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WS1-1

Whole-body Exposure Study: Methods and Value for Inhalation Toxicology

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Whole-body exposure system using inhalation chambers has been widely used to examine adverse health effects of airborne chemicals existing in the environment and work place. It is assumed that experimental animals are exposed to chemicals in inhalation chambers under the circumstance that is similar to human exposure to airborne chemicals in the environment and work place. In this presentation, we attempt to introduce outline of the whole-body exposure method and discuss a difference from the intratracheal administration, which is an aim of this workshop.

Whole-body exposure system is applicable to test various types of chemicals including gas, vapor, mist, fume and dust. Besides, this method can be used for 2-year carcinogenicity studies, because this method does not cause excess stress to animals. In using whole body chambers, the exposed organ is not limited to the lung. We can detect local toxicities of chemicals in the upper respiratory tract, skin and eyes, and totally evaluate the inhalation toxicities of chemicals, considering the deposition of inhaled chemicals at the upper respiratory tract. The data obtained by whole-body exposure studies are useful for setting the exposure limit values in the environment and work place. On the other hand, whole-body exposure studies demand large-scale equipments and facilities, large amount of cost and test chemicals, and technical support. We should select appropriate exposure methods for the toxicological study in accordance with purpose of the study.

WS1-2

Differences of Lung Carcinogenicity on the Conditions of Intratracheal Instillation Using Tobacco Specific Nitrosamine

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Recently, the proportion of lung adenocarcinomas is increasing in Japan and many countries. The prevalence of lowtar filter cigarettes makes smokers inhale more deeply and this is suggested to be a cause for recent increases. In animals, many lung carcinogenesis studies have been conducted using carcinogens in tobacco smoke. However, there are few studies regarding difference of exposure conditions. In the present study, the lung tumorigenicity of tobacco specific nitrosocompound, 4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), was investigated on different exposure conditions through the respiratory tract.

Seven week-old female Wistar rats were intratracheally injected once a week for 8 times with solutions of 0.2 ml saline (group 1), 0.2 ml saline containing 28 mg/ml Fe₂O₃ (group 2), 0.1 ml saline containing 56 mg/ml Fe₂O₃ and 140 mg/ml NNK (group 3), 0.2 ml saline containing 28 mg/ml Fe₂O₃ and 28 mg/ml NNK (group 4), 0.2 ml saline containing 28 mg/ml NNK (group 5). In the group 2, 4 and 5, 0.5 ml of air was also injected with each solution to allow the test chemicals to reach the peripheral lung. In sixty-nine weeks after beginning of the experiment, all rats were killed and lungs were examined histopathologically.

The incidence and the number of lung tumors were 17%, 0.17 in group 2, 33%, 0.33 in group 3, 60%, 0.60 in group 4 and 29%, 0.29 in group 5 respectively. Although the total dose of NNK was lower in group 4 (44.8 mg) than in group 3 (112 mg), the tumors were more developed in group 4. These data suggests that lung carcinogenicity is increased by spreading NNK with entire lung.

In conclusion, it needs to be considered to the exposure conditions of a test chemical on the study of intratracheal injection.

WS1-3

Development of a Repeated Intratracheal Administration Method for Rats

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For intratracheal administration to rats, an agent is sprayed into the trachea with air using a gastric tube or applied directly via a special aerosolizing tip (MicroSprayer™ by PennCentury). However, these methods need to be conducted under light anesthesia condition, and for repeat administration, the anesthetics themselves may influence study results. Therefore, toxicological studies involving long-term intratracheal administration have been limited. With our newly developed technique, an agent is sprayed into the trachea using a DIMS-type aerosolizing tip (provisional name) in unanesthetized rats. To establish the approach, we examined different dose, frequencies of administration, and rat strains. We also developed another DIMS-type aerosolizing tip, which facilitates the administration of viscous compounds.

F344 rats well tolerated 12 sprays of 0.3 ml saline and 28 sprays of 0.3 ml corn oil. Ultra fine titanium oxide particles (particle diameter: 20 nm) were suspended in physiological saline at a concentration of 1.25 mg/0.1 ml, and intratracheally administered to groups of ten 8-week-old rats of 3 strains (F344/N Slc, Slc:SD, and Slc:Wistar Hannover/Rcc), without anesthesia, once a day, 5 times a week, for 4 weeks. There were no marked strain-related differences in the lung lesions (accumulation of foam cells and macrophages, inflammation and foreign bodies) nor their incidences. However the high survival rate, favorable weight gain, and simplicity of administration experienced with Slc:Wistar Hannover/Rcc rats suggests that this is the most appropriate strain for intratracheal administration.

We confirmed that, when the DIMS-type aerosolizing tip was employed, oily (highly viscous) compounds adopted a particle-like structure, reaching the peripheral regions of lung. This aerosolizing tip may facilitate the administration of oily (highly viscous) compounds, which have up to now been almost impossible to administer, especially viscous nitrosamine-based carcinogens.

WS1-4

Challenge for Detection of Lung Toxicity Due to Fine Particles Using an Intratracheal Instillation Bioassay in F344 Rats.

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Recently, the risk of fine particles in the air has been paid much more attention due to the development of nano-technology. Although the bioassay by intratracheal instillation method has both advantages and disadvantages for detection of lung toxicity due to fine particles, it is useful for screening purposes. In order to establish an appropriate bioassay for detection of lung damage after fine particle expose, we examined after intratracheal instillation to quartz, as a typical lung toxic agent, into F344 male rats.

Firstly, it was examined the optimal period of assessment after intratracheal instillation. Sequential analysis of the effects of quartz (DQ-12, 4 mg/rat), a typical lung toxic agent, revealed days 1 and 28 after instillation as most appropriate for detection of acute and subacute inflammatory changes, respectively, with bromodeoxyuridine (BrdU) incorporation on day 1 and inducible nitric oxide synthase (iNOS) levels on day 28 as suitable end-points.

Secondly, the experiment was conducted to determine appropriate doses of instilled quartz as a positive control to detect toxicological changes sensitively for our bioassay as a screening model, and to assess the impact of powdered quartz particle not in suspension. From the results, 2mg quartz suspended in 0.2ml saline was suggested to be the most appropriate dose for sensitive detection.

Finally, the toxicity of a series of different particle types, including nanoparticles and diesel exhaust particles, was also tested in our bioassay. Our experiments indicated the rank order of toxicity by intratracheal instillation to be as follows: CuO > quartz > neutralized Na₂PdCl₄ > NiO > hydrotalcite > MnO₂ > diesel > titanium dioxide > β-cyclodextrin > diesel standard > titanium dioxide (in the other series of experiment) > CaCO₃. On the other hand, our bioassay does suffer from a weakness in that even low doses of some particles may lead to death, for example with K₂PdCl₄, Na₂PdCl₄, CuO nano, NiO nano and C₆H₁₀O₄Pd.

This bioassay is supposed to be used for “a hazard identification” at an early stage with order toxicity of the various particles at a single instillation. This approach is useful for detection of acute and subacute pulmonary particle characters with a histopathological scoring system and markers like BrdU and iNOS for screening purposes.

WS1-5

Respirable Chemical Substances Hazard Assessment in an Intratracheal Instillation Study

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Animal studies involving various routes of exposure including oral toxicity tests and intraperitoneal injection studies have been implemented to assess potential hazards and risks of chemical substances. On the other hand, since correlations between biopersistence and the development of fibrosis and tumor were established for respirable chemical substances notably, asbestos substitutes, exposure via respiratory transairway exposure, a possible route of exposure involving deposition in and clearance from the lung appears to be significantly important in assessing the harmful effects of respirable chemical substances. This study reports the results of the assessment of respirable chemical substances, particularly those of fibrous materials, by using intratracheal instillation: 1) whether the pathological findings of the lung in our intratracheal instillation experiment are consistent with the hazards of chemical substances, 2) whether the pathological findings of the lung are correlated with biopersistence which is deeply related to harmful effects.

1) Relationship between pulmonary pathology and harmful effects of chemical substances in an intratracheal instillation study

Lung tissue sampled at 3 days and 6 months after the intratracheal instillation of 2mg of crocidolite, crystalline silica, titanium dioxide, potassium octatitanate whisker (PT1) and silicon carbide whisker (SiCW) was stained with HE and Elastica van Gieson to make pathological preparations. Images were captured digitally to quantify the degree of inflammation and fibrosis based on a point counting method. Crocidolite and crystalline silica both having high potential of fibronenicity and carcinogenicity showed persistent higher scores of inflammation/fibrosis, the scores of titanium dioxide whose toxicity is low increased transiently, however, those for the physiological saline-exposed negative control and the exposed group decreased to the same level. Inflammation scores for PT1 and SiCW both having lesser fibrogenic potential than asbestos was in the middle of the two groups.

2) Relationship between pulmonary pathological findings in the lung and biopersistence in the intratracheal instillation experiment

At 3 days and 6 months after the intratracheal instillation of 2 mg of crystalline silica, limestone, magnesium sulfate whisker and PT1, inflammation in the lung tissue was quantified and biological half-life was calculated by the measured lung burden to determine biopersistence. Longer retention in the lung tissue resulted in inflammation in the lung. Therefore, the usefulness of intratracheal instillation studies in the hazard assessment of respirable chemical substances was suggested.

WS2-1

What is Molecular PET Imaging ?

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Molecular Imaging is defined as “non-invasive in vivo investigation of cellular molecular events involved in normal and pathological processes (Society for Molecular Imaging HP)” This includes not only nuclear medicine (PET, SPECT) but also, magnetic resonance imaging (MRI), optical imaging, ultrasonic imaging, and so on. Possible application of one imaging modality to various subjects from cell, tissue, animal to human patients, or comparative studies using various modalities in different levels of subjects, allows us to understand the complex network of metabolism, signal transduction as well as cell migration in living organisms.

Molecular imaging research itself has no practical purpose, like astronomy, but it brings us a new insight of life, and various applications for basic life sciences, clinical practices, drug development and so on.

Positron emission tomography (PET) is considered to be a most plausible tool for pharmacological/toxicological studies in clinical practice as well as drug evaluation/development. In this talk, some example of PET studies will be presented.

WS2-2

Optical Imaging of Tumor Metastasis in Animals and RNAi-based Therapeutic Strategy

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RNA interference (RNAi) offers the potential of a novel therapeutic approach for treating cancer. With the increased potential of RNAi as a therapeutic strategy, noninvasive assessment of small interfering RNA (siRNA) and micro RNA (miRNA) delivery to tissues of interest—using clinically relevant imaging paradigms is required to conceive and optimize experimental treatment strategies. We have developed the *in vivo* imaging analysis of siRNA delivery in target tumor tissues by optical imaging of siRNA-mediated silencing. The other obstacle to developing RNAi-based therapies, is the delivery of the RNAs to the tumors. We have studied Atelocollagen-mediated nano-particle delivery system to deliver siRNAs and miRNAs to metastatic animal models of human cancers and found that those RNAi strategies effectively inhibited metastasis of human prostate and breast tumors in mice. Thus, our unique approach to RNAi delivery and imaging strategies creates a foundation for the development and optimization of a new class of promising therapeutics against cancer.

WS2-3

Optical *in vivo* Imaging for Translational Research

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Optical *in vivo* imaging system is very useful tool to non-invasively monitor specific molecular and cellular processes in small animals such as mouse, although it has limitation in tissue penetrance and influence of the autofluorescence. Optical imaging techniques rely on fluorescence absorption, reflectance, or bioluminescence as a source of contrast. Imaging in the near-infrared (NIR) spectrum (700-900 nm) maximizes tissue penetrance in addition to minimizing the autofluorescence from nontarget tissue. Representative advantages of optical imaging system over other modalities are easy handling, safety, speediness and multiplicity. Now optical imaging technique is considered as not only research tools but also clinical ones for next generation.

We have been imaging the activity of HIF-1, which is a transcription factor closely associated with hypoxia. HIF-1 activity is detected in diseases of the three major cause of death; cancer, stroke and myocardial infarction. By using transgenic mice with a HIF-1 dependent luciferase reporter gene, we can monitor growth of tumor, ischemic disease progression and systemic stress response to chemicals. We also have been developing a NIR optical imaging probe specific to HIF-1 active cells. By using this probe we can monitor hypoxic fraction in the xenografts of tumor-bearing nude mice. I will present our recent progress in the studies with optical imaging techniques.

This study is a part of Advancing Technology Excellence “Nano-Medicine” project, which is under Kyoto City Collaboration of Regional Entities assigned by JST.

WS2-4

Neural Stem Cells: Their Involvement in Adult Neurogenesis and CNS-repair

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Neural stem cells (NSCs) are self-renewing, multipotential progenitor cells. A single NSC can give rise to a wide variety of CNS cells, including neurons, astrocytes, and oligodendrocytes. Because of these characteristics, there is an increasing interest in NSCs and neural progenitor cells, both from a basic developmental biology perspective and from a clinical one that is aimed at developing therapeutic applications for the damaged brain. Current research into the nature of the NSCs present in the CNS includes the study of the extracellular factors and signal transduction cascades involved in their differentiation and maintenance, their population dynamics, and their localization in the embryonic and adult brain. These lines of research, combined with other studies intended to permit the prospective identification and isolation of NSCs, and their induction into particular neuronal phenotypes--which will be introduced in my talk --should lead to the development of feasible strategies for manipulating NSC cells *in situ* to treat the damaged brain and spinal cord injury.

WS3-1

The New International Nomenclature Project – INHAND and the GESC

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For the past two years, members of the major Societies of Toxicologic Pathology (JSTP, BSTP, ESTP and STP) have been involved in an international collaborative effort to codify and publish uniform nomenclature for both proliferative and non-proliferative lesions in laboratory rodents.

The project goes under the acronym INHAND (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice). Project oversight is provided by the Global Editorial and Steering Committee (GESC). The GESC currently has 8 members: 2 from the JSTP, 3 from the STP, 2 from the ESTP and 1 from the BSTP. In addition, a liaison from the Fraunhofer Institute (which provides computer support) also participates. Dr Peter Mann from the STP is the current chair of the GESC.

The GESC appoints Organ System Working Groups, whose responsibility it is to write the nomenclature guidelines for both proliferative and non-proliferative lesions for their assigned organ system. Each Organ System Working Group has a Chair and members from each of the participating STPs. A member of the GESC serves as a liaison to each Organ System Working Group.

The draft documents from each Organ System Working Group are placed on the goRENI website (www.goreni.org) for discussion and comments from any member of the participating STPs. After comments have been addressed, the GESC approves the final document, and it is published on the website. In addition, the documents will be published in the journals of the participating STPs.

To date, the following Organ System Working Groups have been formed: Respiratory, Immune, Cardiovascular, Central nervous System, Urinary and Liver. The draft documents for the Respiratory Subcommittee are available on the goRENI site. Other Organ System Working Groups are preparing their documents. Additional Organ System Working Groups will be formed in the coming year.

WS3-2

JSTP Activities in the Project of International Harmonization of Nomenclature and Diagnostic Criteria for Toxicologic Pathology

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In accordance with globalization of use of chemicals including pharmaceuticals and pesticides, toxicological data are shared by many regulatory agencies in the world. However, there are still some controversies over the interpretation of histopathology data because of inconsistency of nomenclature and diagnostic criteria for lesions among pathologists. Since histopathology takes an important part of toxicological data for risk assessment, international standardized nomenclature and diagnostic criteria for toxicologic pathology are essential to improve the reliability of the pathology data. From this point of view, initiatives had been started in the late 1980s by the Society of Toxicologic Pathologists (STP) and the Registry of Industrial Toxicology Animal-data group (RITA). In 1994 the “Joint STPs and ILSI Committee on International Harmonization of Nomenclature and Diagnostic Criteria in Toxicologic Pathology” was established. Consequently, the “Rat Nomenclature Reconciliation Subcommittee” was formed in 1998 to eliminate discrepancies existing among the published nomenclature systems and eventually issued the final version of international harmonization of rat nomenclature in 2000. However, the harmonization was limited to proliferative lesions and not extended to non-proliferative lesions. Thus, the Scientific Regulatory Policy Committee (SRPC) of STP has proposed the initiative “Revision of Standardized Nomenclature for Lesions in the Rat and Mouse”. Corresponding to this proposal, the European Society of Toxicologic Pathology (ESTP) and RITA have discussed the initiative and determined to undertake a project of “International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) for Lesions in Rats and Mice” in cooperation with other major Societies of Toxicologic Pathology including the STP, BSTP, and JSTP in 2005. The project is directed by the Global Editorial and Steering Committee (GESC) consisting of members from the STP, ESTP, BSTP, and JSTP. The 1st Meeting of GESC was held in Vancouver in 2006 and 2nd Meeting in Puerto Rico in 2007. The GESC is designated to appoint “Organ System Working Groups (OWG)” for writing the nomenclature guidelines for both proliferative and non-proliferative lesions in each organ system. The OWGs are currently assigned to the respiratory system, immune system, CNS, cardiovascular system, urinary system, and liver. Additional OWGs will be formed soon. From the JSTP, 2 members are now working for the GESC and 12 members for the OWGs. This project officially has started from 2008 on a 5-year plan with a goal to publish the standardized nomenclature and diagnostic criteria in the official journals of the STP, ESTP, and JSTP.

WS3-3

Present Issues on Nomenclature and Diagnostic Criteria for Lesions in Rodents: Message from an Assessor

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As factors interrupting risk assessment of chemicals, there are inadequate use of nomenclature and diagnostic criteria for lesions and confusion in lack of their unity. Inadequate use is summarized in general as follows: 1) mistranslation, 2) inappropriate findings, 3) no mention about target cells, 4) no mention about sites, 5) lack of comprehensive diagnosis, and 6) too detailed findings. Inadequate summary and discussion may also interrupt proper assessment: 1) absence of comprehensive discussion on systemic effect, 2) no discussion about sequential evaluation for preneoplastic/neoplastic lesions, 3) no discrimination of animals found dead, sacrificed in moribund or scheduled, 4) too intensive discussion based on inadequate data, 5) no characterization of deposited materials. Lack of unity on nomenclature and diagnostic criteria for lesions is a main theme of this workshop. Examples are as follows: 1) lesions resembling to human cases but clearly different in pathogenesis, 2) lesions same in term as human cases but different in histopathology, 3) lesions specific to experimental animals and difficult to extrapolate for human, and 4) unique lesions difficult to classify using existing nomenclature. In the present workshop, such cases are demonstrated and discussed. I hope that our presentation may contribute to revision of the textbook “Toxicologic Histopathology” as well as international harmonization of GESC.

WS3-4

Toxicopathological Diagnosis, Terminology, and Findings: Issues in Professional Practice

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With the aim of evaluating the toxicity risk of chemical substances, many members of our society give toxicopathological diagnoses or make findings using histopathological slides obtained from laboratory animals. Combining these findings with other test data, a risk evaluation of the particular chemical substance is then made in toxicity studies. Pathological data may also be obtained in a relatively objective numerical form (morphometrical, immunohistochemical, or gene analysis), but descriptive textual data (diagnoses, findings, plus pathology reports) in which information is obtained via the pathologist's eyes, that is, textual information that is highly dependent on the individual, constitutes the fundamental data type in this discipline. In this workshop, recent activity concerning international harmonization of diagnostic criteria and terminology will be explained and the requirements by the regulatory and administrative authorities will be discussed. Based on experience in the practical setting of a pharmaceutical company, my session will not only provide case studies of especially challenging diagnoses, but also present some case studies related to diagnosis or terminology. I will also discuss the debate within the working group of the Association's Education Committee in our society that is aiming to develop new diagnostic criteria for hepatocellular nodular lesions. Next, pathological data are usually reported in a standardized format in routine toxicity study reports; I will touch on some issues involved with such standardized documentation. Through the discussion how to use diagnostic / descriptive terminology and grade/severity expression (for more generalized and objective purpose), using some example problems encountered in routine toxicity studies, I will seek a strategy for addressing these issue.

O-1

Comparative Gene Expression Analysis between Differentiated and Undifferentiated Cells of Colon in Mice

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[Introduction] The molecular mechanisms that control homeostatic self-renewal of intestinal epithelium and those that underlies colorectal carcinogenesis are remarkably symmetrical. In the present study, we compared the gene expression profiles of differentiated/quiescent cells and undifferentiated/proliferative cells of the colon in mice.

[Methods] To distinguish undifferentiated cells from differentiated cells, we used transgenic mice expressing histone H2B-GFP under the control of *tet-on* system. The transgenic mice express high levels of nuclear GFP fluorescence in entire colonic crypts by the administration of doxycycline (Dox) and, after the withdrawal of Dox, the fluorescent intensity was decreased at the lower part of crypt, according to the cell proliferation. We prepared single-cell suspensions from crypts of transgenic mice on 2.5 days after the withdrawal of Dox and performed fluorescence-activated cell sorting (FACS) to collect GFP-high cells and GFP-low cells, respectively. Subsequently, we obtained transcriptional profiles for these populations by the microarray analyses.

[Results] Wnt pathway target genes were up-regulated in GFP-low cells and the genes expressed in the upper part of colon, such as *p21*, were up-regulated in GFP-high cells. These results suggest that differentiated cells and undifferentiated cells were sorted successfully.

[Discussion] These profiles might be useful to identify genes that control proliferations and differentiations not only in normal colon but also in colorectal tumors.

O-2

Erc/Mesothelin are Detectable in Sera from Rats with Pancreas Ductal Carcinoma

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Pancreatic adenocarcinoma is a highly lethal disease, which is usually diagnosed in an advanced stage. There is a great need to develop new markers that will increase our ability to diagnose pancreas cancer. Serum represents an ideal diagnostic specimen because of its easy and inexpensive accessibility. We established a transgenic rat model (Hras250) of pancreas ductal adenocarcinomas which were morphologically similar to the human disease. Previous studies suggested that Mesothelin, a homologue of rat Erc found to be elevated in renal carcinoma of Eker rats, is a potential marker of pancreatic adenocarcinoma in humans. In our animal model, expression levels of Erc/Mesothelin gene were much higher in pancreas tumors than in healthy pancreata of Hras250 rats. Recently, we have developed a novel ELISA system for detection of Erc/Mesothelin in the serum. In this study, we evaluate the potential utility of Erc/Mesothelin as a serum marker for the diagnosis of rat pancreas ductal adenocarcinoma. The serum level of Erc/Mesothelin was significantly higher in rats bearing early pancreas carcinoma lesions than in rats with healthy pancreata. Thus, Erc/Mesothelin is a promising marker for early pancreas carcinoma in our rat model and is potentially useful for evaluation of chemotherapeutic agents.

O-3

Enhancement of Pancreatic Carcinogenesis in Hamsters Fed Sugar-rich Diet

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Objective: A number of studies suggest that high-fat diet enhance carcinogenesis in various organs and possible risk factor for pancreatic cancer discussed in relation to increase of serum triglyceride. However, an effect of excess sugar intake, which induces increase of serum triglyceride, to pancreatic carcinogenesis is still obscure. In the present study, we investigated the influence of sugar on pancreatic carcinogenesis by using sugar-rich diet which starch was replaced by sugar in order to administer same calorie and the other nutrient.

Methods: Six weeks male Syrian golden hamsters were given N-nitrosobis-(2-oxopropyl)amine (BOP) at a dose of 50 mg/kg and 20 mg/kg body weight as initiators at 0 and 1 week, respectively. At 2 weeks, animals received a control diet or a sugar-rich diet (65% w/w) which starch was replaced at 100% by sugar. Since some animals fed sugar-rich diet died at 12 weeks, diet was changed sugar-rich diet (37.5% w/w) which starch was replaced at 50% by sugar. Animals were killed 25 weeks after the beginning of the experiment and we examined pancreatic carcinogenesis histologically.

Results and Conclusion: No significant difference were seen in body weight and relative weight of liver, pancreas and body fat at the end of the experiment. The incidence and number of carcinomas were increased in hamsters fed sugar-rich diet than control diet (50.0% vs 6.7% and $0.6 \pm 0.7/\text{animal}$ vs $0.1 \pm 0.3/\text{animal}$, respectively). These results suggest that excess sugar intake may be an enhancing factor for pancreatic carcinogenesis in hamsters.

O-4

Lack of Promotion Activity of Diacylglycerol Oil on 4-Nitroquinoline 1-oxide Induced Carcinogenesis in The Oral Cavity Including The Tongue of SD Rats

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A recent study using c-Ha-ras proto-oncogene transgenic (rasTg) rats demonstrated possible enhancing effects of diacylglycerol (DAG) on 4-nitroquinoline 1-oxide (4NQO) induced carcinogenesis of the tongue. To confirm the effects in their back strain, Sprague-Dawley rats, a two-stage carcinogenesis study using 4NQO as an initiator was performed. Groups of 30 male rats were initially treated with 4NQO at a dose of 10 ppm in the drinking water for the first 10 weeks followed by a 1 week recovery interval by 11% DAG, 5.5% DAG + 5.5% triacylglycerol (TAG), 2.75% DAG + 8.25% TAG, 1.38 DAG + 9.62% TAG, 11% TAG, 11% high linolic acid TAG (HLTG), 5.5% DAG or 2.75% DAG in the diet for 35 weeks. Further groups of animals were treated with distilled water instead of 4NQO followed by 11% DAG, 11% TAG or 11% HLTG in the same manner. Although suppression of body weight gain was observed in all 4NQO-treated rats, there were no significant differences in final body weights among groups. The final survival rates in 4NQO-treated groups were from 50 to 77% and most animals died of tumors in the oral cavity. However, incidences and multiplicities of squamous cell papillomas and carcinomas in the tongue or in the oral cavity induced by 4NQO were not affected by any dietary treatments. Thus, in contrast to the positive data using rasTg rats, DAG had no potential for enhancing 4NQO-induced tumorigenesis in their back strain of rats, which was in line with the previous comprehensive studies for the safety assessment.

O-5

Rapid Induction of Lung Tumor in Hras Transgenic Rats and Identification of Cancer Stem Cells

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Worldwide, lung cancer is the leading contributor to cancer-related death in both men and women. It is important to develop a rapid tumor model in lung. We have established transgenic (Hras250) rats in which expression of a human Hras^{G12V} oncogene is regulated by the Cre/loxP system. In this study, we examined the possibility of rapid induction of lung tumor and identification of cancer stem cells.

Targeted lung activation of the transgene was accomplished by injection of Cre recombinase-carrying adenovirus into the trachea. Six weeks after injection, grossly visible nodules were observed throughout the lung in all Hras 250 rats. Histological examination and immunohistochemistry determined that these nodules were Clara cell adenocarcinoma, adenosquamous carcinoma and squamous cell carcinoma. There were no proliferation of alveolar type I cells or alveolar type II cells.

Here we show that the use of a recombinant adenovirus expressing Cre recombinase to induce Hras^{G12V} expression in the lungs of rats allows control of the timing of tumor initiation. This rat model will provide a new tool for understanding the early stages of lung tumor pathogenesis. Of particular significance, this system has led to the identification of a new cell type contributing to the development of pulmonary adenocarcinoma and squamous cell carcinoma.

O-6

Enhancement of NNK-induced Lung Carcinogenesis and *EGFR* Gene Mutation in *Ogg1* Gene Deficient Mice

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The present study was conducted to assess involvement of oxidative stress in lung adeno-carcinogenesis, using mice deficient in the *8-hydroxyguanine DNA glycosylase (Ogg1)* gene that encodes an enzyme repairing oxidative DNA damage 8-oxoguanine (8-oxoG). Homo- and heterozygously *Ogg1*-deficient and wild type mice (C57BL6/J origin), 6 weeks old, were administered 4-(*N*-hydroxymethylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by a continuous subcutaneous infusion using an osmotic pump at a total dose of 6 mg/mouse for 1 week, then treated with nothing or one of the 4 antioxidants (phenyl *N*-tert-butyl nitrone 0.13% in drinking water, resveratrol 20 ppm in diet, lactoferrin 2% in diet and bilberry powder 2% in diet) for 33 weeks, and sacrificed to obtain the lung. The development of lung adenoma and preneoplastic, atypical hyperplasia was significantly enhanced by the homo- and heterozygous *Ogg1* gene deficiency only in female mice with the accumulation of 8-oxoG. All antioxidants tended to inhibit this enhanced adeno-carcinogenesis. Furthermore, mutations were detected in the *epidermal growth factor receptor (EGFR)* and *K-ras* genes within the NNK-induced lung proliferative lesions in mice, at the same sites as those found in human lung cancers. While demonstrating the human-relevant *EGFR* gene mutation in exogenously induced animal tumors for the first time, the present results indicate that the deficiency in the *Ogg1* gene enhances lung adeno-carcinogenesis in mice initiated with NNK by virtue of accelerated oxidative stress.

O-7

Nitron Anti-cancer Activity in APC^{min} Model of Colon Cancer

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We examined the anti-cancer activity of the nitron α -phenyl-tert-butyl nitron (PBN) in the APC^{min} model of colon cancer. PBN was administered in drinking water to the mice starting at age day 21. Tumor development in three groups of 15-20 mice each were examined using gadolinium probe enhanced magnetic resonance imaging (MRI) taken 4 times (7,12,16 and 18 weeks) during tumor development. Termination pathologic data of tumor size and number was also done. MRI showed in the untreated, a time-dependent increase in tumor number and size, but the PBN-treated mice showed a time-depend significant decrease in tumor volume and number throughout the treatment period. Pathologic data agreed with the MRI data. Human colon cancer cells (HCT-116) were used to determine if PBN would interfere with oxaliplatin killing of colon cancer cells. PBN up to 2 mM had only a small effect (4-5%) on oxaliplatin killing. PBN has previously been shown to have anti-cancer activity in animal models of hepatocellular carcinoma and glioblastoma. Supported in part by OARS grant AR052-041, OCAST fMRI.002 and VA Merit Review Program.

O-8

A Short *in vivo* Assay for Carcinogenic Potential of Chemicals by the Gpx2 / Cytokeratin19 Expression Ratio.

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In order to overcome problems or weak points of chronic rodent carcinogenicity bioassay (long-term, high cost, a large number of animals etc.), alternative short or medium term bioassay models have long been desired.

We previously reported that the expression of Gpx2 have been commonly elevated in mammary cancers induced by different carcinogens in transgenic rats carrying the human c-Ha-ras (Hras128). Furthermore, the ratio of Gpx2 / Cytokeratin19 (CK19) expression in six rat breast cancer cell lines, including RMC-2, derived from DMBA-induced mammary carcinomas in Hras128 rats have been higher than those of rat normal mammary tissues.

In the present study, rat mammary carcinoma cell line RMC-2 were exposed to ten kinds of known carcinogens targeted to various organs, including breast, liver and kidney, and two non-carcinogens for three days. At the day 3, mRNAs were extracted from treated cells. Gpx2 and CK19 expression levels were confirmed by quantitative RT-PCR. As a result, the Gpx2 / CK19 expression ratios were elevated more than twice in RMC-2 cells treated with carcinogens compared to non-treated control cells. In RMC-2 treated with non-carcinogens, Gpx2 / CK19 ratios showed be less than twice compared to control.

In conclusion, this Gpx2 assay could be a new, simple method for detection of carcinogenic potentials.

O-9

Cell Cycle and Apoptosis Regulation in Neural Progenitor Cells of the Etoposide-Treated Developing Fetal Brain

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Etoposide (VP-16), a topoisomerase II inhibitor, is an anti-tumor agent which is also known to show embryotoxicity and teratogenicity. In our previous studies, it was shown that VP-16 induced S-phase accumulation and G2/M arrest, and eventually resulted in apoptosis via p53-related pathway in the fetal brain. Pregnant ICR mice were injected intraperitoneally with VP-16 (4 mg/kg) on day 12 of gestation, and fetuses were collected from 1 to 48 hours after treatment (HAT). Fetal telencephalons analyzed by TUNEL, cell cycle analysis, DNA microarray, real time RT-PCR and Westernblotting.

S-phase accumulation of neural progenitor cells might be induced at 4 HAT by acceleration of G1/S transition rather than the inhibition of DNA replication. VP-16 treatment increased the expression of puma, noxa, bax, fas, p21 and cyclin G in fetal brains. ATM and histone H2AX were concurrently phosphorylated at ser 1981 and ser 139 after VP-16 exposure, respectively. Phosphorylation of p53 (ser 15 and 20) and cdc2 (tyr 15), an increase of p21, cyclin A and cyclin B1, and a decrease of cdc 25A were also confirmed by Westernblotting. The TUNEL-positive cells were not observed in the p53-null fetal brains. In p53-deficient brains, VP-16 treatment induced S-phase accumulation, but did neither G2/M arrest nor apoptosis. The results revealed that VP-16 causes DNA double stranded breaks, and induces S-phase accumulation, G2/M arrest and finally apoptosis through activating ATM-Chk 2-Cdc25A, ATM-p53-p21 and ATM-Chk2-p53 pathways, respectively. In addition, it was shown that p53 is essential for VP-16-induced G2/M arrest and apoptosis, but not necessary for S-phase accumulation.

O-10

Effect of 6-Mercaptopurine on Rat Placenta

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In order to investigate the toxic effects of 6-mercaptopurine (6-MP) on placental development, we examined sequential morphology in the placentas from rats exposed to 6-MP. 6-MP was suspended in olive oil, and was intraperitoneally administered at 60 mg/kg during gestation days (GDs) 11 to 12. The placentas were sampled on GD 13, 15 or 21.

In the 6-MP-treated group, maternal body weight suppression, increased death embryo/fetus ratio and some malformations, such as micrognathia, umbilical hernia/omphalocele, anal atresia, phocomelia, digit anomalies, tail defects, and ectopic pinna, were observed. The placenta weights were decreased on GDs 15 and 21. Macroscopically, placentas on GD 21 were small, brittle and thin with a white peripheral rim. Histopathologically, 6-MP treatment mainly evoked decreased mitosis on GDs 13 and 15, increased apoptotic cells on GDs 13, 15 and 21 in the labyrinth zone, and thinning of its zone on GDs 15 and 21. There were decreased trophoblasts, a reduction in thickness of the trophoblastic septa and irregular dilatation of maternal sinusoids with deposition of fibrin in the treated group. In the basal zone, PAS-positive material in the spongiotrophoblasts was still detected on GD 15, although the spongiotrophoblasts in the control group contained little or no PAS-positive material on GD 15. Thickening of the basal zone was observed with cytolysis of glycogen cells, apoptosis and an increased number of composed cells on GD 21.

In conclusion, 6-MP administration in pregnant rats induces DNA damage in trophoblasts, leads to apoptosis and mitotic inhibition in the labyrinth zone and causes a lack of the cell populations required for later normal histogenesis, resulting in a small placenta, and induces delay in the developmental process in the basal zone. 6-MP-induced fetal abnormalities seem to be caused by its specific direct anti-proliferative effects during organogenesis. However, the fetal intra-uterine growth retardation may not only result in the anti-proliferative effects of 6-MP, but also the disruption of placental functions during the fetal period.

O-11

Neonatal Exposure to DES Induces Dose-dependent Delayed Effects at Doses Showing Estrogenic Activity in Female Donryu Rats

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The neonatal exposure to endocrine disrupting chemicals (EDCs) with estrogenic activity is known to induce irreversible damage to hypothalamo-pituitary-gonadal axis called as androgenization in females, which immediately disrupts reproductive organ development before puberty. Recently, delayed effects of the neonatal exposure to estrogenic EDCs, which are inducible by lower treatment, have been focused as another type of neonatal effects, because these effects can't be detected in short-term bioassays. This type has been already reported as 'delayed anovulatory syndrome' (DAS), but its disruption pathways and endpoint markers remain fully undetermined. We investigated the relationship between occurrence of delayed effects and dose dependency in long-term reproductive organ responses to neonatal exposure to diethylstilbestrol (DES) in female rats. Female Donryu rats treated with single dose of DES at 0.15 to 1500 ug/kg body weight within 24 hours after birth were observed for 12 months. In addition, the rats were initiated with ENNG at 10 weeks of age to investigate uterine carcinogenesis. Typical androgenization was observed at 1500 ug/kg and 150ug/kg before puberty. Although no changes were detected before puberty, early onset of persistent estrus was detected in rats at 1.5 ug/kg body weight (at 21 weeks of age) and higher. Uterine adenocarcinoma development was promoted at 150 ug/kg. In uterotrophic assay, single dose treatments with DES at 1.5 ug/kg and higher exerted estrogenic activity. These results indicate that delayed effects were induced by neonatal exposure to DES at the doses showing estrogenic activity with dose-dependent manner. Estrous cyclicity is a useful marker to detect the effects. In addition, prolonged persistent estrus reflecting higher relative E2 levels might play an important role in promoting effects on uterine carcinogenesis.

O-12

A New Non-diabetic LEA.F-*Xdm1* Congenic Rat Strain with Long-Evans Agouti (LEA) Backgrounds

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The Long-Evans Agouti (LEA) rat is an inbred strain that spontaneously develops pancreatic non-lymphocytic insulinitis at around 8 weeks of age. Whole body X-irradiation induced lymphocytic insulinitis with severe hyperglycemia and hypoinsulinemia within several weeks in LEA rats. In the QTL analysis study, one of the susceptibility loci for X-irradiation-induced hyperglycemia was mapped to D20Rat47-D20Mgh4 (20p12) region. In an attempt to develop a new non-diabetic congenic rat strain, we crossed the LEA/Tj strain with the F344/DuCrj strain to create F1 animals, which were later backcrossed to LEA rats to produce the N2 generation. Using marker-assisted selection, we then performed five generations of backcrossing. Finally, homozygous LEA.F-*Xdm1* congenic rat strain was obtained from intercross of N5 animals. To induce diabetes in these animals, we used a bioassay system for diabetes, in which male rats were X-irradiated at a dose of 2 Gy at week 6 and 8, followed by an oral 2 g/kg glucose tolerance test performed at week 12. In our results, a 30-min postglucose-load blood glucose level was 403 ± 85 mg/dl in LEA rats and 191 ± 15 mg/dl in LEA.F-*Xdm1* rats ($P < 0.001$). Moreover, in the congenic strain, lymphocytic aggregation in the islets was completely suppressed while the spontaneous changes observed in islets of LEA rats were persistent. The present data suggest that the 20p12 region, MHC gene (RT1) locus in rats, plays an important role in the development of X-irradiation-induced diabetes in LEA rats, though the identification of the gene(s) responsible for spontaneous diabetes in LEA rats is worthy of further investigation.

P-1

Nasal Tumor and the Tumor Related Lesion by Long-Term Administration of 1,4-Dioxane in Rats – Comparison of the Nasal Lesions Induced by Inhalation and Drinking Water Routes–

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1,4-Dioxane is known as a contaminant found in both the atmosphere and public water, and may lead human health risks by inhalation and oral exposure. Carcinogenicity studies showed that 1,4-dioxane induced nasal tumors in rats by either inhalation or drinking water exposure. In this study, we compared histopathological types and location of the nasal tumors and the tumor-related lesions induced by 1,4-dioxane in the inhalation and oral routes. **【Methods】** Eight groups of 50 male F344/DuCrj rats each (starting at an age of 6 weeks) were used for the inhalation and oral studies. In the inhalation study, 4 groups of rats were exposed to 1,4-dioxane vapor at a concentration of 0, 50, 250 or 1250 ppm by inhalation route for 2-years. In the oral study, other 4 groups of rats were given 1,4-dioxane-containing water at a concentration of 0, 200, 1000 or 5000 ppm as drinking water for 2-years. The nasal cavity was trimmed at three levels along the frontal plane (Level 1, 2 and 3 from forward).

【Results】 Squamous cell carcinomas and squamous cell hyperplasia in the nasal cavity were occurred by exposure to 1,4-dioxane in both inhalation and oral studies. Squamous metaplasia, inflammation and nuclear enlargement in the respiratory epithelium, atrophy, respiratory metaplasia and nuclear enlargement in the olfactory epithelium, hydropic change and sclerosis in the lamina propria and proliferation nasal gland were also seen in the both routes. The squamous cell carcinomas and squamous cell hyperplasia were located in metaplastic respiratory or olfactory epithelium at dorsal region in both inhalation and oral routes. No remarkable difference in the locations of non-neoplastic lesions were indicated between the inhalation and oral routes, excepting the locations of nuclear enlargement in the respiratory epithelium. The enlarged nuclei of respiratory epithelial cells were mainly localized at Level 1 in the 500 ppm group of inhalation exposure. While, the location of the enlarge nuclei in the 250 and 1250 ppm groups of inhalation exposure and in the 5000 ppm group of oral administration were expanded over the entire respiratory region at Levels 1 through 3. The squamous cell carcinomas, squamous cell hyperplasia and squamous metaplasia showed a similar location in the inhalation and oral routes. The localization of nuclear enlargement observed in the respiratory epithelium lining the anterior portion of nasal cavity in the 500 ppm group of inhalation exposure might be caused by direct contact of this portion to 1,4-dioxane vapor.

P-2

Deposition Process of Eosinophilic Substance in Mouse Nasal Septum

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An eosinophilic substance is usually observed in the mouse nasal septum, and its volume increases with aging. It has been described as amyloid in textbooks. However, our previous report revealed that the eosinophilic substance was not amyloid because it reacted negatively to Congo red and non-branching fibrils were not observed electron microscopically. It has also been reported that when examined electron microscopically, the eosinophilic substance consisted of not only collagen but also of amorphous material, which might be a complex carbohydrate. Furthermore, it was suggested that the amorphous material might be produced by the clear HE stained nasal gland epithelial cells (Doi et al. Vet Pathol 44, 2007). In this study, the process that the amorphous material produced by the nasal epithelial cells deposits in the interstitium was investigated histopathologically and electron microscopically. [Materials & Methods] B6C3F1/Crlj mice purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan) were used. The nasal tissues of 5- and 110-week-old mice (5 males and 5 females each) were stained with HE and reticulin silver impregnation, and were then examined histopathologically. In addition, the clear HE stained nasal glands in 42-week-old mice (3 females) were observed under an electron microscope (H-7600, Hitachi, Tokyo, Japan). [Results & Conclusions] In 5-week-old mice, very little eosinophilic substance was deposited in the clear HE stained nasal gland area, and the nasal glands were almost completely surrounded by the argyrophilic fiber. In 110-week-old mice, the eosinophilic substance was markedly deposited in the clear HE stained nasal gland area, and the argyrophilic fibers surrounding the nasal gland partially disappeared where the border between the nasal glands and the interstitium was indistinct. Furthermore, in some nasal glands, the eosinophilic substance was observed at the space between the nasal epithelial cells and the surrounding argyrophilic fibers. Electron microscopically, the amorphous material was observed at the basal portion of the nasal gland epithelial cells as well as in the interstitium where the fibroblasts were observed. The amorphous material mostly contained free organelle. In some nasal glands, the collagen fibers surrounding the nasal glands partially disappeared and the amorphous material in contact with the rER of the nasal gland epithelial cells continued to the amorphous material in the interstitium. The above-mentioned findings suggested that the amorphous material might be produced in the clear HE stained nasal gland epithelial cells, accumulated at the basal portion, and migrated to the interstitium by partially breaking the basement membrane. The amorphous material might be significant material because no degenerative nasal gland epithelial cells were observed even in the area that the collagen fibers disappeared.

P-3

Acute Pathological Changes of the Lung in Phenylhydrazine-treated Rats

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Phenylhydrazine (PHZ) is well known for its capacity to induce oxidative hemolysis in animals and often selected to develop a hemolytic anemia model. In PHZ-treated animals, histopathological changes in hematopoietic systems, such as spleen and liver, are commonly observed, however, there is little information about other organs. In this study, we reported pathological change of the lung in PHZ-treated rats.

Three male 6-week-old Sprague-Dawley rats were injected intraperitoneally with 50 mg/kg/day PHZ for 3 days. Macroscopical and microscopical examinations were performed at the 24 hr after 3rd administration.

Anemic conditions, such as pale skin, were observed in all rats from 6 hr after 1st administration. Decrease in locomotor activity was observed after 2nd administration, and two of three rats died after 3rd administration. Another animal was terminal sacrificed. Macroscopically, lung of all PHZ-treated rats showed red or dark. Light microscopically, eosinophilic material was observed in the alveolar septa of lung. Edema and hemorrhage were also observed in some part. This eosinophilic material was stained red with Masson trichrome stain, and was weakly positive for PTAH stain. Electron microscopically, fibrin-like fibrils were observed in alveolar capillary and a part of alveolar space. It was revealed that the eosinophilic material observed light microscopically was fibrin thrombus. Thrombus was also observed in left atrium of heart in all PHZ-treated rats. In addition to lung lesions, erythrophagia and increased extramedullary hematopoiesis in liver and spleen, and pigmentation in various organs were observed.

Acute thrombus formation was observed in the lung of PHZ-treated rats. In a therapeutic use of PHZ on human, vascular thrombosis was noted as a side effect, and it has been reported intravascular hemolysis could be the basis for disseminated intravascular coagulation. However, acute thrombus formation in the PHZ-treated rats has not been reported. Now, further studies are carrying out to clarify the mechanism of thrombus formation.

P-4

Prenatal 3,3',4,4',5-Pentachlorobiphenyl Exposure Influence Lung Carcinogenesis in Male Rat by

N-nitrobis(2-hydroxypropyl)amine

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【Purposes】 Porychlorynated biphenyl (PCBs) had been produced and used for commerce abundantly. Recently PCBs are detected in river, air, and soil, and it is indicated that influence human and wild animals through out food chains. We investigated that prenatal exposure 3,3',4,4',5-pentachlorobiphenyl (PCB126) for *N*-nitrobis(2-hydroxypropyl) amine (BHP) induced rat lung carcinogenesis.

【Material and Methods】 SD rats were injected (i.g.) PCB126 (7.5 µg, 250 ng, and 2.5 ng/kg BW) or the vehicle during 13-19th days post-conception. In 5-week-old offspring rats were treated BHP (1,000ppm) for 8weeks, and were dissected at 25-week-old. 【Results】 Histological analysis showed the incidences of adenomas were decreases in PCB126 groups. The incidences of squamous adenocarcinomas were significantly increased at 7.5ug group. Immunohistochemical and westernblotting analysis revealed that the expression of MEK, ERK, and Tpl-2 were higher in PCB126 groups. 【Discussion】 Tpl-2 activates MEK, and participate in activation for MAP Kinase pathway. The present study suggested that PCB126 groups obviously activated these proteins in BHP induced rat lung carcinomas. It might be consider that prenatal PCB126 exposure played an important role in downstream factors of MAP Kinase pathway.

P-5

TGF-β1/smad2/3-mediated Pulmonary Fibrosis Following Intratracheal Instillation of Didecyltrimethylammonium Chlorides (DDAC) in Mice

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An alkyl ammonium compound (cationic surface active agent), DDAC has been widely used as a detergent of germicides, antiseptics, and wood preservatives. The study was conducted in C57BL/6J mice following intratracheal instillation of DDAC to assess pulmonary toxic injuries. We initially intratracheally instilled to the lung with DDAC to determine a dose-related response. The high dose (0.1%) of DDAC treatment caused severe morbidity with pulmonary congestive edema. The middle dose (0.01%) of DDAC exposure increased inflammation, while the low dose (0.001%) did not, when examined inflammatory cells in bronchoalveolar lavages (BAL) from survivors 3 days after instillation. Next we examined time course changes in BAL and lung tissues from mice received the middle dose (0.01%) of DDAC instillation. The numbers of BAL macrophages, lymphocytes, and neutrophils were increases in a time-related manner, reaching peak at 7 days, in coincidence with increased IL-6, but not IL-10, -13, and IFN-γ. Glaucomatous fibrotic foci were strikingly observed in the lung at 3 days, and extended widely 7 days after instillation, with evidence of increased α-smooth muscle actin (α-SMA) and/or vimentin-positive cells. Developing fibrotic foci were likely associated with increased expression of TGF-β1 mRNA, but not TGF-β2 and -β3 mRNA. Phosphorylated smad2/3 was increased in fibrotic lungs, suggesting that there was activation of TGF-β signaling. To further explore the contribution of TGF-β/smad signaling, we isolated fibroblast-like cells from mouse lung, and then treated with a low dose (5 µM) of DDAC. In consistent with *in vivo* data, TGF-β1 mRNA was increased by DDAC treatment *in vitro*, while TGF-β2 and -β3 mRNA were decreased. DDAC cancelled time-related reduction of α-SMA expression which was consistent with prolong phosphorylation of smad2/3. Pretreatment with SD208, a TGF-βRI (ALK5) kinase inhibitor attenuated DDAC-induced α-SMA expression through suppressing phosphorylation of smad2/3. These results suggested that DDAC instillation to mouse lung causes pulmonary fibrosis and these changes might be mediated by TGF-β1/smad2/3 signaling.

P-6

Different Pathological Changes Due to the Suspension in the Intratracheal Instillation of Multi-Wall Carbon Nanotubes (MWCNT)

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[Introduction] Multi-Wall carbon nanotube (MWCNT) is a new industrial material. There has been concern about the potential for toxicity in humans; however, because of its tendency to aggregate, there are only a few reports concerning the toxicity of dispersed MWCNT fibrils. We examined the preparation method to disperse the fibrils for intratracheal instillation of MWCNT and its effect on the pathological changes. [Materials and methods] We prepared two different MWCNT suspensions; MWCNT was ground in an agate mortar and suspended in artificial lung surfactant as a vehicle (ground MWCNT), and MWCNT was suspended in the vehicle without grinding (non-ground MWCNT). To investigate the effect of the grinding, both MWCNT suspensions were examined microscopically and ultrastructurally (transmission electron microscope; TEM and scanning electron microscope; SEM). For the toxicity study, 4 experimental groups, each consisting of 12 rats, were provided. Ground MWCNT (ground group), non-ground MWCNT (non-ground group), Min-U silica suspended in the vehicle as a reference particle, or the vehicle were instilled intratracheally to each group on day 1. There were 3 animals of each group euthanized and necropsied at four time points (days 2, 8, 29, 92). The lung, trachea, and bronchial lymph node were examined histopathologically. The lung was submitted for the TEM examination. [Results] In the observations of the ground MWCNT suspensions, aggregated fibrils decreased and become smaller (microscope), and each fibril shortened (TEM), and distributed equally in the vehicle (SEM) compared to the non-ground suspensions. In the toxicity study, the lung of both MWCNT groups showed accumulation of MWCNT on day 2, mainly in the bronchial lumen of the non-ground group, and in the alveolus of the ground group. In addition, atelectasis with erosion of bronchial mucosa was observed in the non-ground group. On day 8 or later, focal infiltration of MWCNT-laden macrophages were observed in the interstitium of the lung of the non-ground group, compared to the ground group, where macrophages were observed mainly in the alveolus. Ultrastructurally, the macrophages in the alveolus of the ground group had well-developed lysosomes. [Discussions] In this study, the aggregation of MWCNT was inhibited by the grinding. The mechanism thought to have caused the different pathological changes between the two groups is as follows. In the ground group, as the fibrils became dispersed and shorter, the phagocytosis due to the macrophages seemed to progress more easily than in the non-ground group. As a result, the characteristic interstitial change of MWCNT observed in the non-ground group was limited in the ground group.

P-7

Nano-Nickel Oxide Inhalation Study -Direct Elemental Mapping of Particles on HE-Stained Samples and Pulmonary Effect in Rat Lung-

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Background) Nickel compounds are classified as oncogen (class 1) by IARC or Japan Society For Occupational Health. In this study, to establish the hazard assessment for nanoparticles, we examined the biological effects of the nickel oxide (NiO) nanoparticles in an inhalation study.

Materials and Methods) The nanoparticle generation system was composed of an ultrasonic nebulizer and diffusion dryers, and 30 Wistar male rats were exposed to NiO nanoparticles for 4 weeks (6 hrs/day). The NiO nanoparticles were black-colored NiO (99.8%) and APS was 20 nm (Nanostructured & Amorphous Materials Inc.). The geometric mean diameter of the particles and the geometric mean diameter in the chamber and the daily average exposure concentration were 139 ± 12 nm and $1.0 \pm 0.5 \times 10^5$ particles/cc, respectively. At 4 days, 1 and 3 months after the inhalation, each group of 10 rats were sacrificed and histopathological changes in the lung were determined by point counting method (PCM). The elemental mappings of nickel in the HE stained lung tissue or pulmonary macrophages were also analyzed directly by scanning electron microscopy and Energy Dispersive X-Ray Micro Analyzer (HORIBA).

Results) Elemental mappings of nickel showed that nickel particles were located in agglomeration at the mild inflammatory lesion or pulmonary macrophages. Although NiO exposure group showed temporal significant increase in the number of total cells in bronchoalveolar lavage fluid (BALF) at 4 days after the exposure end, the difference were not seen at one month after an exposure end compared with control group. The histopathological change was not severe just after the inhalation nor throughout the observation time by PCM. The deposited amount of NiO nanoparticles in the rat lungs at 4 days after the inhalation was 29 ± 4 μ g.

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P-8

A role of RTP801, a suppressor of the mammalian target of rapamycin (mTOR) pathway, in cigarette smoke-induced pulmonary injuries in mice

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RTP801 is a stress-responsive gene, which inhibits mTOR, and thus affects cell growth, and protein synthesis. We determined whether RTP801 contributes to acute pulmonary injury in mice following cigarette smoke (CS) exposure. CS increased RTP801 mRNA and protein levels in the lung of RTP801 WT mice, and the protein expression was prevented by pretreatment with the antioxidant N-acetylcysteine. Although RTP801 had been expected to be an mTOR repressor, WT mice paradoxically showed increased mTOR activity, as demonstrated by hyperphosphorylation of ribosomal protein S6, a downstream target of mTOR, with evidence of alveolar inflammation and apoptosis. The role of mTOR in alveolar injury caused by CS exposure was supported by the findings that pretreatment with rapamycin, an mTOR inhibitor, prevented CS-induced inflammation, a protection that was associated with hyperphosphorylation of AKT. RTP801 KO mice showed significant protection against CS-induced alveolar injury, along with inactivation of NF- κ B. These effects were also dependent on mTOR as pretreatment with rapamycin restored KO susceptibility to CS. The KO mice had a higher baseline level of mTOR activity than WT mice. Supportive evidence was obtained from isolated lung fibroblasts which showed increased baseline level of phosphorylated S6 and reduced sensitivity to CS extract-induced NF- κ B activation in KO cells when compared with WT cells. These findings suggest that RTP801 regulates mTOR, which has a dual function of promoting alveolar injury after CS exposure and of preventing lung pathology when increased at baseline. Targeting RTP801 might provide a new therapeutic target of CS-mediated pulmonary diseases.

P-9

Pulmonary Injuries by Combined Intratracheal Administration of Oxidative Stress Inducers Chromium (VI) and Arsenate (V) in Mice

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Most wood preservatives developed to protect wood contain toxic or hazardous chemicals that can cause adverse impacts to human health. Amidst a rising tide of concern about the arsenical preservatives, especially Chromated Copper Arsenate, our recent research has been focused on understanding its toxic effects by oral and dermal exposure routes. Whereas chromium (Cr) and arsenic (As) within metal fumes impact respiratory diseases in human, the combined effects of these metals have not been elucidated. The present study was conducted in mice when intratracheally instilled As(V) and/or Cr(VI) to evaluate inflammatory response, cytotoxicity, and oxidative stress on respiratory system. C57BL/6 mice received 1250 μ g/kg of Cr(VI), 1560 μ g/kg of As(V), their mixture, or PBS. Bronchoalveolar lavage (BAL) and lungs were sampled 2 days after instillation. Instillation of As(V) or Cr(VI) increased the number of BAL neutrophils, which was greater in Cr(VI) exposure than in As(V). Concurrent As(V) exposure worsened Cr(VI)-induced BAL neutrophils, which were associated with increasing the numbers of BAL macrophages and lung neutrophils. These findings were supported by evidence of elevated IL-6, total protein, and LDH activity in BAL and caspase-3/7, -8 and -9 activities in the lung, along with enhanced ROS production and increased GSH and GSSG contents caused by co-treatment, when compared with the controls. These stress responses were possibly coordinated by MAPK signaling, as phosphorylation of ERK, SAPK/JNK and p38 MAPK was evident by combined treatment as well as Cr exposure. In vitro MTT assay revealed that Cr(VI) rather than As(V) induced cytotoxicity in a dose-dependent manner and co-treatment of As(V) enhanced Cr(VI)-induced cytotoxicity. These findings suggest that adverse effect of Cr(VI) is more evident than that of As(V) on acute respiratory system, and Cr(VI) exposure with As(V) makes its effect enhanced.

P-10

Review of Pulmonary Nodular Hyperplasia in Beagle Hounds.

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The literature of Multifocal pulmonary nodular hyperplasia in beagle hounds was published by A. Hajdu and G. Rona in 1965. Histologically, the lesion was suggested that scar formation associated with regressive epithelial changes leads to regeneration, proliferation and metaplasia of alveolar and bronchiolar cells, but the obvious cause was unknown. In our laboratory, similar lesions were occasionally observed in toxicity studies of beagle hound. Therefore we reviewed these lesions using 65 toxicity studies of beagle hound (5-25 month old) from 1996 to 2006. In the beginning, we introduced gross and histological characters and incidence of these lesions. [Results] Macroscopically, the lesions consist of small subpleural, grayish-white nodule or patch. These were observed predominantly in the upper lobes in both sexes. Incidences of pulmonary nodular hyperplasia were 13.7 % in males, 11.8 % in females. Age-related changes of the incidence were not obvious. Histologically, Fibrosis and proliferation of epithelial cell were fundamental characters. And squamous metaplasia and thickened pleura were occasionally observed. However, findings of damage of the parenchyma and acute inflammatory reaction were rarely observed. These features were similar to the previous literature. [Conclusion] Although the causes of pulmonary nodular hyperplasia were still uncertain, we also considered reactive change of pulmonary epithelium to some injuries. Because sequence reactions of alveolar repair such as hyperplastic alveolar cell, fibrosis and scar formation were observed.

P-11

Search for Molecules Linked to The White Matter Retardation in Relation with Developmental Hypothyroidism in Rats Exposed Developmentally to Brominated Flame Retardants

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Developmental hypothyroidism causes brain retardation due to impairment of neuronal migration and oligodendroglial myelination. Brominated flame retardants (BFRs), potential environmental pollutants to cause bioaccumulation, usually exert low or no toxicity in conventional toxicity studies, but some of them are known to induce mild hypothyroidism. To search for target genes responsible for possible brain retardation due to BFRs, we performed a global gene expression profiling specific to the cerebral white matter of rat pups developmentally exposed to decabromodiphenyl ether (DBDE). Dams were given DBDE at 10, 100, or 1000 ppm in diet during the period from gestation day 10 to postnatal week 3 (weaning). As a positive control for hypothyroidism, anti-thyroid agent (methimazole or propylthiouracil) was given via drinking water. At weaning, bilateral cerebral white matter and corpus callosum (CC) were selectively microdissected from male pups and subjected to microarray analysis. As a result, 10% of genes showing altered expression by DBDE were identical to those showing fluctuations by anti-thyroid agents involving functions for glial cell differentiation, axon guidance, myelination, and cellular migration. Also, DBDE-alone induced expression alteration of genes related to similar functions as with anti-thyroid agents. After gene screening, vimentin was selected for immunohistochemical analysis in the brains of 3-week-old offspring exposed to hexabromocyclododecane (HBCD) or tetrabromobisphenol A (TBBPA) as well as DBDE. HBCD or TBBPA was given at 100, 1000, and 10000 ppm in the maternal diet. As a result, as well as anti-thyroid agents, DBDE and HBCD increased the number of vimentin-positive cells. At 11 weeks of age, offspring were subjected to brain morphometry regarding white matter components. As a result, as well as anti-thyroid agents, DBDE and HBCD reduced the CC area and the number of CNPase-positive oligodendrocytes suggestive of reduced white matter development. Results thus suggest that the increase of vimentin-positive cells in the white matter may be the good marker for early detection of the retardation of glial differentiation induced by BFRs probably reflecting developmental hypothyroidism.

P-12

The Effects of 5-Fluorouracil (5-Fu)-induced on the Fetal and Placenta Tissues of Wistar Rat

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5-Fluorouracil (5-Fu), a well-known thymidylate synthesis inhibitor, induces developmental anomalies mainly in the craniofacial tissues and limb buds. We examined the effects of 5-Fu in the fetal and placenta tissues of rats. In this study, pregnant rats were treated with 5-Fu (15, 30 or 50 mg/kg) on Day 13 of gestation (GD 13), and their fetuses were examined for histopathological changes at 12, 24 and 48 hours after treatment (HAT).

No deaths occurred in dams or fetuses of any group. The weights of the fetus and placenta were significantly reduced at 48 HAT at 50 mg/kg. But, there were no changes in the fundamental structure of the placenta in any group.

At 15 mg/kg, there were no significant differences in the histology of fetuses between 5-Fu-treated group and the control group. At 30 and 50 mg/kg, pyknotic cells were observed in the brain, spinal cord, mesenchymal tissues of the craniofacial region, hindlimb buds, hematopoietic progenitor cells of the liver, epithelial cells of the lung and digestive tract and cells of the gonads. The incidence of pyknotic cells differed among tissues. The severity of pyknosis differed among tissues and was most prominent in the telencephalon and spinal cord. Almost all of the nuclei of pyknotic cells were positively stained by TUNEL method and showed characteristics of apoptotic cells under electron microscopy. Therefore, these pyknotic cells were considered to be apoptotic ones. In most tissues, enhancement of pyknosis was detected at 12 HAT, and decreased to the control level at 48 HAT. Pyknotic cells tended to decrease in most tissues at 48 HAT. At 50 mg/kg, the cells in the spinal cord were enhanced at 12 HAT, and remained at 48 HAT.

In the fetuses of pregnant rats to which 5-Fu was administered on GD13, apoptotic cell were observed in many kinds of tissues, especially in the telencephalon and spinal cord. The changes in the telencephalon seem to be responsible for the later induction of microencephaly.

P-13

Pathological Assessment of the Nervous System of Rat Offspring Exposed to Acrylamide During the Gestation and Lactation Periods – A Preliminary Study

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To evaluate the developmental exposure effect of acrylamide (ACR) on the nervous system, pregnant Sprague-Dawley rats were given ACR at 0, 50, 100 or 200 ppm in the drinking water from gestational day 10 to postnatal day (PND) 21 and histopathological assessment of offspring was performed at weaning (PND 21) and postnatal week (PNW) 11. Neurotoxicity was quantitatively assessed with reference to nerve fiber density, percentages of degenerated and small caliber axons in the sciatic nerves, and numbers of aberrant dot-like structures immunoreactive for synaptophysin in the cerebellar molecular layer. In addition, a group of rat neonates that received ACR at 50 mg/kg by intraperitoneal injections 3 times a week from PND 2 to 21 was subjected to analysis for comparison with maternal exposure groups. Dams showed dose-related lowered body weights and neurotoxicity, both of them being apparent during the lactation period after delivery. Offspring exposed to ACR maternally also showed lowered body weight from PND 2 through weaning from 50 ppm in males and from 100 ppm in females, the lowered body weight being remained until PNW 11. However, no histopathological changes as well as gait abnormality suggestive of neurotoxicity were observed in these ACR-exposed offspring at both PND 21 and PNW 11. In contrast, offspring given ACR intraperitoneally revealed gait abnormality and peripheral nerve axonal degeneration. Slight reduction of neonatal body weight examined shortly after birth was considered to be due to developmental effect of ACR administered during gestation as reported by others. While ACR injected intraperitoneally caused neurotoxicity, no signs of neurotoxicity were found in offspring exposed to ACR maternally. Therefore, poor lactational ACR-exposure due to maternal toxicity might account for the lack of ACR-induced offspring toxicity other than retarded body growth.

P-14

Toxicological Effects of the Brominated Flame Retardants, Hexabromocyclododecane (HBCD) and Tetrabromobisphenol-A (TBBPA), on Rats after Developmental Exposure. — Effects on Brain Development—

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There is a concern on the risk of the affection of normal development of the nervous and immune systems as well as the sexual differentiation due to hypothyroidism by exposure to agents exhibiting anti-thyroid effects during pre- and early postnatal life. Many of brominated flame retardants with potentials to persist in the environment and bioaccumulate, have been shown to possess a weak anti-thyroid activity. In the present study, we assessed the effect of developmental exposure to HBCD and TBBPA on rat offspring, especially focusing on brain development. Pregnant SD:IGS rats were administered HBCD or TBBPA at doses of 100, 1000 and 10,000 ppm in diet from gestation day 10 to postnatal day 21 (PND 21, weaning), respectively. Offspring at PND 21 and week 11 were subjected to measurements of serum thyroid-related hormone levels (males, T₃, T₄, and TSH) and histopathological assessment (males and females). At week 11 (males), brain morphometry regarding neuronal cell distribution at the hippocampal CA1 region, size area of the corpus callosum (CC) and density of CNPase-positive oligodendrocytes were conducted. As a result, dams exposed to HBCD and killed at weaning showed increases of thyroid weight and incidence of thyroid follicular cell hypertrophy at 10,000 ppm. Pups at this time point showed decrease of the body weight, increases of the liver weight and incidence of hepatocellular vacuolization in both sexes, as well as slightly suppressed T₃ and marginally increased TSH at 10,000 ppm. On week 11, offspring showed increased thyroid weight and slightly decreased T₃ in males from 1000 ppm. Moreover, adrenocortical vacuolization was observed in males at 10,000 ppm. In the brain, CNPase-positive oligodendrocytes were reduced at 10,000 ppm, whereas neuronal cell distribution at the CA1 and area of the CC were unchanged. TBBPA did not show any dose-related changes. The results suggest that developmental exposure to HBCD caused a slight hypoplasia of the white matter reflecting hypothyroidism in the offspring at 1000 ppm and above.

P-15

Development of Clinical Stage and Histopathological Change of Spinal Cord in Experimental Autoimmune Encephalomyelitis model.

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Experimental autoimmune encephalomyelitis (EAE) serves as the animal model of multiple sclerosis and is used for many studies. However, there are few reports that investigated histopathological change of CNS in clinical stage of EAE model.

Female 8-week-old C57BL/6 mice were immunized subcutaneously with myelin oligodendrocyte glycoprotein emulsified in CFA containing *Mycobacterium tuberculosis* H37RA. The mice received intraperitoneally injections with pertussis toxin at the time of immunization and 48 h later. Mice were monitored regularly for clinical signs and were scored on a scale of 0-6: 0, no clinical signs; 1, limp tail; 2, complete loss of tail tonicity or abnormal gait; 3, partial hind limb paralysis; 4, complete hind limb paralysis; 5, hind and fore limb paralysis; 6, death. On Day21, Two mice (mild case of clinical score 1, and severe case of clinical score 4) were perfused with PBS and 4% paraformaldehyde. The brains and spinal cords were removed, and embedded in paraffin. We performed H&E, Kluver-Barrera's stain and used Iba1 antibody for macrophage, CD3 antibody for T-cell.

We didn't find pronounced change in the brain. At spinal cord in each case, CD3 and Iba1 positive monocytes infiltrated. Demyelination was observed at Lumbar and Sacral spinal cord of the mild case mouse, at continuously from Cervical to Sacral spinal cord of the severe case mouse. In conclusion, our findings suggest that demyelination in EAE occurs at caudal side and then progresses cranially and leads to aggravation of paralysis.

P-16

Immunohistochemistry of N-Ethyl-N-nitrosourea Induced Rat Brain Tumors

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To date, N-ethyl-N-nitrosourea (ENU) induced rat brain tumors were reported about immunohistochemical study, but not much has been done to clarify the tumor lesions and protein expression. In this study, we carried out immunohistochemical panel of ENU induced rat brain tumors, and investigated the difference of protein expression in the tumor lesions.

F344/DuCrj rats were injected intravenously via tail vein with 10.0 mg/kg ENU at gestational days 20. Pups were housed for 30 weeks until anesthesia. The immunohistochemistry were done using brain tumors diagnosed astrocytoma, oligodendroglioma, astrocytic microtumor or oligodendroglial microtumor.

In astrocytoma, some tumor cells expressed nestin, stroma and some endothelial cells were weakly reacted with α -internexin or AQP1, respectively. All tumor lesions were positive for GLUT1, GLUT3 and GLUT5.

ENU-induced rat astrocytoma expressed nestin of neuronal stem cell marker and α -internexin of immature neuron differentiation marker. Nestin is known as markers for PNET, glioblastoma, ependymoma and astrocytoma in human and malignant glioma in rat. Results of this study indicate that nestin may take part in the occurrence of astrocytoma, AQP1 relate to brain edema and GLUT families act to transport glucose in ENU-induced rat brain tumor as human. In conclusion, this study demonstrates a similar protein expression between human and ENU-induced rat brain tumor.

P-17

Intracranial Malignant Schwannoma With Invasion in The Brain of A Rat

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[Introduction] Spontaneous trigeminal malignant Schwannoma is rare in rats. We report on malignant Schwannoma which occurred in the trigeminal nerve and invaded the brain.

[Animal case] The animal (died at 28 weeks of age) was a male rat, Crl:CD(SD), in a 26-week toxicity study. The body weight began to reduce a week before death. The rat showed convulsion, prone/lateral position and bradypnea on the day of death.

[Result] At necropsy, grayish-white, partially dark red nodule ($10 \times 10 \times 5$ mm), involving the trigeminal nerve, was observed in the bottom of the cranial cavity. There were two nodules ($10 \times 10 \times 5$ mm, $5 \times 5 \times 2$ mm) in the parietal bone, and a larger one adhered to the neighboring cerebrum. Histologically these 3 nodules resembled each other, and consisted of fusiform cells with eosinophilic cytoplasm and round cells with scant cytoplasm. The fusiform cells were arranged in interlacing fascicles and wavy pattern, and the round cells packed closely. Blood cysts lined by tumor cells were observed. Smaller parietal nodules were surrounded by the stretched bone tissue. In the other nodules, the tumor cells invaded surrounding tissue including pituitary, destroying bone. The cells invading the brain resembled the tumor cells in the nodule of cranial base. The tumor cells infiltrated diffusely in meninges, perivascular space and parenchyma of the brain. In the parenchyma, the borders of lesions were indistinctive, and reactive astrocytes were observed in the lesions. Immunohistochemically, the tumor cells from nodules of cranial base and parietal bone showed positive reactivity for anti S-100 protein and GFAP. On the other hand, the tumor cells invading the brain had partial positive reactivity for anti S-100 protein and had negative reactivity for GFAP. With silver stain, argentaffin fibers surrounding tumor cells were observed.

[Conclusion] From these pathological findings, this tumor was diagnosed as malignant Schwannoma that occurred from the trigeminal nerve. In this case, the infiltrative pattern in the brain resembled those of "malignant reticulosis" or "malignant astrocytoma" in rat. This is an interesting case in which tumor cells invaded the brain from outside the brain.

P-18

Potential of a Noninvasive High-Frequency Ultrasonography for Investigation of Vascular Injury in Comparison with Histopathology

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We evaluated a potential of a high-frequency ultrasonography (US) system, Vevo 770 (VisualSonics) in the continuous non-invasive observation of a development of aneurysm using vascular injury model induced by angiotensin II (AngII) in comparison with histopathology.

Eight weeks old 25 male apolipoprotein E deficient (apoE^{-/-}) mice were used. A high fat diet was fed to mice throughout the study. AngII was infused subcutaneously with osmotic pump at the dose of 1.44 mg/kg/day for 4 weeks. Blood pressure (BP) was monitored by a telemetry system. US observation was performed on the abdominal aorta (AA) and renal artery (RA) periodically. After the 4-week treatment, mice were euthanized and examined grossly. General histopathological examination was conducted on vessels.

AngII-induced vascular dilation was detected with US in the AA and RA from the 1st week of treatment. Thoracic and abdominal aortic aneurysm (vascular dilation of $\geq 200\%$) were observed in 1 and 3 mice out of 15 mice, respectively. US images of vessels were consistent with histopathology in terms of shape. Type of aneurysm (e.g. dissecting aneurysms) could be predicted by US images in case that lesions were severe. Hemodynamic changes could be also detected with US.

In conclusion, vascular remodeling in AngII-treated apoE^{-/-} mice, including vascular dilation, hemodynamic dysfunction and aneurysms, could be estimated with the high-frequency US. Morphological observation with US was consistent with histopathology. Thus, it is suggested that the high frequency US system is a valid method of vascular dysfunction assessment in noninvasive longitudinal rodent studies.

P-19

RNA Extraction and Quantification from Formalin-Fixed Paraffin-Embedded Sections of Rat Liver Specimens

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Formalin-fixed, paraffin-embedded (FFPE) tissue specimens are stored around the world, and a lot of useful information about gene or protein expression is potentially retained in these samples. However, the FFPE samples have been exclusively used to the histochemical studies (e.g. immunostaining or *in situ* hybridization) because the remaining nucleic acids or proteins in FFPE samples were highly degraded and bridged. Here we performed RNA extraction from rat liver FFPE specimens and measured RNA levels by quantitative reverse transcription PCR (qRT-PCR). By using commercially available RNA purification system, we successfully extracted sufficient yield and purity of RNA from the liver sections (5 μ m-thick), and the extracted RNA was readily PCR-amplified. We then prepared both FFPE specimens and intact (RNA preservative solution-fixed) tissues from the liver that were administered by two chemical agents, Wy14643 (PPAR α agonist) and thioacetamide. We quantitatively measured RNA levels in FFPE and intact tissues, and found that both samples showed similar expression profiles. We next examined RNA levels in FFPE specimens that have been stocked over three years. These specimens are the rat liver administered by another PPAR α agonist LY-518674, and the expression profiles of the intact samples were examined three years ago. RNA expression profiles of the FFPE specimens were almost similar to those of intact samples, suggesting that RNAs in FFPE specimens are well-preserved for at least three years. We further tried to extract RNA from microdissected FFPE sections of rat liver administered by thioacetamide. The expression level of HO-1 was selectively increased in the centrilobular region, whereas that of IP-10 was increased both in the centrilobular and peripheral region of the liver. These results together raise the idea that gene expression studies using archived FFPE specimens are possible and that analyses of microdissected FFPE section is useful to reveal the link between pathological changes and gene expression profiles.

P-20

Gene Expression Analysis Methods for Formalin-Fixed Paraffin Embedded (FFPE) Samples

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Recently, there has been an increase in the number of reports concerning gene expression analyses with formalin-fixed, paraffin embedded (FFPE) samples. Application of FFPE for gene expression analysis has many advantages, but it is necessary to take into consideration that it may have an influence on degradation of the RNA. The present study verified the one-step RT-PCR method and MassARRAY (competitive RT-PCR and MALDI-TOF MASS) method for FFPE samples by comparison of fresh samples.

Material and Methods; Testes removed from DEHP-treated rats were fixed by 10% natural buffered formalin (NBF) and an RNA stabilization reagent (RNAlater) respectively. The testes fixed for 1 day or 7 days by NBF were embedded in paraffin wax. The gene expressions of 3 target genes and Actin-beta and GAPDH (housekeeping genes) were measured by the total RNA extracted from the FFPE (7 day-fixed) and RNAlater samples.

Results and Discussion; Although there was an effect of the fixation time noted in quality and quantity of total RNA, a sufficient volume was extracted from 1 day-fixed ($2.75 \pm 0.03 \mu\text{g}$) and 7 day-fixed ($1.32 \pm 0.56 \mu\text{g}$) FFPE. In the RT-PCR method, the gene expression analysis of FFPE was possible by setting the cDNA amplicon size to 90 bp or less, although the expression values of the housekeeping genes in FFPE were 1/500 less than that of RNAlater. In the MassARRAY method, it was possible to detect the difference of the gene expression with high accuracy even if the volume was very little such as 1 aM (3 copies). This study indicated that one-step RT-PCR method and the MassARRAY method are both effective for the FFPE analyses.

P-21

Effects of Spheroid Culture on the Gene Expression of Liver-Enriched Transcription Factors in a Mouse Hepatoblastoma-Derived Cell Line

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Mouse hepatoblastoma is generally considered to be originated from hepatocytes or some sort of undifferentiated cells, but hitherto no sufficient findings to suggest a similarity of this neoplasm to hepatocellular carcinoma or tissues on hepatocytic lineage have been reported. In the present study, we evaluated an effect of spheroid culture on the gene expression in a mouse hepatoblastoma-derived cell line and investigated hepatocyte-like characteristics of this tumor.

MHB-2 cell line, originated from chemically-induced mouse hepatoblastoma, and Hepa 1-6, a mouse hepatoma-derived cell line, was cultured for 10 days in two kind of three-dimensional culture plate (BD Matrigel™ matrix-coated plate and Sumilon celltight spheroid plate) and evaluated mRNA expression of albumin and liver-enriched transcription factors (LETfs) by realtime RT-PCR.

MHB-2 and Hepa 1-6 showed spherical or irregular forms after the culture. RT-PCR analysis revealed mRNA expression of some LETfs (C/EBPalpha, FoxA2, HNF1alpha and HNF4alpha) and albumin was increased in the spheroid colonies of MHB-2. As for Hepa 1-6, increased expression of albumin was observed by spheroid culture, while there was no evident change of the LETfs mRNA expression. Increased mRNA expression of albumin and some LETfs, which have been reported as factors relevant to differentiation of hepatocyte, in MHB-2 under a unique culture condition suggested a differentiating potential of mouse hepatoblastoma into hepatocytic lineage. Considering undifferentiated characteristics of MHB-2 and its differentiating potential into biliary lineage as we previously reported, the present results might suggest that MHB-2 has a nature resembling a liver progenitor cell.

P-22

Hemangiomas and Hemangiosarcomas Induced by Chemicals in Rats and Mice: Analysis of 19 Inhalation Carcinogenicity Studies

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We examined the induction of hemangiomas and hemangiosarcomas in the inhalation carcinogenicity studies of 19 chemicals.

【Materials and Methods】 Groups of 50 mice (B6D2F1/Crlj; Crj:BDF1) and rats (F344DuCrj) of both sexes were exposed by whole body inhalation systems to each test chemicals for 6 hours/day, 5 days/week for 2 years at 3 concentrations or clean air (control group), and examined histopathologically. Test substances were carbon tetrachloride, 1,2-dichloroethane, methylbromide, tetrachloroethylene, chloroform, *p*-dichlorobenzene, dichloroethylene, 1,1,1-trichloroethane, methylchloride, methallyl chloride, dichloromethane, dimethylformamide, crotonaldehyde; propylene aldehyde, glycidol, allylchloride, cyclohexene, 1-bromo-3-chloropropane, *n*-butyl glycidyl ether (butyl 2,3-epoxypropyl ether), 1,2-dichloropropane and propionitrile. The background data of the hemangioma and hemangiosarcoma were used the control data of 19 inhalation 2-year studies (950 rats and mice).

【Results】 Of the 19 chemicals, the following 5 chemicals induced hemangiomas and/or hemangiosarcomas in mice, but not in rats in any chemicals. Glycidol: hemangioma (male: 26%, female: 20%) and hemangiosarcoma (male: 66%, female: 42%) of the nasal cavity in mice. *n*-Butyl glycidyl ether: hemangioma (male: 28%, female: 14%) of the nasal cavity in mice. Tetrachloroethylene: hemangiosarcoma of the liver (male: 10%) and spleen (male: 10%) in mice. 1,2-Dichloroethane: hemangiosarcoma (male: 12%) of the liver in mice. Dichloromethane: hemangiosarcoma (male: 10%, female: 10%) of the liver in mice. In the historical control data of mice, hemangiosarcomas occurred in the spleen (male: 2.4%) and the liver (male: 3.4%, female: 1.5%), and hemangiomas occurred in the spleen (male: 2.2%, female: 1.1%) and the liver (male: 2.6%, female: 1.5%), but the spontaneous incidences of hemangiomas and hemangiosarcomas were less than 1% in other organs. The spontaneous incidences of hemangioma and hemangiosarcoma in rats were less than 1% in all organs.

【Conclusion】 In the mice, the inductions of hemangiomas and hemangiosarcomas by the test chemicals were shown in the nasal cavity, liver and spleen. And spontaneous occurrences of hemangiomas and hemangiosarcomas were more common in the liver and spleen than other organs. In the rat, no induction was found in any of the test chemicals, and spontaneous occurrences of hemangiomas and hemangiosarcomas were rare.

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Pathological Feature of Miniature Swine Arteriosclerosis Model and the Effect of G-CSF Administration for the Model Animal

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Granulocyte colony-stimulating factor (G-CSF) has been reported to be hematopoietic growth factor that acts selectively on cells of the neutrophil lineage. It has been reported recently that in the clinical trial for coronary artery disease (CAD) patients performed by the National Institutes of Health there occurred a serious adverse event which might be associated with G-CSF administration. Patients with CAD are known to have narrowing of the coronary arteries as a result of the progression of atherosclerosis. On the other hand, no systematic study has been performed regarding the safety of G-CSF administration to patients with cardiovascular system disorders. The present study was carried out to evaluate the effects of G-CSF on atherosclerotic lesion, using a miniature swine atherosclerosis model. Gottingen miniature swine (3-months-old, male and female) were fed high-cholesterol diet for 3 months. Animal models for arteriosclerosis were created and received daily s.c. injections of G-CSF in the cervical skin at doses of 0, 5 or 10 µg/kg/day for 10 days. On the day following the final administration, all animals were necropsied. At necropsy, 20 arteries were removed and histopathological evaluation was performed. In the treated group, the pharmacological effect was observed such as an increase in WBC counts. In many arteries, the atherosclerotic lesions, which are similar to type I~V in the classification of human atherosclerosis, were observed. No differences were seen in histopathological findings, incidence, severity, or distribution between the control group and the treated groups. Moreover, no infiltration of neutrophil to the lesions was observed in any animals. The results showed that the administration of G-CSF dose not lead to exacerbation or modification of atherosclerotic lesions.

Polyphenol-Containing Azuki Bean (*Vigna Angularis*) Extract Suppresses Blood Pressure and Macrophage Infiltration in the Heart and Kidney in Spontaneously Hypertensive Rats

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Infiltration by immunocompetent cells such as macrophages and lymphocytes is thought to play a role in the pathogenesis of hypertension. On the other hand, antioxidant treatments reduce the numbers of renal interstitial macrophages in experimental models of hypertension, and ameliorate hypertension. The azuki beans are known to contain proanthocyanidins, which are a group of polyphenolic bioflavonoids with remarkable radical scavenging activities *in vitro*. However, little is known about the beneficial effects of a diet of azuki beans on the progression of hypertension.

The aim of the present study is to investigate the effect of polyphenol-containing azuki bean extract (ABE) on blood pressure (BP) and macrophage infiltration in the heart and the kidney in spontaneously hypertensive rats (SHR).

Different male SHR were fed 0% and 0.8% ABE-containing diets for 8 weeks. Control normotensive Wistar Kyoto rats (WKY) were given the 0% and 0.8% diets. Tail BP, NAD(P)H-stimulated superoxide (O_2^-) production and macrophage kinetics in the heart and kidney were examined.

The BP of the SHR was higher than that of age-matched WKY through the treatment period. After 8 weeks of ABE treatment, the elevation of SBP in the ABE-treated SHR was significantly suppressed compared with the untreated SHR.

NAD(P)H-stimulated O_2^- production increased in the kidney and heart in SHR and WKY groups compared with those in the absence of NAD(P)H. The NAD(P)H-stimulated O_2^- levels in the kidney in the ABE-untreated SHR was significantly higher than that in the ABE-untreated WKY. The O_2^- levels in the ABE-treated SHR was decreased, compared with the untreated SHR.

In the immunohistochemical analyses, the number of macrophages in the heart and in the glomeruli and tubulointerstitium of the kidney were significantly higher than in the ABE-untreated WKY. In contrast, macrophages number in the ABE-treated SHR showed a significant decrease compared with the untreated SHR. There were significant positive correlations between the BP and the macrophages number in the heart and the tubulointerstitial and glomerular areas of the kidney in WKY and SHR.

In conclusion, our results suggested that ABE attenuated the elevation of blood pressure and the increased number of infiltrating macrophages in the heart and in the glomeruli and tubulointerstitium of the kidney in SHR. Moreover, a strong correlation was shown between the elevation of blood pressure and the intensity of macrophage infiltration.

A 12-Week Toxicity Study of Orally Administered Acrylamide in Juvenile Rats

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Acrylamide (AA) has been reported to be formed in fried and baked foods with various concentrations. Human exposure levels to AA from cooked foods in childhood are estimated to be higher than those in adults. Although numerous toxicity studies in adult rats have shown that the nervous system and testes are principal sites of the toxicities of AA, juvenile toxicological data are extremely limited. In the present study, pathological examinations were performed in rats administered AA after birth for 12 weeks. A total of 12 dams of F344 rats were divided into 4 groups and given AA in the drinking water at 0 (control), 10, 20 and 40 ppm during lactation period. The weaned offspring were also given AA at the same dose levels until post-natal week 12. As results, actual AA intakes at 10, 20 and 40 ppm groups after weaning were equivalent to 1.0, 2.1 and 4.4 mg/kg body weight/day, respectively, in males and 1.2, 2.5, 4.9 mg/kg body weight/day, respectively, in females. Treatment with AA caused a slight decrease in body weight in 20 and 40 ppm females as compared to the controls. Histopathologically, AA-related findings were observed in testes and epididymides. In the testes and epididymides, slight degeneration and necrosis of seminiferous epithelium and desquamated epithelium in ducts, respectively, were observed at 40 ppm males. Although no significant findings were recorded in other organs/tissues including sciatic nerve, the incidence and degree of myocarditis in the heart were significantly increased in 40 ppm males. To confirm whether the cardiac changes were related to AA administration or not, an additional study with scaled-up protocol was performed. A total of 10 dams of F344 rats were divided into 2 groups and given AA in the drinking water at 0 and 40 ppm during lactation period. The weaned offspring were also given AA at the same dose level until post-natal week 12. As results, actual AA intakes after weaning were equivalent to 5.0 mg/kg body weight/day in males and 5.5 mg/kg body weight/day in females. Significant reduction of body weight was noted in both sexes. Histopathologically, the incidence and degree of myocarditis in the heart of the treated groups were not significantly different from those in controls of both sexes. In conclusion, juvenile rats were not considered to be more susceptible to AA on the neuro- and testicular toxicities than adults.

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A 90-day toxicity study of L-asparagine in F344 rats

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L-Asparagine is a kind of amino acids and is listed in the list of existing food additives in Japan. This experiment, a 90-day toxicity study in F344 rats, was employed to make the safety assessment and determine no observed adverse effect level (NOAEL) of L-asparagine. [Methods] Five-week old male and female F344/DuCrIcrj rats were randomly assigned to four groups in each composed of 10 males and 10 females. L-asparagine was supplied by Institute of Life Sciences, Ajinomoto Co. Inc. (Kawasaki, Japan). They were given the material at dose levels of 0, 1.25, 2.5 or 5% in diet for 90 days. Rats were observed daily for clinical signs and general conditions. Body weights and food intakes were measured once a week. On day 90, after 16 hours starvation, all animals were sacrificed by exsanguinations after collection of blood sample from the abdominal aorta under deep anesthesia, and hematological, serum biochemical examinations, organ weights were analyzed. Histopathological evaluation was examined of all tissues from 5% treated group and control groups. [Results] Male 5% group was increased significantly in relative organ weights of the brain, kidney and testis than those of control group. In serological examination, GLU, PL, K and ALT were increased significantly in female 5% group, and GLU was increased significantly. In histopathological examination, the number of rats with the lesions was indicated not significant degree between inter groups. [Conclusion] NOAEL was determined to 2.5% in diet (male: 1.65g/kg body weigh/day, female: 1.73g/kg body weight/day).

P-27

A 90-day Repeat Dose Oral Toxicity Test of L-Aspartic Acid in Rats

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A subchronic oral toxicity study of L-aspartic acid (L-Asp) was conducted with groups of 10 male and 10 female Fischer 344 rats that were fed a powder diet containing 0, 0.05, 1.25, 2.5 and 5.0% of L-Asp for 90 days. There were no toxicologically significant, treatment-related changes in body weight, food consumption, water intake or hematology. The serum biochemistry showed dose-dependent decreases of total cholesterol and triglyceride levels of both sexes. These changes are interpreted to be treatment-related but adaptive and thus toxicologically insignificant. Levels of blood urea nitrogen, creatinine and uric acid were also decreased in both sexes, relating to the treatment. In addition, positive incidences of urinary ketone and protein were significantly increased in both sexes, while relative kidney weight was significantly increased, and regenerative renal tubules with tubular dilation were histopathologically observed in male animals. The observed renal injury was confirmed not to be due to the accumulation of α_2 u-globulin. Acinar cell hypertrophy of submandibular and parotid glands was histopathologically observed in male and female treated rats.

In conclusion, L-Asp causes toxic effects on kidneys and possibly salivary glands at high dose levels in male and female Fischer 344 rats. Such toxic effects were observed only in animals given 1.25% and/or higher doses of L-Asp. The no-observed-adverse-effect-level (NOAEL) for L-Asp is, therefore, determined to be a dietary dose of 0.05% (26.9 mg/kg body weight/day for males and 28.7 mg/kg body weight/day for females) under the present experimental conditions.

P-28

A 90-day Toxicity Study of Dietary Cochineal Color in Rats

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Cochineal color is a natural anthraquinone of insect origin and its chief ingredient is carminic acid. Although cochineal is widely used as a coloring in beverages and food, cochineal extract may trigger allergic reactions in some individuals. Recently, protein contaminants in raw cochineal have been identified as allergens. Here, we conducted a 90-day dietary toxicity study to confirm the toxicity of a highly purified cochineal extract.

Groups of 10 male and 10 female F344/DuCrj rats fed the agent at dose levels of 0, 0.15, 0.5, 1.5 and 5.0% for 90 days. No mortalities occurred during the experimental period. Staining of fur and black feces were observed in dosed rats. No-treatment related changes in the body weight, food and water consumption, urinalysis, ophthalmology, hematology, clinical chemistry, gross pathology and organ weights were noted. On histopathological examination, incidence of renal mineralization at the junction of inner and outer strips of the outer medulla in the male rats receiving 5.0% and severity of the lesion in the female receiving 5.0% was increased.

From these results the no-observed-adverse effect level (NOAEL) for cochineal color was estimated to be 1.5% for both sexes (male: 913 mg/kg/day, female: 1062 mg/kg/day).

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A 90-day Subchronic Toxicity Study of Dietary Administered Soybean Saponin in F344 Rats

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Soybean saponin extracted from seeds of *Glycine max* MERRILL is used as a food additive, an emulsifier. This compound has several biological activities, e.g., cell death induction, anti-oxidative and hypocholesterolemic effects, and anti-viral and anticarcinogenic properties. In the present experiment, subchronic toxicity of soybean saponin was evaluated in both sexes of 6-week-old F344 rats with dietary administration at concentrations of 0, 1.25, 2.5 and 5% (w/w) for 90 days. General conditions and mortality were examined daily and bodyweight and food consumption were weighed weekly. At the end of the experiment period, all animals were anesthetized with ether, and blood samples were collected for hematology and blood biochemistry. After complete necropsy, all organ/tissues were routinely processed for histopathological assessment. Neither mortality nor abnormal clinical signs were observed throughout the experimental period in any group. Although there was no variation in food intake among the groups, significant decrease in body weight was observed in 5% males and 1.25% and above females. Significant decrease in red blood cell count (RBC) and hematocrit (Ht) and significant increase in mean corpuscular volume (MCV) in the 5.0% males were noted, suggesting a tendency for hemolytic anemia. Significant increase in blood urea nitrogen (BUN) in 5% males and 2.5% and above females and significant decrease in relative kidney weight in 5% males and 1.25% and above females was observed. Significant increase in total protein (TP) and albumin (Alb) in 2.5% and above males and significant decrease in triglyceride (TG) in 1.25% and above females was observed. Absolute and relative liver weight in 1.25% and above males and relative liver weight in 2.5% and above female were significantly increased, while no histopathological changes were observed in the kidneys and livers. On histopathological observation, atrophy of epithelium in ventral prostate was detected in all 5% males and mucinification, atrophy of mucosal epithelium in vaginas and increased atretic follicles in ovaries were observed in 2.5 % and above female, which might be caused by residual isoflavone after the soybean saponin extraction process. Based on these results, the no-observed-adverse-effect level (NOAEL) of soybean saponin in F344 rats were estimated to be less than 1.25% in both males and females (707.2 and 751.8 mg/kg b.w./day, respectively).

P-30

Assessment of The Carcinogenic Potential of Mitemcinal (GM-611): Increased Incidence of Malignant Lymphoma in a Rat Carcinogenicity Study

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Mitemcinal is an erythromycin derivative, which acts as an agonist of the motilin receptor. For assessment of the carcinogenicity of mitemcinal, we conducted a short-term carcinogenicity study in p53 (+/-) C57BL/6 mice and a 104-week carcinogenicity study in CD(SD)IGS rats. There was no evidence of a carcinogenic potential in mouse when administered for 26 consecutive weeks at levels up to 250 mg/kg/day. In the rat study, an increased incidence of lymphoma was noted in 5/60 males and 8/60 females of the high dose group (60 mg/kg/day) compared to 1/60 and 0/60 in control males and females respectively, with statistical significance in females. Rat lymphomas include different immunomorphologic types (T- or B-cell lineage). Immunohistochemical analysis revealed that lymphomas from mitemcinal-treated rats and spontaneous cases were of T-cell lineage. The overall weight of evidence suggests that the incidence of spontaneous lymphoma was enhanced in the rat study. They also indicate that the increased incidence of lymphomas was based on a non-genotoxic effect with a threshold dose-response and that the tumorigenesis was based on the strain or species specificity of background factors. The high dose in the rat study is approximately 1,600-fold higher (AUC) than that of the clinical dose, a sufficient margin of safety for the clinical dose. We conclude that the risk of carcinogenesis due to mitemcinal in humans can be considered to be minimal and is to represent an acceptable risk for the continued administration of mitemcinal to humans.

P-31

Malignant Lymphoma in WBN/Kob Rats.

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Although spontaneously occurring neoplasms have been often reported in F344, SD and Wister rats that were commonly used strains for toxicologic and carcinogenicity studies, there are only a few reports of malignant lymphoma or lymphatic leukemia except for LGL leukemia in F344 rats. Malignant lymphoma (lymphsarcoma) is thought to be uncommon in F344 rats.

Malignant lymphomas of non-LGL leukemia type with characteristic pathologic features occurred in aged WBN/Kob rats maintained in our laboratory. Thus, we examined the morphologic features and cytological characteristics of malignant lymphoma in rats of this strain.

The onset of disease in all affected 10 rats (6 males and 4 females) was about 60 weeks of age. Common and characteristic clinical signs were abnormal gait of the hind legs. Macroscopically, the enlargement of the lymph nodes, spleen and liver was slight to moderate. Multiple white to gray nodules up to 3 mm scattered encompassing the aorta and took a bead-like appearance near the thoracic and lumbar vertebral. In peripheral blood smears, neoplastic cells had scanty basophilic cytoplasm, cleaved round nuclei with aggregated chromatin and prominent nucleoli. Histopathologically, generalized bone marrow tissues were completely replaced by neoplastic lymphocytes. Tumor cells infiltrated not only in the follicles but also in red pulp on the spleen resulting the loss of the normal architecture. In several lymph nodes, many tumor cells also invaded into adjacent adipose tissue. Most striking histological features were severe infiltration and proliferation of tumor cells in the generalized adipose, periosteal and skeletal muscle tissue and formed large mass in the adipose tissue and skeletal muscle adjacent the thoracic vertebra and lumbar vertebral.

Immunohistochemically, almost all neoplastic cells were positive for PAX-5, one of the B cell markers, and were negative for CD3, one of the T cell markers. From the results of immunohistochemistry and morphological examination, these tumors were diagnosed as malignant lymphomas, lymphocytic (lymphoblastic), B cell lymphomas.

Although infiltrative growth has been described in some T cell lymphoma and LGL leukemia, it was also prominent feature of B cell lymphoma (leukemia) in WBN/Kob rats.

P-32

Investigation of the Histopathological Changes Induced by Complement Activation in Rats

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The complement might be activated by administered medicine or antibody against the administered medicine. In the present investigation, to understand the changes induced by the complement activation, the changes induced by typical complement activators were examined histopathologically in rats.

【Methods】 Zymosan and Inulin known as typical substances that activated the alternative pathway were used as test substances. 5-week-old male Sprague-Dawley rats received intravenously a single injection of Zymosan (25 mg/kg) or Inulin (50 mg/kg), respectively. After 4 hours, 1, 2 and 3 days of injection, 3 rats each in the treated group were sacrificed under the pentobarbital sodium anesthetizing, the complement measurement, hematological examination and histopathological examination were performed. In the histopathological examination, paraffine-embedded sections of liver, spleen, kidney, heart and lung were routinely prepared and stained with hematoxyline and eosin for the light microscopic observation.

【Results】 Immediately after the administration, decrease in locomotor activity was observed in the each group treated with Zymosan or Inulin, but each animal tended to recover in Day 1. The complement values decreased at four hours after the administration in rats treated with Zymosan or Inulin. Although the complement values showed recovery tendency after Day 1, there was not complete recovery by Day 3 in the each group. In the hematological examination, the number of platelets decreased from 4 hours after administration in rats treated with Zymosan or Inulin. The tendency that a decrease in the number of platelets continues was seen in Zymosan compared with Inulin. In histopathological examination, enlargement of Kupffer cells was observed in the liver in each group from 4 hours after administration. A microgranuloma was also seen in rats treated with Zymosan after Day 2, and similar changes were observed very slightly in rats treated with Inulin.

【Conclusion】 The changes induced by the complement activation were enlargement of Kupffer cells and a decrease in the number of platelets in the rats treated with Zymosan or Inulin. It was suggested that not only the Kupffer cells that had the phagocytic capacity but also the platelets affected by the complement activation in rats.

P-33

Histopathological Characteristics of The Tumor Developed by The Implantation of Immortalized Embryal Fibroblasts Originated from *Parp-1* Defect Mice

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[Introduction] Poly (ADP-Ribose) polymerase (PARP) -1 is a housekeeping gene which is involved in DNA repair, gene stability, induction of cell death, differentiation control, tumor formation, etc. In this study, implantation of *Parp-1* defect mice originating immortalized embryal fibroblasts (*Parp-1*^{-/-}MEF) and a wild type MEF to nude mice was associated with tumor formation only by *Parp-1*^{-/-}MEF. The tumor was analyzed histopathologically and immuno-histochemically to clarify the nature of the formed tumor.

[Method] *Parp-1*^{-/-}MEF and wild type MEF (2×10⁶) together with matrigel were implanted subcutaneously (axillary and dorsal areas) to nude mice (BALB/c-*nu/nu*, 12 weeks of age, male) and the formed tumor was resected after 8 weeks. The resected tumor was fixed in formalin, embedded in paraffin, and sections were subjected to H&E staining and immunostaining and observed microscopically under a light microscope.

[Result] Subcutaneous implantation of *Parp-1*^{-/-}MEF was associated with tumor formation, and tumor mass (1 to 6 cm³) was formed during 8 weeks after implantation. The tumor formed consisted of randomly proliferated mesenchymal cells in various forms, mainly in herringbone pattern and storiform pattern. The tumor cells mainly consisted of spindle cells with occasional presence of polygonal to round cells and giant cells with bizarre nucleus, in association with karyokinesis in relatively high amount of cells. Immunostaining revealed positive vimentin, positive α -smooth muscle actin (a part of spindle cells), and positive S-100 (mainly in nucleus, and partially positive in cytoplasm), but negative for myogenin, c-kit, factor VIII and neurofilament.

[Conclusion] The tumor showed the characteristic growth pattern of fibrosarcoma and pleomorphic malignant fibrous histiocytoma and immunohistochemical examination showed differentiation pattern of myofibroblast. However, at this stage, it was thought to be rational to diagnose the tumor as undifferentiated pleomorphic sarcoma. For *Parp-1*^{-/-}MEF, the expression of *insulin-like growth factor 2 (Igf2)* was increased. Therefore, it was suggested that defect of *Parp-1* might not affect the differentiation direction of the tumor developed by MEF, but affect the tumorigenicity by enhancing proliferation via abnormalities in gene expression of *Igf2* etc.

P-34

Mullerian Tumor of Oviduct in the Cynomolgus Monkey

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The cynomolgus monkeys have been used more frequently in toxicological studies, the background data has been accumulated. However, the tumor of oviduct in cynomolgus monkey was rare report. We report on tumors of oviduct were observed in 2 cynomolgus monkeys.

(Material and Method) Case A was ten years old, was born in China. Case B was eleven years old, was born in China. The 2 tumors were fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with Hematoxylin-Eosin (H.E.). And Masson trichrom stain, immunohistochemical stain with anti mouse PCNA antibody and anti human α -SMA antibody were applied.

(Result and Discussion) Case A: The mass like a cauliflower (2.5 cm, grayish white) was observed in left ampulla of fallopian tube at the autopsy. Histopathologically, tumor cells were composed spindle mesenchymal cells have bundle or disarray pattern, covered epithelial cells that have pilus, and presented papillary. Epithelial cells were simple columnar epithelium and germ cells were observed. But locally, cuboidal epithelium, multiseriate, superposition or cribriform pattern were observed. In the Masson trichrom stain, narrow collagen fibers were observed around mesenchymal cells. Most of mesenchymal cells were positive for anti α -SMA. Most of epithelial cells at the papillary portion and mesenchymal cells at the same part were positive for anti PCNA. Case B: The mass like a cauliflower (5 cm, grayish white) was observed in left ampulla of fallopian tube at the autopsy. Histopathologically, tumor cells were composed spindle cells and fascicular mesenchymal cells that proliferate alternatively, covered epithelial cells that have pilus, and presented papillary. Epithelial cells were simple squamous to columnar epithelium and germ cells were observed. In the Masson trichrom stain, collagen fibers were observed at the papillary and basal portion. And mesenchymal cells at the papillary portion were positive for anti α -SMA. Most of epithelial cells at the papillary part were positive and mesenchymal cells at the same part were negative for anti PCNA.

The 2 case of A and B were considered Mullerian Tumor, since both cases were observed neoplastic proliferation of epithelial and mesenchymal cells.

P-35

Arteriosclerosis with Hypercardia in Cynomolgus Monkey

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Arteriosclerosis in humans occurs at the high incidence with reference to ageing, hypercholesterolemia and hypertriglyceridemia. Although atherosclerosis in old monkeys has been described, young monkeys used in experimental study had often only intimal thickening and seldom seen the arteriosclerotic lesions, which contain lipids in the endothelium, fibrous plaque and coronary arterial stenosis. We report a case of coronary artery arteriosclerosis with hypercardia and ischemic changes of myocardial fibers in a young female Malaysian cynomolgus monkey aged 5 years.

At the necropsy, hypertrophy of cardiac wall was recognized in the left ventricular wall and ventricular septum. In light-microscopic examination, arteriosclerotic findings were recognized in almost whole anterior descending branch of coronary artery and its bifurcated arteries. The tunica intima was thickened by fibrous tissues, which consisted of lipid-rich foam cells and modified smooth muscle cells bundled by the proliferated extracellular matrix. Rupture or multiple stratification of elastic laminae of arteries was seen. The stenosis of the vascular lumen by arteriosclerosis was remarkable in left coronary arterial branches more lower than the intermediate part. Focal ischemic changes such as lost of myocardial fibers and fibrosis were recognized in the left anterior ventricular wall and the anterior 1/3 of ventricular septum related the coronary arterial supply. The transverse diameter of myocardial fibers was increased, and the nuclei of myocardial cells were enlarged in whole left ventricular wall. Particularly, hypertrophy of myocardial fibers was remarkable in internal area of fibrosis and the circumference. In the intramural small arteries of the left ventricle, the tunica medial smooth muscle cell proliferation and multiple stratification of elastic laminae of arteries were observed. In addition, the changes that resembled the intramural small arteries were found in cerebrum, lung, cecum and vagina. Sphygmomanometry was not conducted in this case. However, the findings of hypercardia and intramural arterial and several organ arteries seemed to indicate occurring hypertension.

Hypertension plays a significant role in the development of arteriosclerosis. Hypertension reduces the elasticity of blood vessels leads to altered vasoreactivity and vascular endothelial dysfunction. Atherosclerosis causes narrowing of arteries which when combined high blood pressure can put a greater risk of heart attack and stroke. This is the case of the close relation with arteriosclerosis and hypercardia.

P-36

Ovarian Cyst Showing Glandular Appearance in A Cynomolgus Monkey

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The cynomolgus monkey is a common animal species used in pre-clinical toxicity studies, but there is only limited information about spontaneous ovarian lesions. We encountered an uncommon case of ovarian cyst showing glandular appearance in a 4-year-old female cynomolgus monkey.

Case: The animal was used as the control group in a 13-week toxicity study. There were no abnormalities in a series of routine examination items, and the menstruation was regularly observed every 4 weeks. After scheduled necropsy, the ovary as well as other organs and tissues was fixed in 10% neutral-buffered formalin solution, routinely processed, and examined microscopically.

Histopathological Findings and Conclusion: An unusual solitary cystic lesion showing glandular appearance was found in the deep cortex of the unilateral ovary and it compressed the surrounding ovarian tissues. The cyst was surrounded by the theca folliculi consisting of theca cells, smooth muscle cells and vessels with fibrous tissue and the inner surface of the cyst was covered with granulosa cells. The theca folliculi papillary protruding into the cystic cavity like a wrinkle represented the unique glandular appearance. Single cell necrosis and desquamation of the granulosa cells were observed sporadically whereas neither cellular atypia nor abnormal mitotic figures was observed in granulosa cells and theca cells. The granulosa cells had pale eosinophilic and foamy cytoplasm, and the theca cells had vacuolar cytoplasm. Immunohistochemically, the granulosa cells diffusely and strongly and the theca cells weakly reacted with anti-inhibin alpha antibody. Such histopathologic and immunohistochemical findings suggested that the granulosa and theca cells in this lesion were under luteinization. Ovarian follicles under various stages, except mature follicles, were also present in the cortex. There was no histological abnormality in the contralateral ovary, uterus, vagina, pituitary, mammary glands, or other organs and tissues of this case.

Unusual glandular appearance of this case prompted us to suspect a possibility of neoplastic change, such as a granulosa cell tumor. However, the histological and immunohistochemical findings described above suggest that this lesion is a non-neoplastic change and that the granulosa and theca cells organizing the cystic wall are similar in nature to those of the mature corpora lutea. We, therefore, concluded this lesion is a unique form of luteal cyst.

P-37

Jet Lesion (Mural Endocardiosis) in an Aged Beagle Dog

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Jet lesions are observed macroscopically as streaky or dotted focal thickening mainly in the left atrial endocardium, and many of them are considered to be secondary changes caused by mitral regurgitant blood flow. Valvular endocardiosis is frequently observed as irregular or nodular thickening of atrioventricular valves in dogs, and the histopathological findings are described in the textbook. However, there are few histopathological reports on the jet lesions in the left atrial endocardium. Recently we had an opportunity to histopathologically examine jet lesions in an aged Beagle dog, which showed streaky thickening in the left atrial endocardium, while investigating age-related lesions.

Materials and Methods: The animal used for the study was an experimental male Beagle dog, 8 years and seven months old, which had no macroscopic lesions besides a heart.

Results: Macroscopically, atrial endocardium was thickened in the shape of focal elevated streaks, and several layers were formed paralleling to the atrioventricular ostium. No significant lesions in the mitral valves were observed. Microscopically, the atrial endocardium showed elevations of various sizes, and the morphological features were different in degree of thickening. That is to say, the slight thickening merely consisted of fibrotic contents, but the severe thickening mainly consisted of eosinophilic homogenous interstitial elements with large globular cells like cartilage tissue. Liquefactive necrosis of myocardial fibers was observed in the myocardium proximal to the affected endocardium. As mentioned above, jet lesions of endocardium were considered to have such strange features as cause chondrometaplasia.

P-38

Possible Case of Neuroblastoma in Nasal Cavity Observed in A Rat Treated with *N*-Nitrosobis-(2-hydroxypropyl)-amine (BHP)

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[Introduction] It is known that administration of nitroso compounds to rats would be associated with adenocarcinoma, squamous cell carcinoma and neuroblastoma in olfactory epithelium in the nasal cavity; however, there are almost no reports on the induction of neuroblastoma in the nasal cavity by administration of BHP or its spontaneous occurrence. We report a case with possible neuroblastoma in the nasal cavity which was found in rats at 26 weeks of age treated with BHP.

[Materials and Methods] This case is a Wistar strain SPF rat in an experimental carcinogenicity study to which BHP was administered by mixing with drinking water at 2000 ppm for 12 weeks from 6 weeks of age and subjected to necropsy after 8-week withdrawal period. The nose with nasal cavity was removed at necropsy, fixed in 10% neutral buffered formalin, decalcified, embedded in paraffin, subjected to H.E and Masson-Trichrome staining and immunohistochemical examination (Keratin, Vimentin, Desmin, SMA, S-100, NF68, NF120-200, Shinaptophysin, GFAP, NSE, Nestin and Tubulin), and observed with a light microscope.

[Results] The tumor which was centered in the endoturbinat 3 in the posterior part of the nasal cavity showed invasive proliferation involving the ectoturbinat 2 from the ventral surface, and showed compartmentalization of sheets of neoplastic cells into lobules or cords by fibrovascular septa. Rosette-like structure was observed in some regions. The tumor cells had large and poor nuclear chromatin, round to oval nuclei with scant basophilic cytoplasm, poorly defined cell borders and occasional mitotic figures. Immunohistochemical examination revealed positive reactivity for anti Tubulin and NF120/200 in the neurofibril-like structure and cytoplasm, while negative to other antibodies.

[Discussion] It is most likely that the tumor was neuroblastoma in the nasal cavity since the genesis region was the posterior part of the nasal cavity which is the area for distribution of olfactory epithelium, morphological examination indicated a growth pattern associated with rosette-like and neurofibril-like structure that is characteristics of neuroblastoma, immunostaining was positive for Tubulin and NF120/200 in tumor cells.

P-39

A Case of Subcutaneous Tumor in the Neck of a Diet-induced Obese Mouse

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We found a subcutaneous tumor in the neck of a diet-induced obese (DIO) mouse that had been used in a drug efficacy study. The tumor had various histological structures, and we report the histopathological characteristics of the tumor.

[Materials and methods] The case animal was a male C57BL/6J mouse that had been bred with a high fat diet (35.9% fat, 5.578 kcal/g) since age three weeks and had received a drug orally for two weeks since 51 weeks old. The mouse was in the low-dose group of the drug efficacy study. The subcutaneous tumor was observed in the neck at autopsy, and we examined the tumor histologically, immunohistochemically and ultrastructurally.

[Results] The tumor was approximately 1.2 cm in diameter with a well-defined border (i.e. it was clearly distinguishable from surrounding tissue; skin and salivary glands), and it had a capsular structure. The cross section following fixation revealed white solid matter with dark red in color. Anatomically, the tumor seemed to be a submandibular lymph node. Histologically, the tumor consisted of areas with vessel-like structures comprising variously sized blood vessels growing irregularly, and areas with small lymphoid follicle-like structures. The areas with the vessel-like structures were observed throughout the tumor, and a disordered mass of spindle cells and irregular vascularization were observed. Silver impregnation revealed that many fine argyrophilic fibers had surrounded and invaded the vessel-like structures. Electron microscopy revealed a cell adhesion system and basal lamina-like structures in the vascular endothelioid cells. In the areas with the lymphoid follicle-like structures, the following findings were observed. Many fine argyrophilic fibers invading and surrounding the follicle-like structures, to which lymphocytes had accumulated with the silver impregnation stain. Light microscopy revealed almost no mitosis, but immunostaining using anti-PCNA antibody revealed several positive cells throughout these areas. Electron microscopy revealed plasma cells with abundant cytoplasm containing lamellar rough endoplasmic reticula and lymphocytes.

It is not known whether the high-fat diet are related to the tumor occurrence. However, there are no reports of tumor occurrence in DIO mouse. This is a very interesting spontaneous lesion, on which we are conducting further studies, including immunohistochemical analysis.

P-40

Cyst in Submaxillary Region of A Mouse

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A case of submaxillary cyst was observed in a 13-week-old female mouse (CrJ:CD1(ICR)) used in a toxicity study. No abnormalities were observed in the clinical observations and body weights in the administration period. Necropsy revealed a soft fluctuant cyst (8×6×3 mm) which is adjacent to the left salivary glands (submaxillary and sublingual glands), and filled with transparent viscous fluid. No traumatic injury was macroscopically observed on the cervix.

Histopathologically, the cyst was located between the submaxillary and sublingual glands and contained weakly basophilic substance in the cavity. The wall of the cyst was composed of fibrous connective tissue with partial granulomatous tissues and no lining epithelium was observed. Most areas of the wall was edematous and contained weakly basophilic substance similar to that in the cavity. There were macrophages in the wall and cavity but no other inflammatory cells such a neutrophil. The substance was positive for periodic acid-Schiff (PAS), colloidal iron and Alcian blue (pH 2.5) both in the wall and cavity. These staining behaviors were the same as that of secretory granules in mucous cells of the sublingual and submaxillary glands.

The above-mentioned characteristics of the cyst, the location and staining behavior, indicate that it was derived from the salivary glands, submaxillary or sublingual. In the rodent it is known that the salivary glands occurred as a consequence of duct dilatation due to occlusion by foreign body (ex. Calculus) or following the inflammation and such type of cyst has no an epithelial lining. This case was diagnosed as Sialoceles because of no epithelial lining in the wall and the other histological features as described above.

P-41

Case Report: A Primary Hepatic Malignant Mesenchymal Tumor Suspected Leiomyosarcoma in a B6C3F1 Mouse

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Hepatocellular adenomas and carcinomas are the most common hepatic neoplasms in aging B6C3F1 mice. As non-epithelial tumors of the liver, histiocytic sarcomas, hemangiomas and hemangiosarcomas are occasionally observed, whereas Ito cell tumors are rarely seen in aging mice. On the other hand, spontaneous and chemically induced hepatic sarcomas such as fibrosarcomas are uncommon in mice. To our knowledge, there have been no reports on primary leiomyosarcomas of the liver in mice. In our center, we encountered one primary hepatic sarcoma suspected leiomyosarcoma in aging mouse.

The animal was a 110-week-old female Crj:B6C3F1 mouse in two-year carcinogenicity study. At necropsy, sclerous white patches (max width: 20 × 14 mm) were observed mainly in medial lobe. Histologically, the tumor was composed of interlacing fascicles of elongated, spindle-shaped cells displacing the hepatic parenchyma. Neoplastic cells had oval or elongated nuclei and lightly eosinophilic cytoplasm. In posterior right lobe, neoplastic cells grew in the sinusoid, and connected with the vascular wall of the interlobular vein at focal area. Fibrous lesions were widely observed in left lateral lobe. Tumor cells metastasized to the lung, capsule of the spleen and parapancreatic lymph node. There were no histological findings suspected hemangiosarcoma, histiocytic sarcoma, or typical Ito cell tumor. Immunohistochemically, tumor cells were positive for vimentin, α -smooth muscle actin and desmin, but negative for von Willebrand factor, Mac-2, S-100 and cytokeratin. Electron microscopic examination revealed that tumor cells contained abundant rough endoplasmic reticulum and small amounts of thin filaments, but lacked basal lamina. Collagenous fibers in the intercellular matrix were also observed. As a result of ultrastructural examination, this tumor might derive from the myofibroblast (activated Ito cell) rather than smooth muscle cell. At this stage, we diagnosed this case as primary hepatic malignant mesenchymal tumor suspected leiomyosarcoma in a B6C3F1 mouse. We expect to examine immunohistochemical staining for extracellular matrix such as tenascin or laminin to differentiate leiomyosarcoma from malignant Ito cell tumor.

P-42

An Experimental Model of Brain Metastasis of Lewis Lung Carcinoma

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Most metastatic brain tumors originate from lung cancers. However, there has been relatively little progress on developing an experimental model of metastasis of lung cancer to the brain. By injecting Lewis lung carcinoma cells into the right internal carotid artery of C57BL/6NCrj mice, we succeeded in developing a model of metastatic brain tumors. The concentration of tumor cell preparation was adjusted to 2×10^6 cells/mL with PBS. The right common carotid artery was cut and a prepared glass cannula was inserted into the internal carotid artery. After injecting 8 mL of the carcinoma cell suspension, the glass cannula was removed, and the right common carotid artery was ligated. Three days after the operation, carcinoma cells were found in the choroid plexus of the right lateral ventricle. Several carcinoma cells were seen in the blood vessels and around the perivascular sheath in the right cerebral hemisphere. Six days after the operation, small tumors in the coronary sections of the right lateral ventricle were detected. Nine days after the operation, tumors were obvious in the choroid plexus of the right lateral ventricle. Proliferations of carcinoma cells were also found in the perivascular sheath of the right hemisphere and thalamus. Because of bleeding and necrosis in the tumor, the tumor was externally visible through the surface of the brain in some mice. Twelve days after injection, carcinoma cells spread into the left hemisphere. Fifteen days after the operation, carcinoma cells were widespread in the left and right hemispheres, the thalamus, and in the choroid plexus of the lateral and third ventricles in the brains.

P-43

A Murine Immunocompetent Model for Metastatic Mammary Cancer Accessible to Bioluminescence

Imaging

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A repetitive-monitoring metastatic mammary cancer model in living animals is required for many applications, including gene therapy studies, pharmacological therapeutic studies, and transgenic models. To establish such a mammary cancer model accessible to bioluminescent imaging of tumor growth and metastasis in living animals, metastatic mouse mammary carcinoma BJMC3879 cells stably expressing firefly luciferase (BJMC3879luc2) were generated. The BJMC3879luc2 cells were then inoculated into female immunocompetent BALB/c mice. Tumor volumes were correlated to levels of photon counts ($r=0.951$). Sequential analysis of bioluminescent imaging showed strong signals in the axillary, mandibular, inguinal, thoracic and abdominal regions in the mice. Histopathologically, these signals were determined to be due to mammary cancer metastasis. In addition, treatment of the model with luciferase siRNA, which was applied to examine its suitability as a therapeutic model, dramatically reduced the luminescence. Thus, we have established a quantifiable and reliable model of metastatic mammary cancer that can be used for cancer gene therapy and pharmacological therapy studies.

P-44

COX2 inhibitors suppressed osteolysis and osteoclast induction associated with prostate cancer growth in the bone microenvironment

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<Purpose>

Cyclooxygenase (COX) inhibitors have been demonstrated to induce apoptosis in colon and breast cancer *in vivo* and *in vitro* and also to inhibit osteoclast induction *in vitro*. In the growth of prostate cancer at bone metastasis site, osteolysis as well as tumor cell proliferation in the bone microenvironment plays critical roles because the activated osteoclasts expand the space in hard calcified bone tissue to assist tumor growth. Previously, we developed a syngeneic rat model that mimics human prostate cancer bone metastasis with respect to tumor stromal interaction. In the present study, we evaluated the effect of COX inhibitors on prostate cancer growth and osteolysis in the bone microenvironment.

<Materials & Methods>

Rat prostate tumors were transplanted onto the cranial bone of F344 male rats and then administered 50ppm Indomethacin (IM) in drinking water and 400ppm Nimesulide (NIME) in diet. Thirty days after transplantation, we killed rats and evaluated the effects of COX inhibitors on degree of bone destruction, osteoclast induction, induction of apoptosis in tumor cell histologically, in addition to COX1 and COX2 protein expression, prostaglandin E₂ (PGE₂) level in the tumor bone interface (TB-interface) *in vivo*. We also evaluated the effect of PGE₂ on osteoclast differentiation in primary culture of bone marrow cells with receptor activator of nuclear factor- κ B ligand (RANKL).

<Results & Conclusion>

Treatment with IM and NIME significantly reduced PGE₂ level but not COX 1 and COX2 expression at TB-interface. These treatments significantly reduced degree of bone destruction and osteoclast induction. NIME treatment significantly increased apoptosis induction in tumor cells. *In vitro* study revealed that PGE₂ facilitated RANKL-induced osteoclast differentiation. We have demonstrated that COX inhibitors significantly reduced osteolysis and osteoclast induction associated with prostate tumor growth in the bone microenvironment. In particular, NIME may be more effective for growth inhibition of prostate cancer in the bone microenvironment because of induction of apoptosis in tumor cells. Thus, this animal model allows us to develop therapeutic agents effective for bone metastasis of prostate cancer.

P-45

Modifying Effects of *Crassocephalum Crepidioides*, A Herb in The Ryukyu Islands, on Inflammation-Associated Colon Carcinogenesis in Mice

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The aim of the present study was to examine whether dietary feeding of *Crassocephalum crepidioides* (CC) can inhibit inflammation-associated mouse colon carcinogenesis. The CC extract powder was prepared by hot water extraction method. Five-week-old male CD1 mice were divided into seven experimental and control groups. Mice in groups 1-5 were given a single intraperitoneal injection of azoxymethane (AOM, 10mg/kg body weight). Starting 1 week after the injection of AOM, mice in groups 1-5 received 2% dextran sodium sulfate (DSS) in drinking water for 7 days. Mice in groups 2 and 3 were fed a diet containing 0.1 and 0.5% CC, respectively, for 5 weeks, by the start at one week before the injection of AOM. Mice in groups 4-5 were fed a diet containing 0.1 and 0.5% CC for 9 weeks, respectively, starting 2 weeks after the cessation of DSS exposure. Mice in group 6 were fed a diet containing 0.5% CC for 14 weeks. Mice in group 7 were given the basal diet alone and served as untreated controls. Each ten mice of groups 1-3 were sacrificed at week 5 after the start experiment and the number of aberrant crypt foci (ACF) and mucin-depleted foci (MDF) were counted. The other mice were sacrificed at week 14 to determine the chemopreventive effects of CC on colon carcinogenesis. Oral administration of CC at both doses (groups 2 and 3) decreased the numbers of ACF and MDF, when compared with the control group (group 1) although the difference in the numbers of both ACF and MDF were not statistically significant. The incidence of colonic tumors was decreased when mice were fed with 0.1% CC containing diet. However, high dose (0.5%) of CC increased the incidence. Our findings suggest that CC has not tumor inhibitory effect on mouse colon carcinogenesis model for colitis-related colon carcinogenesis.

P-46

Cellular Kinetics Analyses of Early and Neoplastic Lesions in a DMH-DSS Rat Colorectal Carcinogenesis Model

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We recently reported that regenerative mucosa (RM) characterized by Paneth cell metaplasia (PM) and β -catenin accumulation (BCA) is induced for the onset of colorectal carcinogenesis in rats treated with dextran sodium sulfate (DSS) following 1,2-dimethylhydrazine (DMH)-initiation. In the present study, to clarify significance of the RM with PM/BCA on the carcinogenesis, immunohistochemistry for β -catenin/cyclin D1 and NF κ B (p65) as markers for cell proliferation and anti-apoptotic activity, respectively, and cellular kinetics analysis using BrdU were performed. F344 males were given three subcutaneous injections of DMH (40 mg/kg body weight) in a week, followed by free access to drinking water containing 1.5 % DSS for a week. At 4, 8 and 12 weeks after the DSS treatment, rats were euthanized and colorectal samples were collected. Histopathologically, the degree of RM with PM/BCA was not different among three time points. Areas with PM/BCA were significantly more likely to be accompanied by interstitial inflammation. Neoplastic lesions including dysplasias, adenomas and/or adenocarcinomas were observed in each time point, and their multiplicities showed a tendency to increase and 17 of 21 neoplastic lesions were surrounded by RM with PM at week 12. In the RM with PM, BCA/cyclin D1- or p65-positive epithelial cells were scattered, but BrdU-positivities were similar to those in normal-like mucosa. On the contrary, almost all cells showed BCA/cyclin D1-positive along with significantly increased BrdU-positivities, whereas p65-positive cells were scattered in the neoplastic lesions. These findings indicate that RM with PM/BCA is a precursor of neoplastic lesions and inflammation-associated NF κ B-activation possibly involved in selective proliferation of BCA/cyclin D1-positive cells in this DMH-DSS rat model.

P-47

Repair Process of Colon Ulcer induced with Dextran sulfate sodium: Analysis using Aggregation Chimera Mouse of C3H and C57BL/6-Green mouse

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Patients with ulcerative colitis (UC) are at increased risk of colorectal adenocarcinomas. Dextran sulfate sodium (DSS) has been known to cause severe inflammation in rodents resembling UC and to accelerate colon carcinogenesis. To clarify this mechanism, we have analyzed crypt/cell clonality and proliferation during healing stage of ulcer.

Five to six weeks old, male and female C3H \leftrightarrow Green fluorescent protein transgenic mouse (Green mouse, C57BL/6J background) chimera mice were given dextran sulfate sodium (DSS) as drinking water for 7 days. The animals were sacrificed at 8, 10, and 14 experimental days one hour after bromodeoxyuridine (BrdU) administration. Colon tissues were routinely processed and analyzed for the expression of C3H specific antigen (CSA), BrdU, and Cox2 as well as histopathological evaluation including the number of crypt fissions.

Cox2 was expressed especially in erosive (lacking epithelial) region. Cell production was gradually increased after DSS treatment in crypts neighboring erosion both with cell division and crypt fission. Erosion was covered with surface epithelial cells provided by the adjacent crypt. Surface epithelial cells covering erosion began to proliferate in random area rather than in migrating front later in ulcer healing.

P-48

The Effects of Tobacco-derived Carcinogen NNK on AOM and DSS-induced Colon Carcinogenesis in Male A/J Mice

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Smoking may increase the risk of cancer development in certain tissues other than head and neck, as suggested by several epidemiologic studies. However, it is still insufficient to demonstrate any clear association between smoking and colon cancer. Furthermore, there exist only a few reports that carcinogens-derived from tobacco directly enhance colon carcinogenesis in rodents. Thus, we examined the effects of tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) on colon carcinogenesis initiated by azoxymethane (AOM) and promoted by dextran sulfate sodium (DSS) in male A/J mice. NNK (10 μ mol) was administered by a single intraperitoneal (i.p.) injection, and then AOM (10 mg/kg body weight, i.p.) was given after 1 week of NNK administration. One week later, the mice were received 1.5% (w/v) DSS in drinking water for 7 days. All animals were sacrificed at week 22 to examine the pathological lesions in the colon and lung. In the NNK+AOM+DSS group, the multiplicity of colonic tumors was significantly increased by more than 3-folds (4.0 ± 3.6), as compared to the AOM+DSS group (1.2 ± 1.7) at week 22. The incidence of tumors of the NNK+AOM+DSS group (80%) was significantly higher than the AOM+DSS group (40%). Lung tumors were observed in the NNK+AOM+DSS, NNK+DSS, AOM alone and NNK alone groups, but the differences were insignificant. Thus, the tobacco-specific carcinogen, NNK, enhanced the colon carcinogenesis in colitis-associated colon cancer in male A/J mice, suggesting that smoking increases the risk of colon cancer development in patient who have inflamed colon.

P-49

Comparative Effects of Nobiletin and Auraptene on Proliferation and Apoptosis in Human Colon Cancer Cell Lines.

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[Introduction] Nobiletin and Auraptene are citrus phytochemicals which have been shown to act as chemopreventive agents against rat colon carcinogenesis. However, their mechanisms of action remain to be elucidated. Therefore we investigated the effects of these agents on growth of human colon cancer cell lines, and assessed the differences of protein pathway which may be regulated by both agents. **[Methods]** Human colon adenocarcinoma cell lines, Caco-2, DLD-1 and HT-29, were cultured with DMEM medium. EC₅₀ values on day 3 were determined individually and changes of the cell cycle and apoptosis (on day 3) were analyzed at these doses. Cellular proteins which have been reported to regulate colon cell differentiation and proliferation, e.g; β -catenin, PTEN, PPAR γ and cyclin D1 were analyzed on HT-29 by Western blotting. **[Results]** We demonstrated that both agents reduced the proliferation of all three cell lines studied and induced G0/G1 phase arrest in HT-29 and Caco-2 cells. Auraptene induced the apoptosis in HT-29 and DLD-1 cells, significantly. Furthermore, Nobiletin reduced β -catenin activation in HT-29 cells. **[Discussion]** The effectiveness of Nobiletin and Auraptene at inhibiting cell growth show a comparative effects especially in the cell cycle and apoptosis analysis and consequently Nobiletin show specific effect by suppressing the phosphorylation of β -catenin. Further investigations are ongoing to conclude possible protein regulation relevant to the chemopreventive effects of these agents.

P-50

Effect of Lauric Acid on Aberrant Crypt Foci Induced by AOM/DSS in Mice

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Association between fat intake and risk of cancer development has been suggested in epidemiological and animal studies. Lauric acid (LA, C₁₂H₂₄O₂), also called *n*-dodecanoic acid, is a medium chain fatty acid, which forms monolaurin in the human or animal body. The highest content of LA is found in mother's breast milk and coconut oil. LA recently has been reported to induce the expression of cyclooxygenase-2 and inducible nitric oxide synthase in macrophage cells (RAW 264.7). In the current study, we investigated whether LA promotes the occurrence of aberrant crypt foci (ACF) in an inflammation-related mouse colon carcinogenesis model with azoxymethane (AOM) and dextran sulfate sodium (DSS). To induce ACF, male ICR mice were given a single intraperitoneal injection of AOM (10 mg/kg body weight) and then followed by 1% DSS in drinking water for one week, starting one week after dosing of AOM (the AOM/DSS group). Animals were fed with the diet containing 1% LA for 7 weeks, starting one week after cessation of DSS administration (the AOM/DSS/LA group). Other groups included the AOM/LA group, the LA group, and the untreated group. At week 10 (end of the study), the frequency of ACF did not significantly differ between the AOM/DSS group (7.4±3.0) and the AOM/DSS/LA group (8.4±5.0). The value was extremely low in the AOM/LA group (1.0±1.0) and in the AOM alone group (2.4±2.7). No ACF developed in the LA alone and untreated groups. Our findings indicate that dietary LA did not influence the development of ACF in the AOM/DSS-induced mouse colon tumorigenesis, suggesting a lack of modifying effects of LA on the early phase of inflammation-related mouse colon carcinogenesis.

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Preventive Effects of Chrysin on the Development of Azoxymethane-induced Colonic Aberrant Crypt Foci in Rats

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Flavonoids are plant secondary metabolites ubiquitously distributed throughout the plant kingdom, and numerous reports have shown their biological effects, including anti-oxidative and anti-inflammatory activity. We compared the apoptotic induction effects of several flavonoids on human colon cancer cell. Among them, chrysin (5, 7-dihydroxyflavone) exerted powerful pro-apoptotic effect compared with other flavonoids. Therefore we investigated whether chrysin suppresses colon carcinogenesis in vivo.

The modifying effects of dietary feeding with chrysin on the development of azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF) were investigated in male F344 rats. We also assessed the effect of chrysin on mitosis and apoptosis in 'normal appearing' crypts. To induce ACF, rats were given two weekly subcutaneous injections of AOM (20 mg/kg body weight). They also received an experimental diet containing chrysin (10 or 100 ppm) for 4 weeks, starting 1 week before the first dose of AOM. AOM exposure produced a substantial number of ACF (73±13 /rat) at the end of the study (week 4). Dietary administration of chrysin caused significant reduction in the frequency of ACF: 10 ppm chrysin, 37±17 /rat (49% reduction, P<0.001); and 100 ppm chrysin, 40±10 /rat (45% reduction, P<0.001) when compared to the AOM alone group. In addition, chrysin administration significantly reduced the mitotic index and significantly increased the apoptotic index in 'normal appearing' crypts. These findings might suggest a possible chemopreventive activity of chrysin in the early step of colon tumorigenesis through modulation of cryptal cell proliferation activity and apoptosis.

P-52

Gastric Carcinogenesis and Inhibitory Effect of COX-2 Inhibitor, Etodolac in *N*-methyl-*N*-nitrosourea Treated K19-COX-2/mPGES-1 Transgenic Mice

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Helicobacter pylori (*Hp*) infection is closely linked with gastric cancer and induces cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1) in gastric mucosa. To investigate the influence of *Hp* infection and COX-2/mPGES-1 for gastric cancer, we examined the gastric carcinogenesis of *Hp*-infected K19-C2mE Transgenic mice (Tg), which expressed COX-2 and mPGES-1 and developed hyperplastic tumor in stomach. Furthermore, we also investigated the inhibitory effect of COX-2 inhibitor, etodolac for gastric cancer. Tg and Wild type mice (WT) were divided into the groups [*Hp*-infected, *N*-methyl-*N*-nitrosourea (MNU) treated, *Hp* + MNU treated, and none- treated control groups]. As a result, no gastric cancer was observed in *Hp* and control groups. In MNU and *Hp* + MNU groups, all Tg and WT developed gastric cancer in the pyloric region [MNU, Tg: 15/15 (100%), WT: 12/12 (100%), *Hp* + MNU, Tg: 19/19 (100%), WT: 19/19 (100%), respectively]. In the fundic region, Tg in MNU (4/15, 26.7%) and *Hp* + MNU (4/19, 21.1%) groups revealed to develop gastric cancers, whereas WT did not. In groups given etodolac, the incidence of gastric cancer was significantly reduced in pyloric region irrespective of genotypes (Tg: $p < 0.01$, WT: $p < 0.05$). These results suggested that over-expression of COX-2/mPGES-1 promoted gastric carcinogenesis since gastric cancer was developed due to MNU-treatment not only in pyloric but also in fundic region and COX-2 inhibitor might be effective for prevention for gastric carcinogenesis.

P-53

Promotion Effect of *Helicobacter Pylori*-infection on Mouse Gastric Carcinogenesis Model Induced by *N*-methyl-*N*-nitrosourea

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Although Mongolian gerbil has been widely used as a good animal model for *Helicobacter pylori* (*Hp*)-infection, it has disadvantage of poor genetic information. Thus, we tried *Hp* infection to mouse gastric carcinogenesis model induced by *N*-methyl-*N*-nitrosourea (MNU) and analyzed the effect of *Hp* and histopathological character of tumors.

Animals were divided into four groups: *Hp* + 10% NaCl diet, *Hp* + normal diet, 10% NaCl diet, and normal diet groups. We performed histopathological and immunohistochemical analysis including β -catenin, intestinal-alkaline phosphatase, and mucin staining using alcian blue-periodic acid Schiff.

Incidence of tumors induced by MNU were increased in *Hp* + 10% NaCl diet group compared with 10% NaCl diet group significantly ($P < 0.05$). Multiplicity of tumor were increased *Hp* group and *Hp* + 10% NaCl diet group compared with NaCl diet group ($P < 0.005$ and $P < 0.01$, respectively). Immunohistochemical analysis revealed that 38/55 (69.1%) of tumors had accumulation of β -catenin in nuclei and/or cytoplasm at least in part especially in high-grade malignancy, where differentiation markers rarely remained.

These results suggested that *Hp* infection has promotion effect on gastric carcinogenesis, with increasing tumor multiplicity also in mice. In addition, Wnt pathway may play a role at progression stage of gastric carcinogenesis.

P-54

Nordihydroguaiaretic Acid, A Plant Lignan, Inhibits *Helicobacter pylori*-Associated Gastric Carcinogenesis in Mongolian Gerbils

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Epidemiological studies have recently demonstrated that consumption of certain natural products can reduce cancer risk in humans. For, example, plant-derived lignans have been shown to exert chemopreventive effects against cancer *in vitro* and *in vivo*. In the present study, the effects of three such lignans, termed arctiin, arctigenin, and nordihydroguaiaretic acid (NDGA), on the proliferation of *Helicobacter pylori* (*H. pylori*) and the prevention of *H. pylori*-associated gastric cancer were investigated in Mongolian gerbils (*Meriones unguiculatus*). To examine the effects of arctigenin and NDGA on stomach carcinogenesis, specific pathogen-free male, 5-week-old gerbils were infected with *H. pylori*, administered 10 p.p.m. *N*-methyl-*N*-nitrosourea in their drinking water and fed diets containing various concentrations of lignans until they were killed after 52 weeks. At a dietary level of 0.25%, NDGA significantly decreased the incidence of gastric adenocarcinomas. Arctigenin, in contrast, failed to attenuate neoplasia at a level of 0.1%. Both NDGA and arctigenin significantly reduced serum 8-hydroxy-2'-deoxyguanosine levels at doses of 0.25 and 0.05% (NDGA), and 0.1% (arctigenin). Administration of 0.25% NDGA significantly suppressed the formation of intestinal metaplasia both in the antrum and the corpus. Although all three lignans dose-dependently inhibited the *in vitro* proliferation of *H. pylori*, there were no differences in the titers of anti-*H. pylori* antibodies or the amount of the *H. pylori*-specific urease *A* gene among all *H. pylori*-infected groups. These results suggest that NDGA might be effective for prevention of gastric carcinogenesis. The possible mechanisms appear to be related to inhibitory effects on progression of gastritis and antioxidative activity rather than direct antimicrobial influence.

P-55

Immunohistochemical Study of Ghrelin and Neuropeptide Y in Chronic Kidney Failure Rats

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Ghrelin is a novel detected orexigenic gastric hormone that stimulates food intake and growth hormone release from the pituitary. Markedly elevated plasma ghrelin levels and up-regulation of neuropeptide Y (NPY) gene expression in hypothalamus have been found in chronic renal failure in humans and rats. The causes of these abnormalities are still not understood. The present study was carried out to evaluate the immunohistochemical changes in ghrelin in stomach and NPY in arcuate nuclei in rats with chronic kidney failure. Nine-week-old male Sprague-Dawley rats were fed 0.75% adenine-containing diet freely for 4 weeks. During a 9-week recovery period that followed the administration period, the investigation was performed. Body weight and food consumption were measured throughout the study. Hematological and blood chemistry parameters were evaluated at both the end of the dosing period and the end of the recovery period. Gross-pathologic and histopathologic examination was performed on brain, kidney and stomach from all animals. Immunohistochemical expressions for ghrelin and NPY were examined and areas of ghrelin-expressing cells were measured using an image analyzer. The body weight and food consumption were significantly decreased in adenine-fed rats. Serum BUN and creatinine concentration in all adenine-fed rats increased significantly compared with those in control rats. Serum calcium levels decreased and phosphate levels increased significantly in adenine-fed rats. Kidney displayed tubular dilatation, degeneration, necrosis, regeneration, epithelial proliferation with giant cells and deposit of crystal of adenine, and inflammatory cell infiltration in the interstitium. Mucosa and muscular wall in glandular stomach showed remarkable calcification. The progressive increase of density and areas of ghrelin-expressing cells in gland gastric mucosa and NPY positive neurons in arcuate nuclei were confirmed in adenine-fed rats and recovery rats. The present results indicate that uraemic rats made by adenine diet developed overproduction of ghrelin in stomach and NPY in arcuate nuclei.

P-56

Vacuolar Degeneration of Epinephrine-Secreting Cells in the Rat Adrenal Medulla

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Histological alterations in the adrenal medulla are uncommon in short-term toxicity studies. Recently we encountered a vacuolar change in the adrenal medulla of an 8-week old male Sprague-Dawley rats. The animal was from a 4-day exploratory toxicity study and had received repeated high oral doses of a novel pharmaceutical compound. Decreases in body weight, lymphocyte counts, and the potassium level were recorded at necropsy. The adrenals were grossly enlarged. Routine formalin-fixed and hematoxylin and eosin-stained sections showed varying sizes of oval-to-irregular shaped vacuoles in the adrenal medulla, especially in the subcortical zone. Several vacuoles were formed in the cytoplasm of a medullary cell. The vacuolar contents were negative for Periodic acid-Schiff, azan, and alcian blue stains. In the cytoplasm of the vacuolated cells, there were considerably fewer neuroendocrine granules (revealed using Grimelius' method). The vacuolated cells were positive for antibody to phenylethanolamine N-methyltransferase (PNMT), which is a marker for epinephrine-secreting cells. The unaffected cells were negative for PNMT, and there was no change in the number of neuroendocrine granules. Ultrastructurally, the cytoplasm of the vacuolated cells showed dilatation of the membrane-bound compartment, and the contents were electron-lucent. The vacuoles were considered to be distended endoplasmic reticulum formed by the ingress of extracellular fluid with low protein content. Almost devoid of epinephrine granules in the vacuolated cells were confirmed at the ultrastructural level. We diagnosed this change as vacuolar degeneration of the epinephrine-secreting cells accompanied by degranulation in the adrenal medulla. Previously, both vacuolar formation and the degranulation of epinephrine-secreting cells in the adrenal medulla were reported in dogs during surgically-induced hemorrhagic shock. The vacuolar degeneration in the present case, therefore, may be associated with the degranulation of epinephrine granules.

P-57

Investigation of Striated Muscle Fiber in Pineal Gland of Crj:CD(SD)IGS Rats

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There are few chances to observe the pineal gland tissue of experimental animals, especially in the toxicological studies. Previous reports revealed striated muscle fibers in the pineal glands of rats (ex. Diehl BJM. Cell Tiss. Res. 1978; 190, 349-55. Delmas JA. et.al. Age. 1982; 5, 119-26). Some of these reports suggested that the incidence of striated muscle fibers in the pineal gland increases with aging. However, there are no reports comparing the incidence of the fiber at certain age points. In this study, the incidence and feature of the fiber in the pineal glands were examined histopathologically in 223 male and 193 female Crj:CD(SD)IGS rats at ages of 0- to 111 -weeks (w). The pineal glands were fixed in 10% neutral-buffered-formalin and stained with hematoxylin-eosin. Some of them were stained with phosphotungstic acid hematoxylin (PTAH) stain, masson's trichrome stain, and immunohistochemical stains (Desmin, Smooth muscle actin, Myogenin, and GFAP). The striated muscle fibers were found in the connective tissue, especially with fibrosis, and were likely connected to the pineal stalk. The muscle fibers had both short and long spindles, with cross striations. The cross striations were stained with PTAH distinctly. Immunohistochemically, the muscle fibers were only positive for Desmin. Histologically, the muscle fibers did not seem to connect to the pineal cells or glial fibers. The striated muscle fibers were found in total 14 male rats but not in females: 1 of 33 (3%) at 20w, 4 of 59 (7%) at 51w to 58w, 4 of 46 (9%) at 77w to 85w, 5 of 57 (9%) at 111w were found. The incidence of striated muscle fibers in the pineal glands of male rats increased gradually with aging.

P-58

Histopathological Changes and Gene Expression Profile of Testes by Short Term Administration of Di-(2-ethylhexyl) phthalate (DEHP) in Rats

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Testicular toxicity induced by phthalate plasticizers, such as di-(2-ethylhexyl) phthalate (DEHP), have been reported in rodents. However, its mechanism has not yet been sufficiently clarified. To investigate the primary toxic changes in rat testes induced by DEHP, this study evaluated the histopathological changes and gene expression profile of testes.

Materials and Method; DEHP was administered by gavage at doses of 0, 300, and 1,000 mg/kg once a day for 1, 4, and 7 days to groups consisting of 4 or 5 male rats (Crj:CD[SD]) at 5-week-old. All animals were measured for the body weights, organ weights, and blood chemical parameters. Furthermore, histopathological examinations were performed, and the gene expression profiles of the testes were evaluated using Affymetrix GeneChip Array.

Results and Discussion; Although a significant difference was not observed in the organ weight, small testis was noted in 1 male of 1,000 mg/kg the group at Day 7 (after the 7-time treatment). Histopathologically, the single cell necrosis of spermatids was noted of in Stage IX to XI on Day 1, necrotic spermatids on the basolateral surface and degeneration of seminiferous tubules in Stage XII to XIII were found on Day 4. On Day 7, seminiferous tubules without the elongate spermatids were seen in Stage I to VI, and it was assumed that the necrotic spermatid were phagocytosed by the Sertoli cells. On Day 7, seminiferous tubules without the elongate spermatids were seen in Stage I to VI, and it was assumed to be due to phagocytosis of the necrotic spermatid by the Sertoli cells. There were no abnormalities in the spermatocytes, spermatogonia, or Leydig cells. According to the GeneChip analyses, cell damage related genes, such as Free Radical Scavenging, were regulated on Day 2, in addition to the cell growth related genes being regulated on Day 4. On Day 7, alteration of cell function related genes (Cellular Function and Maintenance) were found. Within the regulated genes, *Timp1* and *Hsd11b1* showed specific changes, which were considered to be toxic changes induced by DEHP. Whereas changes in the genes related to the steroid metabolic enzyme and PPAR previously reported were not detected in this study. No alteration of genes was noted in 300 mg/kg the group.

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Spontaneous Osteochondrosis in the Distal Femur of Crj:CD(SD)IGS Rats.

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Spontaneous osteochondrosis has been reported to develop in the proximal humerus and distal femur of SD rats. In the present study, we examined the occurrence of the lesion in Crj:CD(SD)IGS rats, which had been genetically harmonized for use in toxicity studies. A total of 362 rats of both sexes were euthanized at 8, 12, 26, 39, 52, 78 and 104 weeks of age, and distal femurs were pathologically examined. In some selected animals, the proximal tibia was also examined. As a result, a white round plaque with or without partial depression of the articular surface was observed macroscopically in the femoral condyle. In more advanced cases, the whole femoral condyle appeared whitish with a partial defect of the articular cartilage. Microscopically, these lesions were observed as a thickening of the deep zone with small fissures in the cartilage. In more progressed lesions, expansion of the fissure, leading to formation of cartilage flap and detachment, and proliferation of fibrous tissue including large cysts in bone marrow were observed. The incidences of the lesions at 8, 12, 26, 39, 52, 78 and 104 weeks of age were 45.0, 50.0, 0, 16.7, 26.7, 26.5 and 21.4% in males and 45.0, 0, 0, 6.7, 6.7, 2.8 and 12.5% in females, respectively. Thus, the incidence was markedly higher in males than females, and the lesion increased severity with aging. These and histological findings are consistent with previous reports on SD rats. Additionally, severe osteochondrotic lesions accompanied partial depression and detachment of the articular cartilage in the tibia, and detached meniscus tip.

In conclusion, Crj:CD(SD)IGS rats were shown to develop spontaneous osteochondrotic lesions in femoral condyles in the present life-span experiment. Additionally, histological changes in the opposite proximal tibia and meniscus were found in association with severe osteochondrotic lesions. These results can be normal background data, and helpful to toxicologic histopathology.

P-60

Elucidation of the Histogenesis of Malignant Fibrous Histiocytoma (MFH); Mesenchymal Differentiations of a Rat MFH-derived Cell Line (MT-9)

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Malignant fibrous histiocytoma (MFH) is regarded as an undifferentiated pleomorphic sarcoma with unproven histogenesis. We investigated pathobiological characteristics of a newly-established rat MFH cell line (MT-9). Immunocytochemically, MT-9 cells and MT-9-induced tumors reacted to vimentin, A3 (a rat MFH cell-specific antibody), macrophage markers and α -smooth muscle actin (α -SMA; myofibroblastic marker) to varying degrees, indicating that MT-9 showed both histiocytic and (myo)fibroblastic features. Adipogenic supplement-added MT-9 cultures showed increased accumulation of lipid droplets. Addition of BMP-2 or osteogenic supplement to MT-9 enhanced osteoblastic markers such as ALP activity, osteocalcin mRNA expression and calcification. TGF- β 1-treated MT-9 revealed increased numbers of α -SMA-immunopositive cells, and enhanced protein levels of α -SMA, vimentin and fibronectin, indicating myofibrogenesis. Next, we investigated the distribution of A3-immunopositive cells in rat tissues. A3 labeled with immature mesenchymal and perivascular cells in fetuses and neonates, and with bone marrow stem cells in adults. *c-kit* mRNA expression was seen in tissues from bone marrow and MT-9.

Collectively, progenitors of MFH may be involved in the lineage of bone marrow stem cells capable of differentiating into divergent mesenchymal cells, consequently showing heterogeneous histological features depending on surrounding microenvironmental conditions. MT-9 and A3 antibody should provide valuable tools for studies on MFH or mesenchymal differentiation.

P-61

Effects of PDE 4 Inhibitor on Auricular Dermatitis Induced by TNCB-Repeated Application to Mice

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Anti-inflammatory effects of KF66490 (a phosphodiesterase (PDE) 4 inhibitor) in a mouse auricular dermatitis model induced by repeated application of 2,4,6-trinitro-1-chlorobenzene (TNCB) were histopathologically examined.

The dermatitis was induced by repeated TNCB embrocation on the inner side of the right ear of male BALB/c mice (histopathological characteristic was reported by C. Takada et al., Page 274 in the abstract of the JSTP/IFSTP (IATP) 2004). KF66490 (3 and 10 mg/kg/day) and prednisolone (positive control article, 10 mg/kg/day) were administered orally once daily for 23 days. Mice were sacrificed after the day of last dosing. The right ear from each mouse was removed and cut out two narrow longitudinal central strips. One of the strips was fixed in 10 vol% formalin solution and routinely embedded in paraffin, stained with hematoxylin and eosin (H&E), and additionally toluidin blue for identification of mast cells. The other strip was preserved in OCT compound, and frozen by dipping into liquid nitrogen and stored at approximately -80°C. Two frozen sections obtained from each strip were immunohistochemically stained with CD3 and I-A/I-E antibodies.

Repeated application of TNCB induced epidermal and dermal thickening, infiltrations of inflammatory cells (mainly eosinophils and neutrophils) and mast cells, and proliferation of fibroblasts. Immunohistochemical examinations revealed that CD3 positive cells and I-A/I-E positive cells infiltrate in epidermal and dermal layers. The 10 mg/kg/day of KF66490 significantly reduced the severity of epidermal and dermal thicknesses and fibroblast proliferation, and also inhibited infiltration of CD3 positive cells but not I-A/I-E positive cells. The 10 mg/kg/day of prednisolone just only inhibited the mast cell infiltration.

These results suggested that T cell suppression caused by KF66490 treatment may alleviate the TNCB induced dermatitis in mice.

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Investigation of the Local Irritation Study -Comparison with Injection Site Changes Induced by Intramuscular Injection and Subcutaneous Injection-

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【Introduction】 Local irritation of the medicine is often evaluated in the rabbit local irritation study using intramuscular injection regardless of the clinical application route (intramuscular or subcutaneous). However, there are differences of the histological structure in comparison with muscular tissue and subcutaneous tissue. Therefore, there might be differences of the injection site changes induced same medicine in comparison with intramuscular injection and subcutaneous injection. In the present study, histopathological changes induced by intramuscular injection and subcutaneous injection were investigated using rabbit local irritation study method.

【Materials and Methods】 1.7% acetic acid solution, 9.0% saline solution and DPT vaccine were used as test substances. Male rabbits received a single injection of each substance intramuscularly and subcutaneously, respectively. After 2 and 7 days of injection, 3 rabbits each in the treated group were sacrificed under the pentobarbital sodium anesthetizing, necropsy and histopathological examination were performed.

【Result】 < Intramuscular injection > 2 days after injection, wide degeneration/necrosis and cell infiltration were observed in 1.7% acetic acid solution and 9.0% saline solution groups. 7 days after the injection, the severity of the cell infiltration increased, and regeneration of muscular fibers were seen. 2 days after DPT vaccine injection, slight cell infiltration was observed, and 7 days after the injection, cell infiltration was remarkable.

< Subcutaneous injection > 2 days after injection, slight cell infiltration and degeneration/necrosis of muscle cutaneous were observed in 1.7% acetic acid solution groups. 7 days after the 1.7% acetic acid solution injection, the degenerative changes of muscle cutaneous showed restoration. 2 days after DPT vaccine injection, slight cell infiltration was observed. 7 days after the DPT vaccine injection, cell infiltration was remarkable.

【 Discussion 】 Since the differences happened to the histopathological changes of the injection site because of the test substances and the injection route, it was suggested that injection route was an important factor in the irritation evaluation.

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Cytokeratin 8/18 As a Novel Preneoplastic Lesion Marker Regarding Hepatocarcinogenesis in Mice

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Our recent studies have shown that Cytokeratin 8/18 (CK8/18) might became the novel biomarker of preneoplastic lesion developing in rat hepatocellular carcinoma.

However, in mice no preneoplastic lesion marker in the liver has been yet reported. Therefore, in the present study, we have investigated CK8/18 protein expression and cell proliferation in the liver of C57BL/6J and B6C3F1 mice, using single and double immunohistochemistry.

Male and female, 14-days old, C57BL/6J mice and B6C3F1 mice were initiated with diethylnitrosamine (DEN) and sacrificed of weeks 27, 18 and 38 after the initiation. CK8/18 positive foci were found in the mice liver concomitant with the altered foci detected by the HE stain. Cell proliferation index (PCNA) was found significantly increased compared to normal liver area in the areas of CK8/18 positive foci. Furthermore, adenomas and hepatocellular carcinomas were positive for CK8/18. These results indicate that CK8/18 might become a novel preneoplastic lesion marker in mice hepatocarcinogenesis.

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Proteomics of GST-P Positive Foci and Analysis of Potential Biomarkers in Rat Hepatocarcinogenesis

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Nowadays, glutathione S-transferase placental form (GST-P) positive foci are the world-wide recognized endpoint marker and considered the preneoplastic lesions in rat hepatocarcinogenesis, developing after its initiation and reflecting the later formation of tumors. Several of the observed in the liver GST-P positive foci are suggested to develop into hepatocellular adenomas and carcinomas, however, the concrete sequence of events occurred during this transformation is unknown. In the present study, to identify the novel biological markers of rat liver preneoplastic lesions, we have established a protein screening system by a combination of the immunohistochemistry, laser microdissection (LM) with iTRAQ labeling and Q-Star Elite LC-Ms/Ms spectrometry (Applied Biosystems). Protein lysates from microdissected GST-P positive foci in the liver of rats treated with diethylnitrosamine (DEN) followed by phenobarbital (PB) at doses of 0, 2 and 500 ppm were analysed by Q-Star Elite LC-Ms/Ms to generate unique protein profiles. Overexpression of cytoskeleton proteins, including intermediate filaments cytokeratins 8 (CK8), 18 (CK18), 13 (CK13), 14 (CK14) and 17 (CK17), apolipoprotein E (ApoE), alpha-2-macroglobulin (A2M), neurabin 1 (Neb1), mucin 2, septin 9, prohibitin (PHB), microsomal glutathione S-transferase 1 (MGST1), cytochrome b5 (CYB5A), UDP-glucose pyrophosphorylase 2 (UGP2), epoxide hydrolase 1 (EX1), and under-expression of catalase and heat shock proteins in GST-P positive foci of rats treated with PB at 500ppm was found, that predicted the presence and number of hepatocellular adenomas and carcinomas. Ms/Ms data were confirmed by immunohistochemical analysis, and the coordinated potential biomarker proteins overexpression was found in the areas of GST-P positive foci associated with increased cell proliferation. Significant overexpression of CK8/18 complex, was detected in hepatocellular adenomas and carcinomas. Our data imply that CK8/18 might become a novel biomarker of preneoplastic lesions developing into tumors, with important role played by CK13, 14 and 17, ApoE, A2M, Neb1, PHB, septin 9, MGST1, CYB5A, EX1, and UGP2 in rat hepatocarcinogenesis.

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Application of Tissue Microarray Using Prohibitin

Antibody as a Mitochondrial Marker for Assay of Liver

Injuries in Rat —The 2nd Report—

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Prohibitin (PHB) antibody was added in this study as a new immunostain marker in application of tissue microarray (TMA) liver specimens (2mm in diameter of core) for multi-stains micro-assay of liver injuries caused by carbon tetrachloride (CCl₄) in rats. Prohibitin, a homolog protein from yeast to human, is known to be a multi-functional protein regulating cell proliferation and a component of the inner membrane of mitochondrial wall. Its significant roles in apoptosis, cell cycle control, signal transaction and aging are reported (ref. Coates, P.J., et al. Exp. Cell Res. 2001 1;265:262-273).

In contrary to the liver tissue of control rats showing positive localization of PHB in the centrolobular region, the treated rat livers showed a map-shaped positive appearance of PHB one hour after the treatment, followed by the extensive positive distribution over the all hepatic lobules at the later stages (9 hours after CCl₄ treatment). The interesting finding was negative PHB in the areas showing positive by Altmann stain in the liver tissue. Although PHB showed a variety in its appearance, probably depending on the changing control mechanism of cellular proliferation at the variety of stages in liver injuries, its usefulness in assay of liver injury was suggested. Further analyses of PHB appearing at stages related with regeneration or proliferation of the liver cells in liver injuries caused by CCl₄ should be needed.

P-66

Induction of Oxidative Stress in the Liver of Rats Treated with Copper Gluconate, and Possible Modifying Effects of Combined Treatment with Catechol

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Copper gluconate (CuGL) is used as a food additive for the purpose of supplying copper. It has been reported that the excess administration of CuGL causes copper accumulation in rat liver, consequently leading to liver injury. Considering the fact that oxidative stress occurs in the liver of LEC rat, in this study, we investigated oxidative stress in the liver of rats given CuGL administration. In addition, the combined effects of catechol on the oxidative stress were examined. [Exp.1]: Serum biochemistry (ALT, AST and ALP), copper concentration in the serum and liver tissues, and 8-hydroxydeoxyguanosine (8-OHdG) levels in liver DNA of male F344 rats given a diet containing 0.6% of CuGL with or without 0.1% of catechol in the drinking water for 13 weeks were measured. Along with elevation of hepatotoxicity parameters, copper concentration in the liver tissues and 8-OHdG levels of CuGL-treated group were increased. However, there were no modifying effects of the combined treatment with catechol. [Exp.2]: To examine the effects of higher dose of catechol, male F344 rats were given a diet containing 0.6% of CuGL for 2 weeks followed by a single i.g. administration of 150 mg/kg catechol. The levels of TBARS and 8-OHdG formation in the liver were measured at 0, 2, 6, 12 and 24 hr after catechol administration. TBARS levels were significantly increased in CuGL-treated rats through the experimental period except 6 hr. Likewise, the elevation of 8-OHdG levels was found in rats treated with CuGL alone. However, the combined treatment with catechol did not affect the changes. In conclusion, oxidative stress occurred in the livers of rats given CuGL in their diet. *In vitro* finding that catechol is able to give rise to reactive oxygen species in the existence of Cu(II) were not demonstrated under the present experimental condition.

P-67

Effect of Chios Mastic Gum on Pre-neoplastic Lesions Development with Altered Gene Expressions in Rat Liver Median-term Carcinogenesis Bioassay (Ito-test)

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Mastic (*Pistacia lentiscus*) tree is widely native throughout the Mediterranean region and cultivated in Chios island of Greece. Mastic resin and decoction of the leaves are natural food additives from the ancient time due to owning a wide spectrum in biological effects. This time, modifying effect of Chios Mastic Gum (CMG) on rat liver carcinogenesis was investigated by an established liver median-term bioassay (Ito-test). Male F344 rats were fed CRF-1 powdered diet containing mastic at doses of 0, 0.01, 0.1 and 1% during 3-8 weeks. As a result, 1%-mastic did significantly increase the numbers (/cm²) and areas (mm²/cm²) of glutathione *S*-transferase placental form (GST-P)-positive cell foci (□0.2 mm) with a significant increment in larger-sized foci (□0.4 mm). Liver weights were significantly increased in a mastic dose-dependent fashion. BrdU-labeling indices (%) in both background hepatocytes and intra GST-P foci were significantly elevated by any doses, especially 1%-dose caused the highest index intra GST-P foci. Levels of 8-hydroxydeoxyguanosine (8-OHdG) formation in liver DNA did not change. In real-time PCR analysis, 1%-mastic significantly up-regulated several phase-I (CYP1A1, CYP2B2 and 3A2/3) and phase-II (GSTa2, UGT1a1) enzymes, oppositely growth-related genes (VEGF, IGF-IR, or others) were significantly decreased by CMG.

In conclusion, this report is the first that displays a promotion potential of CMG on rat liver carcinogenesis.

P-68

Mechanistic Study on the Carcinogenesis of Fenofibrate in a Two-Stage Carcinogenesis Model in rasH2 Mice.

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To evaluate the carcinogenic susceptibility of rasH2 mice to fenofibrate, 6-week-old male rasH2 mice were subjected to two-third partial hepatectomy (PH), followed by an i.p. injection of N-diethylnitrosamine (DEN) to initiate hepatocarcinogenesis after 24 hrs of PH. One week later, they received a diet containing FFB (0, 1200, or 2400 ppm) for 8 weeks. At the termination of this experiment, mice were killed and their livers were subjected to histopathological examinations and gene expression analyses using real-time RT-PCR. Significant increase in the incidence of centrilobular hypertrophy of hepatocytes and preneoplastic foci (vascular cell foci) were observed in males of the FF-treated groups. By immunohistochemistry, significant increases in proliferating cell nuclear antigen (PCNA)-positive cells and cytokeratin 8/18 positive foci were observed by FF promotion. In addition, most of the genes up-regulated were confirmed by the real-time RT-PCR. These results suggest that the hepato-carcinogenic activity of rasH2 mice to fenofibrate can be detected in this 8-week two stage hepatocarcinogenesis model and up-regulation of gene for ras/MAPK pathway and cell cycle were probably involved in the hepato-carcinogenic mechanism of rasH2 mice to fenofibrate.

P-69

Molecular pathological analysis on the mechanism of liver carcinogenesis in fenofibrate-treated rat

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Our previous studies showed the possibility that DNA damage due to oxidative stress is probably involved in the development of hepatocellular preneoplastic foci induced by fenofibrate (FF). In the present study, further investigations were performed to clarify whether oxidative stress is involved in the development of hepatocellular tumor induced by FF in a medium-term 2-stage hepatocarcinogenesis model of rats. Male F344/N rats were fed diet containing 3000 or 0 ppm of FF for 28 weeks after DEN initiation. Two-third partial hepatectomy was applied one week after treatment of FF. Histopathological examinations revealed significant induction of hepatocellular neoplasms and altered foci, which are not stained with anti GST-P antibody. In mRNA analysis on genes specific to GST-P positive (+) or negative (-) foci and tumors using laser microdissection system, significant down-regulations of *Aco* and *Cyp4a1* related to lipid metabolism were observed in the GST-P(+)/(-) foci and tumors in the liver of rats treated with FF compared to the surrounding tissue in the liver of rats treated with FF. In addition, the fluctuation of *Gpx2* and *Gsta2*, phase II enzyme, were observed in GST-P(+) foci and tumor in the liver of rats treated with FF, but these changes were not observed in the GST-P(-) foci and tumors. No significant changes were observed in the expression level of *Apex1* and *Xrcc5* related to DNA in the foci and tumors compared to the surrounding tissue in the liver of rats treated with FF. In the evaluation using the whole liver, an elevation of 8-OHdG in the liver DNA and a decrease in total GST activity were observed in the liver of rats treated with FF. These results suggest the possibility that oxidative stress is involved in the development of hepatocellular tumors induced by FF and the disturbance of phase II enzyme may be partly responsible for the effect of tumor promotion in rats treated with FF.

The Role of Inducible Nitric Oxide Synthase (iNOS) and Nitric Oxide in Cancer Development

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Nitric oxide (NO) is important in cancer development in both experimental animals and in humans and there is a strong correlation between the presence of iNOS and the occurrence of rapid tumor growth. Our observations on the choline-deficiency rat model of hepatocellular carcinoma show that iNOS/NO is an important factor in cancer development. We have shown that PBN (α -phenyl-tert butylnitron) has potent anti-cancer activity in this model. The anti-cancer action is strongly correlated with observations demonstrating that it depends in part on its action to suppress the expression of iNOS and that this is associated with potent apoptosis exclusively of the cells within the pre-neoplastic nodules. Based upon many experimental observations we have concluded that it is likely that NO-mediate S-nitrosylation of key enzymes and regulatory proteins and that this causes a concerted action of preventing cancer cell apoptosis and simultaneously enhancing oncogenic cell growth and DNA mutations. Research funded in part by NIH grant RO1CA82506, OCAST fMRI-002 and Japan Food Chemical Research Foundation.

Molecular Pathological Analysis of β -Naphthoflavone-Induced Tumor Promotion in Late Stage of Rat Hepatocarcinogenesis

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β -Naphthoflavone (BNF), a novel cytochrome P450 1A inducer, has a potential of tumor promotion in rat liver. Previously, we reported that BNF induced oxidative stress responses via excess production of reactive oxygen species (ROS) that were induced by the process of its metabolism in an early stage of a hepatocarcinogenesis model. To clarify the possible involvement of oxidative stress more precisely, rat two-stage hepatocarcinogenesis model was performed for the 28wk-period of BNF treatment. Male 6-week-old F344/N rats were treated with 1% BNF in the diet after DEN initiation. Two-third partial hepatectomy was applied one week after the start of BNF treatment. Adenomatous lesions have developed in all animals treated with BNF, associated with significant increase of the number and area of GST-P positive foci. BNF also significantly induced mRNA expressions of novel aryl hydrocarbon receptor battery genes (Cyp1a1, Cyp1a2, Cyp1b1, Nqo1, Aldh3a1) and Nrf2-regulated, anti-oxidant genes (Gpx2, Afar, Yc2, Hmox1, Trdx1, Gstm1), confirmed by quantitative real-time RT-PCR. Furthermore, both microsomal reactive oxygen species production and lipid peroxidation (TBARS) were increased, as compared to the group of DEN alone; however, the increase of oxidative DNA damage (8-OHdG formation) was not apparently observed. Immunohistochemical analysis revealed that decreased expressions of CYP1A1 and CYP1B1 in GST-P positive foci, especially in adenomatous lesions, compared to the surrounding hepatocytes. On the other hand, the expressions of phase II enzymes such as Afar, Gpx2, HO-1, TrxR in GST-P-positive foci were relatively increased compared to the surrounding tissue, regardless of BNF treatment. In cell cycle-related genes, BNF fluctuated mRNA expressions of Ccn2, Ccnb1, Cdc2a and p21. Especially in adenomatous lesions, immunohistochemically, cyclin A and p21 were very slightly detected, but PCNA-positive hepatocytes were significantly increased compared to the surrounding tissue. These results suggest that BNF elicits persistent oxidative stress, at least in part, by excess amount of ROS production from induced microsome. Furthermore, adenomatous lesions induced by BNF might acquire some mechanisms to escape from the oxidative stress; these might help preneoplastic foci to develop to cancerous lesions.

P-72

Hepatocarcinogenic Susceptibility of *rasH2* mice to Troglitazone in a Two-stage Hepatocarcinogenesis Model

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We have reported that the carcinogenic susceptibility of *rasH2* mice to troglitazone (TRG) is relatively low and the vascular tumor promoting activity of TRG in *rasH2* mice is extremely weak. On the other hand, TRG induced not only vascular tumors but also hepatocellular tumors in a 2-year carcinogenicity study of mice. Since it has been reported that *rasH2* mice have a hepatocarcinogenic susceptibility to PPAR α agonists, *rasH2* mice may also have a carcinogenic susceptibility to TRG. To examine the hepatocarcinogenic susceptibility of *rasH2* mice to TRG, 6-week-old *rasH2* mice were injected with *N*-diethylnitrosamine (DEN) after partial hepatectomy and given a diet containing 0 or 6000 ppm of TRG for 7 weeks. Five mice of both sexes were not subjected to any treatment to serve as the untreated control. The relative liver weight of female mice significantly increased in the DEN+TRG group as compared with that in the DEN alone group. There were increasing tendencies in the number of γ -glutamyltranspeptidase (GGT) positive cells and anti-PCNA antibody in the DEN+TRG group of both sexes, but such a change was not statistically significant as compared to the DEN-alone group. The gene expressions of VEGFB and VEGF related to angiogenesis, Tmp1 and TGF- β related to ras/MAPK cascade activation, and PCNA related to cell proliferation in females of the DEN+TRG group were significantly higher than those in the untreated control group, but only the Tmp1 gene in the DEN+TRG group was significantly higher than that in the DEN alone group. These results suggest the possibility that the carcinogenic susceptibility of *rasH2* mice to TRG in a two-stage hepatocarcinogenesis model is extremely low.

P-73

Multi Organ Carcinogenicity Using *Ogg1* Knockout Mice

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DMBDD model is a well-known multi-organ carcinogenicity test in the rat. In the present study, we investigated the carcinogenic effect of DMBDD treatment in oxoguanine glycosylase 1 (*Ogg1*) knockout mice (*Ogg1*^{-/-}) and wild type mice (*Ogg1*^{+/+}) of C57BL/6J background.

6 week-old male and female, *Ogg1*^{-/-} (120) and *Ogg1*^{+/+} (100) mice were treated with DMBDD for 6 weeks. Diethylnitrosamine (DEN; 400 ppm) was administered to mice in drinking water for 3 days at the commencement. Injections of *N*-methyl-*N*-nitrosourea (MNU; 20 mg/kg b.w., i.p.) were done 4 times in 2 weeks, and injections of 1,2-dimethylhydrazine (DMH; 10 mg/kg b.w., s.c.) 6 times in 3 weeks. *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN; 0.05%) in the drinking water was administered for 4 weeks after DEN treatment, following by dihydroxybutyl-di-*N*-propylnitrosamine (DHPN; 0.1%) in drinking water for 2 weeks. Pathological analysis in target organs was performed 30 weeks after starting the experiment.

Incidence of tumors in DMBDD-treated male and female mice in *Ogg1*^{-/-} were in the liver: 16% and 12%; colon: 16% and 0%; bladder: 4% and 12%, respectively. Incidence of tumors in DMBDD-treated male and female mice in *Ogg1*^{+/+} were in the liver: 4% and 8%; colon: 8% and 16%; bladder: 8% and 0%, respectively. No tumors were found in concomitant control groups.

Our results indicate that *Ogg1*^{-/-} mice are more sensitive to DMBDD treatment compared to wild type mice. The pathological analysis in target organs is ongoing.

P-74

Existence of Thresholds for Carcinogenicity and *in vivo* Mutagenicity of 1,4-dioxane in Liver of Rats

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1,4-Dioxane is primarily used as industrial solvent for paints, surface-treating agent for artificial leather and is a stabilizer for chlorinated solvent. The purpose of this study is to determine the dose-response relationships for carcinogenicity and *in vivo* mutagenicity of 1,4-dioxane in the liver of rats. In the experiment 1, 3-week-old male, F344 rats were treated with 1,4-dioxane in the drinking water at doses of 0, 2, 20, 200, 2000 and 5000 ppm for 16 weeks. Quantitative analysis of glutathione *S*-transferase placental form (GST-P) positive foci, which are preneoplastic lesions in the rat liver, revealed that 1,4-dioxane significantly increased the numbers of GST-P positive foci at 2000 and 5000 ppm compared to control, but had no effects at doses of 200 ppm and below. In the experiment 2, 6-week-old male, *gpt delta* transgenic rats were treated with 1,4-dioxane in the drinking water at doses of 0, 200, 1000 and 5000 ppm for 16 weeks. *gpt* mutant frequencies showed a tendency to increase at 1000 ppm and significantly elevated at 5000 ppm, but did not differ at 200 ppm from the control. Thus, 1,4-dioxane had no-effect level for carcinogenicity and *in vivo* mutagenicity, suggesting the existence of thresholds for both of them.

P-75

Aggravation of Galactosamine Hepatotoxicity by Albumin in Rats

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The effect of rat albumin (RALB) on D-galactosamine (GalN) hepatotoxicity was investigated in male Crl:CD(SD) rats aged 6 weeks. The animals were divided into the control, RALB, GalN, and RALB + GalN groups. GalN (800 mg/kg) was intraperitoneally administered immediately after an intravenous injection of RALB (100 mg/kg). The animals were euthanized 6 and 24 h later and subjected to laboratory investigations and histological examinations of the liver. Furthermore, to see contributing factors to the effect on the hepatotoxicity, intra- and inter-group comparisons were made between hepatotoxic and normal animals for parameters showing marked fluctuations at 6 h post-dosing. As a result, RALB induced fluctuations in many parameters, but no histological changes in the liver at both 6 and 24 h. GalN induced mild and moderate hepatocyte death at 6 and 24 h, respectively, accompanied by increases in serum ALT and AST levels. Concurrent administration of RALB with GalN markedly aggravated the GalN-induced hepatotoxicity as shown by severe hepatocyte death accompanying further increases in serum ALT, AST, and T-BIL levels in surviving animals at 6 and 24 h, and by very severe hepatocyte death with congestion in dead animals. Moreover, serum tissue necrosis factor α (TNF α) level was also increased at 6 h in the RALB + GalN group, but not RALB or GalN alone group. In conclusion, RALB was shown to aggravate GalN hepatotoxicity in rats and the increased serum TNF α level is considered to be a contributing factor to the aggravation.

P-76

Catechol Caused Oxidative DNA Damage under Mouse Hepatitis Induced by Acetaminophen

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We have shown that catechol generates reactive oxygen species and reactive nitrogen species (RNS) through semiquinone radical formation in the process of the reaction with nitric oxide (NO) leading to oxidative DNA damage in rat forestomach. To confirm whether dietary catechol systemically exerts the same effects under inflammation, we have investigated several oxidative stress markers in the livers of mice treated with catechol in acetaminophen (APAP)-induced hepatitis. Male ICR mice were treated with or without 0.8% catechol in the diet at 2 weeks prior to APAP administration at a dose of 300 mg/kg by singly i.p. injection. Along with several indicators showing APAP-induced hepatitis, 8-hydroxydeoxyguanosine (8-OHdG) levels and immunohistochemistry for 3-nitrotyrosine (NO₂Tyr) in the livers were examined at 1.5, 4 and 24 hr after APAP injection. Additionally, NO₂Tyr formation in the livers was quantified by LC-MS/MS in the same experimental protocol. 8-OHdG was significantly increased at 24 hr only in the co-treatment group. The facts that elevation of serum ALT and AST levels and reduction of reduced glutathione levels being observed at the same extent in APAP-treated groups suggest that co-treatment with catechol did not enhance APAP-induced hepatotoxicity. In view of the positive hepatocytes for NO₂Tyr being found from 4 hr, 8-OHdG formation might result from the chemical reaction of the two compounds. Precise quantitative analysis of NO₂Tyr formation further supported the existence of RNS in the reaction, implying that some antioxidants involving catechol structure might cause oxidative DNA damage in the circumstances of NO generation such as inflammation.

P-77

Hepatocellular apoptosis induces phosphorylation of keratin 8 in rat livers

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Keratin (K) 8 and K18 constitute the intermediate filament cytoskeleton of adult hepatocytes. Recent studies have reported that a site specific phosphorylation of K8 by mitogen-activated protein kinase (MAPK) plays a role in protecting liver injuries in humans and mice. However, the role of K8 in rat livers has not been determined. Here, we examined the relationship between phosphorylated K8 and the rat liver injuries induced by cycloheximide (CHX) and bromobenzene (BB). F344 rats were intravenously treated with 6 mg/kg of CHX. In other groups, rats were intraperitoneally treated with 300 mg/kg of BB. These animals were sacrificed at 1 h and 2 h after the CHX treatment or 12 h and 24 h after the BB treatment, respectively. The number of TUNEL positive nuclei and the amount of hepatic caspase 3/7 activity in the livers were significantly increased, compared with the corresponding control animals at 2 h after the CHX treatment or 24 h after the BB treatment ($p < 0.05$). In a toxicoproteomic analysis using two-dimensional difference gel electrophoresis and mass spectrometry, K8 was present in two forms with similar molecular weights but different pI values: a basic form and a more acidic form. The amount of the more acidic form of K8 in the CHX-treated or BB-treated rat livers was significantly higher than that of the control animals at 2 h after the CHX treatment or 24 h after the BB treatment, respectively ($p < 0.05$ and an average ratio greater than 1.2-fold). The spots shifted to the acidic site were positively stained with Pro-Q Diamond phosphoprotein gel stain. It has been reported that the blockage of K8 phosphorylation predisposes hepatocytes to apoptotic injury by toxicants. These findings suggest that K8 phosphorylation is involved in the regulation of hepatocellular apoptosis in rat livers.

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Chemopreventive Effects of MTBITC and Curcumin Using a Medium-Term Pancreatic Carcinogenesis Model in Hamsters

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The chemopreventive effects of naturally occurring agents were investigated using a 16-week medium-term pancreatic carcinogenesis models in hamsters. Male 6-week-old Syrian hamsters were subcutaneously injected with 10 mg/kg body weight *N*-nitrosobis(2-oxopropyl)amine (BOP) four times within a week, and fed a diet supplemented with 80 ppm or 700 ppm 4-(methylthio)-3-butenyl isothiocyanate (MTBITC), or 2000ppm curcumin(CUR) during the initiation or post-initiation stages. For the initiation stage, each chemical was given for 3 weeks including 1 week before and after the BOP injections, and thereafter fed basal diet. For post-initiation exposure, the groups were changed from basal diet 1 week after the last BOP injection, and then fed each chemical for 14 weeks. All the animals were sacrificed after 16 weeks. The incidence of combined pancreatic lesions including atypical hyperplasias and adenocarcinomas were significantly decreased by 80 ppm MTBITC, and the multiplicity of atypical hyperplasias and combined lesions were significantly decreased by 700 ppm MTBITC given in the initiation but not the post-initiation stage, respectively. On the other hand, CUR, a naturally occurring inhibitor of cyclooxygenase-2 (COX-2), failed to show significant effects on pancreatic carcinogenesis in either the initiation or post-initiation stages under the present experimental conditions. Our data suggest that the naturally occurring isothiocyanate MTBITC exerts effective protection against BOP-induced pancreatic tumors in hamsters.

P-79

Promoting Effects of Madder Color Component and Their Metabolite on Renal Carcinogenesis in a Rat Medium-term Multi-organ Carcinogenesis assay

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It has been shown that madder color (MC), a natural food coloring used in Japan, induces kidney and liver tumors in rats. In our previous short-term study, alizarin (Alz), one of MC component, induced vacuolar degeneration in proximal tubule cells (PTCs) in the renal cortex, and rubiadin (Rub), a metabolite of MC component, caused karyomegaly in PTCs in the outer medulla, however, their carcinogenic potential remain determined. In the present study, a rat medium-term multi-organ carcinogenesis assay was performed to clarify promoting activity of Alz and Rub on the kidney, liver and large intestine where DNA adduct formation has been reported. Particularly, we focused on histopathological analysis for renal lesions.

Male 6-week-old F344 rats initiated with five carcinogens (DEN, MNU, DMH, BBN and DHPN) within the period of 4 weeks in accordance with a standard method for medium-term multi-organ assay were fed diet containing 0.008% or 0.04% of Alz or Rub from week 5 for 23 weeks.

Histopathologically, atypical tubules, atypical hyperplasias and renal cell adenomas/carcinomas were observed in both cortex and outer medulla in all groups including controls. In the outer medulla, a major site of renal cell tumors induction by MC, incidence and numbers of atypical tubules and atypical hyperplasias increased significantly, and incidence of renal cell adenomas/carcinomas tended to increase in the 0.008% and higher Rub groups. Increase in the incidences of atypical hyperplasias with induction of renal cell adenomas and/or carcinomas was also observed in the outer medulla with 0.04% Alz, whereas the treatment did not affect tumor numbers.

These results clearly indicate that both Rub and Alz have promoting effects on renal carcinogenesis. The renal tubular cells in the outer medulla are their target sites. The promoting effect of Alz might be weaker than that of Rub.

A Distinctive Amphophilic-Vacuolar Renal Tubule Tumor Phenotype in Rat Studies Conducted by the National Toxicology Program, NIEHS, NIH

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Carcinogenicity studies, utilizing primarily the Fischer 344 rat, conducted by the National Toxicology Program, NIEHS, NIH were reviewed for the occurrence and distribution of a distinctive renal tubule tumor phenotype. Both control and treated groups were examined. The main features of this tumor included the uniform lobular arrangement of eosinophilic to amphophilic stained, large round to polyhedral cells with finely granular cytoplasm and the presence of numerous variably-sized clear vacuoles and/or minilumens. Central degeneration/necrosis was also prominent in most cases. It is referred to as the "Amphophilic-Vacuolar" renal tubule tumor to help distinguish it from the predominant rat renal tumors which are basophilic staining. Out of 1012 rats surveyed with renal tumors (adenomas, carcinomas or adenocarcinomas), 100 of these had the amphophilic-vacuolar morphology. In a few cases, tumors were multiple and bilateral. These tumors were equally distributed between sexes, did not metastasize, at least to the lungs. The distribution of this tumor was random across studies and dose groups suggesting it was spontaneous and not chemically induced. Results from this review and other published reports of similar appearing tumors, particularly from young and possibly sibling rats, indicate that this tumor may be of familial origin. If this hypothesis can be validated, then tumors of this phenotype could be excluded from final tumor counts when assessing the carcinogenic potential of test chemicals in preclinical studies. This tumor will be briefly compared to Eker and Nihon rats which are familial renal cancer models.

Existence of Thresholds for *In Vivo* Mutagenicity and Carcinogenicity of Potassium Bromate in Kidney of Rats

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The purpose of the present studies was to investigate the dose-response relationship for *in vivo* mutagenicity and carcinogenicity of potassium bromate (KBrO₃), a genotoxic carcinogen in the kidney of rats. To investigate *in vivo* mutagenicity of KBrO₃ in the kidney, male Big Blue transgenic rats were treated with KBrO₃ at concentrations of 0, 0.02, 0.2, 2, 8, 30, 125 and 500 ppm in the drinking water, respectively. After 16 weeks of treatment, 500 ppm KBrO₃ significantly increased the total mutation frequency and frequency of GC to TA transversion of the lacI gene in the kidney compared to control group, but 125 ppm and lower doses of KBrO₃ had no effects. To investigate promoting potency of KBrO₃ on kidney carcinogenesis, male Wistar rats were given 0.05% N-ethyl-N-hydroxyethylnitrosamin (EHEN) for 2 weeks in the drinking water to initiate kidney carcinogenesis. Thereafter, they were treated with KBrO₃ at concentrations of 0, 0.02, 0.2, 2, 8, 30, 125 and 500 ppm (250 ppm after 12 weeks treatment) in the drinking water, respectively, for 24 weeks. There was no significant difference in both the number of preneoplastic lesions and the number of tumors in the kidneys in 125 ppm or below KBrO₃ groups, while both of them were significantly increased in the highest dose group. These results demonstrated the existence of no-effect levels for *in vivo* mutagenicity and promoting effects of KBrO₃ on kidney carcinogenesis, indicating that thresholds exist for both mutagenicity and carcinogenicity of KBrO₃ in rats.

P-82

Histological Background Data of the Urinary Tracts from a 104-week Non-treatment Study of the Wistar Hannover Rat (BrIHan:WIST@Jcl (GALAS))

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[Introduction] It is reported that the Wistar Hannover rat is suitable for the non-clinical safety studies like a carcinogenicity study, because of their limited body weight gain in comparison with the SD rat and higher survival rate (more than 75%) of them over two years. Therefore, we conducted a long-term study of the Wistar Hannover rat (BrIHan:WIST@Jcl (GALAS)) in order to collect the histological background data of the urinary tracts from them under our laboratory environmental conditions.

[Materials and Methods] Ninety nine male and female BrIHan:WIST@Jcl rats are purchased at 4 weeks old from CLEA Japan, Inc. and kept until 110 weeks of age under non-treatment condition. The animals were given low protein pellet food CE-7 (CLEA Japan, Inc.) and ultra-filtered tap water (source: Mikuni-cho, Fukui) ad libitum. Urinalysis (one-hour pooled urine during the early morning) was performed at 84 and 107 weeks of age, and their kidney and urinary bladder were observed histopathologically.

[Results and Discussion] The mean body weights of male and female were 576g and 378g, and the survival rates of male and female were 82% and 74%, respectively. Assessment of the urinalysis showed higher pH, calcium level and ratio of amorphous phosphate granules in females than those in males. The pH showed decrease tendency in males and females, as time passes. Histopathological examination revealed papilloma of the urinary bladder (1/99, male), renal tubule carcinoma and transitional cell carcinoma of the kidneys (1/99 each, female), as neoplastic findings. Regarding non-neoplastic findings, although medullary mineralization of the kidney were frequently observed in females, few of males and females showed the chronic progressive nephropathy, that is frequently observed in the other strains like SD. On the other hand, incidence of the chronic progressive nephropathy in our laboratory was less than that of the same strain (BrIHan:WIST@Jcl) reported from a contract research laboratory (RCC Ltd.). These results indicate that BrIHan:WIST@Jcl rat shows less renal and urinary bladder lesions and is useful for evaluation the drug candidates that have urological effect in the carcinogenicity study. Moreover, it is speculated that our laboratory environment such as drinking water and low protein diet contributed to the decreased incidence of the spontaneous lesions.

P-83

Morphological Differences between Mice and Rats in Glomerular lesions of CPN

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CPN (Chronic Progressive Nephropathy) is a common spontaneous renal disease in aging rats. In aging mice, similar spontaneous renal lesion may be occurred occasionally and diagnosed as CPN. In rats, there are so many reports about mechanism, histological findings or epidemiological data, while there are a few reports about mice. These lesions are described to differ in histological findings from each other in some reports.

Thus, we researched on morphological differences in CPN glomerular lesions between mice and rats.

In our laboratory, mice have lower incidence than rats. In mouse CPN, PAS and PAM positive granular nodule were observed on thickened glomerular basement membrane (GBM) in contrast with diffusely thickened GBM in rat. We could not detect positive reaction on mice GBM to IgG+A+M cocktail antibody by immunohistochemistry. A few similar changes were observed in other age-related renal lesion of mouse, glomerulonephritis and hyaline glomerulopathy.

In electron microscopic findings of CPN, thickened GBM were mostly smooth in rat, while rough surface, high electron density deposits and wash out lesion like changes were observed in mouse.

These results may suggest the differences in etiology or progress of disease in GBM lesion between CPN of mice and rats.

P-84

Early Events Involving Glomerular Calcification Induced by Bolus Injection with Dibasic Sodium Phosphate Solution in Rats

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We have reported on glomerular calcification in rats induced by intravenous bolus administration of dibasic sodium phosphate (Na_2HPO_4) solution for 14 days in the 18th JSTP meeting. To investigate the onset and early stages of the glomerular damage induced by Na_2HPO_4 solution, we examined the morphological and immunohistochemical time-dependent changes of the kidneys and urinalysis. In this study, we identified early events in the glomeruli and describe here the possible pathogenesis of glomerular calcification.

METHODS: 360 mM Na_2HPO_4 solution was administered to the male rats at 8 mL/kg once daily via the tail vein. Urinalysis was carried out on day 1, 3, 5 and 8 of dosing, and the rats were sacrificed on day 2, 4 and 9 for histopathologic and electron microscopic examination of the kidneys.

RESULTS: Urinary protein excretion increased from day 3. Following single dosing, there were no gross or histological findings, but a number of vacuole-like structures were observed scattered within the Bowman's space by electron microscopy. On day 4, minimal and focal mineralization was observed within the parietal epithelium. On day 9, mineralization was minimal to mild and localized within the parietal epithelium and the glomerular basement membrane. Hypertrophy and increased mitotic figures were also frequently observed in the parietal epithelium. Low-density lamellar structures with fusion of podocytes, an increased number of microvilli and large amounts of debris filling the Bowman's space were observed with electron microscopy on day 4 and 9. Immunohistochemically, decreased expression of podoplanin and increased expression of desmin were evident in the glomeruli on day 9. Increased urinary protein excretion was almost correlated with the glomerular changes.

CONCLUSION: These results have shown that phosphate-induced glomerular lesions occur with repeated administration for 3 days. Few morphological changes, which were observable only by electron microscopy, may be important as factors preceding glomerular calcification. Since glomerular changes closely paralleled the onset of marked proteinuria, urinalysis is desired during administration.

P-85

Comparison of Renal Toxicity Induced by Antibiotics

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【 Background 】 In Japan, it is reported that antibiotic-induced renal disorder is one of the most common drug-induced nephropathy in humans and that it becomes a cause of death with relatively high incidence when compared to other drug-induced nephropathies. Rabbit is known as one of the most sensitive animal species to the antibiotic-induced renal toxicity. We evaluated detailed features of renal toxicity induced by several commercially available antibiotics by using rabbits.

【 Materials & Methods 】 Forty male Japanese white rabbits aged 12 to 14 weeks were divided to 10 groups and single intravenous administration of vehicle (saline) or 200 or 400 mg/kg of antibiotics (Cephaloridine (CER), Vancomycin (VCM), Meropenem (MEPM), and Ceftazidime (CAZ)) was performed. The rabbits were sacrificed 48 hours after drug administration. Urine, blood and kidneys were collected and biochemical, and histomorphological examinations were performed. In addition, mRNA levels of certain key proteins involved in renal toxicity were measured by semi-quantitative RT-PCR.

【 Result & Conclusion 】 In the CER group at both low and high doses, increases in BUN, s-creatinine, urine Gamma Glutamyl Transferase (GGT) and urine Total Protein (TP) levels were observed. Histologically, tubular necrosis was seen in kidney.

In the VCM group at both low and high doses, increases in BUN and s-creatinine were observed. Histologically, tubular dilatation and tubular necrosis were seen.

In the MEPM and CAZ groups, tubular basophilia was occasionally seen histologically and slight increases in urine GGT and urine TP were observed at the high dose. There were no histological or biochemical changes at low dose.

The mRNA levels of TNF alpha and iNOS in renal cortex of rabbits in CER group strongly correlated to the severity of the renal lesion. In the VCM and CAZ groups, the mRNA level of TNF alpha tended to show high levels at high dose. The mRNA levels of OAT1 were lower at high doses of each antibiotic-treated group than those of controls. The mRNA levels of CuSOD and TGF beta were similar among all treated and control groups.

The results suggest that additional parameters, such as urine GGT and mRNA levels, could be good tools to detect renal toxicity more clearly at early stage.

P-86

Age of Development of Polycystic Kidney Disease Induced by p-Cumylphenol in Rats

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[Introduction] p-Cumylphenol, p-(α,α -dimethylbenzyl, phenol), (PCP) is a chemical which is widely used as a material for polycarbonate plastics, surfactants, fungicides and preservatives. We reported previously that newborn rats treated with PCP by gavage for 18 days from postnatal day 4 developed cystic dilatation of collecting ducts in the medulla, while rats treated from 6 weeks of age in the same manner did not. In this study, in order to investigate the critical period of cyst formation, PCP was administered to rats at various weeks of age to examine the relationship between the age in weeks and the development of cystic kidney.

[Materials and Methods] Groups of male Sprague-Dawley [CrI:CD(SD)] strain rats at 2, 3, 4, 5 and 6 weeks of age were used. PCP was suspended in olive oil. Each rat was treated with PCP at 300mg/kg/day, at which clear cystic kidney was observed in the previous study, by gastric incubation for 14 days. After the dosing period, animals were euthanized by exsanguination under deep ether anesthesia and autopsied. Then the kidneys were collected for histopathological examination.

[Results] In the week 2 group in which animals were treated from week 2 of age, marked dilatation was detected in collecting ducts in the outer medulla. These cystically dilated ducts were lined by hyperplastic epithelial cells and occasionally showed slight single cell necrosis and mitosis. Dilatation of collecting ducts was detected in inner medulla and cortex, but they were slight changes. Similar changes were observed in the week 3 group, but the severity of the changes was less. There were no such changes in the other groups in which administration was started at week 4 of age or thereafter.

[Conclusion] It became clear that formation of cystic kidney in rats by PCP had a critical period, and it was from the time of birth to week 3 after birth. It was suggested that the kidney maturation process was involved in the formation of cystic kidney.

P-87

Inhibition of Prostate Carcinogenesis by Ellagic Acid *in vitro* and *in vivo*

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Ellagic acid, a plant derived polyphenol was investigated for the anti-proliferative and proapoptotic effects on human prostate cancer cell lines (LNCaP, PC-3 and DU145). Cancer cells were incubated with ellagic acid for 24 and 48 hours, then cell viability and number of apoptotic cell was determined. Ellagic acid (25 – 100 mM) was able to decrease cell viability of hormone sensitive, LNCaP, whereas this effect was not observed in PC-3 and DU145. Ellagic acid had also potential to induce cell death by apoptosis in only LNCaP. In vitro results lead to investigation the modulating effect of ellagic acid on prostate carcinogenesis in transgenic rats developing adenocarcinoma of prostate (TRAP). Three weeks old male transgenic rats were fed phytoestrogen low diet supplement with ellagic acid (0, 0.1 and 1.0% respectively) for 10 weeks then sacrificed. The results showed that rats received ellagic acid had lower relative prostate weight compared to non-treated group whereas the serum level of testosterone, estradiol was similar. The histology alteration prostate tissue was evaluated. The level of malondialdehyde (MDA) was slightly increased during experimental period and in week 10 the level of MDA in serum of rat received 0.1% ellagic acid was lower than control group ($p < 0.05$). The results suggested that ellagic acid had a potential to induce apoptosis in human prostate cancer cell (LNCaP) and might reduce oxidative damage in vivo. The molecular mechanisms on anti-carcinogenesis of prostate will be further studied.

P-88

cDNA Microarray Analysis for Specific Genes for Detection of Prostate Carcinogens.

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Prostate cancer is the second leading cause of cancer in North American men and is associated with significant morbidity and mortality. Recently the incidence of prostate cancer in Japan has been increasing and this increase may involve exposure to unknown prostate carcinogens. To identify the candidate marker of short-term screening assay for detecting unknown prostate carcinogens, we carried out cDNA microarray study and examined commonly up- or down-regulated genes in rat ventral prostate treated with known prostate carcinogens. Correlation to cell proliferation in the ventral prostate was also examined in this study.

<MATERIALS AND METHODS>

We choose three well-known prostate carcinogens, 2-amino-1- methyl-6- phenylimidazo[4,5-b]pyridine (PhIP), 3,2'-dimethyl-4- aminobiphenyl (DMAB) and cadmium. Six-weeks-old male rats were treated with PhIP (100mg / kg body weight, i.g.), or DMAB (50mg / kg b.w., s.c.) or cadmium (3mmol/kg b.w., i.m.) at Day 0 and Day 7, and then rats were killed at Day 10. Total RNA was isolated from the ventral prostate of the rats and the quality of total RNA and fragmented cRNA was easily visualized on a 2100 Bioanalyzer. Affymetrix Rat Genome 230 2.0 Array slides containing > 30,000 probes were used for the analysis. BrdU labeling indices in the ventral prostate and small intestine were evaluated by an image analyzer IPAP.

<RESULTS AND DISCUSSION>

The numbers of the genes significantly up-regulated in the ventral prostate of the rats treated with known prostate carcinogens were 40, 43 and 54 in PhIP, DMAB and cadmium treated group, respectively, and only three genes including oncomodulin (OM) were commonly up-regulated. Among 3 carcinogens, the numbers of the genes significantly down-regulated genes were 725, 744 and 712 in PhIP, DMAB and cadmium treated, respectively, and 594 genes were commonly down-regulated. Quantitative RT-PCR analysis confirmed over-expression of OM in the ventral prostate. Interestingly, BrdU indices in the ventral prostates with three carcinogens were significantly higher than control value. These results suggest that over expression of oncomodulin was correlated with higher cell proliferation in the ventral prostate of the rats treated with prostate carcinogens.

P-89

The Enhancing Effect of Antioxidant Substance NAC on the Injury of Urinary Bladder Induced by DMA Using Intravesical Instillation

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Dimethylarsinic acid (DMA), the major excreted metabolite of inorganic arsenic, is carcinogenic to the rat urinary bladder. Oxidative stress has been proposed as one possible mechanism of DMA-induced carcinogenic action. We determined whether N-acetylcysteine (NAC), which is thought to have antioxidant properties, would inhibit DMA-induced urinary bladder injury in rats. Contrary to our expectations, NAC enhanced damage induced by DMA. Either DMA (10 mg/kg) or NAC (1.6 or 90 mg/kg), or both of them were intravesically instilled into female F344 rats for 2 hours under pentobarbital anesthesia. Intravesical instillation was conducted twice a week at an interval of 3 days. Vehicle control or untreated control groups were set as comparison. All animals were killed at 1 day after the last treatment. In histopathological examination of the urinary bladder, neutrophil infiltration was observed in 1 of 4 or 1 of 6 rats treated with DMA alone or NAC alone, respectively, while the inflammatory response was found in more than half of animals treated with DMA and NAC (high or low). Transitional cell hyperplasia was observed in 2 of 5 rats treated with DMA and NAC (high). The BrdU labeling index was significantly increased in the DMA or NAC (high) alone group compared to the vehicle control group. Co-administration of DMA and NAC (high or low) significantly increased the BrdU labeling index compared to the group administered DMA alone. In immunohistochemical expressions of phosphorylated MAPK (ERK1/2) and Cyclin D1 were found in the area of hyperplasia. Immunointensity of 3-nitrotyrosine, which is known as a biomarker indicating oxidative damage to protein, increased in the DMA+NAC (high) group compared with the vehicle control group. These results suggest that co-administration of DMA with NAC into the rat urinary bladder may increase cell proliferation, along with severe inflammatory response, and the effects may be mediated by excess oxidative stress and ERK signaling.

P-90

Modifying Effects of L-leucine and L-isoleucine on BBN-induced Rat Urinary Bladder Carcinogenesis

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Epidemiological studies have reported that intake of protein-rich diet increased the incidence of bladder cancer in Western countries. The purpose of the present study is to investigate the modifying effects of L-leucine (Leu) and L-isoleucine (Ile) on

N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN)-induced bladder carcinogenesis in rats.

Experiment 1: Groups of male, 6-week-old, F344 rats were administered 0.05% BBN in drinking water for 4 weeks, and then fed Leu or Ile at doses of 0, 0.5, 1.0 and 2.0% in AIN-93G diet. At week 36, no significant differences of incidences and multiplicities of bladder tumors were observed in any amino acid treatment groups compared to control. Experiment 2: Groups of rats were administered 0.05% BBN in drinking water for 4 weeks, and then fed Leu or Ile in MF diet and AIN-93G diet at 0 and 2.0%. Animals in AIN-93G and MF diet groups were sacrificed at weeks 29 and 29 and 36, respectively. There were no significant differences of incidences and multiplicities of bladder tumors in groups treated with Leu or Ile in MF diet compared to control at weeks 29 and 36. However, incidences and multiplicities of TCC and total tumors were significantly increased in Leu and Ile-treated groups, respectively, as compared to control at week 29.

The present studies indicate that Leu and Ile exert promotion effect in BBN-induced rat bladder carcinogenesis when supplemented in AIN-93G diet but not in MF diet.

P-91

Inhibitory Effects of Bowman-Birk Inhibitor (BBI) on Prostate Carcinogenesis

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[Introduction] The soybean-derived protease inhibitor, Bowman-Birk Inhibitor (BBI), may act as a potent chemopreventive agent against several types of tumors. The present study was undertaken to evaluate the effects of BBI on androgen dependent prostate carcinogenesis in the transgenic rats developing adenocarcinoma of the prostate (TRAP) model, and LNCaP cell line to cast light on mechanisms underlying the effects of BBI on prostate carcinogenesis. [Methods] TRAP rats were segregated in 2 groups (12 rats per group), and receiving 3% BBI or the soy-free basal diet both mixed with 2% corn oil. Starting at 3 weeks of age for 10 weeks. In cell culture, LNCaP were exposed to BBI and cell viability was evaluated by WST-1 assay. Connexin 43 (Cx43) and apoptosis expression were investigated by western blot. [Results] BBI feeding resulted in a significant reduction in the body weights ($P<0.05$), absolute whole ($P<0.05$) and dorsolateral prostate (Containing urogenital organs) weights ($P<0.0001$) with increased testes weights ($P<0.05$), but no changes in the serum testosterone and estrogen level, and also significantly reduced the relative epithelial areas ($P<0.01$) and multiplicity of the adenocarcinomas ($P<0.01$), while increased the accumulations of the normal ($P<0.01$) and prostatic intraepithelial neoplasia (PIN) acini ($P<0.05$) in the lateral prostate lobes compared with the control rats. In addition, treatment of BBI on LNCaP cells resulted in inhibition of viability with restoration of Cx43 expression and cleaved caspase-3 induction. [Discussion] These results suggest strong prostate cancer chemopreventive efficacy of soybean diet including BBI, and further provide an insight into the therapeutic implications of BBI for the treatment of prostate cancer by Cx43 restoration without affecting hormone level.

P-92

Suppression of Rat Prostate Carcinogenesis by Angiotensin Receptor Blocker.

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[Introduction] Renin-angiotensin system is involved in prostate carcinogenesis and we performed an in vivo experiment to explore the effect of telmisartan, an angiotensin receptor blocker, in our Transgenic Rat for Adenocarcinoma of Prostate (TRAP) model rats. **[Methods]** Three weeks old TRAP rats were given telmisartan (2 and 10 mg/kg/day) in drinking water for 7 weeks. Histopathological examination of prostate was performed and the percent area of epithelium to each acinus was determined **[Results and Discussion]** Significantly, telmisartan decreases the incidence of prostatic adenocarcinoma in the lateral lobes. Evaluation of the proportion of preneoplastic and neoplastic lesions showed that telmisartan shifted the progression of neoplastic growth by suppressed the number of adenocarcinoma and high grade PIN and consequently, increased low grade PIN in the ventral lobes. The numbers of apoptotic cell in the prostate of rats treated with telmisartan were significantly increased in the ventral lobe compared with those in the controls although Ki-67 labeling indices were not different. MAPK pathway was suppressed and cleaved caspases 3 and 7 were increased by telmisartan treatment in ventral prostate tissue. Thus, these results indicate that angiotensin receptor blocker is a candidate for prostate cancer chemopreventor that suppresses cancer growth through the inhibition of MAPK signal pathway.

P-93

Modifying Effects of Soybean Isoflavone on Mammary Gland Carcinogenesis and Its Estrogenic Activity in the Uterus of Donryu Rats

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The soybean isoflavone have been shown to possess estrogenic activity in human and laboratory animals, however, its effects on mammary gland and uterine carcinogenesis are still unclear. The maximum of its intake for human has been recently established by Japan Food Safety Commission. In the present study, we evaluated the effects of soybean isoflavone on proliferation capacity of mammary gland epithelium, estrous cycle, and its estrogenic activity in uterus of Donryu rats treated with the dose equal to the established maximum intake for human. In experiment 1, 5-week-old female Donryu rats were administered 50 mg/kg 7,12-dimethylbenz[α]anthracene (DMBA) by gavage (i.g.) and fed 0 (control) or 0.2% soybean isoflavone (SoyAct, Kikkoman, Japan) in the phytoestrogen low diet (NIH-07PLD) for 4 weeks. Cell proliferation (BrdU labeling index) was found to be significantly increased in the group of rats treated with DMBA and isoflavone as compared to initiation control. In the experiment 2, 5-week-old female Donryu rats were ovariectomized and administered 0.2% soybean isoflavone in the same diet from day 14 to 28 after the ovariectomy. The maintenance of estrous phase in smear examination and significant increase of uterus weight signifying the potential estrogenic activity were found in 0.2% soybean isoflavone-treated group. These results indicated that soybean isoflavone administered at a dose established as maximum intake for human exerts estrogenic activity in the uterus and induces cell proliferation in the mammary gland of Donryu rats.

Effects of Different Diets in 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) Induced Rat Mammary Carcinogenesis

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Japanese commercial basal diets have been used in the most of carcinogenesis studies in Japan. High fat diet has been reported to enhance the colon and mammary carcinogenesis, however Japanese commercial diets have been used in those studies. The comparing study of diets in carcinogenesis has not been reported. Modifying effects of diets (high fat diet and Japanese basal diet (CE-2)) on 2-amino-3-methylimidazo[4,5-*b*]pyridine (PhIP)-induced mammary carcinogenesis were investigated in female Sprague-Dawley (SD) rats. Eighty rats were divided into 4 groups. Starting at 6 weeks of age, rats were fed the high fat diet (Groups 1 and 4) or CE-2 (Groups 2 and 3). At 7 weeks of age, Groups 1 and 2 were given PhIP in corn oil (85 mg/kg body weight, 8 times for 11 days) by intragastric intubation. Diets were continued to given to the termination. All rats were sacrificed at 24 weeks after the start of experiment. At the termination, mammary tumors were recognized in Group 1 (PhIP + High fat diet) and 2 (PhIP + CE-2). All of tumors were adenocarcinomas; their incidences were 59.4 % in Group 1 and 20.0 % in Group 2 ($P<0.001$) and multiplicities of tumors were 1.72 ± 2.26 in Group 1 and 0.23 ± 0.50 in Group 2 ($P<0.001$). Mean sizes of the tumors were 10.4 ± 6.2 mm in Group 1, and 10.4 ± 4.7 mm in Group 2. No mammary tumors were observed in rats of groups 3 and 4. CE-2 shows low incidence of PhIP-induced mammary carcinogenesis compared with the high fat diet. These results indicate that the high fat diet is suitable for the diet of mammary carcinogenesis induced by PhIP in female SD rats. The basic ingredients of CE-2 are not informed, although the main components of CE-2 have been published. More analysis of CE-2 need for the elucidation of the effect of basal diets.