results] RT-PCR analysis in wild mice revealed that iNOS was hardly expressed in control livers whereas iNOS was moderately expressed in the livers fed a CDAA diet for 21 and 44 weeks, and in the resected tumors and surroundings at 80 weeks. Immunohistochemically iNOS protein was weakly - moderately positive in steatotic hepatocytes scatteringly and some of altered foci at 21 and 44 weeks, and in adenomas and HCCs at 80 weeks. Serum NOx levels in iNOS -/- mice were significantly lower than those of wild at 80 weeks. Nevertheless, no significant differences were observed between wild and iNOS -/- mice, in the incidence and multiplicity of altered foci, adenomas and HCCs at 80 weeks. These results suggested that iNOS might not work as a driving force for the hepatocarcinogenesis at least in the present CDAA diet model.

P-86

Modifying effects of Terminalia catappa on azoxymethane-induced colon carcinogenesis in male F344 rats

Takamitsu MORIOKA[1], Masumi SUZUI[1], Viengvansay NABANDITH[1], Morihiko INAMINE[1], Tatsuya KANESHIRO[1], Tatsuya KINJO[1], Takahiro SHIMIZU[1], Miyuki AONAHATA[1], Naoki YOSHIMI[1]

Tumor Pathology, University of the Ryukyus Faculty of Medicine, Okinawa 903-0215, Japan[1]

The modifying effects of dietary administration of *Terminalia catappa* (TC) was investigated on the rat colon carcinogenesis induced by azoxymethane (AOM). The number of aberrant crypt foci (ACF) and β-catenin accumulated crypts (BCAC) in the colon, and proliferating cell nuclear antigen (PCNA) labeling index in the colonic epithelium were examined in a total of 36 male F344 rats. At 6 weeks of age, rats in groups 1, 2 and 3 were given s.c. injections of AOM once a week for 2 weeks at a concentration of 20 mg/kg body weight. One week before the first injection of AOM, rats in groups 2 and 3 were fed a diet containing TC at concentrations of 0.02 and 0.1%, respectively, throughout the experiment. Rats in group 4 were fed a diet containing 0.1% TC. Rats in group 5 were served as untreated controls. All animals were sacrificed at the week 5 after the start of the experiment. Oral administration of TC at both doses significantly decreased the numbers of both ACF ($P<0.05$ for 0.02% TC, $P<0.005$ for 0.1% TC) and BCAC/cm^2/rat ($P<0.05$ for both 0.02 and 0.1% TC), when compared with the control group (group 1). PCNA labeling indices of rats in groups 2 and 3 were also significantly lower than that of rats treated with AOM alone ($P<0.001$ for 0.02% TC, $P<0.005$ for 0.1% TC). These results suggest that TC has a potent chemopreventive effect for colon carcinogenesis and this effect may be associated with the inhibition of the development of ACF and BCAC.

P-87

Lovastatin inhibits tumor growth and metastasis in a mouse mammary carcinoma model by p53-independent mitochondrial-mediated induction of apoptosis

Masa-aki SHIBATA[1], Yuko ITO[1], Junji MORIMOTO[2], Yoshinori OTSUKI[1]

Department of Anatomy & Biology, Osaka Medical College, Osaka 569-8686, Japan[1]
Laboratory Animal Center, Osaka Medical College[2]

The effects of lovastatin, a potent inhibitor of HMG CoA reductase, were studied in a mouse metastatic mammary cancer model incorporating mutation in p53. Mammary tumors, induced by inoculation of syngeneic BALB/c mice with BJMC3879 cells, were subsequently treated with lovastatin at 0, 25 and 50 mg /kg three times a week. Significantly reduced tumor volumes were observed for the 25 and 50 mg/kg groups by experimental week 2 and thereafter throughout the 6-week study. There was an associated increase in TUNEL-detected apoptosis and a decrease in DNA synthesis, as assessed by BrdU incorporation. Lovastatin at 50 mg/kg significantly inhibited lung metastasis. In a separate in vitro study, flow cytometric analyses of mammary cancer cells treated with lovastatin showed arrest at G1 as well as decreases in S and G2/M fractions and a significant elevation of p53-independent apoptosis. Apoptosis was further confirmed by ultrastructural analysis demonstrating fragmented nuclei with condensed chromatin that occasionally assumed a characteristic half-moon shape. Elevated caspase-3, -8 and -9 activities were observed in lovastatin-treated cells. Furthermore, lovastatin decreased the mitochondrial membrane potential with subsequent release of cytochrome c. These results suggest that lovastatin may be useful both as an adjuvant therapy in and a chemopreventative of breast cancers having p53 mutations due to its suppression of DNA synthesis and induction of p53-independent mitochondrial-mediated apoptosis.

P-88

Prevention of H. pylori associated gastric carcinogenesis in N-methyl-N-nitrosourea-treated Mongolian gerbils using concentrate of Japanese apricot (Ume)

Harunari TANAKA[1], Ken-ichi INADA[2], Toru NIWA[1], Takafumi OTSUKA[1], Mitsuo GOTO[1], Hirotoshi UTSUNOMIYA[3], Tetsuya TSUKAMOTO[1], Masae TATEMATSU[1]

Division of Oncological Pathology, Aichi Cancer Center Research Institute, Aichi 464-8681, Japan[1]
1st Department of Pathology, Fujita Health University[2]
Department of Pathology, Wakayama Medical University School of Medicine[3]

[Background] Helicobacter pylori (H. pylori) is considered to be a cause of gastric and duodenal ulcer, and its persistent infection is an important risk factor of gastric cancer. Since H. pylori infection has been treated with antibiotics in combination with a proton pump inhibitor, several problems have been issued such as adverse effects or emergence of resistant bacteria. Some natural foodstuffs such as garlic, cinnamon, and green tea are known to have bactericidal effect and have been shown suppression effect against growth of H. pylori and H. pylori induced gastritis. A Japanese traditional folk medicine, concentrate of Japanese apricot (CJA, Ume, Prunus mume), is said to have bactericidal effect and to be effective to sickness of a digestive organ. Thus, we investigated whether CJA was effective to relieve gastritis and *N*-methyl-*N*-nitrosourea (MNU)-induced gastric carcinogenesis using H. pylori infected Mongolian gerbils.
[Methods] One hundred and forty one 5-week-old male Mongolian gerbils were divided into 8 groups. They were treated with 30 ppm MNU every other week for ten weeks (groups A, B, C, and D), inoculated with H. pylori intragastrically (groups A, B, C, E, F, and G) and administered CJA at 3 (groups A and E) or 1% (groups B and F). Animals in groups C, D, G, and H received no CJA. Gerbils were sacrificed at 52 experimental week. Stomach mucosae were routinely processed and histologically evaluated for severity of inflammation using modified Sydney system and emergence of tumors.
[Results] Inflammation in stomach mucosae were suppressed significantly ($P<0.0001$) by administration of CJA at both 3 and 1%. Thirteen adenocarcinomas were found in 9 animals (9/25=36%) in MNU+H. pylori group (group C) and 1 cancer in 1 animal (7%=1/15) from MNU alone group (group D). CJA was significantly effective ($P<0.005$) to reduce incidence of adenocarcinomas in both 3 and 1% CJA groups; no cancers were found in group A (0/30) and in group B (0/24). No tumors were found in control groups (groups E, F, G, and H).
[Conclusion] CJA could be effective to alleviate gastric inflammation and consequently to prevent gastric carcinogenesis associated with H. pylori infection.

P-89

The effect of N-acetylecysteine on low dose 2-amino-3,8dimethylimidazo[4,5-f] quinoxaline(MeIQx)-induced rat hepatocarcinogenesis

Natsuko MIYAZI[1], Hideki WANIBUCHI[1], Masaharu MOKU[1], Keiichirou MORIMURA[1], Yoshiaki TAGAWA[1], Shoji FUKUSHIMA[1]

Department of Pathology, Osaka City University Medical School, Osaka 545-8585, Japan[1]

Human is exposed to low dose MeIQx in cooking meat and fish. In this study, we examined whether N-acetylecysteine (NAC), one of water soluble organosulfur components contained in garlic, can prevent low dose MeIQx-induced hepatocarcinogenesis. 320 F344/Ducrj male rats at the age of 21 days were devided to 14 groups. The rats in groups 1 to 7 were given 1.5 ml/mg NAC drinking water. The rats in groups 8 to 14 were given tap water. They were fed a diet containing 0, 0.01, 0.1, 1, 10, 30, 100 ppm of MeIQx for 32 weeks. The numbers of GST-P possive foci in the liver increased significantly between 0 ppm rats and 30 ppm, 100 ppm treated rats, however no significant difference was observed compared with NAC treated group and MeIQx alone group, respectively. The 8-OH formation, PCNA expression, Glutathione S-transferase activity and Glutathione levels were no significantly different. In conclusion, NAC has no chemopreventive effect in this model.

P-90

Glycogen granule, glycogen phosphorylase, and CYP1 after exposure to DMBA in rat liver

Shin WAKUI[1], Masakuni FURUSATO[2]

Department of Toxicologic Pathology, Azabu University School of Veterinary Medicine, Kanagawa 229-8501, Japan[1]
Department of Pathology, Kyorin University School of Medicine[2]

The changes in rat liver after a single oral dose of 7,12-dimethylbenz[a]anthracene (DMBA) were examined. Ultrastructually, glycogen granules of centrilobular hepatocytes increased with time to peak on Day 1. After this, they sharply decreased on Day 2, and on Days 5-10 returned to the normal levels. Proliferation of smooth endoplasmic reticulum (sER) of the centrilobular hepatocytes also gradually increased with time to peak on Day 2, after which it decreased, and on Days 5-10 returned to the normal levels. While the activity of hepatic glycogen phosphorylase a was decreased at 6-12 hrs and on Day 1, on Days 2-10, it was elevated to a level similar to the normal levels. Thus, the high proliferation of sER and the glycogenolysis concurrently occurred on Day 2 following DMBA administration. DMBA is an indirect carcinogen that enlists the host metabolism to produce its ultimate carcinogenic form, and it is known that this metabolism is conducted by cytochrome P450 1A1 and/or 1B1(CYP1). It has been recognized that the increase of glycogenolysis, glucolysis, is required to sustain the induction levels of CYP1. Moreover a proliferation of sER may be associated with increased quantities of CYP1 activity in the liver. Expression of liver CYP1 mRNA was observed at 6 hr and gradually increased with time to peak on Days 1-2, but then disappeared on Day 5. Expression of CYP1A1 protein was first observed at 12 hr, peaked on Day 2 and decreased on Day 5, while expression of CYP1B1 protein was first observed on Day 2 and decreased on Day 5. Results from the present study indicate that exposure of rats to DMBA could induce a reversible initial change in the hepatic glycogen metabolism. Moreover, the glycogenolysis of hepatocytes with high proliferation of sER and the high CYP1 enzyme production that occurred concurrently on Day 2. Therefore DMBA metabolism might occur in rat liver substantially on Day 2 following DMBA administration.

P-91

Comprehensive gene expression profiles of hepatocellular carcinomas induced in rats fed a choline-deficient, L-amino acid-defined diet

Fumiyuki UEMATSU[1], Naoto WATANABE[2], Maki IGARASHI[2], Masakazu TAKAHASHI[1], Midori YOSHIDA[1], Akihiko MAEKAWA[3], Dai NAKAE[1]

Department of Pathology, Sasaki Institute, Sasaki Foundation, Tokyo 101-0062, Japan[1]
Department of Food and Nutritional Sciences, Graduate School of Agriculture, Tokyo University of Agriculture[2]
Director, Sasaki Institute, Sasaki Foundation[3]

A choline-deficient, L-amino acid-defined (CDAA) diet induces hepatocellular carcinomas (HCCs) in rats at a high rate through the endogenous molecular mechanisms that are different from the exogenous mechanisms due to carcinogenic xenobiotics but remain still largely obscure. The morphological, biochemical and molecular biological characters of the carcinogenic processes of rat hepatocarcinogenesis by the CDAA diet resemble, at least partly, those of human hepatocarcinogenesis cases including that derived from viral chronic hepatitis. On the other hand, human carcinogenesis in general occurs by virtue of not only extrinsic but also intrinsic factors. It is thus meaningful to elucidate the molecular mechanisms underlying the CDAA diet-associated rat hepatocarcinogenesis. In this context, the present study assessed comprehensive gene expression profiles of HCCs induced in rats fed the CDAA diet.
Total RNA was extracted from 5 HCCs and their surrounding non-cancerous liver tissues of 5 individual male Fischer 344 rats fed the CDAA diet from the age of 6 weeks for 70 weeks, and from 5 liver tissues of 5 individual strain-, sex- and age-matched untreated rats. The RNA was then subjected to an oligonucleotide microarray analysis involving 3757 genes. After normalization, 148 genes were detected to be differentially expressed among the 3 classes of tissues by an ANOVA procedure. Based on the differences in the expression patterns, these genes were classified into 4 clusters either by hierarchical or k-means clustering procedures. The members of each cluster became identical by these 2 clustering procedures, and the numbers of the genes in Clusters 1, 2, 3 and 4 were 54, 18, 47 and 29, respectively.
While each cluster included genes with various functions, notable genes were related to apoptosis, transcriptional regulation and alternative splicing. Among these, several changes were suggested to be human relevant. For instance, two CYP isozymes were down-regulated in HCCs compared to their surrounding liver tissues, while these genes are down-regulated also in human HCCs. The *tcf1* gene encoding hepatocyte nuclear factor 1 was down-regulated in HCCs compared to the untreated liver tissues, and the transcription factor plays crucial roles in the development and malignant conversion of human hepatocellular adenomas. It is thus indicated that the present results provide information contributable to elucidate human hepatocarcinogeic mechanisms and to draw an evidence-based strategy to control human HCCs.

P-92

Enhanced preneoplastic lesion induction in the livers of gap junction disrupted transgenic rats

Naomi HOKAIWADO[1], Makoto ASAMOTO[1], Kazunari TSUJIMURA[1,2], Kumiko OGAWA[1], Tadashi OGISO[1], Tomoyuki SHIRAI[1]

Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Aichi 467-8601, Japan[1]
Chemicals Evaluation and Research Institute, Chemicals Assessment center[2]

We have established transgenic (Tg) rats with a dominant negative mutant of connexin 32 gene under control of the albumin promoter, in order to investigate the role of gap junctions in hepatocarcinogenesis in vivo. Connexins are subunits of gap junction channels, which allow direct transfer of ions, secondary messenger molecules and other metabolites between contacting cells. Gap junctions are reported to be involved in tissue homeostasis, embryonic development and control of cell proliferation. In the livers of the male Tg rats, the normal membrane localization of endogenous connexin 32 protein appeared disturbed and gap junction capacity measured by scrape dye-transfer assay in vivo was found to be markedly decreased as compared with the wild-type case. In normal rats, d-galactosamine and carbon tetrachloride induced severe necrosis of hepatocytes and marked elevation of serum AST and ALT, while, only scattered single cell necrosis of hepatocytes and slight elevation of enzymes (AST and ALT) were detected in the Tg animals. These findings suggest that the Tg rats have marked resistance to hepatotoxicity of these compounds. Important roles of gap junctional intercellular communication (GJIC) in multistage carcinogenesis are supported by several pieces of evidence. Gap junctional proteins are often decreased in tumor tissue, and overexpression of connexins suppresses tumorigenicity. Furthermore, connexin 32 knock out mice demonstrate increased susceptibility with regard to hepatocarcinogenesis. Therefore we examined the effects of functional inhibition of gap junction on hepatocarcinogenesis in our Tg rats. Animals were given a single intraperitoneal administration of 200 mg/kg diethylnitrosamine (DEN) at 8 weeks of age and killed sequentially at time intervals up to 20 weeks later. Liver tissues were taken for histological and immunohistochemical examination and RT-PCR analysis. Glutathione S-transferase placental form (GST-P) positive foci were semiquantatively measured with the aid of an image analyzer. No differences between Tg and wild rats in DEN-induced inflammatory responses and necrotic changes were revealed by histological assessment. In male Tg rats, a significant increase in the numbers and areas of GST-P positive foci was evident at 20 weeks compared with the wild type case. Interestingly, there was also a clear tendency for Tg rats to develop larger foci. These results indicate that GJIC plays important roles in the development of liver preneoplastic lesions. Now a one year long-term experiment is underway in order to analyse differences in tumorigenic sensitivity between Tg and wild rats at the level of hepatocellular tumor development.

P-93

In situ hybridization of interferon-gamma mRNA in concanavalin A-induced hepatitis in mice using the polymerase chain reaction-derived cRNA probes

Hideki TANAKA[1], Atsushi FUKUNARI[1], Tomomichi IWAKI[1], Shiro TAKAGI[1]

Mitsubishi Pharma Corporation, Kanagawa 227-0033, Japan[1]

The information obtained by immunohistochemistry (IHC) regarding intracellular protein synthesis might be limited; especially cytokines are rapidly secreted and hardly deposited in cells. As in situ hybridization (ISH) using cRNA probes (riboprobes) detects gene transcription products, many laboratories have reported success in hybridizing cytokines mRNA. However, constriction of plasmid templates for the synthesis of riboprobes with phage RNA polymerases is often a complicating and time-consuming step. Additionally, since most investigations use frozen sections for ISH, some problems exist, i.e. poor structural resolution and/or instability of mRNA. Recently, a rapid and efficient generation of the polymerase chain reaction (PCR)-derived riboprobe template has been introduced, and the riboprobes can be applied in ISH to routinely fixed and paraffin-embedded sections. Concanavalin A (Con A) is known to induce T-cell activation and an acute hepatitis, and this experimental system is reported to provide a model for investigating the regulation of a set of cytokines. We thus analyzed the spatial and temporal expression of interferon-gamma (IFN-γ) using a mouse model of hepatitis induced by Con A injection. Con A (20mg/kg) was administered to male BALB/c mice via the tail vein. Total RNA was extracted from the injured liver 8 h after Con A-injection. Reverse transcription PCR was performed for mouse IFN-γgene. T7 promoter adaptor was directly added to IFN-γ cDNA-specific PCR product by using a PCR promoter addition kit. This cDNA was then amplified by PCR using IFN-γgene-specific primer in combination with T7 promoter adaptor primer sequence and used as a template of the riboprobes. Digoxigenin-labeled riboprobes were synthesized by in vitro transcription method using RNA polymerase. ISH was performed using formalin-fixed and paraffin-embedded sections. Striking increase in ISH signals of IFN-γ were recognized in lymphoid cells within the hepatic sinusoids during 2 - 12 h after Con A-injection. Time course of change in the signals was well correlated to that of the plasma IFN-γlevels. The present results indicate that ISH using the PCR-derived riboprobes is a reproducible and efficient technique even in the case of detecting cytokines, very small amounts of biomarkers.

P-94

Rapid induction of type-1 diabetes mellitus by X-irradiation in Long-Evans Agouti (LEA) rats

Tokiko NAKAI[1], Akiko KUBO[1], Eiko TAKISHITA[1], Tamotsu TAKIZAWA[1], Takeshi TSUCHIGAUCHI[1], Keiji KODAMA[1], Keisuke IZUMI[1]

Department of Molecular and Environmental Pathology, The University of Tokushima School of Medicine, Tokushima 770-8503, Japan[1]

The Long-Evans Agouti (LEA) rat established from a Long-Evans rat closed colony has been used for the control strain of the Long-Evans Cinnamon (LEC) rat, an animal model of Wilson's disease. It is reported that both LEA and LEC rats are sensitive to ionizing radiation. Initially, the aim of the present study was to examine the effect of X-irradiation on carcinogenesis. In Experiment 1, male F344, LEC and LEA rats (n= 15) were X-irradiated at 6 and 8 weeks of age (Group 1) and at 6 weeks of age (Group 2) at a dose of 2 Gy /day. Control rats were sham-irradiated (Group 3). In Experiment 2, male LEC and LEA rats (n=9) were irradiated at 6 and 8 weeks of age at a dose of 2 Gy/day and the control rats were sham-irradiated. At week 6, rats were submitted to an oral 2 g/kg glucose tolerance test. In Experiment 1, all LEA rats irradiated 4 Gy became diabetic by week 10, and the survival rates were 7/15 (47%) at week 10 and 3/15 (20%) at week 30. Thirteen of 15 (87%) LEA rats irradiated 2 Gy showed glycosuria by week 30 and the survival rate was 13/ 15 (87%). Fourteen % of the control LEA rats showed glycosuria at week 30, but hyperglycemia and glycosuria did not appear in F344 and LEC rats in any group. Histologically, the pancreas in X-irradiated LEA rats showed islet cell loss with hemosiderosis. In Experiment 2, 30-min postglucose load blood glucose in all non-irradiated LEA rats were diabetic levels (159-249 mg/dl). By 4 Gy irradiation, fasting blood glucose in 3 of 8 LEA rats and 30-min blood glucose in 5 of 8 LEA rats were 250 mg/dl, but not in LEC rats. Serum insulin levels in LEA rats were decreased more severely by irradiation. In conclusion, LEA rats are prone to develop type-1 diabetes mellitus and X-irradiation accelerates the development of the disease. Radiation-induced islet injury may be a cause of diabetes in LEA rats.

P-95

Porcine-serum-induced hepatic fibrosis in Brown Norway and Wistar rats

Yasuko BABA[1], Koji UETSUKA[1], Hiroyuki NAKAYAMA[1], Kunio DOI[1]

Department of Veterinary Pathology, Graduate School of Agricultural and Life Science, The University of Tokyo, Tokyo 113-8657, Japan[1]

[Background] In human, hepatic fibrosis and cirrhosis are severe diseases. Many hepatic fibrosis models have been developed, but detailed fibrogenic process and mechanisms of hepatic fibrosis are still obscure. Porcine serum (PS) induces hepatic fibrosis with little hepatocyte damage, although many models accompany prominent hepatic damage.
[Aim] Strain differences in PS-induced hepatic fibrosis were examined in Brown Norway (BN) and Wistar rats.
[Materials and Methods] Twenty-four 6-week-old male rats each of BN and Wistar strains were used. The rats of each strain were divided into two groups. One group was injected i.p. with 0.5ml sterile PS twice a week for 1, 2, 3 and 4 weeks and the other group was treated with physiological saline in the same way and served as controls. At 1, 2, 3 and 4 weeks, three rats of each group were killed by blood sampling from abdominal aorta under ether anesthesia at 24 hr after the last PS or saline injection. Liver was divided into two pieces. One piece was fixed by 10% neutral buffered formalin and the other was frozen by liquid nitrogen. Four-μm paraffin sections were stained with hematoxylin and eosin. Total RNA was extracted from frozen liver and used for Affymetrix GeneChip microarray analysis, and reverse transcription-polymerase chain reaction (RT-PCR).
[Results] Histologically, the development of PS-induced hepatic fibrosis differed between BN rats and Wistar rats, and formation of hepatic fibrosis occurred earlier in the former. In GeneChip microarray analysis, the expression of genes linked to antigen presentation (CD74, major histocompatibility complex (MHC) class II alpha chain and beta chain) was elevated earlier in BN rats than Wistar rats. The changes in the expression of these genes were confirmed by RT-PCR in BN rats, but not in Wistar rats.
[Discussion] CD 74 and MHC class II are well known to be closely correlated with exotic-antigen presentation and antibody production. Therefore, the present results suggest that antigen presentation is an important process in PS-induced hepatic fibrosis and that the difference in the development of hepatic fibrosis between BN and Wistar rats may be at least due to the difference in the MHC class II-related antigen presentation.

P-96

Induction of mRNA related with hepatic injury response to oxidative stimuli in hepatocytes and non-parenchymal cells

Toshinobu YAMAMOTO[1], Hiroyuki UTSUMI[1], Naoko SHIMADA[1], Tetsu OGATA[1], Naohisa TSUTSUI[1]

Toxicology Laboratory, Mitsubishi Pharma Corporation, Chiba 292-0818, Japan[1]

Oxidative stress and inflammatory reactions may lead and promote hepatic injury. However, the gene expression of hepatocytes and non-parenchymal cells response to oxidative stimuli was not well known. In the present study, two liver injury models induced oxidative stress, acetaminophen-administration or ischemia /reperfusion, were designed, and the mRNA expression (oxidative and inflammatory related genes: HO-1, HSP70, iNOS, TNF α, IL-1 β, and IL-6) of liver were analyzed by RT-PCR. Moreover, hepatocytes and non -parenchymal cells were divided by collagenase perfusion method, and both cells were suspected to RT-PCR analysis.

Treatment of male SD rats with a hepatotoxic dose of acetaminophen (1000 mg/kg, p.o.) resulted in an induction of HO-1, HSP-70, iNOS, TNF α, IL-1 β, and IL-6 in the liver. Under the experimental condition, HSP70 was mainly increased in both hepatocytes and non-parenchymal cells in contrast to mRNA of HO-1, which was rather induced in hepatocytes. Other mRNAs, iNOS and inflammatory cytokines, were mainly increased in the non-parenchymal cells. Elevation of ALT in serum, and centrilobular necrosis of hepatocytes were also observed.

In ischemia(45 min)/reperfusion experiments, HSP70 and IL-6 were induced in liver at 1hr reperfusion. At this point, ALT in serum was elevated but no histopathological change was observed in liver. At 3hr reperfusion, HO-1, HSP70, iNOS and IL-6 were induced in liver. Elevation of ALT in serum, and periportal necrosis of hepatocytes were also observed. HO-1 was increased in hepatocytes, and iNOS and IL-6 were increased in non-parenchymal cells. While, HSP-70 was mainly induced in both hepatocytes and non-parenchymall cells.

These observations suggest that oxidative stress causes a common spectrum of changes in gene of liver. In those two injured liver models, hepatocytes would be more sensitive than non-parenchymal cells to the effects of oxidative stress on HO-1. In contrast, non-parenchymal cells would be more sensitive than hepatocytes on iNOS and inflammatory cytokines.

P-97

Persistence of liver cirrhosis is associated with altered location of α-smooth muscle actin (α-SMA) positive cells

Jin Seok KANG[1], Hideki WANIBUCHI[1], Keiichirou MORIMURA[1], Elsayed I. SALIM[1], Shoji FUKUSHIMA[1]

Department of Pathology, Osaka City University, Osaka 545-8585, Japan[1]

Liver cirrhosis is one of the strongest risk factors for hepatocellular carcinoma in human. To investigate whether liver cirrhosis is irreversible after cessation of stimuli, we used thioacetamide (TAA)-induced liver cirrhosis model. Male F344 rats were randomly allocated to three groups. Animals of group 1 (n=15) were received normal water as control, and those of group 2 (n=15) and group 3 (n=15) were received 0.015% or 0.03% TAA in drinking water for 12 weeks, respectively. During the treatment of TAA, there were four animals death in group 3, but there were no death in group 1 and group 2. At 12 weeks, 10 animals of groups 1 and group 2, and 6 animals of group 3 were sacrificed, and remaining animals were maintained for a further 4 weeks without TAA treatment, and were sacrificed. Liver cirrhosis was induced in 30% and 100% of animals in group 2 and group 3, respectively, at 12 weeks of TAA treatment, and this lesion was also found at 4 weeks after cessation of TAA treatment. Collagen contents of liver in group 2 and group 3 were significantly increased at 12 weeks ($p<0.01$), and there was significant increase of collagen content in group 3 after cessation of TAA treatment ($p<0.05$). Number and area of GST-P positive foci and 8-OHdG formation in group 3 after cessation of TAA treatment showed significant higher level compared with that of control ($p<0.01$, $p<0.01$, and $p<0.05$, respectively). Although PCNA index of hepatocytes in group 2 and group 3 were reduced, those of non-parenchymal cells were increased significantly after cessation of TAA treatment ($p<0.01$). RT-PCR analysis of α-smooth muscle actin (α-SMA) also showed significant higher level in group 3 after cessation of TAA treatment ($p<0.05$). Immunohistochemical staining of α-SMA represented that positive cells were mainly existed around regenerating hepatic nodules at 12 weeks of TAA treatment, however, they were focused into enlarged portal areas consisting of plentiful fibrous tissues and pseudo-bile ductular cells after cessation of TAA treatment. Taken together, we conclude liver cirrhosis is irreversible at 4 weeks after cessation of TAA treatment, and we suggest that persistence of liver cirrhosis could be associated with the proliferation of non-parenchymal cells and altered location of α-SMA positive cells.

P-98

Spontaneous development of maxillary incisor lesions in aged poly(ADP-ribose) polymerase-1 (Parp-1) knockout mice

Osamu KUSUOKA[1], Masahiro TSUTSUMI[2], Toshifumi TSUJIUCHI[2], Kazutoshi TAMURA[1], Kohsuke HORIGUCHI[1], Kazumi SHIRAIWA[3], Nobuo KAMADA[4], Hitoshi NAKAGAMA[5], Takashi SUGIMURA[5], Mitsuko MASUTANI[5]

Gotemba Laboratories, Bozo Research Center Inc., Shizuoka 412-0039, Japan[1]
Department of Oncological Pathology, Cancer Center, Nara Medical University[2]
Institute for Life Science Research, Asahi Kasei Corporation[3]
Chugai Research Institute for Medical Sciences, Inc.[4]
Biochemistry Division, National Cancer Center Research Institute[5]

Parp-1 is activated by DNA strand breaks and is involved in DNA repair and maintenance of genomic stability. Furthermore, alterations of gene-transcription, apoptosis, and cell differentiation have been reported in *Parp-1*$^{-/-}$ deficient cells and animals. In the present study, a spontaneous occurrence of maxillary incisor lesions in aged *Parp-1*$^{-/-}$ mice is documented.

[Materials and methods] *Parp-1*$^{-/-}$ and *Parp-1*$^{+/+}$ mice of a mixed genetic background of ICR and 129Sv were used in this study. Mice were killed and autopsied at the ages of 16 (5 each of *Parp-1*$^{-/-}$ and *Parp-1*$^{+/+}$ mice), 41 (7 *Parp-1*$^{-/-}$ mice and 5 *Parp-1*$^{+/+}$ mice) and 110 weeks (9 *Parp-1*$^{-/-}$ mice and 13 *Parp-1*$^{+/+}$ mice). Organs were fixed in neutralized 10% formalin solution. Histopathological analysis of incisors was performed after decalcification by a formic acid/formalin solution, and the routine process of embedding the tissue in paraffin and staining sections with hematoxylin and eosin.

[Results and discussion] Maxillary incisor lesion was observed in 9 out of 9 *Parp-1*$^{-/-}$ mice and 4 of 13 *Parp-1*$^{+/+}$ mice at the age of 110 weeks. No pathological changes were observed either in 16 or 41 week-old *Parp-1*$^{-/-}$ and *Parp-1*$^{+/+}$ mice. Lesions were observed in the pulp cavity and characterized as a disarrangement of odontoblast, hypertrophy of dentin, and distortion of tooth architecture caused by denticles impacting. The incidence of incisor alteration was higher in *Parp-1*$^{-/-}$ than *Parp-1*$^{+/+}$ mice, and much severe changes were observed in *Parp-1*$^{-/-}$ mice. These results suggest that *Parp-1* deficiency may be attributable to the development of dental dysplasia in mice by perturbing normal tooth development during the aging process.

P-99

Pancreas anomaly and intestinal tumorsin the mouse small eye mutants, Pax6 Sey3H and Pax6Sey4H

Yumiko NITTA[1]

Res. Inst. Radiat. Biol. Med., Hiroshima University, Hiroshima 734-8553, Japan[1]

Deletions of the central regions of chromosome 2 are associated with radiation-induced acute myeloid leukemias in mice. While, there are some murine small eye mutants, which genetically delete the central segments of chromosome 2. Expecting a predisposition to acute myeloid leukemia, we examined the tumorigenicity of two small eye mutants, Pax6Sey3H and Pax6Sey4H. Both mutants were missing a segment of 3.2Mb between 106.0Mb and 109.2Mb from the centromere, where the Wilm's tumor 1 (Wt1), Reticulocarbin (Rcn), Paired box gene 6 (Pax6), Elongation protein homolog 4 (Elp4) and other fourteen novel genes are located. E!-rays and N-methyl-N-nitrosourea shortened the latency but did not increase the frequency of the hematopoetic tumors in the mutants. Hemizygous deletions of the 3.2Mb-segment of chromosome 2 did not contribute to the development of hematopoietic tumors. Instead, both mutants developed intestinal tumors spontaneously (42.9% and 52.0% for Pax6Sey3H and Pax6Sey4H, respectively), which were not observed in normal sibs. They impaired to elevate the blood glucose level when loaded with insulin, no matter how the glucagons level was normal. They showed some morphological anomaly of the Wirsung duct. Chronic pancreatitis or fatty degeneration of the pancreas was observed with age. Hemizygosiy of the Pax6 gene might contribute to the intestinal tumorigenicity.

P-100

Establishment and characterization of cancer cell lines from glandular stomach carcinoma induced by MNU in p53-deficient mice

Masami YAMAMOTO[1], Hayao NAKANISHI[1], Tetsuya TSUKAMOTO[1], Akihiro HIRATA[1], Hiroki SAKAI[2], Norimitsu SHIRAI[1], Takeshi IIDAKA[1], Tokuma YANAI[2], Toshiaki MASEGI[2], Masae TATEMATSU[1]

Laboratory of Pathology, Aichi Cancer Center Research Institute, Aichi 464-8681, Japan[1]
Department of Veterinary Pathology, Gifu University[2]

[Purpose] We previously established stomach cancer cell lines from N-methyl-N-nitrosourea (MNU)-induced glandular stomach carcinoma in BALB/c mouse, which were non-tumorigenic. In the present study, we newly established additional malignant cancer cell lines with both tumorigenecity and metastatic potential from MNU-induced glandular stomach carcinoma in p53 knock out (KO) mouse.
[Materials and Methods] Mouse glandular stomach carcinomas generated in a male p53 (+/-) KO mouse of C 57BL strain at 30 weeks after oral administration of 30 ppm MNU for alternate 10 weeks (for total exposure of 5 weeks) were used as a tumor source in this study. From this tumor, we established two cloned cell lines, designated MGT53-1E and MGT53-1G. Biological characteristics of these cell lines, including growth, morphology, tumorigenicity, metastatic potentials and genotype were examined.
[Results] The primary glandular stomach tumor was a well-differentiated adenocarcinoma. MGT53-1E cells were keratin positive and grew as an epithelial monolayer in culture, whereas MGT53-1G cells were keratin positive but contained abundant giant cells. Flow cytometry analysis revealed that DNA content of MGT53-1 G cells was significantly larger than MGT53-1E cells. The genotype of the primary stomach tumor was p53 (+/-), but cell lines showed loss of wild type allele. When subcutaneously injected into a p53 (+/-) KO mouse, both cell lines produced tumors with 100% tumor take rate and without difference in growth rate. Spontaneous lung and lymph node metastasis were observed in both cell lines, but MGT53-1E cells showed higher metastatic potential than MGT53-1G cells. Histology of both subcutaneous tumors in p53 (+/-) KO mice were moderately differentiated adenocarcinoma with undifferentiated or sarcomatous change.
[Conclusion] These cell lines derived from glandular stomach adenocarcinoma in a p53 (+/-) KO mouse proved highly malignant and phenotypically unstable in terms of appearance of giant cells and sarcomatous component. These results suggest that these malignant phenotypes are related to the genetic instability via p 53 inactivation. These cell lines would be useful model for the malignant progression of gastric cancer.

P-101

Influence of p53 gene deficiency on spontaneous tumor development in TCRs$^{-/-}$/p53$^{-/-}$ mice

Kana HASHIMOTO[1], Shoichi KADO[1], Kazumi UCHIDA[1], Shin IWATA[1], Yuriko NAGATA[1], Minoru ANDO[1], Hideyuki FUNABASHI[1], Masaharu ONOUE[1]

Yakult Central Institute for Microbiological Research, Tokyo 186-8650, Japan[1]

TCRβ;$^{-/-}$/p53$^{-/-}$ mice (T cell receptor β chain and p53 gene-deficient mice = Double Knock Out mice; DKO mice) develop adenocarcinoma in the large intestine by the age of 17 weeks at a rate of 100%. We have demonstrated that adenocarcinoma of DKO mice is initiated by TCR β chain gene deficiency, and p53 gene deficiency accelerates the tumorigenesis. DKO mice develop not only adenocarcinoma of the large intestine, but also spontaneous tumors in lymphatic tissues, soft tissues and many other organs, although thorough histopathological analysis of these tumors has not yet been performed. We investigated non-treated male DKO mice (mean age; 17.1 weeks), to examine the occurrence rate and histological tumor type of tumors other than adenocarcinoma in the large intestine, and compared them with those reported in the literature on p53$^{-/-}$ mice. We also investigated non-treated male TCRβ$^{-/-}$/p53$^{+/+}$ mice (mean age; 17.0 weeks), to examine the influence of p53 gene deficiency on the tumorigenesis of spontaneous tumors in DKO mice. Lymphoma was observed in 45% (21/47) of DKO mice, and the histological type was malignant lymphoma, lymphoblastic type. It was confirmed by immunohistochemical study that the tumor cells were of T cell origin, as they were CD3 ε antibody positive and CD45Ra antibody negative. On the other hand, sarcomas were observed in 15% (7/47) of DKO mice, and the tumor type was leiomyosarcoma (3/7), hemangiosarcoma (2/7) or liposarcoma (1/7). Other than sarcomas, teratoma of the testis was observed in one case. These results on the occurrence rate and tumor type correspond to the literature on lymphomas and sarcomas of p53$^{-/-}$ mice. These findings indicate that deficiency of the p53 gene plays a critical role in the tumorigenesis of tumors other than adenocarcinoma of the large intestine in DKO mice, and deficiency of the TCR β chain gene has a small influence on it.

P-102

Progression of glomerulonephritis in cynomolgus monkeys caused by autoimmunity to NC1 domain of type Ⅳ collagen [K35]

Masatoshi YAMAMOTO[1], Iori ITAGAKI[1], Masami HIRUMA[1], Yoshihiro TAKEI[1], Norio MUTO[1], Flordeliza P. DE VILLA[2], Tsukao YOKOYAMA[3], Syunji HATTORI[4], Hidekazu SHIGEMATSU[5]

Ina Research Inc., Ina-shi, Nagano, 399-4501 Japan, Nagano 399-4501, Japan[1]
INA RESEARCH PHILIPPINES, INC.[2]
Collagen Research Center[3]
Nippi Reseach Institute of Biomatrix[4]
Department of Pathology, Shinsyu University School of Medicine[5]

K35, NC1 domain of type Ⅳ collagen, is an antigen purified from bovine glomerular basement membrane (GBM). Ina Research and INARP have developed a primate model of human autoimmune anti-GBM nephritis. The procedure involves immunization of K35. The purpose of this study is to evaluate the histopathological features in the renal tissues.
[Materials and methods] To induce the glomerulonephritis (GN), two female cynomolgus monkeys (*Macaca fascicularis*) were immunized once with K35 (4 mg/monkey) in Freund's complete adjuvant by intradermal injections to dorsal skin. Two additional females were likewise immunized at 1 mg and boosted at 3 mg three weeks later. All animals were observed for clinical signs daily. Determinations were conducted on serum anti-NC1 antibody titers, urinalysis, hematology and blood chemistry. Renal tissues were collected 4 weeks (biopsy sample) and 6 weeks after the single or first immunization, respectively. Paraffin sections were stained with HE, PAS and PAM for morphological evaluations. Additional sections were also stained by indirect immunoperoxidase method to detect the expression of CD68, as a macrophage marker and α-SMA (α-smooth muscle actin), as a phenotypic marker of myofibroblasts. Immunofluorescent staining was conducted to detect IgG, IgA and C3 from cryostat sections.
[Results] No unusual clinical signs were evident. Circulating anti-NC1 antibodies were first detected 2 weeks after the single or first K35 immunization. High titers were evident in all animals at Weeks 3 to 6. Proteinuria and hematuria were detected from Weeks 4 to 6. Anemia and low serum albumin were evident at Week 6. Autologous IgG deposition was noted along the GBM in all animals at Weeks 4 and 6. Intraglomerular hypercellularity with focal and segmental GBM destruction and extracapillary efflux of fibrin were evident in three animals at Week 4. These animals developed slight to marked crescentic and necrotizing glomerulonephritis at Week 6. Epithelioid cell proliferation was also noted in the Bowman's space and tubular lumens. In addition, CD68-positive cells and α-SMA-positive cells were detected in the glomeruli and peri-glomerular regions as well as in the cortical interstitium.
[Conclusion] These findings suggest that the macrophage accumulation and transdifferentiation of glomerular and tubular cells may play a critical role in the progression of more severe renal damage.

P-103

Histological changes of pancreatic islets in Zucker Diabetic Fatty rats, with progress in the type 2 diabetes-like disease

Naoshi SHIMOJI[1], Wakako TOGA[1], Satoko TOMIOKA[1], Haruyo SUGIYAMA[1], Kaori SASA[1], Nobutaka DEMURA[1], Haruko KAMEDA[1], Yusuke NAGAE[1]

Preclinical Development Department, Novartis Pharma K. K., Ibaraki 300-2611, Japan[1]

The Zucker Diabetic Fatty (ZDF) rat is known as an animal model for type 2 diabetes mellitus. The ZDF rat shows the progressive hyperinsulinemia, followed by hypoinsulinemia with severe hyperglycemia. This phenomenon suggests that the ZDF rat is available as a spontaneous model of beta cell dysfunction. This study was carried out to characterize the morphologic features of spontaneous changes in pancreatic islets observed in the ZDF rat at the early and progressed stages of diabetes.
Five weeks old male ZDF rats were purchased from Charles River Genetic Models Inc., as well as ZDF lean rats as control animals. They were bred in an animal room under well-controlled conditions, and fed with Purina diet. Plasma glucose and insulin levels were monitored. Animals were sacrificed under anesthesia 30 minutes after injections of 5-bromo 2'-deoxyuridene (BrdU), at 11 and 16 weeks of age, respectively. The pancreases were removed, weighed, fixed with 10% buffered formalin, embedded in paraffin and sectioned at 5 μm. Staining was performed with hematoxylin and eosin and Masson's trichrome stain for light microscopic examination. Immunohistochemical approaches using anti-insulin, anti-ssDNA and anti-BrdU antibodies were also applied.
Prominent histological findings observed in the pancreas of the ZDF rats were hyperplasia and/or fibrosis of the islets. Size of islets evidently increased when compared with those in lean rats. Irregular and obscure islet margins and fibrous tissues within and/or surrounding islets were noted. Entrapped exocrine pancreatic tissues were often seen within the islets. Ratio of the insulin-positive area to the whole islet's area was reduced, which suggested a dysfunction of the beta cells. The ssDNA positive islet cells in the ZDF rats were observed at higher rate than in the lean rats, though BrdU labeling indices remained unchanged. Noteworthiness of this study was also the observation of insulin positive cells in the outside of the islets such as ductal epithelial cells and endocrine acinar cells. The present results, therefore, imply the potential ability of the redifferentiation of the non-endocrine cells to the beta cell-like endocrine cells in the ZDF rat pancreas.

P-104

The mouse rasH2/BHT model as an in vivo rapid assay for lung carcinogens

Takashi UMEMURA[1], Yukio KODAMA[2], Keita KANKI[1], Yasuki KITAMURA[1], Takayoshi IMAZAWA[1], Akiyoshi NISHIKAWA[1], Masao HIROSE[1]

Division of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan[1]
Division of Toxicology, National Institute of Health Sciences[2]

We have demonstrated the applicability of a 9-week *in vivo* two-stage model for screening lung cancer initiating agents, using transgenic mice carrying the human prototype c-Ha-ras gene (rasH2 mice) and butylhydroxytoluene (BHT) as a potent lung tumor-promoter (rasH2/BHT model). The first aim of the present study was to establish a standard protocol for this model, especially with regard to appropriate BHT administration. Subsequently, according to the established protocol, validation studies were performed with well-known genotoxic murine lung carcinogens. [Methods] Experiment 1 Male rasH2 mice received by gavage one to six consecutive administrations of 400 mg/kg of BHT. The interval of administration was set to be 1 week. Controls were treated with corn oil alone. They were killed 3 days after each final treatment with BHT. All animals were intraperitoneally injected with bromodeoxyuridine (BrdU: 100 mg/kg) once 2 h before killing. Experiment 2 Male and female rasH2 mice were intraperitoneally injected with urethane (UR) at a dose of 250 mg/kg and then given weekly administration of BHT at a dose of 400 mg/kg starting 1 week after the UR treatment for 3, 5 or 8 weeks. Control animals were given corn oil instead of BHT. All animals were killed 9 weeks after the UR treatment. Experiment 3 Validation was tested with six chemicals as follows: 3-methylcholanthrene (100 mg/kg), *N*-ethyl-*N*-nitrosourea (120 mg/kg), dimethylnitrosamine (15 mg/kg), diethylnitrosamine (100 mg/kg), benzo(*a*)pyrene (80 mg/kg), 7,12-dimethylbenz(*a*)anthracene (5 mg/kg). [Results and Discussion] Whereas the BrdU-labeling index (LI) of alveolar type II cells was increased dramatically with the initial BHT administration, the efficacy was gradually diminished with further exposures, and finally six BHT treatments failed to induce cell proliferation. In fact, in Experiment 2 using UR as an initiator, although up to five consecutive doses of BHT were able to enhance the promoting effects in terms of lung adenoma yield, no more increment was evident with further treatments. Therefore, it can be proposed that as a standard protocol for rasH2/BHT model rasH2 mice are treated with test chemicals, starting 1 week later given BHT weekly five times at a dose of 400 mg/kg by gavage, and then killed at week 9. Under this protocol, initiating activities of all the chemicals examined in Experiment 3 were substantially detectable. Thus, the rasH2/BHT model could be a powerful tool to detect environmental carcinogens acting as initiating agents to the lung.

P-105

The utility of EHBR for toxicity study of organic anion drugs

Chitose KUWAYAMA[1], Hiroyasu NABA[1], Chihaya KAKINUMA[1], Takuo OGIHARA[1], Shuhei OHNISHI[1], Akihito SHIMOI[1]

Phamaceutical Research Center, Mochida Phamacetical Co., Ltd., Tokyo 160-8515, Japan[1]

Some organic anion drugs are actively transported to bile by the action of multidrug resistance-associated protein 2 (MRP2), which is a major ATP-dependent canalicular organic anion transporter. EHBR (Eizai hyperbilirubinemic rat) is characterized by the deficiency of MRP2, and show higher plasma concentrations of organic anion drugs than the normal rats when treated with those drugs. Hence, we assessed the utility of EHBR for toxicity study from the results of pravastatin or methotrexate induced toxicities. [Method] 12-weeks old EHBRs were used. SDs (Sprague-Dawley rats) were also prepared for comparison. Study 1: Animals were administrated orally with pravastatin at 200mg/kg/day or 400mg/kg/day for 7 days or 14 days. Skeltal muscles were examined histopathologically and plasma concentration of pravastatin was determined. Study 2:Animals were administrated orally with methotrexate at 0.6mg/kg/day for 7 days. Main organs were examined histopathologically, and plasma concentration of methotrexate was determined. [Result] In both studies, plasma concentrations of drugs in EHBRs showed higher than those in SDs. Study 1:EHBRs with 200mg/kg/day for 14 days showed rhabdomyolysis, but SDs didn't. Study 2:EHBRs with 0.6mg/kg/day for 7 days showed severe affections in liver, hematopoietic organs and digestive tracts, but SDs showed slight affections in bone marrow and thymus. [Discussion] From the result of the toxicity study for organic anion drugs, EHBRs showed higher sensitivities than SDs. Taking these facts together, we concluded that EHBR is useful for the evaluation of toxicities induced by organic anion drugs.

P-106

Morphological evaluation of bone dynamics using in vivo micro-CT analysis and histomorphometry in mice osteoporosis model

Anbo XIANG[1], Masahiro KANEMATSU[1], Mana MITAMURA[1], Hideo KIKKAWA[1], Kiyoshi KOBAYASHI[1], Hiroyuki HIGASHIYAMA[1], Mine KINOSHITA[1], Satoshi ASANO[1]

Pharmacology Department, GlaxoSmithKline KK., Ibaraki 300-4247, Japan[1]

For the purpose of comprehensive evaluate the bone dynamics in mice osteoporosis model, we quantified the parameters of bone resorption and formation, the bone architecture and the trabecular connectivity by using in vivo micro-CT and histomorphometry. Furthermore, the influence of repetitive X-ray irradiation by micro-CT in vivo scanning on the immunological function, haemopoiesis function and bone density was examined in time course study. The 6-week-old female mice of BALB/c strain were either sham or ovariectomized (OVX) on day0. The OVX mice were daily treated with vehicle or Estradiol-17beta (E2) for 2 weeks. On day 14, the left femur bones were isolated and then analyzed using micro-CT and resin embedded bone tissue specimens. For the detection and analysis of osteoclasts and osteoblasts, we applied TRAP (tartrate-resistant acid phosphatase)/ALP (alkaline phosphatase) double staining. Using morphometric analysis, TRAP and ALP positive cells were quantified. For the in vivo micro-CT study, the body of each mouse was protected by lead sheet, and only the left femur of mice was scanned by micro-CT (X-ray: 1.68 Gy/min for 2.78 min) for once irradiation on day 0 or twice irradiation on day 0 and 7. On day 14, the animals were killed and the left femur bones were scanned. As the results, the number of osteoclasts per bone surface, one of the parameter revealed bone resorption, were significantly increased in OVX group, and prevented by E2 administration. In both of micro-CT and morphometrical analysis, the significant decrease of bone volume, collapse of trabecular bone was observed in the same way. In addition, the deterioration of connectivity of trabecular bone was observed in OVX mice compared with sham mice using micro-CT analysis. These alterations induced by OVX were recovered to the sham level after E2 treatment. In the study of in vivo micro-CT scanning, X-ray irradiation did not affect the body weight change, spleen weight, bone marrow cell number and hematological parameters. The bone volume did not change in once irradiation group, but significantly decreased in twice irradiation group. Micro-CT and histomorphometry could be the high content and high throughput (micro-CT) tools for evaluating the effect of compounds in the models of osteoporosis and related bone diseases. Using in vivo micro-CT, high sensitive examination would be applied in these models for the development of chemicals for bone disease and the detection of bone toxicity.

P-107

Expression of a system L amino acid transporter at testis of rats after prenatal exposure to di(n-butyl)phtalate

Tomoko MUTO[1], Yoshikatsu KANAI[1], Hitoshi ENDOU[1]

Department of Pharmacology and Toxicology, Kyorin University, Tokyo 181-8611, Japan[1]

L-type amino acid transporter 1 (LAT1) has been proposed to be one of the major nutrient transport system. The expression of LAT1 in rat testis following prenatal exposure to di(n-butyl) phtalate (DBP) was examined. SD rats were injected (i.g.) with 1g of DBP/kg or vehicle, on days 12-21 postconception. At 25 to 65 days old, testicular weights of DBP group were significantly lower, and showed degenerated suminiferous tubules with Leydig cells hyperplasia and/or Leydig cell tumor lesions. At 100 to 150 days old, testicular weight and many suminiferous tubules and Leydig cells were similar to those of the vehicle group. But some number of Leydig cells hyperplasia and/or Leydig cell tumors were also observed. A polyclonal affinity purified antibody against the C-terminus of LAT1 was generated in chickens and used in immunohistochemical analysis. At 25 to 65 days old of DBP group, LAT1 expressed on the hyperplastic and /or neoplastic Leydig cells and the capillary endothelial cells. At 100 to 150 days old of DBP group, no-expression of LAT1 revealed in testis except Leydig cells hyperplasia and/or Leydig cell tumor lesions. Previous studies have been showed the DBP induced reversible Leydig cell hyperplasia that is involved in an underproductivity of testosterone in Leydig cells. These results suggest that the expression of LAT1 might be involved in Leydig cells proliferation and malignant transformation as well as in amino acid transport in rat testis induced by prenatal exposure to DBP.

P-108

Infiltrating CD8+ T and NK cells, and IL-10 and TGF-β 1 cytokines expression in chemically induced neoplasias in a medium-term alternative bioassay using male Wistar rats

Ana Lucia Tozzi SPINARDI-BARBISAN[1], Luis Fernando BARBISAN[1], Joao Lauro Viana de CAMARGO[1], Maria Aparecida Marchesan RODRIGUES[1]

Dept. Pathology, UNESP Medical School, SP 18618-000, Brazil[1]

An initiation-promotion assay with Wistar rats was adopted by the Brazilian Institute for the Environment (IBAMA) as a source of evidence of the carcinogenic potential of pesticides. This bioassay protocol has been originally proposed as an alternative/complementary assay for the standard 2-year rodent assay for chemical carcinogenesis. In order to better understand this model, the present study aimed to evaluate the CD8+ T and natural killer (NK) infiltrating cells and the expression of interleukin-10 (IL-10) and transforming growth factor-beta 1 (TGF-beta1) in the chemically induced neoplasias. Male Wistar rats were sequentially treated with N-nitrosodiethylamine, N-methyl-N-nitrosourea, N-butyl-N-(4-hydroxybutyl)nitrosamine, dihydroxy-di-N-propylnitrosamine and 1,2-dimethylhydrazine during 4 weeks. Two groups were subsequently exposed through diet to phenobarbital (0.05 %) or 2-acetylaminofluorene (0.01 %) for 25 weeks. An untreated group was used as control. Immune cells and cytokines were immunohistochemically evaluated in neoplasias and in surrounding normal tissues of the liver, kidney, lung, and small and large intestines. When compared to the respective normal tissues an increased number of NK cells was verified infiltrating the colon, lung and kidney neoplasias, being the number of CD8+ T cells decreased in the intestine and lung neoplasias. Expression of IL-10 was found mainly in tumors of kidney and small and large intestines. TGF-beta 1 was expressed mainly in liver and kidney tumors. The results indicate a differential immune activity within neoplastic and in normal tissues which may be dependent of tumor development and characterizes its microenvironment.

P-109

Green fluorescent protein (GFP) as a marker of aryl hydrocarbon receptor (AHR) function in transgenic zebrafish (Danio rerio)

Seung H. SEOK[1], Jong-H. PARK[1], Sun A. CHO[1], Min W. BAEK[1], Hui Y. LEE[1], Dong J. KIM[1], Yong S. LEE[2], Jae H. PARK[1]

Department of Laboratory Animal Medicine, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea (South)[1]
Department of Public Health, College of Veterinary Medicine, Seoul National University[2]

Zebrafish (*Danio rerio*) is a very attractive experimental model system because it has short generation time (12 weeks), long life span (2-3 years), and relatively small diploid genome. We are developing transgenic zebrafish lines in which DNA motifs that respond to selected environmental pollutants are capable of activating a reporter gene that can be easily assayed. Aromatic hydrocarbon response elements (AHREs) respond to numerous polycyclic hydrocarbons and halogenated coplanar molecules. Each of these substances is known to be bioconcentrated in fish to varying degrees and would act upon the AHRE motif and turn on the green fluorescent protein (GFP) reporter gene. AHRE is a ligand-activated transcription factor that mediates the toxic actions of environmental contaminants such as beta-naphthoflavone (BNF). Induction of cytochrome P4501A1 (CYP1A1) is an early biomarker of AHRE activation. Injected fish exposed to BNF exhibited induction of GFP in the liver and vertebrae of zebrafish compared to vehicle controls, which did not express GFP.

P-110

Pathology of Minamata disease (methylmercury poisoning) - special reference to the peripheral nerves

Komyo ETO[1], Akira YASUTAKE[1], Motohiro TAKEYA[2], Michio AKIMA[3]

National Institute for Minamata Disease, Kumamoto 867-0008, Japan[1]
Graduate School for Medicial and Pharmaceutical Science, Kumamoto University[2]
Toho University, School of Medicine[3]

Minamata disease was first discovered about 50 years ago in Minamata Bay, Kumamoto Prefecture, Japan. A study group of Kumamoto University School of Medicine found that the disease was caused by human ingestion of a large amount of methylmercury (MeHg) contaminated fish or shellfish from Minamata Bay. The mercury came from a nearby factory that manufactured acetaldehyde and vinyl compounds. The factory used manganese dioxide as a cocatalyst from 1932 to 1951 in its acetaldehyde production plant. In 1951, the factory changed the cocatalyst used in the process of producing acetaldehyde from manganese dioxide to ferric sulfide. This resulted in the production of MeHg chloride, which the factory dumped directory into Minamata Bay from 1951 to 1968. Over those 17 years, Minamata Bay was contaminated with a high level MeHg in their meat and internal organs.

Sensory disturbance is a very important symptom of Minamata disease patients with constrictive visual fields, hearing disturbance, ataxia, and dysarthria. It is well known that peripheral neuropathy is a common but non-specific clinical and histopathological entity. The peripheral nervous system alone was found to susceptible to MeHg in rodents, while swine and common marmosets showed lesions both in the central and peripheral nervous system similar to those we have seen in Minamata disease.

To evaluate peripheral nerve involvement in Minamata disease patients, we examined the peripheral nerves of human autopsy and control cases, and also experimental MeHg poisoning in common marmosets. For histochemical examination, we used antibodies to PG-M1, KP-1 (CD 68, macrophage), protein product 9.5 (PGP-9.5), neurofilament 210 (NF-210) and S-100. The infiltration of macrophages in the spinal posterior root was stronger than the anterior root in the Minamata disease patients, and also the infiltration of macrophages was prominent in the sciatic nerves of severe cases of MeHg poisoning in the common marmosets. Spinal posterior root showed stronger degeneration than the anterior root by the staining of PGP-9.5 and NE-210. Schwann cells' proliferation is prominent in the spinal posterior root.

Our studies of MeHg poisoning in common marmosets and the autopsy studies on patients with Minamata disease showed peripheral nerve damage, which at least in part contribute to the clinical sensory symptoms. We conclude that pathogenesis sensory nerve lesions were derived from axonal degeneration and the pathological changes of the spinal posterior ganglions. For the study of the complete or incomplete degeneration of peripheral nerves, it is important to use an electron microscope.

P-111

Mechanisms and disease control of N-methyl-N-nitrosourea-induced retinal degeneration in animals

Katsuhiko YOSHIZAWA[1], Katsuji KIUCHI[3], Kaei MORIGUCHI[3], Yuji OISHI[1], Nobuaki SHIKATA[2], Airo TSUBURA[2]

Department of Toxicologic Pathology, Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka 532-8514, Japan[1]
Department of Pathology II, Kansai Medical University[2]
Department of Ophthalmology, Kansai Medical University[3]

Retinitis pigmentosa (RP) is a human disease characterized by loss of photoreceptor cells leading to visual disturbance and eventually to blindness. Establishment of reliable animal models is essential for better understanding of this disease and approaches to therapeutic intervention. N-Methyl-N-nitrosourea (MNU)-induced retinal degeneration is highly reproducible, and involves photoreceptor cell loss that ends approximately 7 days after a single systemic administration of MNU to mice (60mg/kg), rats (60 to 75mg/kg), hamsters (90mg/kg), shrews (65mg/kg), and monkeys (40mg/kg). Although the triggering mechanisms of pathogenesis are different and the apoptosis cascade may differ from human RP, MNU-induced photoreceptor cell loss is due to apoptosis with down-modulation of Bcl-2 protein, up-regulation of Bax protein, and activation of caspase families. Therapeutic approaches to control MNU-induced photoreceptor cell loss have been evaluated. The first strategy we attempt to suppress photoreceptor cell loss was shutting down the apoptosis cascade; caspase-3 inhibitor (4000ng Ac-DEVD-CHO injected intravitreally twice) partially suppressed disease progression. Next, we tested the ability of nicotinamide (a water-soluble B-group vitamin; 25 to1000mg/kg nicotinamide injected subcutaneously) to expect damaged DNA repair or by other mechanisms, and found to be effective. Also, dietary supplementation of 9.5% docosahexaenoic acid counteracted photoreceptor cell loss. Suppression of MNU-induced photoreceptor apoptosis in animals may give therapeutic information for human RP control.

Key words: Retinitis pigmentosa, Retinal degeneration, Caspase-3 inhibitor, Apoptosis, Nicotinamide, Docosahexaenoic acid, N-Methyl-N-nitrosourea

References:

1.Tsubura A, Yoshizawa K, Miki H, et al. Phylogenetic and ontogenetic study of retinal lesions induced by N-methyl-N-nitrosourea in animals. Anim. Eye Res. 17: 97-103, 1998 (in Japanese; abstract in English).
2.Yoshizawa K, Nambu H, Yang J, et al. Machanisms of photoreceptor cell apoptosis induced by N-methyl-N-nitrosourea in Sprague-Dawley rats. Lab. Invest. 79: 1359-1367, 1999.
3. Yoshizawa K, Yang J, Senzaki H, et al. Caspase-3 inhibitor rescues N-methyl-N-nitrosourea -induced retinal degeneration in Sparague-Dawley rats. Exp. Eye Res. 71: 629-635, 2000.
4. Kiuchi K, Yoshizawa K, Shikata N, et al. Nicotinamide prevents N-methyl-N-nitrosourea- induced photoreceptor cell apoptosis in Sprague-Dawley rats and C57BL mice. Exp. Eye Res. 74: 383-392, 2002.
5. Moriguchi K, Yuri T, Yoshizawa K, et al. Dietary docosahexaenoic acid protects against N-methyl-N-nitrosourea-induced retinal degeneration in rats. Exp. Eye Res. 77: 167-173, 2003.

P-112

Neuronal cell death in the fetal rat spinal cord following NMDA-treatment to dams

Hirotake TAKAI[1], Tsuneo ITO[1], Kiyoka KATSUYAMA[1], Masami SUZUKI[1], Kei-ich KATAYAMA[2], Kunio DOI[2]

Safety Assessment Department, Chugai Pharmaceutical Co., Ltd., Shizuoka 412-8513, Japan[1]
Department of Veterynary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo[2]

The N-methyl-D-aspartate receptor (NMDAR), which is one of the glutamate receptors, is considered to have a close relation with synaptic plasticity in the rat brain. Recently we clarified that apoptosis was induced in the fetal rat brain following NMDA administration to the dams (Exp Toxic Pathol 2003; 55: 33-37). In addition, it is also reported that NMDARs are expressed in the spinal cord as well as in the brain. In this study, we carried out a histopathological study on the fetal rat spinal cord after NMDA administration to dams.
[Materials and Methods] Pregnant rats at embryonal day of 20 (E20) were injected with 150 mg/kg of NMDA, NMDAR agonist, intraperitoneally. Dams were sacrified at 24 hours after the treatment, and the fetuses were collected. The spinal cords from the fetuses were fixed in 10% neutral-buffered formalin. Paraffin sections were stained with hematoxylin and eosin. Some cases were stained for the TUNEL method, a widely used method for the in situ detection of apoptotic nuclei. Immunohistochemical staining for MAP-2 was performed for the control group.
[Results and Discussions]In the control group, morphological differences were observed between the dorsal and ventral neurons in the spinal cord. The neurons in the dorsal horn, which were positive for MAP-2, were small and round-shaped with large oval nuclei and scant cytoplasm. On the other hand, many of the cells in the ventral horn, which were also positive for MAP-2, were large and multiangular-shaped with relatively abundant cytoplasm. In the NMDA-treated group, a large number of cells characterized by pyknotic or karyorrhectic nuclei, almost all of which were stained by the TUNEL method, were mainly detected in the dorsal horn while a smaller number was observed in the ventral horn. The cytoplasm of these cells was eosinophilic and somewhat glanular. Thus, differences in the morphology and the sensitivity for NMDA were seen between the dorsal and ventral neurons in the fetal spinal cord. Differences in the stage of cell differentiation and/or distribution of NMDARs among the regions may contribute to the different changes observed in the present study.

P-113

Analysis of cell cycle, migration and apoptosis of neural stem cells in the 5-azacytidine (5AzC)-treated rat fetal brain

Masaki UENO[1], Kei-ichi KATAYAMA[1], Hiroyuki NAKAYAMA[1], Kunio DOI[1]

Department of Veterinary Pathology, The University of Tokyo, Tokyo 113-8657, Japan[1]

(Background) 5-azacytidine (5AzC), a cytidine analogue, is suggested to induce p53-dependent apoptosis and cell cycle arrest in the rat fetal brain. In the fetal brain, neural stem cells are proliferating in the ventricular zone (VZ), the area around the ventricles. In VZ, the nuclei of neural stem cells undergo a typical migration, "elevator movements", whose positions are correlated with the cell cycle phases. Namely, S phase nuclei located in the outer area of VZ translocate inward during G2 phase and mitosis occurs at the ventricular surface. Then, the nuclei migrate outward during G1 phase and enter into S phase again. In the present study, to clarify the detailed mechanism of 5AzC-toxicity to the neural stem cells, we histopathologically examined the change of their migration and further used flow cytometric methods for investigating the alteration of cell cycle distribution and the cell cycle position of apoptotic cells.
(Materials and methods) 5AzC (10 mg/kg) was injected intraperitoneally (i.p.) into pregnant Jcl:Wistar rats on day 13 of gestation and sacrificed at 1, 3, 6, 9, 12, and 24 h after treatment. Forebrains of fetuses were obtained under stereoscopic microscope, and then subjected to flow cytometric methods. Cells were stained with propidium iodide (PI) for cell cycle analysis and detection of fragmented DNA was performed using TUNEL method. For detecting migration, we used BrdU-incorporating method. In brief, 5AzC (10 mg/kg) and BrdU (20 mg/kg) were injected into pregnant rats i.p simultaneously and BrdU-positive cells in the fetal brain were sequentially examined immunohistochemically.
(Results) In the BrdU-incorporating method, BrdU-positive neural stem cells in S phase firstly migrated inward and remarkably accumulated along the ventricle at 6 h. Then, they migrated outward and underwent apoptosis from 9 to 24 h. The migration mostly delayed from 6 to 12 h compared with controls. These observations suggested that 5AzC induced cell cycle arrest in some phases and apoptosis after M phase. Using flow cytometric methods, G2/M phase cells were remarkably increased at 6 to 9 h and then decreased, suggesting an occurrence of cell cycle arrest in G2/M phase. Apoptotic cells were increased at 9 to 24 h. The cells undergoing apoptosis were mainly in G1 phase, however apoptosis was also observed in G2/M and S phase cells.
(Conclusion) In the rat fetal brain, 5AzC induced cell cycle arrest in G2/M phase and apoptosis mainly in G 1 phase. This was coincided with the delay of nuclei migration.

P-114

T-2 toxin-induced changes in the rat fetal brain

Shinya SEHATA[1], Naoki KIYOSAWA[2], Toshihiko MAKINO[2], Fusako ATSUMI[2], Kazumi ITO[2], Takashi YAMOTO[2], Munihiro TERANISHI[2], Sunao MANABE[2], Koji UETSUKA[1], Hiroyuki NAKAYAMA[1], Kunio DOI[1]

Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan[1]
Medicinal Safety Research Laboratories, Sankyo Co., Ltd.[2]

T-2 toxin is a trichothecene mycotoxin produced by various species of *Fusarium spp*. We reported that T-2 toxin induces apoptotic cell death in the lymphoid tissues, gastrointestines, liver, placenta and fetal tissues in the pregnant rats at 24 or 48 hours after treatment (HAT). The mechanism of T-2 toxin-induced toxicity is still unclear. In the present study, we focused on the changes observed in the fetal brain as one of the target organs of T-2 toxin-induced fetotoxicity. The purpose of the present study was to investigate sequential morphological changes and gene expression changes in the fetal brain induced by T-2 toxin.
Wistar rats (3 rats/group) on day 13 of gestation were orally administered with T-2 toxin at a single dosage of 2 mg/kg. At 1, 3, 6, 9, 12 and 24 HAT, rats were sacrificed. The fetal brain was fixed and histopathological examination was conducted. TUNEL staining was performed, and morphometry was done in the telencephalon (labeling index: number of positive cells/500 cells). Microarray analysis was performed for the samples at 6, 12, and 24 HAT using the Affymetrix Rat Genome U34A oligonucleotide chip (Affymetrix, USA). Real time RT-PCR was also performed for selected genes.
Sequential histopathological examination showed that the number of apoptotic neuroepithelium in the telencephalon was increased by T-2 toxin. Morphometry using TUNEL staining showed that the labeling index was increased from 1 HAT. The highest labeling index was observed at 12 HAT. From the histopathological results, we selected the time point (6, 12 and 24 HAT) for the microarray analysis. We are now conducting the analysis, and will also perform a real time RT-PCR analysis for some selected genes obtained from the results of microarray analysis. We will present the result at the meeting.

P-115

Examination of discriminative diagnosis of brain tumors difficult to be stained with hematoxylin-eosin

Yoshihumi KANEKO[1], Akiko IKEDA[1], Makiko TAKAHASHI[1], Sumihisa SUEYOSHI[1], Yasukazu SATO[1], Yoshihiro MASUMOTO[1], Kiyoyuki TSURU[1]

Kyorin Pharmaceutical Co., Ltd. Research Center, Tochigi 329-0114, Japan[1]

Generally, lesions suspected of tumors as well as non-tumor lesions are diagnosed on morphological standpoints with stained tissue preparations. Discriminative diagnosis between difficult-to-discriminate lesions requires additional information, for example, based on some special staining or other technique. We examined several staining methods to select an applicable one for such diagnosis of well-known brain tumors (atrocytoma and oligodendroglioma) in rats, in combination with ultra-structural observation on an electron microscope. Brain tumors were induced to pregnant rats by giving a transplacental dose of ethyl nitroso-urea (ENU, 50 mg/kg) during the days 14 to 20 of gestation and then discriminatively diagnosed on their morphological observation. Animals found dead were included in these examinations to consider their postmortem changes.

Examination showed that Luxol fast blue (LFB) and Aldehyde fuchsin (AF) each stained characteristically the cytoplasm of astrocytoma in survivals, and distinguished between astrocytoma and oligodendroglioma. In addition, transmission electron microscopy revealed that Rosental fibers were present in astrocytoma, and that in oligodendroglioma, intermediate filaments were present and rough endoplasmic reticula (RERs) increased. Lipid markers were traced but found not useful for discriminative diagnosis, because phosphatidylinositol 4-monophosphate (PIP) was located in the cytoplasm of both tumors and phosphatidylinositol 4,5-biphosphate (PIP2) also in the nuclear membrane of both tumors. Meanwhile, LFB stained the cytoplasm of astrocytoma in dead animals whereas AF did not. Transmission electron microscopy revealed that characteristic ultrastructures were present in both tumors in dead animals but less than in survivals. Grial fibrillary acidic protein (GFAP:astrocytoma's marker) and Ki-67 (marker for cell malignancy) were found not useful for tumor discrimination in this study.

In conclusion, the results suggest that conventional LFB and AF staining methods may be used for distinction between astrocytoma and oligodendroglioma in survival animals, and that LFB may be used for their distinction in dead animals within one day after death.

P-116

A spontaneous cholesterol granuloma of choroid plexus in a beagle dog

Shuji HAYASHI[1], Satoshi SUZUKI[1], Fumiko NINOMIYA[1], Kazuo HAKOI[1], Shuji YAMAGUCHI[1], Kenji IRIMURA[1], Shoji FUKUSHIMA[2]

Drug Safety Research Laboratory, Taiho Pharmaceutical Co., LTD., Tokushima 771-0194, Japan[1]
Department of Pathology, Osaka City University Medical School[2]

A cholesterol granuloma of the choroid plexus was identified within the fourth ventricle of a male 72-month-old beagle dog. No remarkable clinical signs were observed. Macroscopically, a nodule was 5×4×3 mm in size and tan in color. Some pearl-like spots were observed in the nodule. The aqueduct of the mid brain was slightly dilated. Other remarkable findings were not observed. Histopathologically, the nodule was mainly composed of numerous cholesterol clefts, lipid-laden macrophages, granulomatous tissues and cystic structures in the choroid plexus. Focal hemorrhage, mononuclear inflammatory cell infiltration, hemosiderin-laden macrophages and giant cells were scattered. In addition, proliferation, hyalinization and calcification of connective tissue, and edema in interstitium were occasionally observed. The cystic structures were lined with cuboidal, columnar or stratified squamous epithelium. The lumens of the cysts were filled with cholesterol clefts, lipid-laden macrophages, erythrocytes, neutrophils, eosinophilic serous fluids and cell debris. Immunohistochemically, the epithelial cells of cystic structures were positive for cytokeratin, and the spindle cells around cholesterol clefts were positive for smooth muscle actin. No adhesions and invasions to the surrounding nervous tissues were observed. This is a rare case report of the cholesterol granuloma of the choroid plexus in a beagle dog.

P-117

A study on the developmental process of distal axonopathy caused by acrylamide

Naofumi TAKAHASHI[1], Maki KUWAHARA[1], Yukiko TAKEUCHI[1], Toshinori YOSHIDA[1], Akiko ENOMOTO[1], Nobuaki NAKASHIMA[1], Yasufumi SHUTOH[1], Sayaka ISHIMINE[1], Toshiaki KITAZAWA[3], Keizo MAITA[2], Takanori HARADA[1]

Toxicology Division II, The Institute of Environmental Toxicology, Ibaraki 303-0043, Japan[1]
Division of Study Planning and Consultation, The Institute of Environmental Toxicology[2]
Contract and Research Management Division, The Institute of Environmental Toxicology[3]

Acrylamide (ACR) is a prototypical neurotoxicant that produces distal axonopathy and is recommended by regulatory agencies as a positive control substance for neurotoxicological studies of chemicals. We conducted a 28-day validation study of ACR in rats. In the validation study, ACR was repeatedly administered by gavage to male and female Crj:Wistar rats at dose levels of 0, 10, 20, and 40 mg/kg/day, but the duration of exposure to the high-dose level was shortened to 14 days because of severe intoxication. As the results of detailed clinical observations and functional tests, dysfunctions of the central-peripheral nervous and neuromuscular systems were observed in the 40 and 20 mg/kg/day groups. Neuropathological examinations revealed moderate necrosis of Purkinje cells and slight axonal degeneration of peripheral nerves in the 40 mg /kg/day group. Minimal Purkinje cell necrosis and severe axonal degeneration were also observed in the 20 mg/kg/day. These results were consistent with previous reports. It is generally believed that ACR primarily produces nerve cell damage in the central nervous system (CNS) when exposed to a high-dose level, whereas a low-dose exposure induces more prevalent lesions in the peripheral nervous system (PNS). However, recent investigations by Lehning et al. have suggested that ACR intoxication may produce early and progressive nerve terminal degeneration regardless of dose levels, and distal axonal degeneration is a secondary effect related to duration of exposure. Thus, the toxic profile of ACR at high- and low-dose levels is still open to discussion. In order to confirm this toxic profile, we have conducted a further 14-day study of ACR in rats at a dose of 50 mg/kg/day. In the additional study, animals showing abnormal gait were selected based on its severity and killed at a certain interval during the study to examine the developmental process of CNS lesions caused by ACR. A contemporary amino-cupric-silver stain method was used to detect degeneration of neuronal dendrites, cell bodies, terminals and axon tracts. In addition, electron microscopic examination was conducted. Histopathologically, degeneration of Purkinje cells was observed in the ACR-treated rats and the occurrence of the lesion was consistent with the time when the abnormal gait appeared. Detailed examination of the cerebrum is now underway.

P-118

Screening test for central neurotoxicity by a newly developed human hybrid neuron

Min-Cheol LEE[1], JK RYU[2], SU KIM[2], KH YANG[3]

Department of Pathology, Chonnam National University Medical School, Chonnam 501-190, Korea (South)[1]
Brain Disease Research Center, Ajou University[2]
Department of General Toxicology National Toxicology Institute[3]

A human hybrid neuronal cell line A1 has been generated by somatic fusion between a human fetal cerebral neuron and a human neuroblastoma cell, and tested neurotoxicity for beta amyloid. RT-PCR, immunochemical, and electrophysiological studies of the hybrid cells indicated that the cells express faithfully of morphological, immunochemical, physiological, and genetic features of human cerebral neurons. A1 hybrid neurons express neuron-specific markers such as neurofilament-L (NF-L), NF-M, NF-H, MAP-2, and beta tubulin III. A1 human hybrid neurons express messages for various cytokines and cytokine receptors which are similar to parental human CNS neurons and different from the other parental cell line, SK-SH-SY5Y neuroblastoma. A1 hybrid neurons also express messages for choline acetyltransferase (ChAT), tyrosine hydroxylase (TH), and glutamic acid decarboxylase (GAD), indicating that they could differentiate into various subsets of neuronal types. Whole-cell patch clamp experiments showed that A1 hybrid neurons expressed Na+ currents, which were completely blocked by tetrodotoxin. In addition, depolarizing and hyperpolarizing voltage clamp steps evoked respective outward and inward K+ currents in these cells. When A1 hybrid neurons were exposed to beta amyloid for 72 hr, there was three-fold increase in TUNEL positive cells over controls, indicating that beta amyloid is neurotoxic to A1 hybrid neurons. The present study indicates that the A1 human hybrid neuronal cell line should serve as a valuable cell for the screening of central neurotoxicity in vitro as well as for the studies of biology, physiology, and pathology of human neurons in health and disease.

P-119

Developmental neurotoxicity of domoic acid in rat

Min-Cheol LEE[1], YS KIM[1], JY WOO[1], J KIM[1], KH YANG[2]

Department of Pathology, Chonnam National University Medical School, Chonnam 501-190, Korea (South)[1]
Department of General Toxicology National Toxicology Institute[2]

Domoic acid (DA), an amnestic shellfish poisoning, has been known a kainate-receptor agonist resulting excitotoxic injury of the brain. It has been known that the hippocampus had well-developed glutamate receptors. To investigate neuronal injury of the hippocampus, DA (0.8, 1.0, 2.0, 2.5 mg/Kg) was administrated intravenously via tail vein in the different developmental periods of rats consisting of fetal stage; E15, neonatal stage; P14, actively growing stage; P35, and young adult stage; P70. Histopathological and immunohistochemical studies, ultrastructural studies, and Western blot analysis were performed from the hippocampus at 3, 7, 14, and 28 days after DA treatment. Presence of neurobahavioural abnormalities and electroencephalography were evaluated at 6 weeks after DA administration. Acute mortality rate was 40-50% in rats treated with 2.5 mg/Kg of DA. Histopathologic features of neuronal injury of the hipocampus demonstrated in all developmental periods of rats, and significantly severe injury observed in growing and young adult stage rats and over 1.0 mg/Kg of DA treated rats. There were two types of neuronal injury, swollen or shrunken degenerating neurons, especially in the CA1 and CA2 regions. The shrunken neurons positively stained with c-FOS, and some of the neurons demonstrated ISEL positivity indicating apoptotic cell death. Electron microscopically, clumping of heterochromatin of nucleus and shrinkage of cytoplasmic organelles with increased number of mitochondria, and subsequent neuronal condensation were noted. Western blot analysis revealed amplification of Bax, Bcl-2, and Caspase 3 proteins on 3 to 7 days after DA administration. Various stages of convulsive seizures with epileptic spikes were developed. In conclusion, neurotoxicity of DA was developed all of the experimental period from fetal to young adult stage, especially administrated dosages over 1.0 mg/Kg. The hippocampal neuronal injury was predominant on CA1 and CA2 regions which resulted abnormal convulsive seizures. DA-induced seizures might be a valuable animal model to study epileptogenesis related to excitotoxic injury.

P-120

Newly-established tumor lines from a spontaneous malignant schwannoma in F344 rats

Jyoji YAMATE[1], Hisae YASUI[1], Mitsuru KUWAMURA[1], Takao KOTANI[1], Sadashige SAKUMA[1], Jonathan LAMARRE[2]

Laboratory of Veterinary Pathology, Osaka Prefecture University, Osaka 599-8531, Japan[1]
Department of Biomedical Sciences, University of Guelph[2]

F344 rats are an inbred strain with genetically homogeneous nature, and have been widely used in chronic toxicity and carcinogenecity studies. In order to develop animal tumor models, we have attempted to establish various transplantable tumor lines from spontaneous tumors found in F344 rats in terms of studies on biological behavior, morphological characteristics and paraneoplastic manifestation in relation to neoplasia. Schwann cells are derived from the neuroectodermal neural crest that can differentiate towards meso-ectoderm in embryogenesis. The histogenesis and cellular characteristics of Schwann cell tumors remain to be investigated. The frequency of naturally occurring malignant schwannomas in the soft tissue of F344 rats, that had been maintained to 2 years of age, was less than 4%. In this study, transplantable tumor (KE) and cloned cell (KE-F11) lines were established from a spontaneous malignant schwannoma found in a 24-month-old F344 rat. The primary tumor and KE tumors consisted of oval or spindle cells arranged in ill-defined bundles. Cultured KE-F11 cells exhibited polygonal or spindle configurations. Immunohistochemically, neoplastic cells in KE and KE-F11 reacted to vimentin, S-100 protein, NSE, MBP, and GFAP in varying degrees, indicating neurogenic features; occasional cells reacted to alpha-smooth muscle actin. Cells positive for lysosomal enzymes (acid phosphatase and non-specific esterase), and ED1 (rat macrophage- specific) were observed in KE-F11, and electron microscopically, cells with many lysosomes were frequently present, indicating expression of macrophage-like phenotypes. Bioassay analysis revealed that KE-F11 cells produced high levels of NGF. DNA synthesis was inhibited by TGF-beta1 addition, and Northern blot analysis revealed that expression of c-myc, a cell cycle-related immediate early gene, was depressed by TGF- beta1. Likely, TGF-beta1 is a factor capable of inhibiting cellular growth of Schwann cells. mRNA expression of the low density lipoprotein receptor-related protein (LRP) was seen in KE-F11 cells by Northern blot analysis, and the level was decreased by lipopolysacccharide (LPS) treatment. LRP may be attributable to regulation of Schwann cell functions. KE-F11 cells seeded on laminin-coated dishes exhibited more extended cytoplasmic projections than on collagen type I-coated dishes. The present study provides evidence that biological properties of malignant schwannoma-derived cells might be affected by exogenous factors such as TGF-beta1, LPS and laminin. These tumor lines would become useful for studies on pathobiological characteristics of Schwann cells.

P-121

Spontaneous malignant schwannoma in F344 rat

Bang Hyun KIM[1], Dong Deuk JANG[1], Mina CHOI[1], Beom Seok HAN[1], Ki Taek NAM[1], Chul Kyu KIM[1], Kook Kyoung LEE[2], Ki Dae PARK[1], Wan Seob CHO[1], Ki Hwa YANG[1]

Department of General Toxicology, National Institute of Toxicological Research, Seoul 122-704, Korea (South)[1]
Department of Veterinary Medicine, Cheju National University[2]

During the carcinogenicity study with diisodecyl phthalate (DIDP), we incidentally found one male F344 rat which showed dyspnea and severe flexured lumbar vertebrae in the 0.2 % DIDP treated group at the age of 23 week. Two white to yellow masses were observed in the subcutis of the lumbosacral region and the left inguinal region of abdominal cavity, 25*17*8 mm and 16*14*8 mm in size, respectively. Microscopic findings were varied. Highly cellular and spindle cells were growing in sweeping fascicles, herringbone pattern, and locally rossette-like pattern. They were pleomorphic and had more than 2 nucleoli. Characteristically, extensive necrosis was observed over the whole masses. Immunohistochemically, the neoplastic cells were positive for S-100 but negative for smooth muscle actin, desmin, etc. According to these results, these masses in male F344 rat were diagnosed as malignant schwannoma. This case might be a good reference of malignant schwannoma.

P-122

Histopathological characterization of nasal airway lesions induced by intranasal challenge with ovalbumin in sensitized guinea pigs

Emiko KUWASAKI[1], Koshirou KATOKU[1], Takafumi OSHIKATA[1], Yutaka NAKAHARA[1], Hideyuki WATANABE[1], Masao HAMAMURA[1], Kosuke MORIZUMI[2]

Department of Pathology, Panapharm Laboratories Co., Ltd., Kumamoto 869-0425, Japan[1]
Department of Parmacology, Panapharm Laboratories Co., Ltd.[2]

Allergic rhinitis induced by challenge with ovalbumin in sensitized guinea pigs is commonly known. The histopathological examination of the nasal airway lesions for the above pathological model was, however, shown in very few reports. We made an allergic rhinitis model induced by intranasal challenge with ovalbumin in sensitized guinea pigs. In this study, we investigated the histopathological features due to the suggested time-dependent nasal airway lesions. Male guinea pigs, 6 weeks old, were sensitized by a single subcutaneous administration of saline containing ovalbumin and aluminum hydroxide as the adjuvant. The sensitized animals were challenged by intranasal instillation with ovalbumin 14 days after the sensitization, and they received a second challenge with ovalbumin 7 days after the first challenge. 5 animals were euthanized at 0.5, 4 and 8 hours after the second challenge. The nasal cavity, the trachea and the lung were fixed in 10% buffered formalin. The nasal cavity was decalcified in 10% EDTA. The paraffin sections of these organs were stained with hematoxylin and eosin, Luna, Alcian blue pH2.5 and periodic acid-Schiff, and these sections were processed for light microscopy. All guinea pigs challenged with the above method were observed to have eosinophil infiltration in the mucosa of the nasal cavity. At 0.5 hours after the second challenge, eosinophils infiltrated into the lamina propria underling the respiratory epithelia of the nasal septum, the ventral wall and the nasoturbinates. At 4 and 8 hours after the second challenge, eosinophils infiltrated from the lamina propria into the respiratory epithelia. Eosinophil infiltration into the lamina propria and the respiratory epithelia of the nasal septum, the ventral wall and nasoturbinates tended to be most intense 8 hours after the second challenge. At 4 and 8 hours after the second challenge, eosinophils exudated in the nasal cavity. At all time points, several mast cells, stained positively with Alcian blue pH2.5, were found in the lamina propria of the nasal septum. The results of this study suggested that the eosinophils left the lumen of the nasal cavity from the lamina propria through the respiratory epithelia in this model. Eosinophil infiltration in this model was similar to that in human allergic rhinitis. In addition, the time of eosinophil infiltration in this model was in agreement with the time of late nasal airway responses in allergic rhinitis.

P-123

Hydroxyurea(HU)-induced apoptosis and changes in apoptosis-related genes expression in the mouse fetal lung

Gye-Hyeong WOO[1], Eun-jung BAK[2], Koji UETSUKA[1], Hiroyuki NAKAYAMA[1], Kunio DOI[1]

Department of Veterinary Pathology, The University of Tokyo, Tokyo 113-8657, Japan[1]
Department of Biomedical Science, The University of Tokyo[2]

Hydroxyurea (HU), a potent mammalian teratogen, affects proliferating embryonic cells and inhibits DNA synthesis. The teratogenic potential of HU has been well known in experimental animals for several decades. In human beings, cases of respiratory distress have been reported. In this study, we assessed the cytotoxicologic effects of HU on the fetal lung by exposing pregnant mice to HU on day 13 of gestation. The number of TUNEL-positive cells began to increase at 3 hours after treatment (HAT), peaked at 6 HAT, and rapidly decreased at 24 HAT. This positive reactivity for TUNEL was seen mainly in mesenchymal cells. On the other hand, the number of immunohistochemically p53-positive cells peaked at 3 HAT and decreased at 12 HAT, and the expression of *p53* mRNA was significantly elevated at 6 HAT. In addition, the expression of apoptosis-related genes (*p21* and *bax*) was also significantly elevated. Significant increase in the expression of *p21* mRNA was detected at 1 HAT to 12 HAT and that of *bax* mRNA was noted at 6 HAT. These results suggest that p53 may play an important role in HU-induced apoptosis in the fetal lung.

P-124

In vivo detection of lung tumors in mice by high-resolution X-ray microtomography

Horst WEILER[1], Kris MEURRENS[2], Nora M. DE CLERCK[3], Andrei A. POSTNOV[3], Piter M. TERPSTRA[2]

Philip Morris Research Laboratories, GmbH, Cologne 51149, Germany[1]
Philip Morris Research Laboratories Bvba[2]
University of Antwerp[3]

The major application of micro-CT in biomedical research has been the imaging of calcified tissues. In vivo high-resolution X-ray microtomography (micro-CT) is a promising technique for the non-invasive imaging of soft tissues of small laboratory animals. For example, detection and evaluation of structural lung disorders has up to now required many animals to be killed at many timepoints, and for emerging lesions, such as small tumors, time-consuming step-serial histological sectioning is unavoidable. As a possible alternative, the application of X-ray micro-CT to detect lung tumors was investigated in live mice at an early and more advanced stage of tumor development. A/J mice were treated with urethane 1000 mg/kg bodyweight ip and examined at 3.5 and 9 months post treatment. Healthy age-matched groups of mice were used as control. The chest area of anesthetized mice was scanned for lung tumors by X-ray micro-CT (Skyscan 1076, Aartselaar, Belgium) without gating for cardiac or respiratory motion. Positioning the mice on their backs in the X-ray micro-CT and banding the chest area reduced motion artifacts. Scans lasted 17 minutes per mouse: spatial resolution was 35 μm and radiation load was 0.4 Gy. All mice survived the procedure with no apparent ill effects. Reconstructed virtual CT cross sections of the lungs (Feldkampf cone beam algorithm) confirmed the macroscopic evaluation. Tumors in the hilar region or at the periphery of the lungs were more difficult to localize on the CT cross sections. In step-serial sections (every 100 μm) from one pair of lungs, the tumors detected by CT were seen plus a few additional, very small tumors (≤ 150 μm diameter). The in vivo X-ray micro-CT method opens broad perspectives for non-invasive imaging. Continued improvements in resolution and detection in soft tissue imaging may make this a powerful research tool for monitoring tumor development and progression.

P-125

Eosinophilic change of the lining cells of the nasal cavity in rasH2 mice administered di(2-ethylhexyl)phthalate

Takatoshi KOUJITANI[1], Kaoru TOYOSAWA[1], Izumi MATSUMOTO[1], Kazuo OKIMOTO[1], Mami KOUCHI[1], Koji KUROKI[1], Kohji TANAKA[1], Nobuo MATSUOKA[1]

Safety Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Osaka 564-0053, Japan[1]

[Introduction] Administration of Di(2-ethylhexyl)phthalate (DEHP), a peroxisome proliferator, induces hepatocellular adenomas and enhances eosinophilic change of the respiratory epithelium in the nasal cavity in transgenic mice carrying a human prototype c-Ha-*ras* gene (rasH2 mice)(1). In the present study, eosinophilic change in the olfactory region of the nasal cavity was examined in addition to the lesion in the respiratory region.
[Materials and methods] Thirty-three male rasH2 mice were purchased at 6-week old. After 2-week acclimation period, mice were divided into a control group (n=12) and a DEHP group (n=21). Mice in the DEHP group were treated DEHP 6000 ppm in diet for 26 weeks. At necropsy, the head was fixed in 10% neutral buffered formalin, and decalcified by 5% formic acid solution. Thereafter, the nasal cavity was trimmed according to the method described by Nagano *et al* (2). Sections from the nasal cavity were stained with hematoxylin and eosin, PAS, Alcian blue, Toluidine blue and Masson-trichrome, and examined microscopically.
[Results] Respiratory region: Eosinophilic change characterized by appearance of eosinophilic globules was observed in 10 of 21 mice in the control group and in all of 21 mice in the DEHP group, and respiratory metaplasia of glands in 11 of 21 in the DEHP group. Histologic grade of the eosinophilic change was greater in the DEHP group than in the control group. Eosinophilic globules in the respiratory epithelium were stained in red-orange by Masson-trichrome, however, were negative by PAS, Alcian blue and Toluidine blue. Olfactory region: No histopathological change was found in the control group. Eosinophilic change was observed in all of 21 mice, and respiratory metaplasia in 4 of 21 mice in the DEHP group. The eosinophilic change in the olfactory region was exclusively located in the olfactory epithelium of the dorsal meatus in the nasal cavity. Eosinophilic globules in the olfactory epithelium were stained in pale-purple by Masson-trichrome, however, were negative by PAS, Alcian blue and Toluidine blue.
1) K. Toyosawa *et al.* Toxicol Pathol 29:458-466, 2001.
2) K. Nagano *et al.* J Toxicol Pathol 1:115-127, 1988.

P-126

Biological persistence and pathological changes of potassium octatitanate of two different shapes.

Akira OGAMI[1], Takako OYABU[1], Yasuo MORIMOTO[1], Hiroshi YAMATO[1], Izumi AKIYAMA[1], Isamu TANAKA[1]

Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Fukuoka 807-8555, Japan[1]

The biological effects (bio-persistence and pathological changes) in the rat lung were evaluated with two different types of potassium octatitanate. The materials used in this study are; Potassium Octatitanate whisker (PT1), and Potassium Octatitanate Particle (PTP). The rats had the intratracheal instillation of PT1 and PTP with dose of 2mg were sacrificed at the time of 3days, 1week, 1 month, 3 months and 6 months after the instillation. The bio-persistence of PTP was much shorter than PT1in the lung. Inflammation score showed that the inflammation of PT1 after instillation was much more persistent than in that of PTP. These results suggests that the scale-shaped potassium octatitanate is much less hazardous to the rat lung than the fiber-shaped.

P-127

Sequential change in acute lung damage due to intratracheal instillation of Quartz in F344 male rats establishment of a biological bioassay for detection of lung toxicity by fine particles

Masanao YOKOHIRA[1], Hijiri TAKEUCHI[1], Keiko YAMAKAWA[1], Kousuke SAOO[1], Mico IKEDA[1], Yoko HOSOTANI[1], Zeng YU[1], Kyoko HOSOKAWA[1], Katsumi IMAIDA[1], Makoto SHIRAISHI[2]

Department of Onco-Pathology, Kagawa University, Kagawa 761-0793, Japan[1]
Diagnostic Pathology and Cytology Institute, Shikoku Cytopathologic Institutes[2]

There are many toxicants in our environment, including air pollutants, and it is an urgent priority to establish biological bioassay for detection of hazards of fine particles, which can be inhaled into deep lung tissue by human being. Quartz instillation is known to produce an inflammatory reaction followed by histological changes characteristic of lung fibrosis. In order to establish an appropriate bioassay for detection of lung damage after particle inhalation, sequential histopathological changes were here examined using Quartz as a typical lung toxic chemical.
A total of 50 F344 male, 10-week-old rats, were separated into two groups. 25 rats were exposed by intratracheal instillation to Quartz (DQ-12, 4mg/rat) suspended in saline (0.2ml) and subgroups of 5 rats were killed on Days 1, 3, 7, 14, and 28. The remaining rats were exposed by intratracheal instillation to saline (0.2ml) as a control group and 5 rats were killed on the same days as for the Quartz exposure group. Histopathological changes were observed as follows; neutrophil infiltration in the walls and spaces of the alveoli, histiocyte infiltration, pulmonary edema and pulmonary fibrosis, and restructure of the walls of the alveoli in all lungs of Quartz treated rats. These parameters could be scored. For detection of acute inflammatory changes, neutrophil infiltration in the alveoli appeared to be an important parameter. Furthermore, as subacute inflammatory changes, histiocyte infiltration in the alveoli, areas of pulmonary edema and pulmonary fibrosis and restructure of the walls of the alveoli were readily identifiable. Lungs of rats in the Quartz treated group killed on Day 1 demonstrated the strongest signs of acute inflammation, whereas, lungs of rats killed on Day 28 had the most pronounced evidence of subacute inflammation. Additionally, granulation with giant cells and macrophages in the alveoli were significantly observed. According to the results, Days 1 and 28 after intratracheal instillation of test fine particles were suggested to be the most appropriate days for detection of acute and subacute inflammatory changes, respectively. This bioassay may be useful for detection of acute or subacute lung toxicity due to inhaled fine particles.

P-128

Immunohistochemical demonstration of IL-13 protein in rat late athmatic response model

Kiyoshi KOBAYASHI[1], Osamu KURUSU[1], Hiroyuki HIGASHIYAMA[1], Anbo XIANG[1], Hideo KIKKAWA[1], Satoshi ASANO[1], Mine KINOSHITA[1]

Pharmacology Department, High Throughput Biology, TRL, GlaxoSmithKline K.K., Tsukuba 300-4247, Japan[1]

[INTRODUCTION] IL-13 is an immunoregulatory and effector cytokine in the allergic diseases such as bronchial athma. A variety of immune and non-immune cells are known as IL-13 producers. In the present study, we immunohistochemically analyzed the IL-13 expressing cells in the lung tissue of ovalbumin(OVA)-sensitized and challenged Brown-Nowway (BN) rat known as athma model.
[MATERIALS AND METHODS] Nine weeks old BN rats were intraperitoneally injected with OVA at a dose of 100 mg/kg in $Al(OH)_3$ (100 mg/kg) in 0.9% saline on 3 consecutive days. Three weeks after the final OVA injection, rats were exposed to aerosol containing OVA at 1% (w/v) for 20 minutes. Animals were killed under anesthesia 24 h after aerosol exposure. Inflammatory cell counting and ELIZA analysis for IL-13 protein was performed using bronchioalveolar lavage fluid (BALF). In a separate experiment, lung tissue was removed, fixed in zinc fixative, embedded in paraffin followed by histological and immunohistochemical analysis using anti-rat IL-13 monoclonal antibody.
[RESULTS] In OVA-challenged rats, number of eosinophils and neutrophils in BALF was markedly elevated being accompanied by the elevation of IL-13 protein concentration. Histological examination revealed perivascular, peribronchiolar and alveolar infiltration of inflammatory cells consisting of neutrophils, eosinophils and macrophages. Immunohistochemically, eosinohils infiltrated in alveolar spaces were strongly positive for IL-13 whereas those in perivascular/peribronchiolar area were negative or scantly positive. In addition, alveolar macrophages were also stained positive for IL-13. Phagocytosis of degenerated eosinophils and other materials by alveolar macrophages were observed suggesting the possible IL-13-positive reaction in macrophages due to the phagocytotic reactoin.
[DISCUSSION] IL-13 is known to play an important role in various allergic diseases, and eosinophil infiltration is one of the most characteristic findings in athma. Thus, IL-13 production by eosinophils with advances in alveolar infiltration may play an important role in the pathogenesis of present animal model through an autocrine mechanism.

P-129

One case of pleural mesothelioma in male F344/DuCrj rat

Hideki SENOH[1], Tetsuya TAKEUCHI[1], Yumi UMEDA[1], Taku KATAGIRI[1], Shigetoshi AISO[1], Kasuke NAGANO[1]

Japan Bioassay Research Center, Kanagawa 257-0015, Japan[1]

A case of mesothelioma spontaneously developing in the thoracic cavity of a male F344/DuCrj rat (Charles River Japan, Kanagawa), found from 5,000 males used in 25 studies of 2-yr carcinogenic bioassay carried out following the Japanese Guideline in the past 20 years is presented.
The present case was survived up to the end of a 2-yr study and received a complete necropsy. Gross observation revealed numerous white nodules, from 2 to 5 mm in size, on the pleural surface of lungs, the diaphragm and the mediastinum accompanied by hemorrhagic fluid in the thoracic cavity. No abnormal finding was seen in the abdominal or scrotal cavity. Specimens of nodules were fixed in 10% buffered formalin solution and embedded in paraffin. Each 5 μm thick sections were stained with hematoxylin and eosin, and examined microscopically.
Multiple nodules developing on the pulmonary surface, parietal thoracic wall and diaphragmatic surface showed same histological characteristics as same as mesotheliomas, usually occurring in the abdominal or scrotal cavity of male rats. Nodules were composed of both cuboidal epithelial-like cells and mesenchymal components. Proliferation of cuboidal epithelial-like cells were seen covering the surface areas and in the inner part of the mesenchymal area. The epithelial-like growth was mostly arranged in a single layer, occasionally showing a papillary projection. However, multiple layer-arrangement and solid growth were also observed. Mesenchymal components, mainly much of fibrous tissue, were found in the almost all areas.
The thymus was atrophic, but remained intact. The microscopic examination confirmed that no mesothelioma was found in the abdominal or scrotal cavity of the present case.
From the above-described findings, this tumor was diagnosed as a rare mesothelioma probably originated from the pleura in the rat.

P-130

Incidence of cardiomyopathy in rats treated with PhIP varies with the rat strain

Shoji KASHIWABARA[1], Naoki KASHIMOTO[1], Toshihiro UESAKA[1], Osamu KATOH[1], Keiji WAKABAYASHI[2], Hiromitsu WATANABE[1]

Department of Cellular Biology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima 734-8553, Japan[1]
National Cancer Center Research Institute[2]

It is well known that 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is a heterocyclic amine derived from cooked meat and a potent environmental carcinogen for rat colon, prostate and mammary glands. We have reported that the cardiomyopathy was induced in Donryu rats treated with PhIP. The aim of this study is to clarify the rate of cardiomyopathy induced by PhIP in several strains of rats.
[Methods] The following seven strains of rats were used in this study: Crj:Donryu, Crj:CD, Crj:Wistar, WKY /NCrj, SHR/NCrj, LEW/Crj and BN/Crj. Each strain was divided into two groups. One was used as a PhIP treatment group and the other as a non-treatment group. PhIP (75 mg/kg/day) was administered by gavage 3 times per week at two-day interval to a total of 10 doses. Saline was administered as a control instead of PhIP. Animals in a moribund state were killed and autopsied, and all others were killed 53 weeks after the initial treatment. The incidence of cardiomyopathy in each strain was investigated.
[Results and summary] No significant difference in the mean survival was observed among the groups, except for in the Donryu rats. Donryu rats began to die due to cardiomyopathy after the first PhIP treatment, reaching 45% at 98 days and 80% at 310 days. The body weights of Donryu, CD, LEW and BN rats in the treated groups were significantly decreased in comparison with non-treatment. The relative heart weights (heart weight/body weight) of LEW/Crj in the treated groups were significantly greater than those in the non -treated groups. The blood pressure showed no significant difference between the treated and non-treated groups of each strain. Cardiomyopathy was observed to develop only in the Donryu strain. These results indicate that induction of cardiomyopathy by administration of PhIP occurs only in Donryu, but not in any of the other treated strains.

P-131

Histopathological effect of Enalapril maleate on fetal heart development in rat.

Arash KHAKI[1], Iraj SOHRABI HAGHDOST[1], Amir Afshin KHAKI[2], Mahnaz HAYDARI[3]

Department of Veterinary Pathology, Islamic Azad University, Tabriz/Iran, Tabriz 51388, Iran[1]
Department of Histology in Medical College of Tabriz University/Iran[2]
Department of Pharmacology, Shahid Beheshty Medical College Tehran/Iran[3]

Enalapril maleate is an antihypertensive drug, which could reduce blood pressure by dilating blood vessels and diminishing systolic of diastoles pressure. Since there is little information about enalapril maleate side effect on of heart rat embryo. This preliminary study was planed to see what kind of changes occurs in development of heart in Rat embryo. The aim of present study was to determine the histopathological effects of enalapril maleate on heart of rat fetal. Twenty male Wistar Rat & twenty female Wistar Rats were selected & matched together & after pregnancy, randomly divided into two groups; control (n=10) and test (n=10). The test group has been received 0.4mg/kg (PO) enalapril maleate daily during pregnancy. However, the control group just received plate. After delivery, heart tissue of fetal in both groups were taken and prepared for light microscopy. Staining method was H&E. Microscopic study of heart tissue slices showed that in the test group myocardial cell were smaller and nucleus were denser when controlled to control group. Weight of fetal in test group was reduced in compared to control group ($P<0.05$). Since enalapril maleate had side effect on heart of rat fetal. It is not suggested that enalapril maleate has been used during pregnancy in human.

P-132

Human heart glutamate receptors (GluRs) - possible effector sites: An opportunity for drug discovery.

Olga PULIDO[1], John VEINOT[2], Ruedi MUELLER[1], Meghan KAVANAGH[1], Colin ROUSSEAUX[3], Santokh GILL[1].

Pathology Section, Toxicology Research Division, Food Directorate Bureau of Chemical Safety, HPFB, Health Canada, Ontario K1A OL[2], Canada[1]
Department of Pathology and Laboratory Medicine, University of Ottawa, Civic Hospital Campus[2]
Department of Cellular and Molecular Medicine, Faculty of medicine, University of Ottawa[3]

Excitatory amino acids (EAAs) mediate their effects through the glutamate receptors (GluRs) in the brain. GluRs play an important role in the pathobiology of a variety of neuropsychiatric conditions and in their treatment; they are also central to the neurotoxicity of EAAs such as domoic and kainic acid. Previously we demonstrated the presence of GluRs in the heart of rats and nonhuman primates, where they are preferentially localized within the different components of the conducting system, nerve terminals and intramural ganglia cells. The findings of severe cardiac lesions in Californian sea lions that died from domoic acid (DA) intoxication suggest that DA and other glutamate analogs may be potentially cardiotoxic and these effects may be mediated through the GluRs. Since the GluRs are present in cardiac and other peripheral tissues, we have hypothesized that EAAs can exert systemic effects through a common mechanism mediated by GluRs. We investigated the presence of GluRs in human hearts using immunohistochemistry. Unstained histological preparations of human heart tissues were obtained from archived specimens collected during postmortem examinations following the standard autopsy procedures established at the Ottawa Civic Hospital. All tissues were fixed in formaldehyde, embedded in paraffin, sectioned at 5-6μ mounted on salinated slides and stored until stained. Preliminary results showed that several subtypes (ionotropic and metabotropic) of GluRs tested are differentially expressed in all the human hearts assessed, and the distribution and stain intensity varieg with of each receptor subtype. In contrast to non-human primates, GluRs are more widely expressed in humans, where the specialized structures of the conducting system, the working myocardium, the wall of blood vessels, intramural nerves and ganglia cells stained for GLuRs. Slides stained with H&E were used for the histopathological assessment and for the anatomical identification of specific cardiac structures such as the different components of the conducting system. This is the first report of GluRs in human heart. Hence, they suggest that in human these receptors may be involved on important cardiac functions such as contraction, rhythm, coronary circulation and thus the pathobiology of some cardiac disease. Further functional and pharmacological characterization of these GluRs in the heart could lead to the development of therapeutic agents designed specifically for heart GluRs.

*This study was supported by a grant from the Office of the Chief Scientist and approved by the Health Canada Research Ethics Board

P-133

Enzyme- and immuno-histochemical changes in rat salivary glands induced by theophylline

Satoru KAJIKAWA[1], Kenji NAKANO[1], Aisuke NII[1], Hiroyuki NAKAYAMA[2], Kunio DOI[2]

Safety Research Labs., Yamanouchi Pharmaceutical Co., Ltd., Tokyo 174-8511, Japan[1]
Laboratory of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo[2]

Introduction Theophylline (1,3-dimethylxanthine), a phosphodiesterase inhibitor, is widely used as a smooth muscle relaxant and its efficacy may be associated with increased cellular cAMP as a result of inhibition of phosphodiesterase. A high dosage of theophylline is also known to induce salivary gland hypertrophy. It was previously shown that a single administration of theophylline induces salivation and time-dependent decreases in organ weight and the number of secretory granules in the salivary glands up to 4 hours after the treatment. However, the precise mechanisms of salivary changes by theophylline still remain unclear. In the present study, the changes in salivary glands induced by theophylline were examined using immuno- and enzyme-histochemical methods.
Materials and Methods Male F344 rats were given theophylline at 50 mg/kg intraperitoneally, sacrificed at 1 and 4 hours after the treatment, and the submaxillary and parotid glands were excised (n=3). As a vehicle control, physiological saline was given in the same fashion. Enzymehistochemistry for phosphodiesterase and 5'-nucleotidase was performed using frozen sections. Immunohistochemistry for aquaporin 5, a water channel protein, and α-amylase was done using paraffin sections.
Results In the submaxillary glands of the control group, the activity of phosphodiesterase was diffusely detected and that of 5'-nucleotidase was observed in the peri-acinar and peri-intercalated duct areas. The expression of aquaporin 5 was evident in the luminal area of the acinus and intercalated duct. Alpha-amylase was not detected. At 4 hours after the treatment, the activity of phosphodiesterase was reduced, while that of 5'-nucleotidase was increased. Aquaporin 5 signals were increased and also detected in the cytoplasm of acinar cells. In the parotid glands of the control group, the activities of phosphodiesterase and 5'-nucleotidase were detected exclusively in the intercalated ducts. Alpha-amylase was expressed in the secretory vesicles. Aquaporin 5 was not detected in any portion of the parotid glands. In the theophylline group, the signals of α-amylase were reduced time-dependently, while the activities of the two enzymes were unchanged.
Discussion Reduced activity of phosphodiesterase by theophylline was demonstrated in the submaxillary glands. Subsequently increased cAMP might induce the expression of water channels, leading to saliva secretion. By contrast, the activity of phosphodiesterase was unchanged in the parotid glands despite the time-dependent reduction of α-amylase. Therefore, there might be different mechanisms of saliva secretion and different responses to theophylline between the submaxillary and parotid glands.

P-134

Histopathological study of the time course changes in the PTHrP-induced incisor lesions of rats.

Atsuhiko KATO[1], Masami SUZUKI[1], Yayoi KARASAWA[1], Tetsuro SUGIMOTO[1], Kunio DOI[2]

Safety Assessment Dept.Chugai Pharmaceutical Co., Ltd., Shizuoka 412-8513, Japan[1]
Department of Veterinary Pathology, Faculty of Agriculture, University of Tokyo[2]

Parathyroid hormone related peptide (PTHrP) was known as a causative factor of humoral hypercalcemia of malignancy (HHM). Previously we have reported our HHM model showed incisor fracture with symmetrically localized odontoblast lesions. In this study, the early response of odontoblats to elevated PTHrP, its progression, and relation to the fracture are described, and the possible role of PTHrP in the odontogenesis is discussed. Twenty HHM nude rats were prepared by implantation of PTHrP-expressing human tumor cell line (LC-6), and each 5 of them were sacrificed at 2, 5, 8 and 10 weeks after implantation, along with 3 untreated control animals. The plasma and incisors were collected for measurements of blood parameters (Ca2+, PTHrP), and for preparation of paraffin embedded H&E staining sections, which were evaluated histopathologicaly and histomorphmetricaly. In histomorphometry analysis, both columnar- and high-columnar-odontoblastic cell heights were measured in each sampling point. Blood Ca2+ and PTHrP levels were increased in week-5 and later HHM rats. All week-8 and -10 HHM rats fractured its incisor in 7 weeks after tumor implantation. In histopathology, shortening of odontoblast was observed in week-5 and later HHM rats. In week-8 and -10 HHM rats, shortening of odontoblast was accompanied by thinning of dentin, and was the only change, which reached to the fracture area. In histomorphometry, cell heights were decreased in culumnar-odontoblats of week-5, and in both columnar- and high-columnar-odontoblasts of week-8 and -10 HHM rats. From these results, as an earliest response to the elevated PTHrP, columnar-odontoblasts are decreased its cell height. This change is progressed to more mature high-columnar phenotype, and accompanied by thinning of dentin in later stage. Finally, thinning of dentin deteriorate the strength of the incisor, and is caused fractures. Since it is known PTHrP delays the maturation of chondrocytes, it is suggested that the response of odontoblasts to elevated PTHrP is not mere decrease of the cell height, but a maturation delay.

P-135

Spontaneous ectopic sebaceous glands (Fordyce's granules) in the oral mucosa of Sprague-Dawley rats

Masako IMAOKA[1], Hiroshi SATOH[1], Kiyonori KAI[1], Tetsuyo KAJIMURA[2], Kazuhisa FURUHAMA[1]

Drug Safety Research Laboratory, Daiichi Pharmaceutical Co., Ltd., Tokyo 134-8630, Japan[1]
Research Planning & Administration Department, Daiichi Pharmaceutical Co., Ltd.[2]

Although Sprague-Dawley rats are most frequently used in numerous toxicological studies, there have been few reports dealing with the spontaneous occurrence of ectopic sebaceous glands (Fordyce's granules) in the oral tissue so far. Hence, to clarify the morphological characteristics of Fordyce's granules and the incidence in this strain, 110 male and 110 female CD(SD)IGS rats aged 19 to 112 weeks were examined microscopically. As results, Fordyce's granules were identified in the upper molar gingiva, and most of them were located around the first and third molars. The granules consisted of one or more sebaceous glands without hairs and hair follicles, and had some lobules comprising acini with adipose vacuoles. The short excretory duct was lined with stratified squamous cells and drained to the gingival surface. The sebum constituting disintegrated acinar cells in the duct was also noted. In some cases, the granules were accompanied by cystic dilatation in the ducts and/or inflammatory reactions, without neoplastic or pre-neoplastic lesions. The overall incidence (9.1%) of Fordyce's granules in males was higher than that (0.9%) in females, and it somewhat increased with ascending ages, demonstrating sex- or age-specific difference. Based on the above findings, the nature of Fordyce's granules seen in CD(SD)IGS rats was similar to those of other strains and humans.

P-136

Acquisition of a gastric or duodenal phenotype on heterotropic transplantation of esophagus diaphragm, trachea and bladder tissues in F344 rats

Hiromitsu WATANABE[1], Takashi OCHIYA[2], Seiichi KAWAMATA[3], Tomoyuki KUROSE[3], Yoko KOMINAMI[1], Masayo NISHIKI[1], Atsushi SASAKI[1], Miho SHIRAISHI[1], Rina GON[1], Masaomi HAYASHI[1], Shoji KASHIWABARA[1], Naoki KASHIMOTO[1], Toshihiro UESAKA[1], Osamu KATOH[1]

Department of Cellular Biology, Research Institute for Radiation Biology & Medicine, Hiroshima University, Hiroshima 734-8553, Japan[1]
Department of Cancer Metastesis, National Institute of Cancer[2]
Institute of Health Science, Faculty of Medicine, Hiroshima University[3]

Cell differentiation is very important but not well understood. We have reported that intestinal metaplasia can be induced by X-irradiation in rats, and proposed that an elevation of the pH of gastric juice due to the disappearance of parietal cells in the fundic gland mucosa is one of the principal factors responsible for its development. We also found that grafted colon mucosa differentiates into pseudogastric mucosa in the gastric region and, more recently, that female stomach grafts differentiate into 'intestine' with goblet cells in the male duodenum. In the present study the possibility that such trans-differentiation might similarly occur with other tissues transplanted into the fundus or the duodenum in rats was tested. Pieces of esophagus, diaphragm and trachea from 8-week-old male F344 rats were transplanted into the gastric fundus or duodenum of females and examined especially after 3 or 6 months. While the diaphragm was not recognizable as a muscle layer in either the stomach or the duodenum, the esophagus and trachea persisted, the latter with the presence of cartilage. Esophagus grafts transplanted into the glandular stomach and duodenum, trans-differentiated into gastric and duodenal mucosa, respectively. Pieces of bladder were transplanted into the duodenum and observed after 1, 3, 6 and 12 months. Goblet cells with alcian-blue positive mucin appeared in bladder tissue implanted into the duodenum three months after operation. Six months after the operation, their numbers had increased and cytoplasm alkaline phosphatase (ALP) positivity was noted. Twelve months after operation number of goblet cells were decreased and ALP was not observed. Some donors were used in transgenic F344 GFP rats. Positive cells against anti-GFP antibody were observed in recipient tissues. Gastrointestinal and also bladder stem cells may have multipotential ability for differentiation and to be able to trans-differentiate when transplanted into different environments in the gastrointestinal tract.

P-137

Proliferative changes in upper digestive duct of rats treated with a single dose of Alloxan

Kiyokazu OZAKI[1], Yasushi KODAMA[1], Tetsuro MATSUURA[1], Isao NARAMA[1]

Research Institute of Drug Safety, Setsunan University, Osaka 573-0101, Japan[1]

Male rats of WBN/Kob strain are one of the diabetic model animals that develop long-lasting diabetic symptoms and some complications from about 40 weeks after birth without any treatment. In order to accelerate the diabetic condition, animals were received a single dose of Alloxan and developed proliferative lesions of squamous epithelium in forestomach. The lesions of forestomach progressed to squamous cell carcinoma 50 weeks after Alloxan treatment. Histopathologic and immunohistochemical examinations were performed to clarify carcinogenic mechanisms and distribution of digestive lesions.

Methods & Materials

Male and female WBN/Kob rats, 7-36 weeks old, were dosed singly with Alloxan (50 and 40mg/kg BW respectively) via tail vein. The animals were sacrificed 50 weeks after dosing. Immunohistochemistry was applied in addition to routine histopathological examination.

Results

Hyperplasia of squamous epithelium was observed in the central part of tongue, hard palate, posterior parts of esophagus and whole part of forestomach, and was often associated with hyperkeratosis in esophagus and forestomach. About 20% of animals had squamous cell carcinoma (SCC) in the forestomach, and some animals also had SCC in palate and/or posterior parts of esophagus. SCC was well-differentiated but invaded into submucosal and/or muscular layer, and metastases to regional lymphonodus was also observed in some cases. Proliferative changes of squamous epithelium were constantly accompanied with erosion or ulceration, and moderate to severe infiltration of lymphocytes and plasma cells in underlying mucosal propria. *Candida albicans* and *E. coli* infection were also frequent in the superficial mucosal layer. In cancer tissue, E-Cadherin and s-Catenin showed partial loss, but p53 and Ras/p21 were not amplified. COX-2 was positive for fibroblast and macrophage in the lamina propria near to the lumen without any topographic relation to the neoplastic transformation, and was negative for neoplastic cells. iNOS was positive in suppurative inflammatory lesion, but was negative for neoplastic cells.

Conclusion

It is strongly suggested that inflammatory process caused by fungous and bacterial infection play important role in the pathogenesis of Alloxan-induced squamous cell carcinoma.

P-138

Gastric carcinoma in mustard gas factory workers in the Ohkuno island

Takamitsu SASAKI[1], Tomonori SASAHIRA[1], Wataru YASUI[2], Hiroki KUNIYASU[1]

Department of Oncological Pathology, Nara Medical University Cancer Center, Nara 634-8521, Japan[1]
Department of Molecular Pathology, Hiroshima University Graduate School of Biomedical Sciences[2]

Gastric carcinomas occurred in persons who had worked in the mustard gas factory during World War II are subjected. In follow up study, 56 mustard gas workers and 91 non-mustard gas workers in Ohkuno island showed higher frequency of well-differentiated type adenocarcinoma than patients treated in National Cancer Center Hospital (as a control). However, no significant difference was found between mustard gas workers and non-mustard gas workers. Five cases of mustard gas workers who were received pathological diagnosis on the resected materials were examined immunohistochemically. All cases showed no abnormal accumulation of p53 protein. No point mutation was confirmed by genome sequencing on exon 5-8 of the p 53 gene. E-cadherin production was reduced in all cases. Four cases showed reduced production of beta-catenin, and one cases showed abnormal accumulation of beta-catenin in cytosol, whereas nuclear staining of beta-catenin was not detected in all cases. By methylation sensitive restriction enzyme assay showed DNA methylation of E-cadherin promoter. These data suggested that gastric carcinoma in mustard gas workers might possess distinctive properties on genetic and epigenetic alterations from usual cases.

P-139

Gastric carcinoma in a Japanese macaque (Macaca fuscata)

Tokuma YANAI[1], Masato KOBAYASHI[1], Shunji GOTOU[2], Akino KATOU[2], Hiroki SAKAI[1], Akihiro HIRATA[1], Toshiaki MASEGI[1]

Department of Veterinary Pathology, Gifu University, Gifu 501-1193, Japan[1]
Primate Research Institute, Kyoto University[2]

Gastric carcinoma is one of the most common malignant tumors in humans, especially in Japanese. The tumors frequently occur in the lesser curvature of the antropyloric region, and less frequent in the cardiac region in humans. There have been only a few reports of gastric carcinomas in nonhuman primates which have a lot of similarity in anatomy and physiology with humans. We examined a case of gastric carcinoma occurred in the cardiac region in a Japanese macaque, and tried to compare it's morphological features to those in humans. An 18-year-old male Japanese macaque had gradual weight loss with occasional vomits about 1 year before death. The animal became to have frequent vomits and severe anorexia 2 weeks before death. Grossly, there was a tumor mass, 2 cm in the diameter, in the esophagus-cardia (EC) junction with a severe degree of cardial stenosis. The tumor bulged irregularly with a severe ulcer on the surface, and was ill -defined in the mucosa. Histologically, there was an infiltrative growth of poorly differentiated carcinoma in the mucosa of the cardiac region, invading deeply through the muscularis mucosae to the submucosa and muscular layers. The tumor cells showed marked cell atypia with various-sized nuclei and prominent nucleoli, and formed occasional glandular structures and squamous differentiation. There were frequent mitotic figures in the tumor. The tumor cells contained mucinous material in the cytoplasm, when the Alcian blue stain and periodic acid- Schiff (PAS) reaction were applied. There was no neoplasm except the gastric tumor. This tumor was diagnosed as gastric carcinoma originating from the cardiac region based on the site of occurrence and morphological features. We previously reported an advanced gastric carcinoma originating from the cardiac region, characterized by infiltrative growth with prominent glandular formation in a Brazza's guenon (Cercopithecus neglectus).1) Other three cases of gastric tumors (one adenocarcinoma and two squamous cell carcinomas) were reported in the literature, all of which were originated from the cardia. It is still uncertain why gastric carcinomas favored to occur in the cardiac region in nonhuman primates? 1) Yanai T, Noda A, Sakai H, Murata K, Hama N, Isowa K, Masegi T; Advanced gastric carcinoma in a de Brazza's guenon(Cercopithecus neglectus) J. Med. Primatol 1997; 26: 257-259

P-140

One case of Ito cell tumor in female F344/DuCrj rat

Yumi UMEDA[1], Tetsuya TAKEUCHI[1], Hideki SENOH[1], Taku KATAGIRI[1], Shigetoshi AISO[1], Kasuke NAGANO[1]

Japan Bioassay Research Center, Kanagawa 257-0015, Japan[1]

One case of Ito cell tumor, spontaneously developing in the liver of a female F344/DuCrj rat (Charles River Japan, Kanagawa), found from 5,000 females used in 25 studies of 2-yr carcinogenic bioassay carried out following the Japanese Guideline is presented. The present case was survived up to the end of the 2-yr study and received a complete necropsy. A mass, approximately 5 cm in size and with varying colors from white to red, was observed in the central lobe of liver. Multiple white areas were also found in the lungs.
Organs were fixed in 10% buffered formalin solution and embedded in paraffin. Each 5 μm thick sections were stained with hematoxylin and eosin, and examined microscopically. Unicentric large mass was found histologically in the liver and well demarcated from the surrounding liver tissue at the low magnification. This nodule was predominantly composed of clear stained cells having a round, ovoid or spindle-shaped nucleus. The cytoplasm, varying from abundant to scanty and often having vacuoles, exhibited chromophobic tinctorial appearance in hematoxylin and eosin staining. The cell border was not clear. Mitotic figures were occasionally seen.
The fluorescence microscopic examination on frozen sections of the liver mass revealed fluorescent contents in the cytoplasm of clear cells, suggesting a vitamin A being characteristic of the Ito cell. These clear cells were arranged in a cord-like structure covered with the sinusoidal endothelium. A part of cell-cords was found to be continuous with the surrounding hepatic cell cords at high-power magnification. A few nest of clear cells with the cord-like structure was found to grow in parenchymal hepatocytes associated with the sinusoidal endothelia and Ito cells keeping the normal liver architecture. Prominent amount of collagenous matrix was observed among the cord-like components of the tumor.
Several small foci consisting of clear cells were observed in the lungs and regarded as metastasis of this tumor.
From the above-described findings, this tumor was diagnosed as a rare Ito cell tumor in the rat liver.

P-141

Effects of bandage of the torso to hepatomegaly induced by phenobarbital and clofibrate in rats

Kazuhiro HAYAKAWA[1], Satoru HOSOKAWA[1], Toyohiko AOKI[1], Atsushi INAGAMI[1], Akira INOMATA[1], Jiro SONODA[1], Satoru MOTOOKA[1], George LOSOS[2], Kazuo TSUKIDATE[1]

Drug Safety Research Laboratories, Eisai Co., Ltd., Japan, Gifu 501-6195, Japan[1]
Asia Pacific Consulting Company[2]

There are many drugs which induce hepatomegaly. In some cases of hepatomegaly we have observed focal coagulative necrosis usually confined to focal subcapsular areas of the liver. The lesions are compatible with focal ischemic necrosis but it is difficult to confirm if these focal lesions are primary or secondary drug effects. It has been demonstrated that in experiments which require wrapping of the torso by a bandage focal ischemic liver lesions characterized by centrilobular coagulative necrosis with inflammatory cell infiltration or fibrosis, and these were generally located near capsular surface (Parker GA: Toxicol Pathol, 23, 507-512, 1995). It was postulated that some forms of physical manipulation predispose to the development of focal ischemic liver lesions in the cases of drug-induced hepatomegaly in toxicity studies. A study was conducted applying a bandage around the abdomen of rats administered either phenobarbital (PB) or clofibrate (CF) to induce hepatomegaly. Thirty male SD rats were divided into 6 groups and 100 mg/kg of PB and 300 mg/kg of CF were administered orally for 11 days to each treatment group; the groups were: non-bandage control, bandage control, non-bandage PB, bandage PB, non-bandage CF and bandage CF groups. The control group received an equivalent volume of the vehicle (corn oil). The bandage was applied during the final 4-days (4 hours in first day, and 1 hour per day for 3-days). Liver enzymes, ALT and AST, were measured and liver was weighed and all liver lobes were processed for microscopic examination. Local subcapsular ischemic liver necrosis associated with ALT/AST elevation were observed in all bandage groups including control, but the severity of the lesions was clearly greater in the PB/CF groups in which the liver weights were 1.3 to 1.5 times higher than control group. Histopathologically the liver lesions were characterized by centrilobular coagulative necrosis with inflammatory cell infiltration predominantly located in the subcapsular areas, and in severe case the necrosis extended between central veins without affecting portal areas. In less severe case, hyaline droplet in hepatocytes, possibly derived from serum protein, and centrilobular mononuclear cell infiltration were observed in subcapsular regions, and centrilobular hepatic vacuolation was also observed in some bandage PB group. These findings indicated that the liver lesions were ischemic in nature. There were no necrotic lesions in non-bandaged groups. Theses results indicate that physical manipulation of abdomen can induce focal subcapsular ischemic liver lesions in the toxicity studies of drugs that cause hepatomegaly.

P-142

Different susceptibilities of rat liver lobes in carbon tetrachloride-induced hepatotoxicity

Takeki UEHARA[1], Takashi MURAI[1], Satoshi INOUE[1], Akira TOUCHI[1], Satoru MORI[1], Toshiyuki MARUYAMA[1]

Developmental Research Laboratories, Shionogi & Co., Ltd., Osaka 561-0825, Japan[1]

It is well known that carbon tetrachloride (CCl_4) is metabolically activated with cytochrome P-450 (CYP), and the reactive intermediates cause hepatocellular damage. It is also well established CCl_4-induced hepatotoxicity is enhanced by the pretreatment with several agents that induce hepatic CYP activity. We have previously demonstrated that the differences in hepatic CYP activity among the lobes are closely correlated to the differences of hepatotoxic effect of CCl_4 among various lobes in the rat liver. In the present study, we investigated the correlation between hepatic CYP activity and CCl_4-induced hepatotoxicity enhanced by the pretreatment with two typical hepatic enzyme-inducing agents, phenobarbital (PB) or beta-naphthoflavone (NF) among the lobes.

Male Wister rats (10-11 weeks old) were allocated to 6 groups: (1) vehicle-treated alone, (2) PB-pretreated alone, (3) NF-pretreated alone, (4) CCl_4-treated alone, (5) PB-pretreated + CCl_4-treated and (6) NF-pretreated + CCl_4-treated. Animals were given a single oral administration of CCl_4 (0.1 mL/kg, dissolved in liquid paraffin) or vehicle alone following the pretreatment, intraperitoneal injection for 3 days of PB (40 mg/kg, dissolved in sterile saline) or NF (40 mg/kg, dissolved in sesame oil). One day after the CCl_4-administration, all animals were necropsied, and the liver samples were obtained from the left and the median lobes. Each sample was used for histopathological examination and 7-alkoxycoumarin O-dealkylase activities assay to evaluate the CYP activity.

PB-pretreatment significantly enhanced 7-alkoxycoumarin O-dealkylase activities as compared to the vehicle group, and induced hepatocellular hypertrophy in centrilobular region. NF-pretreatment significantly enhanced some of the enzyme activities without any histopathological change. The enzyme activities in the median lobe were higher than those in the left lobe in both the vehicle and PB groups. All CCl_4-treated animals revealed typical liver damage, such as the presence of hydropic degeneration and necrosis in centrilobular hepatocytes, and the depression of the enzyme activities. PB-pretreatment remarkably enhanced the extent of the liver damage, which was also greater in the median than in the left lobe. NF-pretreatment only slightly enhanced the extent of the liver damage.

These results indicate that different susceptibilities among the lobes in CCl_4-induced liver damage may be closely related to the heterogeneity of hepatic CYP activity.

P-143

Characterization of drug metabolic reaction involved in the acquired resistance to bromobenzene-induced hepatotoxicity

Kohji TANAKA[1], Satoko SATO[1], Isao IGARASHI[1], Naoki KIYOSAWA[1], Toshiyuki WATANABE[1], Munehiro TERANISHI[1], Sunao MANABE[1]

Medicinal Safety Research Laboratories, Sankyo Co., Ltd., Shizuoka 437-0065, Japan[1]

Previously, it was demonstrated that the initial hepatic insult caused by bromobenzene (BB) was no longer detected in rats despite subsequent dosing, indicating that the liver acquired resistance to BB-induced hepatotoxicity. Changes in drug metabolic reaction including phase I (CYP), phase II (GST) and phase III (drug transporters) contributed to the resistance with the greatest contribution considered to be from an increased phase II response. In this study, changes in drug metabolic reactions were characterized by hepatic localization of the proteins using immunohistochemistry. The experimental design used in a previous study, in which rats had acquired resistance to BB-induced hepatotoxicity (The Japanese Society of Toxicological Pathology, 17th annual meeting, 2001: The Japanese Society of Toxicology, 30th annual meeting, 2003) was employed. Briefly, F344 rats were treated intraperitoneally with BB (225 mg/kg). At 24 hr post-dose, hepatic injury was confirmed by measuring AST values in the blood obtained from the tail vein, and then dosing was continued at the same dose for an additional 8 days. Immunohistochemical staining using antibodies was conducted in the liver on Days 4 and 8. As a result, positive staining for CYP3A and 2E antibodies was observed in the central zone of the hepatic lobule on both days. However, the staining intensity of CYP3A and 2E was weaker in the whole hepatic lobule of the treatment groups than in that of the vehicle-treated controls. Regarding GST antibodies, strong intensity of GST staining was observed in the central-to-mid zonal hepatocytes. There were no remarkable differences in the intensity between Days 4 and 8. Decreased CYP 3A and 2E, increased GST and multidrug-resistance protein (MRP) 3 were observed as changes in protein content and mRNA levels. Thus, the results in this study corresponded with the changes in protein content and mRNA levels of CYP and GST. In conclusion, GST induction was observed centrilobularly, but CYP inhibition was not observed in a specific zone of the hepatic lobule. Presently, changes in protein content and immunohistochemical localization of drug transporters involved in the phase III response are being examined.

P-144

Depletion of tumor-infiltrating macrophages is associated with amphoterin expression in colon cancer

Tomonori SASAHIRA[1], Takamitsu SASAKI[1], Hiroki KUNIYASU[1]

Department of Oncological Pathology, Nara Medical University Cancer Center, Nara 634-8521, Japan[1]

Macrophage infiltration into colon cancer and amphoterin expression in cancer cells was examined in 42 subserosa-invading human colon cancers. The mean number of infiltrating macrophages was significantly higher in Dukes' B cases than that in Dukes' C cases ($p=0.0065$). Tumors with few infiltrating macrophages (macrophage depletion) were significantly more frequent in Dukes' C cases than that in Dukes' B cases ($p=0.0014$). No Dukes' C cases with relevant macrophage infiltration showed macrophage-cancer cell contact, whereas 5 Dukes' B cases showed such contact ($p<0.0001$). In human colon cancer cells implanted in the cecum of nude mice, KM12SM (high metastatic) tumors yielded less macrophage infiltration and more liver metastases than were yielded by KM12C (low metastatic) tumors (14 ± 3 vs. 78 ± 32 and 24 ± 6 vs. 5 ± 3 per liver, respectively). Amphoterin expression was detected at high frequency in both Dukes' B and C cases ($p=0.0684$). In macrophage-depleted cases, frequency of amphoterin expression was significantly higher than that in non-depleted cases ($p=0.0015$). To confirm biological effects of amphoterin on macrophages, cell-seeded Boyden-chamber assay was done. Infiltration of PMA-treated U937 monocytes through the KM12SM cell-layer was increased by pretreatment of KM12SM cells with amphoterin antisense S-oligodeoxynucleotides exposure. Moreover, extracted amphoterin inhibited PMA-U937 monocytes infiltration in a dose-dependent manner. Thus, amphoterin may play an important role in the inhibition of macrophage infiltration into colon cancer.

P-145

A new medium-term bioassay system for the detection of colon carcinogenesis modifiers

Young-Man CHO[1], Jun-ichi ONOSE[1], Toshio IMAI[1], Mai HASUMURA[1], Makoto UEDA[1], Masao HIROSE[1]

Division of Pathology, National Institute of Health Sciences, Tokyo 158-8501, Japan[1]

Bioassay systems utilizing aberrant crypt foci (ACF) as a presumable preneoplastic marker have been widely used for the detection of carcinogens/modifiers for colon carcinogenesis within a short period in rats. However, it was documented that in number of cases, ACF do not correlate with tumorigenic potential of test chemicals. Furthermore, some compound which reduced the ACF induction, have been found to enhance the development of colon cancers. In these circumstances, we recently established a rapid colorectal 2-stage carcinogenesis model in rats initiated with 1,2-dimethylhydrazine (DMH) followed by dextran sodium sulfate (DSS), applying neoplastic lesions as endpoint markers. In the present study, we validated this new model using known colon carcinogenesis modifiers. F344 male rats were given three s.c. injections of DMH (40 mg /kg b.w.) in a week and administered drinking water containing 1% DSS ad libitum for a week after the initiation period. Chemicals used were 0.3% deoxycholic acid (DCA) as a promoter, 1.5% 1-hydroxyanthraquinone (1-HA) as a carcinogen, and 0.04% nimensulide and 2% lactoferrin as inhibitors of carcinogenesis. They were dietary administered from 3rd week to 20th week of the experiment. Animals were killed at weeks 10 and 20. At the 10th week of the experiment, the number of ACF in the control group was 20.7 ± 4.5/rat, and it was significantly ($p<0.01$) decreased and increased in the group treated with nimensulide (13.7 ± 3.6) and DCA (31.1 ± 11.2), respectively, but lactoferrin (19.1 ± 6.18) and 1-HA (24.1 ± 26.4) showed no effect. Histopathologically, dysplastic foci, adenomas and adenocarcinomas were observed in all groups. At week 10, in 1-HA group, the incidence of adenomas and the multiplicity of both dysplastic foci and adenomas were significantly ($p<0.05$ or 0.01) increased, while in nimensulide and lactoferrin groups, the incidence and combined multiplicity of adenomas adenocarcinomas were significantly ($p<0.05$ or 0.01) decreased as compared to controls. At week 20, both the incidences and multiplicities of the proliferative lesions obviously increased in DCA and 1-HA groups and decreased in nimensulide and lactoferrin groups. Particularly the multiplicities of adenomas and adenocarcinomas were significantly ($p<0.01$) increased in 1-HA group, while the incidence and multiplicity of adenocarcinomas were decreased in nimensulide group ($p<0.01$) as compared with control. It is concluded that this medium-term bioassay system is useful for the detection of promoters and inhibitors of colorectal carcinogenesis.

P-146

Renal damage in newborn rats treated with p-Cumylphenol

Yuko YAMAGUCHI[1], Nobuo NISHIMURA[2], Megumi YAHATA[2], Hiroshi EDAMOTO[1], Shinichiro IKEZAKI[1], Kazutoshi TAMURA[1], Eiichi KAMATA[4], Makoto EMA[4], Ryuichi HASEGAWA[3]

Division of Pathology, Gotemba Laboratory, Bozo Research Center Inc., Shizuoka 412-0039, Japan[1]
Division of The Second Laboratory for Safety Evaluation, Gotemba Laboratory, Bozo Reseach Center Inc.[2]
Division of Medicinal Safety Sciences, National Institute of Health Sciences[3]
Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences[4]

To explore renal toxicity of p-Cumylphenol (PCP) in newborn rats [Crj:CD(SD)IGS], single and repeated dosing studies were conducted. In the single dosing study a total of 50 newborn rats, 4-days old, were given doses of 0, 300 and 600mg/kg/day, and were terminated at 1, 3 and 7 days after treatment. Seven males and 3 females at 600mg/kg died by 24 hours after administration. These animals showed tubular damage including slight dilatation of collecting ducts and renal tubules, and slight necrosis of papillary ducts and neutrophilic infiltration in the tip of papilla. Similar changes were observed on days 1 and 3 in each treated group, while no animals showed histological change on day 7 in any group.
In the repeated dosing study a total of 50 rats were given doses of 300mg/kg/day from 4 days to 21 days after birth, and were terminated on days 4, 8, 15 and 18, and 7 days after withdrawal. On days 4 and 8, slight dilated and hyperplastic collecting ducts were frequently observed in medulla, prominently in inner medulla, and also neutrophils infiltrated in the tip of papilla. On days 15 and 18, marked cystic dilated ducts were mainly observed in outer medulla, tending to be progressive with time. These cystic dilated ducts were lined by hyperplastic epithelial cells and occasionally contained exfoliated epithelial cells. The collecting ducts in papilla were dilated, but not with cystic formation or hyperplastic epithelial cells. Focal neutrophilic infiltration in the tip of papilla was also noted in some animals on day 15. Seven days after withdrawal, although the dilated ducts in outer medulla were still persistent, they were slightly reduced in size with decrease of epithelial cell density. However, in cortex, basophilic tubules with lymphoid infiltration appeared multifocally. Similar change was noted in our previous study, in which the lesions existed at 9 weeks after withdrawal when cystic dilated ducts were mostly diminished.
In conclusion, a single high dose of PCP induced papillary damage, and it may not be related to form polycystic lesions. Predilection site of polycystic lesions was a collecting duct in outer medulla, and the lesions may be primarily associated with proliferation of epithelial cells, although pathogenesis of cystic lesions including a hyperplastic change and after-withdrawal changes in cortex are still undetermined.

P-147

A photographic spectrum of non-neoplastic renal tubule and transitional epithelial structures within selected renal neoplasm of the rat

John Curtis SEELY[1], Gordon C HARD[2]

Experimental Pathology Laboratories, Inc., Research Triangle Park, North Carolina 27709, USA[1]
Consultant in Toxicology, Pathology, Carcinogenesis.[2]

The identification and characterization of non-neoplastic renal tubule and transitional epithelial structures are important in the diagnosis of several tumors of the rat kindly;i.e.,renal mesenchymal tumor (RMT) and nephroblastoma(NB). Controversy still exists over the interpretation of these structures. Some pathologist consider these structures to represent pre-existing and entrapped renal tubules or transitional epithelium which have undergone hyperplastic and/or metaplastic (embryonal) transformation. Other pathologists consider these structures to represent a neoplastic component of the tumor. Indeed, these are some tumors in which these structures represent a challenging and often puzzling dilemma for the pathologist. We present a number of microphotographs which illustrate the spectrum of changes that we encountered during a review of large database of RMT and NB. Our diagnostic criteria contend that these structures represent a hyperplastic to metaplastic transformation. Although the mechanism for these changes are not known, the unique relationship during renal development between the primitive metanephric mesenchyme and the epithelial structures derived from the ureteric bud might support a hypothesis of a 'genetic component' or the presence of 'tumor factors' which could account for the spectrum of changes observed within these neoplasms.

P-148

Effect of water deprivation or furosemide on cefalotin and glycerol-induced proximal tubular toxicity in rats

Miyoko OKADA[1], Naoki OHYAMA[1], Shun-ichiro ISHII[1], Naoya MASUTOMI[1], Fumiko SANO[1], Jiro SUGIMOTO[1], Shiro TAKAGI[1]

Toxicology Laboratory, Mitsubishi Pharma Corporation, Chiba 292-0818, Japan[1]

In combination with cephalosporin antibiotics and glycerol are known to cause acute renal failure such as proximal tubular necrosis. Dehydration has been reported to be an important risk factor to induce acute renal failure. In this study, we investigated the effect of water deprivation or a diuretic drug, furosemide, on cefalotin and glycerol-induced proximal tubular toxicity in rats. Experiments were performed in six groups using male CD(SD)IGS rats aged between 6- and 11-week-old; Group 1: physiological saline was injected intravenously (i.v.) as controls, Group 2: cefalotin sodium (CET) was injected i.v. at a dose of 3000 mg/kg, Group 3: glycerol (GLY) was injected subcutaneously (s.c.) at a dose of 1000 mg/kg, Group 4: furosemide (FUR) was injected s.c. at doses of 20, 50, 100 or 200 mg/kg, Group 5: CET (2000 or 3000 mg/kg) were concomitantly treated with GLY (1000 mg/kg), Group 6: FUR (20, 50, 100 or 200 mg/kg) were combined with CET (2000 mg/kg) and GLY (1000 mg/kg). All administrations were performed at once on the same day. All groups, except Group 6, had a water-supplied or -deprived subgroups. Animals in the water-deprived condition were deprived of water 24 hours before administration to the sacrifice. After 24 hours of the final administration, all animals were killed under anesthesia and the kidneys were examined histopathologically. In Group 1, no treatment-related change was observed in the water-supplied or -deprived rats. In Groups 2 and 3, slight degeneration/necrosis of proximal tubular (PT) epithelium was noted in the water-deprived rats while no treatment-related change was seen in the water-supplied rats. In Group 4, slight PT damages were observed in the water-supplied rats at 200 mg/kg of FUR and slight to moderate changes were seen in the water-deprived rats at all dose groups of FUR. In Group 5, slight PT degeneration/necrosis was noted in the water-supplied rats and slight to severe changes were observed in the water-deprived rats. In Group 6, slight to severe renal damages were noted in rats at all dose groups of FUR. It is concluded that the water-deprived condition or FUR-treatment enhances the PT damage induced by CET or GLY alone, or the combination of CET and GLY.

P-149

Pathological observation of karyomegalic cells in the kidney on male rats treated with Paecilomyces japonica

Yong-Bum KIM[1], Chang-Su HA[1], Hwa-Young SON[2], Sung-Whan CHO[2], Boo-Hyon KANG[1]

Korea Institute of Toxicology, KRICT, Yuseong, Daejeon 305-600, Korea (South)[1]
College of Veterinary Medicine, Chungnam National University[2]

Background. Paecilomyces japonica(P. japonica) is a fungus used as a traditional medicine in China, Japan and Korea. We recently observed that P. japonica induced renal failure with karyomegaly. The purpose of this study was to investigate the pathogenesis of karyomegaly induced by P. japonica and the possibility as a progenitor of kidney tumors.
Methods. 40 male SD rats, 5 weeks old, were orally treated with P. japonica at dose levels of 0, 250, 500 or 1000 mg/kg/day for 5 weeks. Kidneys were observed with light and electron microscopy. Immunohistochemical detection of proliferating cell nuclear antigen(PCNA) and bromodeoxyuridine(BrdU) were performed to assess renal epithelium proliferation. To detect apoptosis, TUNEL method was adopted.
Results. Karyomegaly was observed in the outer stripe of outer medulla in all treatment groups. Karyomegalic cells contained enlarged, irregularly shaped nuclei, showed invaginations of nuclear membrane and formed apparent nuclear inclusions. The number of mitotic cells significantly increased in all treatment groups (15,6, 24,1 and 5,3) compared with control group (0.2). The number of PCNA(5.4, 8.8 and 5.2 times of control) and BrdU (2.9, 3.1 and 2.5 times of control)-positive cells significantly increased in all treatment groups, but the cells with karyomegalic nuclei had a lower rate of positivity on both BrdU and PCNA reaction. TUNEL-positive cells significantly increased in all treatment groups (13, 17 and 22.7) compared with control group (1.7). Karyomegalic cells with inclusions showed predominantly positive immunoreaction with the TUNEL method.
Conclusions. The karyomegalic renal tubular cells underwent neither abnormal cell division nor high proliferation rates, in spite of sharing enlarged nuclei of tumor -like morphology. The possibility of a renal tumor progenitor from karyomegaly was considered to be low.

P-150

Long-term observation of anti-Thy-1 glomerulonephritis in uninephrectomized rats: Relationship between morphological changes and renal function

Shunji NAKATSUJI[1], Kenjiro TSUBOTA[1], Yoshimasa OKAZAKI[1], Masahiro MATSUMOTO[1], Shiro FUJIHIRA[1], Yuji OISHI[1], Masayuki TOMITA[2], Hajime SOGABE[2], Shoko NAKAZATO[2], Hiroshi KAWACHI[3], Fujio SHIMIZU[3]

Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka 532-8514, Japan[1]
Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.[2]
Institute of Nephrology, Niigata University Graduate School of Medical and Dental Sciences[3]

A progressive renal injury accompanied by continuous proteinurea is induced by administration of anti-Thy-1 antibody to unilaterally nephrectomized rats; however, the mechanism of progression to end-stage kidney has not fully understood. In the present study, we investigated this progressive renal injury in uninephrectomized rats for 47 weeks, and discussed the relationship between morphological changes and renal functional parameters.

Methods. A progressive glomerulonephritis was induced in unilaterally nephrectomized Wistar rats (8 weeks of age) with a single injection of anti-Thy-1 antibody (1 mg). Animals were sacrificed on Day 2 and Weeks 1, 2, 4, 6 and 8, then every 4 weeks by 47 weeks after the injection of the antibody. Urine and blood samples were collected to determine proteinurea, blood urea nitrogen (BUN) and serum creatinine concentrations. Kidneys were processed for light and electron microscopy according to standard procedures. Paraffin sections were stained with hematoxylin-eosin and periodic acid-Schiff (PAS), and immunohistochemistry was performed using anti-desmin, anti-alpha-smooth muscle actin and anti-vimentin antibodies.

Results. Initial stage (Day 2 to Week 8 after the injection): Urinary protein excretion peaked on Week 1 and then decreased gradually, but it began to increase again after 4 weeks. Histologically, glomerular mesangial cell proliferation and matrix expansion were observed on Week 1, followed by interstitial inflammation and tubular regeneration by Week 8. Glomerular alpha-smooth muscle actin expression was strongly appeared on week 1 and then decreased thereafter. In contrast, glomerular desmin expression was noted from Weeks 2 to 8. Later stage (Weeks 12 to 47 after the injection): Progressive proteinurea was noted during the observation period until 47 weeks, and high levels of BUN and serum creatinine were maintained from Weeks 12 to 47. After 24 weeks, tubulointerstitial lesions including interstitial fibrosis and tubular atrophy was remarkably appeared in addition to glomerular screrotic changes.

Conclusion. These data suggest that the renal functional changes in the early stage in this model are associated with the morphological changes of glomerular epitherial and mesangial cells, and that the tubulointerstitial lesions are closely involved in the deterioration of renal function in the later stage.

P-151

Spontaneous glomerulonephritis in cynomolgus monkeys

Xiuying YANG[1], Shigeru SATAKE[1], Yasuhiro KAMIMURA[1], Kimiaki HIRAKAWA[1], Hiroshi MAEDA[1], Hiroaki MIYAJIMA[1]

Shin Nippon Biomedical Laboratories, Ltd., Kagoshima 891-1394, Japan[1]

[Background] Spontaneously occurring glomerulonephritis, to our knowledge, is very rare in non-human primates and has not been documented in cynomolgus monkeys. We present 7 cases of glomerulonephritis in cynomolgus monkeys that we encountered during safety studies.
[Materials and Methods] The kidneys of 7 cynomolgus monkeys aged 4-6 years old were collected from the control groups of repeated studies. They were sectioned routinely and stained with H.E., PAM, PAS and Masson trichrome. Immunohistochemical staining was performed with IgG. Sections for electron microscopic examination were prepared from paraffin-embedded specimens.
[Results] Histologically, the glomeruli were characterized by expansion of the mesangial areas with hypercellularity and increased mesangial matrix. Except for 1 case, there were no remarkable changes in the tubules or intersitium. Immunohistochemically, granular IgG deposition was confirmed in 2 cases. Increased mesangial cells and matrix, infiltration of the macrophages in the mesangial areas, electron dense deposits in the mesangial and paramesangial areas were confirmed by electron microscopy.
[Conclusion] As in humans, immune-mediated glomerulonephritis can spontaneously occur in cynomolgus monkeys, which have histological, immunohistologial and electron microscopic features that are similar to humans. Since monkeys used in safety studies are usually very young, the occurrence of the disease is considered rare. This accounts for the infrequent documentation of the disease.

P-152

Spontaneous collagen glomerulopathy in a cynomolgus monkey

Kae FUJISAWA[1], Nobuo TAKASU[1], Noriko TSUCHIYA[1], Shuuichi MATSUSHIMA[1], Haruhisa INAGAKI[2], Mikinori TORII[1]

Developmental Research Laboratories, Shionogi & Co., Ltd., Osaka 561-0825, Japan[1]
Discovery Research Laboratories, Shionogi & Co., Ltd.[2]

Collagen glomerulopathy is one of rare glomerular lesions and has been known in human, however, it has not been reported in monkey to our knowledge. We report here light microscopic and electron microscopic findings in a young male cynomolgus monkey that was diagnosed as collagen glomerulopathy by histopathologic examination.
An intact male cynomolgus monkey showed mild proteinuria and hypoproteinemia at 4 years and 5 months of age and was necropsied under anesthesia with sodium pentobarbital. Kidneys were fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin. Paraffin section was stained with hematoxylin and eosin, Mallory azan, Congo-red, Periodic Acid-Schiff reagent (PAS) and anti-type III collagen antibody for immunohistochemistry. Electron microscopic examination was routinely carried out using a piece of formalin-fixed kidney tissue. Kidneys were macroscopically pale and granular on the surface. Histopathology revealed diffuse glomerular swelling accompanying with deposition on eosinophilic amorphous material in the glomerular basement membrane and mesangial area. Amorphous material was negative for amyloid and PAS staining, but was positive for collagen fiber by azan staining.
Immunohistochemistry revealed strongly positive reaction with anti-type III collagen. Mesangial area was widened, but there was no increase in number of mesangial cells. There was no remarkable lesion in the efferent/afferent artery, small or medium-sized artery. Mild tubular degeneration was only observed in the cortex, occasionally with cholesterin crystal and interstitial lymphocytic infiltration. Electron microscopy revealed various-sized collagen fibrils with periodicity in the glomerular basement membrane and mesangial area.
These findings draw a conclusion of spontaneous collagen glomerulopathy in a young cynomolgus monkey.

P-153

Expression of somatostatin mRNA and peptides in C-cell tumours of the thyroid gland in Han Wistar rats

Andrew PILLING[1], Stewart JONES[2], John TURTON[3]

Huntingdon Life Sciences, Huntingdon PE28 4HS, UK[1]
GlaxoSmithKline[2]
School of Pharmacy, University of London[3]

C-cell tumours are among the most common spontaneous neoplasms of the laboratory rat. With the exception of calcitonin, little attention has been paid to the secretory peptides of C-cells and their possible role in the development of neoplasia. Of these peptides, somatostatin (SS) is of particular interest because it has been shown to regulate the growth and secretion of thyroid follicular cells in vitro. In the present study, in situ hybridization and immunohistochemistry were used to investigate the expression of SS mRNA and SS peptides, in both normal C-cells and a range of spontaneous proliferative C-cell lesions in the Han Wistar rat. It was confirmed that a small minority of C-cells in the normal rat thyroid gland produce and store SS peptides, however approximately half of all C-cell adenomas and C-cell carcinomas carcinomas stained positively for SS mRNA and peptides. SS expression was also observed in all metastatic deposits of carcinomas in drainage lymph nodes. The discrepancy in the extent of SS expression between the normal and neoplastic C-cell population can possibly be attributed to the lack of differentiation of the neoplastic cells, with the assumption of a more primitive phenotype that is capable of producing alternative peptides. In addition, the mean percentage of cells that stained positively for SS mRNA and peptides was significantly higher in smaller (4mm) suggesting that the presence of SS was exerting a growth controlling influence on these lesions.

P-154

A spontaneous dwarf mutation in BrlHan:WIST@Jcl (GALAS) rat - Histopathological characteristics of the endocrine system

Takuya DOI[1], Masato NAMIKI[1], Michiko ASHINA[1], Naoto TOYOTA[1], Hiroko KOKOSHIMA[1], Yuki TOMONARI[1], Junko SATO[1], Takeshi KANNO[1], Yumi WAKO[1], Minoru TSUCHITANI[1]

Mitsubishi Chemical Safety Institute Ltd., Ibaraki 314-0255, Japan[1]

We found a spontaneous dwarf mutation in BrlHan:WIST@Jcl(GALAS) rats. This report describes the histopathological characteristics of the endocrine system in the affected animals.
[Background] Two males produced from externally normal parent BrlHan:WIST@Jcl(GALAS) rats, purchased from Clea Japan Inc. (Shizuoka, Japan), showed external abnormality (small body, delicate fur, micrognathia, brachyury, and exophthaloms). One of them was successfully mated with a normal littermate, and dwarf and normal rats were produced. Presently, we know that the dwarf animal was produced by brother-sister mating between normal littermates.
[Material & methods] Eight- and forty-five-week-old dwarf (D) rats, 45-week-old normal littermates (NL), and eight- and forty-five-week-old normal (N) rats purchased from Clea Japan Inc. (Shizuoka, Japan) were used in the present study. The thyroid, parathyroid, pituitary, adrenal and pancreas were removed, fixed in 10% neutral phosphate-buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for microscopic examination.
[Result] Body weights of the D rats were markedly lighter than those of the same age NL and N rats. Macroscopically, an increased size of the thyroid in the D and NL rats, and discoloration (grayish) of the pituitary anterior lobe were observed in the D rats. Microscopically, the following changes were observed. **Thyroid:** Little colloid formation and decreased follicular size in the D rats. Vacuolar change in thyroid follicular cells in the D and NL rats. **Pituitary:** Few acidophilic cells in the D rats. **Adrenal:** Marked lipofuscin deposition in 45-week-old D rats only. **Parathyroid and pancreas:** No remarkable change.
[Discussion] Little colloid formation and decreased follicular size in the thyroid of the D rats indicated that they had been affected by hypothyroidism. The *rdw* rat was reported by Koto et al., which is a hereditary dwarf model caused by hypothyroidism derived from Wistar-imamichi strain (Experimental Animals 37: 21-30, 1988). The vacuolar change in thyroid follicular cells in the D rats was similar to the change caused by retention of thyroglobulin in the dilated rER in the *rdw* rat (Anatomical Record 259: 60-66, 2000). Vacuolar change, however, is occasionally observed in BrlHan:WIST@Jcl(GALAS) rat, and is not accompanied with hormonal abnormality (Journal of Toxicologic Pathology 14: 253-257, 2001). In the present study, vacuolar change was also observed in the NL rats. Hence, detailed analysis is needed to determine whether or not the vacuolar change in the D rats is related to hypothyroidism.

P-155

Association of adrenal pheochromocytoma and lung pathology in inhalation studies with particulate compounds in the male F344 rat--the National Toxicology Program experience.

Keisuke OZAKI[1], Joseph K. HASEMAN[2], James R. HAILEY[3], Robert R. MARONPOT[3], Abraham NYSKA[3]

Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Osaka 554-8558, Japan[1]
Biostatistics Branch, National Institute of Environmental Health Sciences (NIEHS)[2]
Laboratory of Experiment Patholgy, National Institute of Environmental Health Sciences (NIEHS)[3]

Systemic hypoxemia, occurring in space-occupying lung pathologies such as inflammation and neoplasms. reduces the gas exchange area and stimulates catecholamine secretion from the adrenal medulla where chronic endocrine hyperactivity may lead to hyperplasia and neoplasia. We investigated the possible correlation between nonneoplastic chronic pulmonary lesions and adrenal pheochromocytoma in 9 recent, NTP, 2-year particulate inhalation studies in male F344 rats. Re-evaluation for chronic active inflammation, interstitial fibrosis, alveolar epithelial hyperplasia, squamous metaplasia, proteinosis, and histiocytosis revealed significant associations of pheochromocytoma only with the severity of inflammation and fibrosis. Nickel oxide, cobalt sulfate, indium phosphide, talc, and nickel subsulfide studies showed chemical-related incidences of adrenal pheochromocytoma and significant ($p < 0.01$) associations with inflammation and fibrosis. Gallium arsenide, vanadium pentoxide, molybdenum trioxide, and nickel sulfate hexahydrate studies revealed an increased incidence and/or severity of nonneoplastic lung lesions, but no increased incidence of pheochromocytoma. Although gallium arsenide and molybdenum trioxide showed no dose-related increase in pheochromocvtoma, a significant ($p < 0.01$) correlation of the latter with the severity of fibrosis and inflammation occurred. In the vanadium pentoxide and nickel sulfate hexahydrate studies, no relationship between nonneoplastic lung lesions and pheochromocytoma was manifested. Our investigation assessed the strength of these various associations and supports the possible roles of 2 chronic pulmonary lesions-fibrosis and inflammation-and hypoxemia in the induction of pheochromocytoma in the F344 male rat.

P-156

Renal toxicity induced by folic acid is directly responsible for the enhancement of male reproductive toxicity of di (n-butyl) phthalate in male rats

Toshio ICHIHARA[1], Tomoko TSUTSUMI[1], Mayumi KAWABE[1], Hiroko YOSHINO[1], Makoto ASAMOTO[2], Syugo SUZUKI[2], Tomoyuki SHIRAI[2]

Daiyu-kai Institute of Medical Science, Aichi 491-0113, Japan[1]
Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences[2]

Di (n-butyl) phthalate (DBP) is a phthalate ester with extensive industrial use in plastic piping, various varnishes and lacquers, safety glass, nail polishes, paper coatings, dental materials pharmaceuticals, and plastic food wrapping, there is a high potential for human exposure in work place and home environment. DBP has been shown to possess antiandrogenic action, which results in testicular atrophy (seminiferous degeneration, Leydig cell hyperplasia and adenomas) and blocks male reproductive development. The pharmacologic actions of chemicals may be modified, strengthened or diminished, under certain pathologic conditions. For example, decreased hepatic or renal function can influence chemical activation / detoxification or excretion resulting in toxicity of a given chemical. Usually, bioassay for toxicity of chemicals are carried out using health experimental animals and there is always a question as to how observed toxicities would be modified under pathologic conditions such as lowered liver or renal functions. This type of data might be very important for risk evaluation and risk management for human health. In this study, we investigated whether testicular toxicity of DBP is influenced by diminished renal function. To generate an experimental condition reflecting chronic renal disease in man, six week old male F344 rats were given 5 consecutive weekly subcutaneous injections of folic acid at a dose of 300 mg/kg and then the diet containing 1200, 5000 and 20000 ppm of DBP for 4 weeks. These concentrations roughly correspond to 60, 250 and 1000 mg/kg/day/rat, respectively. Urine volume, specific gravity and osmotic pressure were also measured as markers of renal function. All animals were killed under anesthesia at experimental week 9 and blood samples were collected from the aorta for measurement of serum creatinine and BUN. And spermatogenesis (account of sperm, sperm mobility and morphological abnormality) were investigated. The liver, kidneys, testes, epididymis and prostate of each rat were weighed after removal. Folic acid clearly induced interstitial nephritis accompanied by impairment of renal function. Enhanced testicular toxicity with seminiferous degeneration, diminished spermatogenesis and increase in the number of morphologically abnormal sperm was evident in rats given folic acid and then 20000 ppm DBP as compared to those given DBP alone. The data suggest that DBP male reproductive toxicity can be increased by renal dysfunction. We thank Dr. Shibutani, Division of Pathology, National Institute of Health Sciences, Tokyo, for his kind comments and discussion for this study.

P-157

Motional and pathological analysis for rat testicular toxicity induced by boric acid

Masakazu KAKUNI[1], Naoya KIMOTO[1], Tsuyoshi TAKEDA[1], Masato MIZUTANI[1], Kazuo SUZUKI[1], Hitoshi SATO[1], Katsumi TAKABA[1], Takuji HARA[1]

Toxicological Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Yamaguchi 755-8501, Japan[1]

Boric acid is known to induce the male reproductive toxicity. We had some supportive data of the male reproductive toxicity induced by boric acid with motional and pathological analysis. In the study boric acid was administered daily by oral gavage to male Crj:CD(SD)IGS rats of 6 wks old at dose level of 500 mg/kg for 4 weeks. In necropsy, testes and epididymidis were macroscopically atrophic, and the weights of these organs were significantly decreased as compared with control group. Motional analysis, which evaluated Percentage of motile sperm, Curvilinear velocity, Linearity, Amplitude of lateral head displacement (A.L.H.) max, Amplitude of lateral head displacement (A.L.H.) mean and Beat cross frequency, revealed that significant decrease was observed in Percentage of motile sperm, Linearity and Beat cross frequency. A further histopathological examination is in progress.

P-158

Early ultrastructural changes of the Sertoli cells with vacuole formations

Yoshiaki SAITO[1], Kenji USUMI[1], Noriko OSAWA[1], Tomoko NAGATA[1]

Food and Drug Safety Center, Hatano Research Institute, Kanagawa 257-8523, Japan[1]

Among histological alterations of the testis caused by various chemicals in the toxicity studies, we frequently observed vacuoles in the germ cell linings as the first noticeable changes and recognized later as scattered groups of degeneration and/or depletion of germ cells that assumed to originate from Sertoli cell degeneration. To investigate early ultrastructural changes on the Sertoli cells that accompany with vacuoles as well as grouped depletion and/or degeneration of germ cells under light microscopy, we chose three chemicals as follows; di-(2- ethylhexyl)phthalate (A), (-)-hydroxycitric acid-calcium salt (B) and aminophenylnorharman (C). Rats of Sprague-Dawley strain (Crj:CD SPF) were purchased and used for experiments at the age of 10-11 weeks. After a single oral administration of 2800 mg/kg of A, rats were anesthetized with sodium pentobarbital at 3, 6, 24 and 48 hours after dosing. Rats treated with single oral dosing of 100 or 200 mg/kg of C were anesthetized at 1, 2, 3, 4 or 5 days after dosing and for B, 0.13, 0.66 or 3.31% containing powder diet (CE-2, CLEA Japan) were given for 28 days and were anesthetized. The testes of all animals were fixed by perfusion via aorta with fixatives, postfixed with osmium tetroxide and embedded in epoxy resin and subjected to the light and electron microscopic observations. Under light microscopy, vacuoles were observed 6 hours after administration of A, 2 days after treatment of 200 mg/kg group and 4 days after treatment of 100 mg/kg groups of C and 28 days treatment of B. By electron microscopy, dilation of the rough endoplasmic reticulum (ER) associated with ectoplasmic specialization which consisted of the blood-testicular barrier was observed in animals treated with A, dilation of extracellular space as well as fragmentation of the cell membrane were observed in B and dilation of smooth ER of the Sertoli cells were recognized in C. From these results, vacuolar formation in the Sertoli cells may possibly be caused by different mechanisms among these chemicals, though the light microscopy showed similar morphological changes. Thus, it is important to examine early ultrastructural changes of the Sertoli cells to clarify pathogenesis of the testicular toxicity.

P-159

International advancement in education in veterinary & comparative pathology

D. Reid PATTERSON[1]

Reid Patterson Consulting, Inc., Grayslake 60030, USA[1]

With over 3,000 member-pathologists in more than 80 countries around the world, the C. L. Davis, D.V.M. Foundation has become the most effective educational institution in veterinary and comparative pathology in the world. Founded in 1970 by an endowment from the family of famed American pathologist and educator, Charles Louis Davis, this charitable, not-for-profit corporation has recruited over 100 renowned educators and researchers in pathology from Canada, Europe, the United Kingdom and the United States. This Faculty of Discussants has produced hundreds of video-recorded lectures for its members and institutions and personally present at 12 highly acclaimed annual symposia, seminars and workshops in Africa, Europe and the U.S. Topics range from toxicological pathology, to avian, wildlife, domestic and companion animal, and comparative pathology, plus unique lectures in mutant animal models, laboratory animal medicine, molecular pathology , medical photography and pharmaceutical safety assessment. Presentations accompany those organized such as the American College of Veterinary Pathologists, the Society of Toxicologic Pathology, the European Society of Veterinary Pathology, the Royal College of Pathologists, the American Association of Zoo Veterinarians, and the Armed Forces Institute of Pathology, or are hosted independently. To advance knowledge in zoo and wildlife species, the Foundation established a decade ago a pathology research program, in collaboration with the American Association for Zoo Veterinarians. The Foundation has the largest collection of pathology specimens of any single organization, enabling an extensive circulation of study sets and the establishment of 6 independent study centers within the U.S. With a mission to advance the study of the diseases of animals and the comparison of diseases manifested by diverse species of animals, including humans, the Foundation strives to improve the health and well being of animals and humans throughout the world. To realize this mission, the Foundation seeks to share its wealth of information to all 404 veterinary schools in the world, to to sponsor externship programs that have already benefited 46 international veterinary students and to recognize the exceptional teaching and/or scholarship of over 550 international veterinarians. This poster is offered to understand interest within Asia for participation in this unique educational program. The Foundation is presently offering seminars within Eastern Europe and South America. A recent Foundation conference in Vietnam was well received. The Foundation is excited about sharing with scholars in Asia.

P-160

Histopathology, immunocytochemistry and electron microscopy of spontaneous uveal melanoma in two HAN Wistar rats

Alexandra MORAN[1], Christopher BARTON[1]

Pathology Department, Covance Laboratories, Harrogate HG3 1PY, UK[1]

Uveal melanoma in rats are unilateral neuroectodermal tumours derived from uveal melanocytes. They occur in numerous species of animals, including laboratory animals, and are most frequently recorded in cats. Here, we describe the histopathologic, immunocytochemical and electron microscopic characteristics of uveal melanomas in two HAN Wistar rats.

The tumours occurred in two, one year old HAN Wistar rats that were involved in a long term drug study. Fresh tissues were routinely fixed and processed. Paraffin embedded sections were stained with haematoxylin and eosin. Additional sections were immunohistochemically examined for reactivity to Vimentin, S100, Neuron Specific Enolase (NSE), Melan A, Ki-67 and Proliferating Cell Nuclear Antibody (PCNA). In addition, sections were also processed for Transmission Electron Microscopy.

Histologic examination in each case revealed an unencapsulated, infiltrative, densely cellular mass composed of sheets and nests of cells supported by a fine fibrovascular stroma. The tumour cells were round to spindloid, with variably distinct cell borders. Occasional cells contained a dark brown granular pigment within their cytoplasm. The tumour cells were positive for Vimentin, S100, NSE, and Melan A. Cell proliferation was apparent with both Ki-67 and PCNA.

Using histopathology, immunohistochemistry and electron microscopy, these tumours were conclusively diagnosed as malignant uveal melonomas. These are relatively rare tumours in laboratory rodents and to our knowledge this is the first report combining these techniques in the diagnosis of this tumour in HAN Wistar rats.

P-161

Case report: naturally occuring proliferative vitreoretinopathy with multiple ocular alterations in a rabbit

Osamu KATSUTA[1], Takeshi YAMAGUCHI[1], Kenji TAKASE[1]

Drug Safety/Drug Metabolism Group, Research and Development Center, Santen Pharmaceutical Co., Ltd., Nara 630-0101, Japan[1]

One of the most serious complications of retinal detachment is proliferative vitreoretinopathy (PVR). The pathogenesis of the disease is explained in humans as follows: After retinal detachment, the cells derived from the retinal pigmental epithelium infiltrate in the vitreous cavity through a retinal hole and retinal glial cells grow through the internal limiting membrane. These cells make preretinal proliferative membranes. The membrane exerts a contraction on the retina, resulting in the retinal folding and its entire detachment. This disease often occurs in failures in retinal surgery. Recently, we came across a young rabbit with naturally occuring PVR. Now, we describe the pathological features of this case.

Animal: A six-week-old male Japanese white rabbit (Kbl: JW) (Conv.) purchased from Kitayama Labes Co., Ltd., Nagano, Japan.

Clinical findings: The animal showed protrusion of both eyes with unilateral pupillary dilatation of the left eye at the time of arrival. This case also did not react to light stimulation. So this was regarded as one of congenital glaucomas like buphthelmos, and was sacrificed at eight weeks of age. Ophthalmologic examinations were not performed.

Necropsy findings: The intraocular examination revealed retinal detachment of both eyes and a whitish coagulated substance on the posterior surface of the lens. In the left eye, the subretinal space was filled with a whitish gelatinous substance. Other organs and tissues were normal.

Microscopic findings: In both eyes, major findings were heavy foldings of the detached retina, posterior subcapsular cataracts with partial rupture, preretinal proliferative membranes, and choroidal hypoplasias. The retina of both eyes showed a funnel-shaped detachment. The retinal foldings were connected to each other by fibrous membrane. The membrane contained a few pigmented cells, glial cell-clusters, and well-developed capillaries. Preretinal neovasculization was conspicuous in the right eye. Marked serous exudation was observed under the retinal space in the left eye. No particular findings were seen in the anterior part of the eyes.

Based on these observations, this case was diagnosed as a late stage PVR. However, we could not explain the pathogenesis of this case. Of course this case has not received any surgery. It is possible that the PVR of this case occurred bilaterally almost at the same time. Congenital disorders such as congenital cataracts might be associated with the retinal detachment. Also, in some degree the choroidal hypoplasia might be concerned with the event. Further examination is necessary to define the pathogenesis.

P-162

Histopathological findings of cleft palate in rat embryos induced by triamcinolone acetonide

Satoshi FURUKAWA[1], Koji USUDA[1], Masayoshi ABE[1], Izumi OGAWA[1]

Biological Research Laboratories, Nissan Chemical Industries, LTD., Saitama 349-0294, Japan[1]

Triamcinolone acetonide (TAC), a synthetic glucocorticoid, induces cleft palate resulting from poor development of palatal shelves in mice. However, TAC has no effect on medial edge epithelial cells (MEE cells) in secondary palatal shelves. In the present study, we examined the relationship between the pathogenesis of cleft palate and the effects on MEE cells and palatal mesenchymal cells in rat embryos exposed to TAC. Pregnant Wistar Hannover rats were given TAC intramuscularly at 0.5 mg/kg at gestation days (Day) 12, 13, and 14, then embryos were harvested on Days 14.5, 15, and 16. The effects of TAC were as follows; an inhibition of palatal mesenchymal cell proliferation on Day 14.5, a decrease in the density of palatal mesenchymal cells and expression of epidermal growth factor (EGF) receptors in MEE cells on Day 15, and a reduction of stratified squamous differentiation of MEE cells with expression of cytokeratin and EGF receptors on Day 16. These findings indicated that TAC inhibited the proliferation of mesenchymal cells and affected the differentiation of MEE cells into stratified squamous epithelia in the palatal shelves of rat embryos. However, these stratified squamous MEE cells partially fused with each other. Thus, we suspected that a major contributing factor to the formation of TAC-induced cleft palate might not be the altered differentiation of MEE cells, but the inhibition of mesenchymal cell proliferation.

P-163

Spontaneous neural crest tumors of pinna in mice

Hiroshi EDAMOTO[1], Mariko NAGATANI[1], Rie ANDO[1], Sachiko TAMAI[1], Kenichiro KASAHARA[1], Shuzo OKAZAKI[1], Kazutoshi TAMURA[1]

Division of Pathology, Gotemba Laboratory, Bozo Research Center Inc. Shizuoka, 412-0039, Japan[1]
Division of the Second Laboratory for Safety Evaluation, Gotemba Laboratory, Bozo Research Center Inc.[2]

Neural crest tumor (NCT) in pinna, also referred to as a melanotic / amelanotic melanoma, is a rare tumor in mice. The purpose of the present study is to provide information about the frequency and some aspects of the pathological features of NCTs arising spontaneously at pinna in mice. A total of 7688 B6C3F$_1$ mice used in long-term studies including studies for background data collection in our facility were surveyed. The pinna NCTs occurred in 0.2% of males and 0.5% of females. The earliest occurrence was recorded at 87 weeks of age in a male, which was observed externally as a solitary small nodular lesion. Most NCTs arose at the basal portion of the pinna as a solid tumor pale in color. It is noteworthy that most NCTs were observed on the side of ear-tag application. Some NCTs grew rapidly with partial necrosis and ulceration, and the biggest reached to 35x30x25mm. Seventeen out of 30 (54.8%) mice died from NCTs including eating disturbance from the markedly grown tumor. Histologically, most tumors were mainly composed of spindle cells which proliferated with interlacing fascicular and striform patterns. Moreover, round and pleomorphic cells which had a large nucleus or multiple atypical nuclei were also seen in various ratios. Mitosis also varied in the cases. In 8 out of 30 (26.7%) cases a few melanin pigments were observed but they could not be detected in other cases. However, various numbers of premelanosomes and/or melanosomes were confirmed in all cases examined electron microscopically. Almost all tumor cells destructively invaded the surrounding tissues, sometime involving the eyes. Distant metastasis was observed in 8 out of 30 (26.7%) cases, and the main sites of metastasis were lungs, liver, kidneys, submandibular lymph nodes and heart, and only a few cases were seen in spleen, adrenals, thoracic cavity and tongue. Although pinna NCT is a rare tumor, it often kills the animals directly or indirectly, and persistent and/or repeated inflammation and regeneration caused by trauma may play a role in development of tumors.

P-164

Histological changes in the dorsal skin of YPC mice irradiated with UVB for 4 weeks

Taro OKADA[1], Akira YASOSHIMA[1], Kaoru TAKANO[2], Junichiro MATSUDA[2], Koji UETSUKA[1], Hiroyuki NAKAYAMA[1], Kunio DOI[1]

Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, the University of Tokyo, Tokyo 113-8657, Japan[1]
Department of Veterinary Science, National Institute of Infectious Diseases[2]

[Background] YPC is an autosomal recessive mutant mouse strain which expresses poor and wavy hair coat and curly whiskers, and has been detected first in a female of Swiss albino mice colony at National Institute of Infectious Diseases, Japan. In this study, YPC mice were irradiated with ultraviolet (UV) B daily for up to 4 weeks, and histological changes in the dorsal skin were examined.
[Materials and Methods] A total of 16 seven-week-old mice (male:8; female:8) were irradiated with UVB at a daily dose of 3 or 6 kJ/m^2 × 6 days/week for 2 or 4 weeks(w). They were sacrificed 2 hours after subcutaneous injection of bromodeoxyuridine (BrdU; 50mg/kg), and dorsal skin samples were served to histological, immunohistochemical (cytokeratin 10 (CK10), involucrin, and BrdU), and ultrastructual studies.
[Results] In mice irradiated with 6kJ/m^2 UVB, thickening of the epidermis and infundibulum of hair follicles due to proliferation of keratinocytes and dermal thickening due to proliferation of connective tissues were observed in both sexes at 2w. At 4w, infundibular keratinocytes became more proliferated and some cystic structures were observed in those areas. Cystic structures were more frequently found in females than in males. Many keratinocytes making up these structures were labeled with BrdU, and keratinocytes lining the inside surface of the cyst were positive for CK10 and involucrin. Ultrastructually, infudibular keratinocytes showed cornification as seen in the epidermis, and some of these keratinocytes anchoring the basement membrane had small cytoplasmic projections into dermis. Degeneration of collagen fibers and vessels was also observed in the upper dermis. On the other hand, in mice irradiated with 3kJ/m^2 UVB, epidermal and dermal thickening was observed at 2w and 4w. However, no changes were observed in the infundibulum and hair structures.
[Conclusion] In this study, the thickening of the epidermis and infundibulum with formation of cystic structures and dermal thickening were found in the dorsal skin of YPC mice exposed to UVB-irradiation for 4 weeks. In addition, degeneration of collagen fibers and vessels in the dermis were also observed. In addition, proliferation of keratinocytes was induced not only in the epidermis but also in the infundibulum, and such phenomena have not been reported yet. Thus, YPC is considered to be a useful experimental animal in the field of photodermatology.

P-165

Effects of scratching on the onset and exacerbation of dermatitis in NC/Nga mice

Yutaka NAKANISHI[1], Atsushi NAKAMURA[1], Yuki HASHIMOTO[2], Iwao ARAI[2], Hiroto MIYATA[1], Masaaki KIMURA[1]

Toxicology Laboratory, Medical Research Laboratories, Taisho Pharmaceutical Co., Ltd., Saitama 331-9530, Japan[1]
Phamacology Laboratory, Medical Research Laboratories, Taisho Pharmaceutical Co., Ltd.[2]

Introduction:
NC/Nga mouse is known as an atopic dermatitis model. The onset of dermatitis in NC/Nga mouse admits at conventional breeding, whereas it doesn't admit at SPF (specific pathogen free) breeding. The scratching behavior increases immediately after conventional breeding, since then the onset of dermatitis is observed in NC/Nga mouse. Although it is thought exacerbation of atopic dermatitis in human relate to scratching behavior, the proof of evidence using experimental animals is not understood. So we established an experimental model to avoid the effect of scratching by claw cutting in NC/Nga mouse at conventional breeding. In this study, to estimate the effect of scratching on the dermatitis pathogenesis in NC/Nga mouse, we carried out histopathological verification.
Method:
Animal experiment: The dermatitis was induced after conventional breeding in NC/Nga mice (male, 4 weeks, Charles River). The dermatitis was observed macroscopically, and judged with skin severity score (minimum 0 to maximum 12). The claw of their hinds was cut with toenail clippers and nail files twice in a week. The experimental design was as the following.
Prophylactic test: Divided the NC/Nga mice at 4 weeks age into 3 groups.
i) SPF breeding, ii) conventional breeding and iii) claw cutting + conventional breeding. These mice were sacrificed after 8 weeks breeding.
Therapeutic test: Divided the NC/Nga mice with severe dermatitis at 15 weeks age into 2 groups.
i) conventional breeding and ii) claw cutting + conventional breeding. These mice were sacrificed after 7 weeks breeding.
Histopathological study: The back skin in NC/Nga mice were fixed and embedded by conventional methods, and stained with hematoxylin and eosin(H.E.) and toluidine blue.
Result and discussion:
In prophylactic test, the scratching behavior in NC/Nga mice increased immediately after conventional breeding, and then the dermatitis was observed macroscopically within 4 weeks. In therapeutic test, the dermatitis improved macroscopically for one week. Microscopically, several findings including acanthosis, ulcer/erosion, crust, inflammatory cell infiltration, infestation of ectparasite, fibrosis were noted. These histopathological findings gave a good correlation with skin severity score. Skin severity score of dermatitis showed improvement by claw cutting in these test. Furthermore, both of acanthosis and inflammatory cell infiltration were also improved histopathologically on treatment with claw cutting. These results showed that scratching behavior plays an important role for dermatitis pathogenesis in NC/Nga mice as well as atopic dermatitis in human.

P-166

Morphological changes of auricular tissue in the mouse TNCB-repeated application model.

Chie TAKADA[1], Daisuke HARADA[1], Yukihito TSUKUMO[1], Toyoko KASHIWAGI[1], Haruhiko MANABE[1], Satoshi NISHIKAWA[1], Kazuo SUZUKI[1], Katsumi TAKABA[1]

Kyowa Hakko Kogyo Co.,Ltd., Shizuoka 411-8731, Japan[1]

2,4,6-trinitro-l-chlorobenzene (TNCB)-repeated application model is well-known as chronic dermatitis model. However, it has not been reported the pathological analysis of this model in detail. In this study, we examined morphological changes of auricular tissue in the mouse TNCB-repeated application model.
Right ear auricles of BALB/c mice were sensitized with 0.3 w/v%TNCB and challenged three times per week for 21 days starting from 7 days after sensitization (first challenge: day 0). The animals were sacrificed at 0, 3, 6, 9, 24 and 48 hours after each challenge on days 0, 7, 9, 14 and 21, and at 48 hours after each challenge on days 2 and 16. The right auricles were removed and were fixed in 10 % buffered formalin. Then, the tissues were embedded in paraffin. The sections were stained with Hematoxilin and Eosin, and fast green FCF for light microscopy examination.
Crust formation, erosion, microabscess in epidermis, inflammatory cell infiltration into epidermis and dermis, and edema in dermis were observed and/or getting more serious from day 7. On the other hand, the thickening of epidermis and eosinophil infiltration increased gradually from the first challenge. Some of them were time-dependent changes. Microabscess and increased eosinophils were most prominent at 6 to 9h after each challenge. Erosion and crust formation attained the maximum magnitude at 24 hours, and edema at 6 hours, respectively.
This model indicated that the lesions aggravated abruptly from day 7 except for the thickening of epidermis and eosinophil infiltration.

P-167

Application of special staining to decalcified skeletal tissues in routine toxicity studies

Aisuke NII[1], Kenji NAKANO[1], Takanori HANADA[1]

Safety Research Laboratories, Yamanouchi Pharmaceutical Co., Ltd., Tokyo 174-8511, Japan[1]

Although relatively rare compared with other major organs, the skeletal system can be a target in toxicity studies of drug candidates. The skeletal system is different from other organs in that it needs to be decalcified before tissue preparation. Decalcified and paraffin embedded sections stained with hematoxylin and eosin (H&E) are routinely used in toxicity studies. Other special techniques including non-decalcified sections, X-ray microanalysis, bone labeling with fluorochrome markers such as tetracycline etc., are not usually incorporated in study design. We here present the usefulness of some special staining for decalcified skeletal sections.

The important points when examining bone slides are cellular components and the intercellular matrix. For the former, there are three major types of cells: osteoblasts, osteocytes, and osteoclasts. Toxic effects on osteoblasts and osteoclasts are often encountered and sometimes result in lesions which resemble metabolic bone diseases such as osteomalacia, osteoporosis, osteopetrosis, etc. Alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) are the best-known histochemical markers of the osteoblast and osteoclast, respectively. For the osteoclast, some macrophage markers for immunohistochemistry and lectin histochemistry are also useful. It is infrequent that the osteocyte is affected; however, the changes of the cell, once occurred, are easily recognized since the cell is located in the lacunae and difficult to be eliminated. Osteocyte processes can be appreciated by the network structure of bone canaliculi using silver staining like Bodian's method. Apoptosis of the cellular components can be seen by TUNEL or single-strand DNA staining.

For the intercellular bone matrix, information on inorganic components is limited due to decalcification. For organic matrices, immunohistochemistry can be applied for collagens, while that for non-collagenous proteins is uncommon. Osteoid layers on the bone surface are seen with Masson's trichrome, and cement lines in the trabeculae with Giemsa or alcian blue-PAS stain. Cartilagenous components are stained with alcian blue or toluidine blue. In addition, even in H&E stained sections, birefringent collagen fibers can be observed with a polarized microscopy. For drug-induced skeletal changes, several special staining can be applied to decalcified sections in routine toxicity studies, and the obtained results will be help in evaluating the toxicity. Photomicrographs of some staining will be presented.

P-168

Histopathological characterization of the skeletal myopathy in rasH2 mice

Takayuki TSUCHIYA[1], Fumiko SANO[1], Kazuhiro GOTO[1], Mamoru MUTAI[1], Toshimi USUI[2], Jiro SUGIMITO[1], Shiro TAKAGI[1]

Toxicology Laboratory, Mitsubishi Pharma Corporation, Chiba 292-0818, Japan[1]
Central Institute of Experimental Animals[2]

Skeletal myopathy was found approximately 100% in the rasH2 mice. Microscopically, this is essentially similar to the features of muscular dystrophy. To confirm the detail features of rasH2 skeletal myopathy, HE, Gomori trichrome and NADH-TR stain and electron microscopic examinations were applied to the muscles obtained from male rasH2 and non-transgenic littermates aged 10-13 weeks. To evaluate the possible involvement of dystrophin and its associated protein in the pathogenesis of this skeletal myopathy, the expression of dystrophin and α-sarcoglycan was assessed immunohistochemically. As the result, variation of the muscle fiber size was widespread in the skeletal muscle. Scattered degeneration/necrosis and regeneration of muscle fibers were observed. Myopathic grouping of regenerating muscle fibers was not a feature of the rasH2 mice myopathy in this age. The results of NADH-TR stain demonstrated no fiber-type grouping that suggests denervation of the muscle fiber. Muscle fibers showing disorganized intermyofibrillar network and necrotic change were clearly demonstrated in NADH-TR stain. No specific morphological changes, like rod structure or tubular aggregation suggesting specific type of myopathy, were noted in Gomori trichrome and NADH-TR stains. Immunohistochemically, the expression pattern of dystrophin and α-sarcoglycan (positive around the rim of muscle fibers) were comparable between rasH2 and non-transgenic mice. Electronmicroscopically, occasional muscle fiber degeneration/regeneration and invaded phagocytic cells were observed. These results indicated that myopathic changes in rasH2 mice is based on the gradual progress of degeneration and regeneration process of muscle fibers without specific structural changes seen in the specific type of human myopathy. Abnormality of dystrophin and α-sarcoglycan, major components of dystrophin-glycoprotein complex, was not considered to be involved in the pathogenesis of myopathic changes in rasH2 mice. Therefore, the mechanism of muscular degeneration in the rasH2 mice is thought to be different from that of dystrophin-deficient model such as the *mdx* mice.

P-169

Spontaneous degenerative lesions of the femoral growth plate in rats

Shinichiro IKEZAKI[1], Osamu KUSUOKA[1], Mizuho TAKAGI[1], Katsuhiko WARITA[1], Maiko TSURUKAME[1], Kayoko KUDO[1], Sachiko TAMAI[1], Atsushi NAKAMURA[1]

Division of Pathology, Bozo Research Center Inc., Shizuoka 419-0101, Japan[1]

Degenerative lesions of cartilage, such as chondromucinous degeneration (CMD) or chondromucinous cystic degeneration, are often seen in the sternum but also in articular and growth plate of the femur in rats. Histologically, the lesions consist of various stages of progression from beginning of dissolution of the cartilage to lysis with cystic formation. It is reported that the incidence of CMD increases with advancing age, with lesions first becoming noticeable at about 130-180 days of age. However, we have recently found that similar changes spontaneously occur in distal growth plate of the femur of young adult Crj:CD(SD)IGS rats. The present study was performed to clarify the historical information about the spontaneous degenerative lesions in rats. Over two thousand male or female rats between 8 and 45 weeks of age, which had been purchased from commercial breeders as Crj:CD(SD)IGS, were surveyed laying emphasis on incidence of the degenerative lesions in distal growth plate of the femur. These animals were collected from non-treatment and vehicle control groups in various toxicological studies and were divided into four different age groups of 8 (group 1), 10-14 (group 2), 19-23 (group 3) and 32-45 (group 4) weeks old. All samples were cut into sagittal sections and stained with hematoxilin and eosin for microscopical examination. Histology characteristics of the degenerative lesions in this study were: (a) lysis of cartilage matrix in growth plate, (b) disorder of differentiation in the chondrocytic columns, (c) resulting in disarrangement of the growth plate line. These lesions generally occurred in opposite side of the patella and in rats of over 10 weeks of age. The incidence of the lesions in groups 1 to 4 by the above criteria were respectively 0, 1.7, 10.5 and 7 % in male, and 0, 0.7, 2, and 3.4% in female. Thus lesions in males showed a tendency to occur more frequently compared with females. The incidence in rats over 19 weeks of age was higher than that in rats under 14 weeks of age. Furthermore, the present survey showed the degenerative lesions of cartilage such as CMD have also occurred in younger rats than that in the past reports, although they are of low incidence.

P-170

Early pathophysiological feature of arthropathy in juvenile dogs induced by ofloxacin, a quinolone antimicrobial agent

Koichi YABE[1], Hiroshi SATOH[1], Toshimasa JINDO[1], Tadaki SUGAWARA[1], Kazuhisa FURUHAMA[1], Masanobu GORYO[2], Kosuke OKADA[2]

Drug Safety Research Lab., Daiichi Pharmaceutical Co., Ltd., Tokyo 134-8630, Japan[1]
Veterinary Pathology, Iwate University[2]

Arthropathy in dogs induced by ofloxacin, a quinolone antimicrobial agent, was pathophysiologically investigated to elucidate the early morphological characteristics in both in vivo and in vitro systems. In the in vivo studies, ofloxacin was orally administered once or twice at 20 mg/kg/day to male juvenile (3-month-old) or adult (36-month-old) dogs. Unlike adult dogs, unifocal or multifocal fluid-filled vesicles were macroscopically observed on the articular surfaces of juvenile dogs from 24 hours after a single treatment with ofloxacin. Microscopically, fissures or cavity formations in the middle zone of the articular cartilage were noted only in juvenile dogs. Further, the cartilage matrix from the abnormal area to the articular surface showed a decreased safranin-O staining intensity, suggesting proteoglycan depletion. Ultrastructurally, chondrocytes in the middle zone of juvenile dogs displayed dilatation of the cisternae in the rough endoplasmic reticulum as an initial hallmark, in conjunction with depletion in the proteoglycan granules and edema in the territorial matrix. In the in vitro studies, chondrocytes isolated from the articular cartilage of naive juvenile dogs were exposed to ofloxacin at 6.3 to 100 μg/ml for 24 hours. Although no changes were noted in the DNA synthesis, protein synthesis or proteoglycan release at concentrations of up to 100 μg/ml, the proteoglycan synthesis was evidently decreased in a dose-dependent manner from 12.5 μg/ml. The results obtained suggest that the inhibitory action of ofloxacin on proteoglycan syntheses in the chondrocytes would largely contribute to the early morphological changes in the articular cartilage of the juvenile dog, and consequently the proteoglycan depletion in the matrix may play a critical role in the cavity formation, preceded by dilatation of the cisternae in the rough endoplasmic reticulum.

P-171

Organ calcification in monkeys treated intradermally with rhPTH1-34 for 6 months followed by a 1-month recovery period

Jihong YANG[1], Peng WANG[1], Xiangting XU[1], Guoping XU[1], Ling ZHANG[1], Liuyi ZHANG[1]

Department of Pathology, Yunnan Pharmacological Laboratories of Natural Products, Kunming Medical College, Kunming City P. R. 650031, China (PRC)[1]

ABSTRACT: Parathyroid hormone (PTH) is the key endocrine factor regulating extracellular Ca^{2+} homeostasis. In recent years, PTH has been used for prevention and treatment of osteoporosis. Human parathyroid hormone 1-34 (rhPTH1-34) is the segment which includes the extracellular Ca^{2+} homeostasis function.

Objective: The purpose of this 6-month study was to investigate the potential toxicity of $rhPTH_{1-34}$ administered once daily in Cynomolgus monkeys (macaqa fascicularis) for safety evaluation. Based upon the clinical dose, $rhPTH_{1-34}$ was administered via intradermal (i.d.) injection for six months at dose levels 5, 15, and 45μg/kg/day. Solvent administered i.d. to an additional group of monkeys served as control. The solvent control group and the $rhPTH_{1-34}$ groups at 5 and 15μg/kg/day consisted of six monkeys (3F, 3M), while the $rhPTH_{1-34}$ group at 45μg/kg/day included seven monkeys (3F, 4M).

Histopathology Results: One male monkey in the high dose group, showing diffuse multifocal calcification of heart and kidneys, was sacrificed after 117days.

① After 6-month treatment followed by 1-month recovery, calcium deposits, verified in situ by the staining methods of von Kossa and Dahl, were observed in various regions of cortex and medulla of the kidneys in one monkey of the median dose and two monkeys of the high dose.

② Bone with decreased bony spicules of decreased size was not demonstrated in vertebrae, sternum, ribs and thighbone.

③ After 6-month treatment the number of chief cells of the parathyroid glands was noted to decrease with an accompanying interstitial fibrosis in altogether three monkeys of the 15μg/kg/day and 45μg/kg/day groups. One monkey in the high dose group showed atrophy of the parathyroid glands, but following 1-month recovery this was restored to normal. No changes were observed in the solvent control group or the $rhPTH_{1-34}$ 5μg/kg/day group.

Conclusion:6-month administration at doses of 15μg/kg/day and 45μg/kg/day of rhPTH1-34 would induce lesions in organs of Cynomolgus monkeys. Despite the fact that the clinical dose, 0.8μg/kg/day, is in the safety range, great care has to be exerted during administration of rhPTH1-34 to avoid organ lesions.

P-172

Physiological parameters and background histopathology findings from chronic (2 year) carcinogenicity studies in the HAN wistar rat

Christopher BARTON[1]

Department of Pathology, Covance Laboratories, North Yorkshire HG31PY, UK[1]

The HAN Wistar rat is becoming increasingly popular for use on regulatory toxicology studies.
The HAN rat strain tends to have a lower incidence of spontaneous background histopathology, when compared with some other rat strains; most notably those tumours involving endocrine and hormone-associated tissues.
The HAN rat generally has a low mortality rate, with good survival over the course of a 2 year study and the potential for reducing group sizes. In addition, the small adult body weight can result in considerable reductions in bulk drug requirement.
There are few publications detailing basic physiological data: Bodyweight gain, food consumption, survival data and spontaneous tumour incidences for the HAN Wistar rat in chronic (2 year) carcinogenicity studies.
The control data in this poster presentation are from carcinogenicity studies conducted recently at Covance Laboratories, Harrogate, England

Author Index

AUTHOR INDEX

S : Symposium P : Poster

I

J

K

L

M

N

O

P

R

S

T

U

V

W

X

Y

Z

MEMO

MEMO

MEMO

MEMO

MEMO

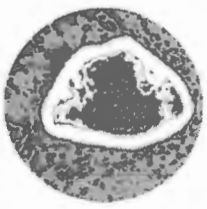
CTBR - Building Relationships Based on Trust and Commitment

CTBR is pleased to have been involved with the Japanese health care industry for more than 15 years, and to recognize Japan as our second largest geographic market after the United States. Our staff of more than 1000 professional, scientific, and technical personnel conduct more than 850 GLP preclinical studies per year, with 98% On-Time Reporting performance. Our commitment to you is unchanged from our beginnings in Japan in 1984 - to provide the highest levels of quality and service in support of your successful product development.

CTBR Pathology Experience and Expertise

- 13 Veterinary Pathologists (9 ACVP Certified)
- 160 pathology scientific and technical staff
- Pathology evaluation on more than 550 GLP studies per year
- More than 750,000 paraffin tissues processed and evaluated per year
- Specialized bone laboratory processing more than 12,500 specimens annually
- Scientific Directors supervise specialized pathology areas
- Specialized pathology experience and background data

-Bone diseases including osteoporosis and arthritis
-Biomaterials histological preparation and evaluation
-Biomechanical strength evaluation
-Carcinogenicity studies
-Electron microscopy
-Imaging, including DXA and pQCT densitometry
-Immunohistochemistry
-Infusion site pathology
-Male Reproductive Assessment (MRA)
-Neuropathological evaluation
-Histomorphometry
-Respiratory pathology
-Veterinary Clinical Pathology



- Contract Pathology services, in addition to *in vivo* studies

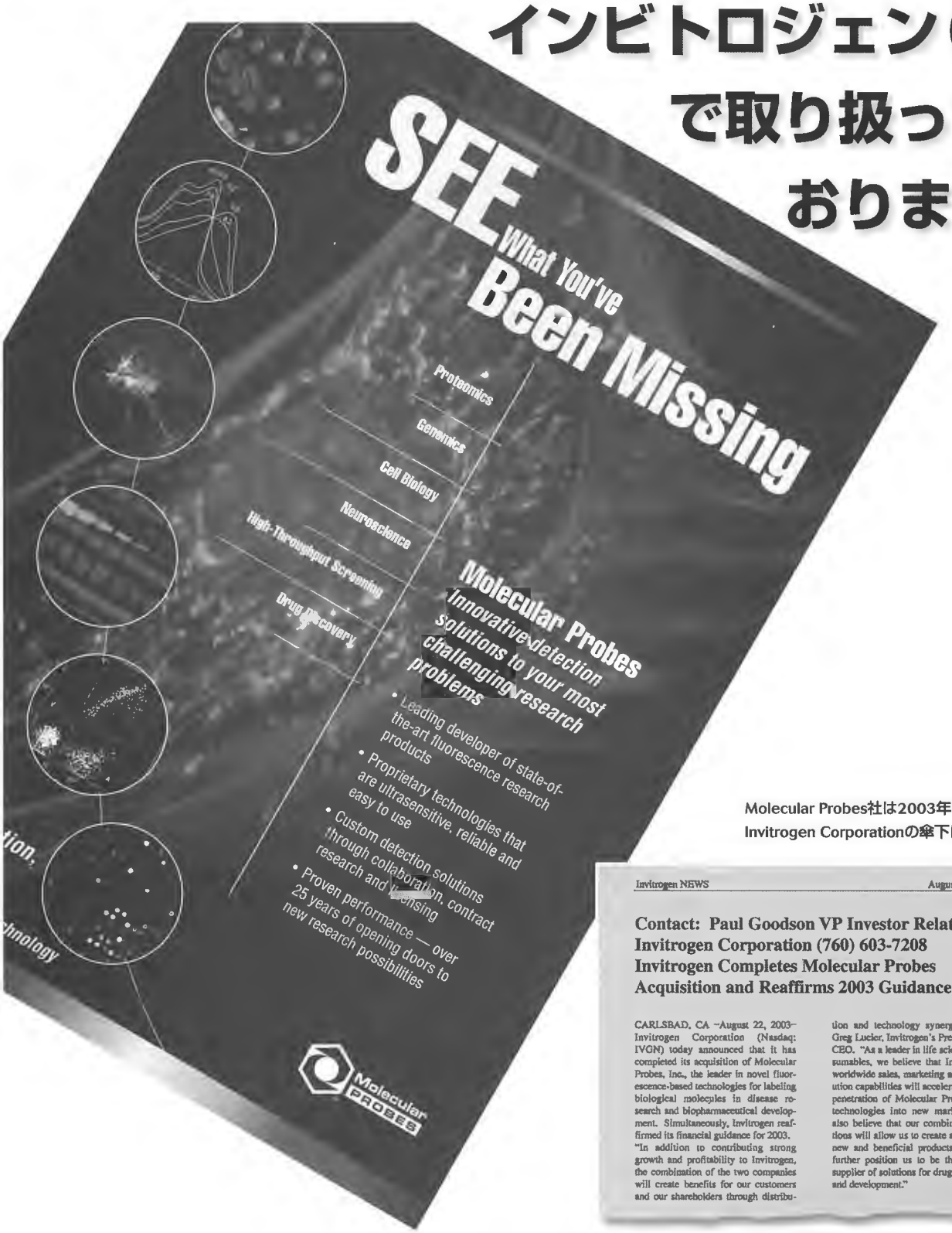
Please contact CTBR directly or through LSG Corporation.

エルエスジー株式会社
〒162-0814 東京都新宿区
新小川町 6-36 S&S ビル3階
TEL: 03 (3513) 6534
FAX: 03 (3513) 6535

CTBR
A Member of the Inveresk Research Group
87 Senneville Road, Senneville, Quebec, Canada, H9X 3R3
Tel: (514) 630-8200 Fax: (514) 630-8230
E-mail: japanmarketing@ctbr.com Web site: www.ctbr.com

Invitrogen™
life technologies

モレキュラープローブ製品：
インビトロジェン(株)
で取り扱って
おります。



Molecular Probes社は2003年8月に
Invitrogen Corporationの傘下に入りました。

Invitrogen NEWS August 22, 2003

Contact: Paul Goodson VP Investor Relations
Invitrogen Corporation (760) 603-7208
Invitrogen Completes Molecular Probes Acquisition and Reaffirms 2003 Guidance

CARLSBAD, CA –August 22, 2003– Invitrogen Corporation (Nasdaq: IVGN) today announced that it has completed its acquisition of Molecular Probes, Inc., the leader in novel fluorescence-based technologies for labeling biological molecules in disease research and biopharmaceutical development. Simultaneously, Invitrogen reaffirmed its financial guidance for 2003.
"In addition to contributing strong growth and profitability to Invitrogen, the combination of the two companies will create benefits for our customers and our shareholders through distribution and technology synergies," said Greg Lucier, Invitrogen's President and CEO. "As a leader in life sciences consumables, we believe that Invitrogen's worldwide sales, marketing and distribution capabilities will accelerate the penetration of Molecular Probes' core technologies into new markets. We also believe that our combined operations will allow us to create an array of new and beneficial products that will further position us to be the premier supplier of solutions for drug discovery and development."

---

http://www.invitrogen.com/

インビトロジェン株式会社
〒103-0007　東京都中央区日本橋浜町2-35-4 日本橋浜町パークビル
マーケティングコミュニケーション TEL(03)3663-8143 FAX(03)3663-8242

TaKaRa

新機能を搭載し、バージョンアップ!!

Smart Cycler® II System

TaKaRa Code SC200N

Smart Cycler® Systemが、
お求めやすい価格は据え置いて、
バージョンアップしました。
コンパクトなボディに凝縮された、
多彩な機能はさらに充実。
リアルタイムモニタリングが可能な
定量PCRシステムの
ファーストチョイスです。

特長

- 異なる4種類の蛍光を同時に検出
- 高速加熱および冷却により、増幅時間を短縮
- 1台で同時に16通りのプログラムを実行可能
- 6台まで増設可能（基本システムでは、3台まで増設可能）。
- インターカレーター（SYBR*1 Green I）や各種蛍光標識プローブ（サイクリングプローブ、TaqMan*2プローブ、Molecular Beaconなど）を用いた既存の検出システムに対応可能。
- Advanced to Next Stage機能によりSYBR® Green Iによる検出精度がアップ。

*1 Molecular Probes社の登録商標です。
*2 Applied Biosystems社の登録商標です。

【Smart Cycler® II Systemで使用可能な蛍光試薬】

検出チャンネル	励起波長（nm）	検出波長（nm）
1	450～495	510～527
2	500～550	565～590
3	565～590	606～650
4	630～650	670～750

【仕様】

本体寸法	305（W）×305（D）×250（H）mm
重量	10 kg
電源	100～240 V AC, 50/60 Hz, 350 W
温度制御能力	加熱時（最大）：10℃/秒（50～95℃） 冷却時（最大）：2.5℃/秒（95～50℃）
温度制御精度	±0.5℃（60～95℃）

仕様は、改良のため予告なしに変更することがあります。

本製品はCepheid社の製品です。

We are actually required to include the authorization notice in its entirety which is:
"Practice of the patented polymerase chain reaction (PCR) process requires a license. The Smart Cycler® System is an Authorized Thermal Cycler and may be used with PCR licenses available from Applied Biosystems. Its use with Authorized Reagents also provides a limited PCR license in accordance with the label rights accompanying such reagents. Purchase of this instrument does not convey any right to practice the 5′ nuclease assay or any of the other real-time methods covered by patents owned by Roche or Applied Biosystems."

タカラバイオ株式会社

東日本販売課
TEL.03-3271-8553 FAX.03-3271-7282

西日本販売課
TEL.077-565-6979 FAX.077-565-6978

TaKaRa テクニカルサポートライン
製品についての技術的なご質問に専門の係がお応えします。
TEL.077-543-6116 FAX.077-543-1977

Visit us at
www.takara-bio.co.jp

組織標本作製、組織検査から最終報告書まで

■よきパートナー・サイエンティフィックテクニシャンを目指して■

受託業務

組織標本作製

- ヘマトキシリン・エオジン重染色標本作製
- 特殊染色標本作製
- 免疫染色標本作製
- その他

組織学的検査

- ＧＬＰに則った病理組織学的検査
- 開発スクリーニングにおける病理組織学的検討
- 作用メカニズム、作用部位等の基礎検討における組織学的検討
- 病態動物における組織学的検討
- その他

安全性研究コンサルタント

- 研究企画から最終報告書まで

秘密は完全に守ります 迅速、廉価です

このような時に御用命下さい

- 標本作製、組織学的検査を一括委託したいとき
- 実験が立て込んで期限に間に合わないとき
- 病理要員が急に不足したとき
- 安全性研究以外で病理設備・要員のいない研究室

※切り出し、包埋、薄切、染色、検査のどの段階からもお引き受けいたします。

サンプル、標本、報告書の受発送は

航空便で当日着きます。
宅配便で九州・四国・関西・中部地区は翌日、関東以北は翌々日に着きます。
※輸送中に固定液の組織へのほど良い浸透が行えます。

株式会社 **バイオ病理研究所**

〒873-0511
大分県東国東郡国東町小原1200-2
TEL（0978）72−0454
FAX（0978）72−2320
E-Mail:biopathology@muj.biglobe.ne.jp

大分空港から車で10分
（打ち合わせに便利）

Tissue-Tek®

One name found on *more* products,
in *more* histology labs—worldwide.

The most trusted name in histology.
Instrument by instrument. Specimen by specimen. Cassette after cassette.
Blade after blade. Proven reliability.
Consistent, dependable, long-life performance. Greater value. Sakura Tissue-Tek.
A tradition of quality for over 130 years.

Proven Reliability

SAKURA®

Sakura Finetek U.S.A., Inc.
1750 West 214th Street
Torrance, CA 90501 U.S.A.
Phone:(800)725-8723
Fax: (310)972-7888
www.sakuraus.com

Sakura Finetek Japan Co., Ltd.
1-7 Honcho 2-chome, Nihombashi
Chuo-ku, Tokyo 103-0023 JAPAN
Phone:(03)3270-1666
Fax: (03)3270-2779
www.sakurajp.co.jp

Sakura Finetek Europe B.V.
Hoge Rijndijk 48a
2382 AT Zoeterwoude
The Netherlands
Phone:+31-71-58 98 300
Fax: +31-71-58 98 488
www.sakuraeu.com

Schedule for Conference

Sunday February 15, 2004

	Registration	Main Hall	Reception Hall	Room 301	Room 401+402	Room 501	Room 502	Other
10:00am								
11:00am								
12:00pm	11:30am							
1:00pm		JSTP Microexamination Explanation						
2:00pm	|	Opening Remarks						
3:00pm		IATP Educational Session						
4:00pm	5:00pm							
5:00pm								JSTP Board of Directors Meeting (Room 505)
6:00pm								
7:00pm								Welcome Reception (Portopia Hotel)
8:00pm								
9:00pm								

Monday, February 16, 2004

	Registration	Main Hall	Reception Hall	Room 301	Room 401+402	Room 501	Room 502	Other
8:00am								
9:00am	8:30am			Poster Set-up	Poster Set-up			
10:00am		Symposium 1-1						
11:00am								
12:00pm		Luncheon Seminar 1				Luncheon Seminar 2	Luncheon Seminar 3	
1:00pm	|		Exhibition	Poster Discussion	Poster Discussion			
2:00pm		John Faccini Memorial Keynote Lecture						
3:00pm	6:00pm							
4:00pm		Symposium 1-2						
5:00pm								
6:00pm								

Tuesday, February 17, 2004

	Registration	Main Hall	Reception Hall	Room 301	Room 401+402	Room 501	Room 502	Other
8:00am								
9:00am	8:30am							
10:00am		Symposium 2-1						
11:00am								
12:00pm		Luncheon Seminar 4				Luncheon Seminar 5	Luncheon Seminar 6	JSTP Council (Room 505)
1:00pm	|		Exhibition	Poster Discussion	Poster Discussion			
2:00pm		Plenary Lecture						
3:00pm	6:00pm							
4:00pm		Symposium 2-2						
5:00pm								
6:00pm								
7:00pm								Gala Reception (Portopia Hotel)
8:00pm								

Wednesday, February 18, 2004

	Registration	Main Hall	Reception Hall	Room 301	Room 401+402	Room 501	Room 502	Other
8:00am								
9:00am	8:30am							
10:00am		Symposium 3						
11:00am								
12:00pm		JSTP General Meeting						
1:00pm	|	Luncheon Seminar 7	Exhibition			Luncheon Seminar 8	Luncheon Seminar 9	
2:00pm				Poster Discussion	Poster Discussion			
3:00pm	5:00pm	Symposium 4						
4:00pm				Poster Removal	Poster Removal			
5:00pm		Closing Remarks						