Hanada, K.[*], Nakai, K.[*], Tanaka, H.[*], Suzuki, F.[*], Kumada, H.[*], Ohno, Y., Ozawa, S., Ogata, H.[*]: **Effect of nuclear receptor downregulation on hepatic expression of cytochrome P450 and transporters in chronic hepatitis C in association with fibrosis development**

Drug Metab. Pharmacokinet. **27**, 301-306（2011）

Analysis of mRNAs from liver biopsy samples of patients with chronic hepatitis C revealed that the levels of nuclear receptor expression were correlated with those of drug-metabolizing enzymes and transporters in relation to the development of fibrosis. Overall, the median mRNA level was largely dependent on fibrosis stage（F）, and that for stage 3 patients（F3）was about 50% less than that for F1 patients. Levels of expression of AhR, together with CAR and PXR, were lowest in livers of F3 patients. Multivariate linear regression analysis revealed that AhR expression appeared to be involved in the regulation of CYP1A2, 2E1, 2D6, UGT1A, MDR1/3, MRP2/3, NTCP and OCT1 in the livers of patients with chronic hepatitis C. These results suggest that downregulation of AhR during the progression of liver fibrosis is associated with decreased expression levels of these phase I and II enzymes and drug transporters during inflammation-related signal transduction between AhR and other nuclear receptors.

Keywords: nuclear receptor, hepatitis C, gene expression, P450

[*] Meiji Pharmaceutical University

Low, Y.[*1], Uehara, T.[*2], Minowa, Y.[*3], Yamada, H.[*3], Ohno, Y., Urushidani, T.[*3,4], Sedykh, A.[*1], Muratov, E.[*1], Kuz'min, V.[*1], Fourches, D.[*1], Zhu, H.[*1], Rusyn, I.[*1], Tropsha, A.[*1]: **Predicting drug-induced hepatotoxicity using QSAR and toxicogenomics approaches**

Chem. Res. Toxicol. **24**, 1251-1262（2011）

Quantitative structure-activity relationship（QSAR）modeling and toxicogenomics are typically used independently as predictive tools in toxicology. In this study, we evaluated the power of several statistical models for predicting drug hepatotoxicity in rats using different descriptors of drug molecules, namely, their chemical descriptors and toxicogenomics profiles. The records were taken from the Toxicogenomics Project rat liver microarray database containing information on 127 drugs（ http://toxico.nibio.go.jp/datalist.html）. The model end point was hepatotoxicity in the rat following 28 days of continuous exposure, established by liver histopathology and serum chemistry. First, we developed multiple conventional QSAR classification models using a comprehensive set of chemical descriptors and several classification methods（k nearest neighbor, support vector machines, random forests, and distance weighted discrimination）. With chemical descriptors alone, external predictivity（correct classification rate, CCR）from 5-fold external cross-validation was 61%. Next, the same classification methods were employed to build models using only toxicogenomics data（24 h after a single exposure）treated as biological descriptors. The optimized models used only 85 selected toxicogenomics descriptors and had CCR as high as 76%. Finally, hybrid models combining both chemical descriptors and transcripts were developed; their CCRs were between 68 and 77%. Although the accuracy of hybrid models did not exceed that of the models based on toxicogenomics data alone, the use of both chemical and biological descriptors enriched the interpretation of the models. In addition to finding 85 transcripts that were predictive and highly relevant to the mechanisms of drug-induced liver injury, chemical structural alerts for hepatotoxicity were identified. These results suggest that concurrent exploration of the chemical features and acute treatment-induced changes in transcript levels will both enrich the mechanistic understanding of subchronic liver injury and afford models capable of accurate prediction of hepatotoxicity from chemical structure and short-term assay results.

Keywords: Prediction, hepatotoxicity, QSAR, toxicogenomics

[*1] University of North Carolina
[*2] Shionogi & Co., Ltd.
[*3] National Institute of Biomedical Innovation
[*4] Doshisha Women's College of Liberal Arts

Sumida, K.[*1], Igarashi, Y.[*2], Toritsuka, N.[*1], Matsushita, T.[*1], Abe-Tomizawa, K.[*1], Aoki, M.[*1], Urushidani, T.[*2,3], Yamada, H.[*3], Ohno, Y.: **Effects of DMSO on gene expression in human and rat hepatocytes**

Hum. Exp. Toxicol. **30**, 1701-1709（2011）

Dimethyl sulfoxide（DMSO）is a very common organic solvent used for dissolving lipophilic substances, for example for in vitro cell-based assays. At the same time, DMSO is known to be cytotoxic at high concentrations. Therefore, it is important to define threshold concentrations of DMSO for cells but relevant data at the molecular level are very limited. We have focused on conducting microarray analyses of human

and rat hepatocytes treated with more than 100 chemicals in attempts to identify candidate biomarker genes. In the present study, the effects of DMSO on gene expression and cytotoxicity were assessed in human cryopreserved hepatocytes and rat primary cultured hepatocytes. A cytotoxicity test with lactate dehydrogenase（LDH）activity demonstrated DMSO to be noncytotoxic up to a concentration of 2%（v/v）in both cases and there were only few effects on the gene expression profiles up to 0.5%（v/v）. The observed differences from controls were considered to be of little toxicological importance, but still need to be taken into account in interpretation of findings when DMSO is used at high concentration.

Keywords: DMSO, gene expression, human, rat, hepatocytes, toxicogenomics

[*1] Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.
[*2] National Institute of Biomedical Innovation
[*3] Doshisha Women's College of Liberal Arts

Okuno, Y.[*1], Minowa, Y.[*2], Yamada, H.[*2], Ohno, Y. and Urushidani, T.[*2,3]: **In Silico Toxicology Prediction Using Toxicogenomics Data**

General, Applied and Systems Toxicology, Online（2011）

Toxicogenomics holds the promise of unprecedented advances in two broad, overlapping fields: mechanistic or investigative toxicology, and predictive toxicology. The progress of toxicogenomics has been supported by DNA microarray technology, a powerful tool for directly monitoring patterns of cellular perturbations through the identification and quantification of global shifts in gene expression resulting from pathological alterations within cells and tissues. Microarrays provide a large amount of transcriptional expression data for thousands of individual genes under various experimental conditions. Bioinformatics technologies can determine which genes are meaningful, facilitating the analysis of huge pools of toxicogenomics data in mechanistic and predictive toxicology. This chapter is devoted to computational approaches for the data mining of biomarker genes from toxicogenomics data, leading to toxicity prediction. Many algorithms have been developed for feature gene selection. Most studies on feature selection have found that wrapper methods are superior to filter methods, but many of these studies have over-emphasized prediction accuracy and overlooked the robustness of the selected genes. In fact, this study illustrates that intensity-based moderated t-statistics - support vector machine（SVM）produces more stable gene lists than recursive feature elimination - SVM. Therefore, we have to carefully gauge not only prediction performance but also the robustness of gene sets in feature gene selection.

Keywords: Toxicogenomics, systems biology, In silico, prediction

[*1] Kyoto University
[*2] National Institute of Biomedical Inovation
[*3] Doshisha Women's College of Liberal Arts

Yudate, HT.[*1], Kai, T.[*1], Aoki, M.[*1], Minowa, Y.[*2], Yamada, T.[*1], Kimura, T.[*1], Ono, A., Yamada, H[*2]., Ohno, Y., Urushidani, T.[*2,3]: **Identification of a novel set of biomarkers for evaluating phospholipidosis-inducing potential of compounds using rat liver microarray data measured 24-h after single dose administration**

Toxicology **295**, 1-7（2012）

Phospholipid accumulation manifests as an adverse effect of cationic amphiphilic drugs in particular. Detection, however, by histopathology examination is time-consuming and may require repeated administration of compounds for several weeks. To eliminate compounds with potential for inducing phospholipidosis from the discovery pipeline, we have identified and validated a set of biomarkers for predicting the phospholipidosis-inducing potential utilizing a comprehensive rat transcriptome microarray database created by the Japanese Toxicogenomics and Toxicogenomics Informatics Projects（TGP/TGP2）together with in-house data. The set of biomarkers comprising 25 Affymetrix GeneChip probe sets was identified using genetic algorithm optimization on 24-h time-point microarray data from rats treated with single doses of hepatotoxic compounds including amiodarone, clomipramine, haloperidol, hydroxyzine, imipramine, and perhexiline. The set of novel biomarkers represents an early time-point gene-expression pattern characteristic for a condition eventually leading to phospholipidosis. This implies significant advantages in terms of time and resources over currently published biomarkers derived using repeated-dosing late time-point data. The biomarker set was validated by 11 independent compounds. Accuracy, sensitivity, and specificity values were 82%, 67%, and 100%, respectively and the area under the receiver operating characteristic curve was 0.97. These results show that the biomarker set possesses a high classification accuracy for novel compounds. Pathway analysis was carried out for the biomarkers and the detection of pathways related to lipid-metabolism was statistically significant. These

pathways most probably reflect lipid metabolism changes associated with phospholipidosis supporting the validity of our novel biomarkers.

Keywords: phospholipidosis, prediction, toxicogenomics

[*1] Dainippon Sumitomo Pharma Co., Ltd.
[*2] National Institute of Biomedical Innovation
[*3] Doshisha Women's College of Liberal Arts

Yoshida, H., Nishikawa, M.[*], Kiyota, T.[*], Toyota, H.[*], Takakura, Y.[*]: **Increase in CpG DNA-induced inflammatory responses by DNA oxidation in macrophages and mice**

Free Radic. Biol. Med., **51**, 424-431（2011）

Unmethylated CpG dinucleotide（CpG motif）is involved in the exacerbation of DNA-associated autoimmune diseases. We investigated the effect of DNA containing 8-hydroxydeoxyguanosine（oxo-dG）, a representative DNA biomarker for oxidative stress in the diseases, on CpG motif-dependent inflammatory responses. ODN1668 and ODN1720 were selected as CpG-DNA and non-CpG DNA, respectively. Deoxyguanosine in the CpG motif（G9）or outside the motif（G15）of ODN1668 was substituted with oxo-dG to obtain oxo（G9）-1668 and oxo（G15）-1668, respectively. Oxo（G15）-1668 induced a significantly higher amount of tumor necrosis factor（TNF）-α from RAW264.7 macrophage-like cells than ODN1668, whereas oxo（G9）-1668, oxo（G8）-1720, or oxo（G15）-1720 hardly did. CpG DNA-induced TNF-α production was significantly increased by addition of oxo（G8）-1720 or oxo（G15）-1720, but not of ODN1720. This oxo-dG-containing DNA-induced increase in TNF-α production was also observed in primary cultured macrophages isolated from wild-type mice, but not observed in those from Toll-like receptor（TLR）-9 knockout mice. In addition, TNF-α production by ligands for TLR3, TLR4, or TLR7 was not affected by oxo-dG-containing DNA. Then, the footpad swelling induced by subcutaneous injection of ODN1668 into mice was increased by coinjection with oxo（G8）-1720, but not with ODN1720. These results indicate that oxo-dG-containing DNA increases the CpG motif-dependent inflammatory responses, which would exacerbate DNA-related autoimmune diseases.

Keywords: 8-hydroxydeoxyguanosine containing DNA, Toll-like receptor-9, inflammatory response

[*] 京都大学大学院薬学研究科

Izutsu, K., Yomota, C., Kawanishi, T.: **Impact of heat treatment on the physical properties of non-crystalline multi-solute systems concentrated in frozen aqueous solutions**

J. Pharm. Sci., **100**, 5244-5253（2011）

The purpose of this study was to elucidate the effect of heat treatment on the miscibility of multiple concentrated solutes that mimic biopharmaceutical formulations in frozen solutions. The first heating thermal analysis of frozen solutions containing either a low-molecular-weight saccharide（e.g., sucrose, trehalose, and glucose）or a polymer（e.g., polyvinylpyrrolidone and dextran）and their mixtures from −70◦C showed a single transition at glass transition temperature of maximally freeze-concentrated solution（T_g'）that indicated mixing of the freeze-concentrated multiple solutes. The heat treatment of single-solute and various polymer-rich mixture frozen solutions at temperatures far above their T_g' induced additional ice crystallization that shifted the transitions upward in the following scan. Contrarily, the heat treatment of frozen disaccharide-rich solutions induced two-step heat flow changes（T_g' splitting）that suggested separation of the solutes into multiple concentrated noncrystalline phases, different in the solute compositions. The extent of the Tg' splitting depended on the heat treatment temperature and time. Two-step glass transition was observed in some sucrose and dextran mixture solids, lyophilized after the heat treatment. Increasing mobility of solute molecules during the heat treatment should allow spatial reordering of some concentrated solute mixtures into thermodynamically favorable multiple phases.

Keywords: freeze-drying, phase separation, stabilization

梶村計志[*]，川口正美[*]，四方田千佳子：**難溶性製剤の溶出試験に界面活性剤として使用されるラウリル硫酸ナトリウムの品質に関する研究**

医薬品医療機器レギュラトリーサイエンス，**42**，626-632（2011）

ラウリル硫酸ナトリウム（SDS）の試薬としての品質が，経口製剤の溶出試験に及ぼす影響を検討したところ，13種類の市販SDSのうち，C_{12}の他にC_{14}やC_{16}を含む試薬の場合に，やや溶出率が高くなる傾向が認められた．試薬の入手には一定品質のものを確保する必要があることが示唆された．

Keywords：溶出試験，難溶性医薬品，ラウリル硫酸ナトリウム

[*] 大阪府立公衆衛生研究所

Miyazaki, T., Aso, Y. and Kawanishi, T.: **Feasibility of atomic force microscopy for determining crystal growth rates of nifedipine at the surface of amorphous solids with and without polymers**

J. Pharm. Sci., **100**, 4413-4420（2011）

Amorphous nifedipine, which has a smooth surface immediately after preparation, was shown to have structures resembling clusters of curling and branching fibers approximately 1 μm wide by atomic force microscopy（AFM）after storage at 25 ˚C. The size of the cluster-like structures increased with storage over time, implying crystal growth. The average elongation rate of the fibers determined by AFM at ambient room temperature was 1.1×10^{-9} m/s, and this agreed well with the crystal growth rate of 1.6×10^{-9} m/s determined by polarized light microscopy. The crystal growth rate of nifedipine in solid dispersions with 5 % polyethylene glycol was found to be 5.0×10^{-8} m/s by AFM. Although this value was approximately the same as that obtained by polarized light microscopy, three-dimensional information obtained by AFM for the crystallization of NFD in a solid dispersion with PEG revealed that the changes in topography were not a consequence of surface crystal growth, but rather attributable to the growth of crystals formed in the amorphous bulk. For solid dispersions with α, β-poly（N-5-hydroxypentyl）-L-aspartamide, acceleration of nifedipine crystallization by tapping with an AFM probe was observed. The present study has demonstrated the feasibility and application of AFM for interpretation of surface crystallization data.

Keywords: amorphous, crystallization, microscopy

Maitani, Y.[*], Nakamura, A.[*], Tanaka, T.[*], Aso, Y.: **Hydration of surfactant-modified and PEGylated cationic cholesterol-based liposomes and corresponding lipoplexes by monitoring a fluorescent probe and the dielectric relaxation time**

Int. J. Pharm., **427**, 372-378（2012）

For the optimization of plasmid DNA（pDNA）-cationic lipid complexes and lipoplex delivery, proper indexes of the physicochemical properties of lipoplexes are required. In general, the characteristics of lipoplexes are defined by particle size and zeta-potential at various mixing ratios of cationic liposomes and pDNA. In this study, we characterized the hydration level of surfactant-modified and PEGylated cationic cholesterol-based（OH-Chol）liposomes and their lipoplexes by monitoring both the fluorescent probe laurdan and the dielectric relaxation time. Fluorescence measurement using laurdan detected hydration of the headgroup of lipids in surfactant-modified liposomes and PEGylated DOTAP-liposomes, but hardly any fluorescence was detected in PEGylated OH-Chol-liposomes because the PEG layers may extend and cover the fluorescent maker. On the other hand, the measurement of dielectric relaxation time of water molecules revealed total hydration, including hydration of the PEG layer and the headgroup of cationic lipids. Furthermore, we found an inverse correlation between hydration level and cellular uptake of PEGylated lipoplexes（R = 0.946）. This finding indicated that the dielectric relaxation time of water molecules provides an important indicator of hydration of liposome and lipoplexes along with the fluorescence intensity of laurdan.

Keywords: Hydration, liposome, dielectric relaxation time

---

[*] 星薬科大学

Yoshioka, S.[*], Forney, K.M.[*], Aso, Y., Pikal, M.J.[*]: **Effect of Sugars on theMolecularMotion of Freeze-Dried Protein Formulations Reflected by NMR Relaxation Times**

Pharm. Res., **28**, 3237-3247（2011）

Purpose To relate NMR relaxation times to instability-related molecular motions of freeze-dried protein formulations and to examine the effect of sugars on these motions. **Methods** Rotating-frame spin-lattice relaxation time（T1ρ）was determined for both protein and sugar carbons in freeze-dried lysozyme-sugar（trehalose, sucrose and isomaltose）formulations using solid-state ^{13}C NMR. Results The temperature dependence of $T_{1\rho}$ for the lysozyme carbonyl carbons in lysozyme with and without sugars was describable with a model that includes two different types of molecular motion with different correlation times（τ_c）for the carbon with each τc showing Arrhenius temperature dependence. Both relaxation modes have much smaller relaxation time constant（τ_c）and temperature coefficient（E_a）than structural relaxation and may be classified as β-relaxation and γ-relaxation. The τ_c and Ea for γ-relaxation were not affected by sugars, but those for β-relaxation were increased by sucrose, changed little by trehalose, and decreased by isomaltose, suggesting that the β-mobility of the lysozyme carbonyl carbons is decreased by sucrose and increased by isomaltose. Conclusion $T_{1\rho}$ determined for the lysozyme carbonyl carbons can reflect the effect of sugars on molecular mobility in lysozyme. However, interpretation of relaxation time data is complex and may demand data over an extended temperature range.

Keywords: Stability, Protein, NMR relaxation time

---

* School of Pharmacy, University of Connecticut

Katori, N., Sai, K., Saito, Y., Fukushima-Uesaka, H., Kurose, K., Yomota, C., Kawanishi, T., Nishimaki-Mogami, T., Naito, M., Sawada, J., Kunitoh, H.*, Nokihara, H.*, Sekine, I.*, Ohe, Y.*, Yoshida, T.*, Matsumura, Y.*, Saijo, N.*, Yamamoto, N., Okuda, H., Tamura, T.*: **Genetic Variations of Orosomucoid Genes Associated with Serum Alpha-1-Acid Glycoprotein Level and the Pharmacokinetics of Paclitaxel in Japanese Cancer Patients**

J. Pharm. Sci., **100**, 4546-4559（2011）

Alpha-1-acid glycoprotein（AGP）encoded by orosomucoid genes（*ORM1* and *ORM2*）is an acute-phase response protein and functions as a drug-binding protein that affects pharmacokinetics（PK）/pharmacodynamics of binding drugs. To explore the effects of genetic variations of *ORMs* and a role of AGP on paclitaxel（PTX）therapy, we analyzed the duplication and genetic variations/haplotypes of *ORMs* in 165 Japanese cancer patients and then investigated their associations with serum AGP levels and the PK parameters of PTX. No effects of *ORM* duplications on serum AGP levels at baseline or PK of PTX were observed, but close associations of *ORM1* −559T > A with the increases of AGP levels and area under the curve（AUC）of PTX metabolites were detected. In addition, a significant correlation between the serum AGP level and the AUCs of PTX metabolites was observed, suggesting that AGP may function as a carrier of PTX from the blood into the liver via putative receptors. This study provided useful information on the possible clinical importance of *ORM* genetic polymorphisms and a novel role of AGP in PTX therapy.

Keywords: alpha-1-acid glycoprotein, paclitaxel, pharmacogenomics

---

* National Cancer Center

Yamamoto, Y.*1, Fukami, T.*2, Koide, T., Suzuki, T.*2, Hiyama, Y., Tomono, K.*2: **Pharmaceutical evaluation of steroidal ointments by ATR-IR chemical imaging: Distribution of active and inactive pharmaceutical ingredients**

Int. J. Pharm., **426**, 54-60（2012）

We recently used micro attenuated total reflection infrared（ATR-IR）spectroscopy to conduct imaging analysis of ointments and evaluate the distributions of the active pharmaceutical ingredient（API）and excipients. An alclometasone dipropionate（ALC）ointment was used as a model product. Almeta, a brand-name product, had a domain with absorbance at 1656 cm^{-1} attributable to the carbonyl group of ALC, the API. Absorbances at 1040 and 3300cm^{-1} were also noted in this domain, indicating the presence of the solubilizer, propylene glycol. Data also suggested the presence of benzyl alcohol in this domain. More detailed analysis showed the distribution of surfactants and other excipients in the base. Similar results were obtained for Vitra, a generic version of Almeta. Imaging analysis with micro ATR-IR confirmed that both ointments are liquid droplet dispersions with ALC dissolved in propylene glycol and dispersed in a base. However, minor differences in the ingredient distributions of the two ointments were detected and reflect differences in excipient concentrations and type, or manufacturing differences. In summary, we used micro ATR-IR for imaging analysis of an original ointment, Almeta, and its generic form Vitra, and established a method for visually evaluating the distributions of the API and excipients in these ointments.

Keywords: ATR, Chemical Imaging, Ointment

---

*1 平成帝京大学薬学部

*2 日本大学薬学部

Sakai-Kato, K., Ota, S.*1, Takeuchi, T.*2, Kawanishi, T.: **Size separation of colloidally dispersed nanoparticles using a monolithic capillary column**

J. Chromatogr. A., **1218**, 5520-5526（2011）

We developed a method to separate colloidally dispersed nanoparticles on monolithic capillary columns. Silica nanoparticles were eluted according to their sizes, and the plots of the logarithm of the size of nanoparticles against their elution volume showed good linearity（r = 0.992）over wide range of sizes. Because of the high porosity of the monolithic column（porosity; 88%）, the column's length could be increased without clogging of the dispersed samples and the pressure in a long column（500 mm × 0.2 mm i.d.）was low, with a value of 5.8 MPa at a flow rate of 1 μL/min. We demonstrate that this method using monolithic capillary columns could be used as a powerful tool for size separation of nanometer-size materials, which will open a new pathway to quality control of nanomaterials in nanotechnology applications.

Keywords: Nanoparticles, Monolithic column, Size exclusion chromatography

---

*1 ジーエルサイエンス（株）

*2 岐阜大学工学部

Sakai-Kato, K., Ota, S.[*1], Hyodo, K.[*2], Ishihara, H.[*2], Kikuchi, H.[*2], Kawanishi, T.: **Size separation and size determination of liposomes**

J. Sep. Sci., **34**, 2861-2865（2011）

We developed a method for separating liposomes by size and determining their average diameters. Liposomes with different average diameters were separated on a monolithic silica capillary column, and the size of the liposomes corresponding to each peak was determined online with a dynamic light scattering detector coupled to the capillary liquid chromatography system. The calculated diameters for the separated liposomes were similar to the diameter values measured in batch mode. We demonstrate that this combination of a monolithic capillary column and light scattering detection could be used for size separation of liposomes and could provide more details about average diameters than batch-mode analysis.

Keywords: Capillary liquid chromatography, Light scattering detection, Liposomes

[*1] ジーエルサイエンス（株）
[*2] エーザイ（株）

Sakai-Kato, K., Ishikura, K., Oshima, Y., Tada, M., Suzuki, T., Ishii-Watabe, A., Yamaguchi, T., Nishiyama, N.[*1], Kataoka, K.[*1,2], Kawanishi, T., Okuda, H.: **Evaluation of intracellular trafficking and clearance from HeLa cells of doxorubicin-bound block copolymers**

Int. J. Pharm., **423**, 401-409（2012）

New technologies are needed to deliver medicines safely and effectively. Polymeric nanoparticulate carriers are one such technology under investigation. We examined the intracellular trafficking of doxorubicin-bound block copolymers quantitatively and by imaging doxorubicin-derived fluorescence using confocal microscopy. The polymers were internalized by endocytosis and distributed in endosomal/lysosomal compartments and the endoplasmic reticulum; unlike free doxorubicin, the polymers were not found in the nucleus. Moreover, the ATP-binding cassette protein B1（ABCB1）transporter may be involved in the efflux of the polymer from cells. This drug delivery system is attractive because the endogenous transport system is used for the uptake and delivery of the artificial drug carrier to the target as well as for its efflux from cells to medium. Our results show that a drug delivery system strategy targeting this endogenous transport pathway may be useful for affecting specific molecular targets.

Keywords: Intracellular trafficking, Transporter, Endocytosis

[*1] 東京大学大学院医学系研究科
[*2] 東京大学大学院工学系研究科

Sakai-Kato, K., Nanjo, K., Kawanishi, T., Okuda, H.: **Rapid and sensitive method for measuring the plasma concentration of doxorubicin and its metabolites**

Chem. Pharm. Bull., **60**, 391-396（2012）

Doxorubicin is an anti-cancer drug with a wide therapeutic range. However, it and its metabolites cause severe side effects, limiting its clinical use. Therefore, measuring the plasma concentration of doxorubicin and its metabolites is important to study the dosing regimen of doxorubicin. We developed a rapid and sensitive method by ultra-high-performance liquid chromatography with fluorescent detection for measuring the plasma concentration of doxorubicin and its metabolites in small volumes（around 10 μL）, enabling repeated measurements from the same mouse. The sensitivity of 7-deoxydoxorubicinolone, a major metabolite of doxorubicin,increased about 5 times than those ever reported using conventional HPLC, and the run time was within 3 min. The area under the curve（AUC0－24 h）of doxorubicin was 5.9 μg h/mL similar to the value of 4.16 μg h/mL obtained previously using a conventional HPLC method. This method would provide information that could be used to refine the therapeutic approach to doxorubicin use.

Keywords: Doxorubicin, Metabolite, Pharmacokinetics

Un, K., Kawakami, S.[*1], Higuchi, Y.[*1], Suzuki, R.[*2], Maruyama, K.[*2], Yamashita, F.[*1], Hashida, M.[*1,3]: **Involvement of activated transcriptional process in efficient gene transfection using unmodified and mannose-modified bubble lipoplexes with ultrasound exposure**

J. Control. Release, **156**, 355-363（2011）

We have developed ultrasound（US）-responsive and mannose-modified gene carriers（Man-PEG_{2000} bubble lipoplexes）, and successfully obtained a high level of gene expression following gene transfection using Man-PEG_{2000} bubble lipoplexes and US exposure. In the present study, we investigated the involvement of transcriptional processes on enhanced gene expression using Man-PEG_{2000} bubble lipoplexes. The transcriptional process related to activator protein-1（AP-1）and nuclear factor-κB（NFκB）was activated by US exposure, and involved in enhanced gene expression obtained by this gene transfection method. On the other hand, activation of AP-1 and NFκB pathways followed

by US exposure was hardly involved in the inflammatory responses.

Keywords: Transfection, Ultrasound, Transcription factor

*1 京都大学大学院薬学研究科
*2 帝京大学薬学部
*3 京都大学物質細胞統合システム拠点

Un, K., Kono, Y.[*1], Yoshida, M.[*1], Yamashita, F.[*1], Kawakami, S.[*1], Hashida, M.[*1,2]: **Enhancement of gene expression by transcriptional activation using doxorubicin-loaded liposome/pDNA complexes**

Pharmazie, **5**, 400-405（2012）

The transcriptional process is activated by doxorubicin（DXR）, and gene expression efficiency followed by gene transfection can be enhanced by the combination-use of DXR. Therefore, co-encapsulation of plasmid DNA（pDNA）and DXR into non-viral gene carriers can enhance gene expression. In the present study, we prepared DXR-loaded liposome/pDNA complexes（DXR-loaded lipoplexes）. Gene expression was enhanced by DXR encapsulation into lipoplexes in colon-26 cells and cultured mouse macrophages, and this gene expression level was significantly higher than that obtained in combination with free DXR. Moreover, the activation profiles of transcriptional factors induced by DXR-loaded lipoplexes were different from those induced by free DXR; suggesting that co-encapsulation of pDNA and DXR into gene carriers might be contributed to effective enhancement of gene expression.

Keywords: Transfection, Doxorubicin, Transcriptional activation

*1 京都大学大学院薬学研究科
*2 京都大学物質細胞統合システム拠点

Harazono, A., Kobayashi, T., Kawasaki, N., Itoh, S., Tada, M., Hashii, N., Ishii, A., Arato., T.[*1], Yanagihara, S.[*2], Yagi, Y.[*2], Koga, A.[*3], Tsuda, Y.[*3], Kimura, M.[*3], Sakita, M.[*3], Kitamura, S.[*4], Yamaguchi, H.[*4], Mimura, H.[*4], Murata, Y.[*4], Hamazume, Y.[*5], Sato, T.[*5], Natsuka, T.[*6], Kakehi, K.[*7], Kinoshita, M.[*7], Watanabe, S.[*7], Yamaguchi, T.: **A comparative study of monosaccharide composition analysis as a carbohydrate test for biopharmaceuticals**

Biologicals, **39**（3）, 171-180（2011）

The various monosaccharide composition analysis methods were evaluated as monosaccharide test for glycoprotein-based pharmaceuticals. Neutral and amino sugars were released by hydrolysis with 4-7N trifluoroacetic acid. The monosaccharides were N-acetylated if necessary, and analyzed by high-performance liquid chromatography（HPLC）with fluorometric or UV detection after derivatization with 2-aminopyridine, ethyl 4-aminobenzoate, 2-aminobenzoic acid or 1-phenyl-3-methyl-5-pyrazolone, or high pH anion exchange chromatography with pulsed amperometric detection（HPAEC-PAD）. Sialic acids were released by mild acid hydrolysis or sialidase digestion, and analyzed by HPLC with fluorometric detection after derivatization with 1,2-diamino-4,5-methylenedioxybenzene, or HPAEC-PAD. These methods were verified for resolution, linearity, repeatability, and accuracy using a monosaccharide standard solution, a mixture of epoetin alfa and beta, and alteplase as models. It was confirmed that those methods were useful for ensuring the consistency of glycosylation.

It is considered essential that the analytical conditions including desalting, selection of internal standards, release of monosaccharides, and gradient time course should be determined carefully to eliminate interference of sample matrix. Various HPLC-based monosaccharide analysis methods were evaluated as a carbohydrate test for glycoprotein pharmaceuticals by an inter-laboratory study.

Keywords: monosaccharide composition analysis, glycoprotein pharmaceuticals, inter-laboratory study

*1 Pharmaceuticals and Medical Device Agency
*2 Production Division, Kyowa, Kyowa Hakko Kirin Co., Ltd.
*3 Analytical Technology Research Department, Chugai Pharmaceutical Co., Ltd.
*4 Pharmaceutical Analysis, Pharmaceutical Research & Technology Laboratories, Astellas Pharma Inc.
*5 Technology Research and Development, Dainippon Sumitomo Pharma Co., Ltd.
*6 College of Science, Niigata University
*7 School of Pharmacy, Kinki University

Gotoh, Y.[*1], Ishizuka, Y.[*2], Matsuura, T.[*3], Niimi, S.: **Spheroid Formation and Expression of Liver-Specific Functions of Human Hepatocellular Carcinoma-Derived FLC-4 Cells Cultured in Lactose-Silk Fibroin Conjugate Sponge**

Biomacromolecules, **12**（5）, 1532-1539（2011）

This study presents a hepatic tissue engineering application of three-dimensional（3D）porous sponges composed of lactose-silk fibroin（SF）conjugates（Lac-CY-SF）bearing β-galactose residues, hepatocyte-specific ligands. Lac-CY-SF

sponges were prepared by freeze-drying, followed by immersion in a series of methanol aqueous solutions. Lac-CY-SF sponges showed heterogeneous pore structure with round pores about 100 μm in diameter and elongated pores 250-450 μm in length and 100-150 μm in breadth. To employ a 3D Lac-CY-SF culture system, human hepatocellular carcinoma-derived FLC-4 cells were seeded in Lac-CY-SF sponges and cultured up to 3 weeks. FLC-4 cell culture in collagen and SF sponges was also performed for comparison with the cell response to Lac-CY-SF sponges. Within 5 days of culture, FLC-4 cells cultured in Lac-CY-SF sponges, as well as the cells cultured in collagen sponges, formed multicellular spheroids with diameters from 30 to 100 μm more efficiently than did the cells cultured in SF sponges. After 3 weeks of culture, WST-1 viability assay revealed that shrinkage suppression of Lac-CY-SF sponges enabled the maintenance of viable FLC-4 cells for a long time, while the shrinkage and disintegration of collagen sponges prevented the maintenance of the cells. FLC-4 cells cultured in Lac-CY-SF sponges exhibited greater elevation of albumin secretion and sustained a higher albumin level compared with the cells cultured in collagen and SF sponges during the 3 week cultivation period. FLC-4 cells cultured in Lac-CY-SF sponges for 3 weeks expressed genes related to liver-specific functions such as transferrin and HNF-4α. On the other hand, the cells cultured in collagen and SF sponges for 3 weeks did not express these genes. These results indicated the very promising properties of Lac-CY-SF sponges as a scaffold for long-term culture of functional FLC-4 cells to study drug toxicity and hepatocyte metabolism in humans and develop a bioartificial liver model.

Keywords: FLC-4 cells, Lac-CY-SF, liver-specific functions

[*1] (独)農業生物資源研究所
[*2] (株)エーシーバイオテクノロジーズ
[*3] 東京慈恵会医科大学

Yuan, Y.[*], Maeda, Y.[*], Terasawa, H.[*], Monde, K.[*], Harada, S.[*], Yusa, K.: **A combination of polymorphic mutations in V3 loop of HIV-1 gp120 can confer noncompetitive resistance to maraviroc**

Virology, **413**, 293-299 (2011)

Maraviroc binds to the pocket of extracellular loops of the cell surface CCR5 and prevents R5 HIV-1 from using CCR5 as a coreceptor for entry into CD4-positive cells. To evaluate the contribution of the V3 loop structure in gp120 to maraviroc resistance, we isolated maraviroc-resistant variants from the V3 loop library virus (HIV-1(V3Lib)) containing a set of random combinations of 0-10 polymorphic mutations in vitro. HIV-1(V3Lib) at passage 17 could not be suppressed even at 10 μM (>1400-fold resistance), while HIV-1(JR-FL) at passage 17 revealed an 8-fold resistance to maraviroc. HIV-1 (V3Lib-P17) contained T199K and T275M plus 5 mutations in the V3 loop, I304V/F312W/T314A/E317D/I318V. The profile of pseudotyped virus containing I304V/F312W/T314A/E317D/I318V in V3 loop alone revealed a typical noncompetitive resistance, although T199K and/or T275M could not confer noncompetitive resistance. This type of library virus is useful for isolation of escape viruses from effective entry inhibitors.

Keywords: HIV-1, entry inhibitor, antiviral drugs

[*] 熊本大学大学院

Nonaka, M.[*1], Yong Ma, B.Y.[*1], Imaeda, H.[*2], Kawasaki, N.[*1],Kawabe, K.[*1], Hodohara, K.[*2], Kawasaki, N., Andoh, A.[*1], Fujiyama, Y.[*2], Kawasaki, T.[*1]: **DC-SIGN recognize a novel ligand Mac-2BP characteristically expressed on human colorectal carcinomas**

J. Biol. Chem., **286**(25), 22403-22413 (2011)

Dendritic cell (DC)-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) is a type II transmembrane C-type lectin expressed on DCs such as myeloid DCs and monocyte-derived DCs (MoDCs). Recently, we have reported that DC-SIGN interacts with carcinoembryonic antigen (CEA) expressed on colorectal carcinoma cells. CEA is one of the most widely used tumor markers for gastrointestinal cancers such as colorectal cancer. On the other hand, other groups have reported that the level of Mac-2-binding protein (Mac-2BP) increases in patients with pancreatic, breast, and lung cancers, virus infections such as human immunodeficiency virus and hepatitis C virus, and autoimmune diseases. Here, we first identified Mac-2BP expressed on several colorectal carcinoma cell lines as a novel DC-SIGN ligand through affinity chromatography and mass spectrometry. Interestingly, we found that DC-SIGN selectively recognizes Mac-2BP derived from some colorectal carcinomas but not from the other ones. Furthermore, we found that the α1-3,4-fucose moieties of Le glycans expressed on DC-SIGN-binding Mac-2BP were important for recognition. DC-SIGN-dependent cellular interactions between immature MoDCs and colorectal carcinoma cells significantly inhibited MoDC functional maturation, suggesting that Mac-2BP may provide a tolerogenic microenvironment for colorectal carcinoma cells through DC-SIGN-dependent

recognition. Importantly, Mac-2BP was detected as a predominant DC-SIGN ligand expressed on some primary colorectal cancer tissues from certain parts of patients in comparison with CEA from other parts, suggesting that DC-SIGN-binding Mac-2BP bearing tumor-associated Le glycans may become a novel potential colorectal cancer biomarker for some patients instead of CEA.

Keywords: DC-SIGN, Mac-2BP, CEA

[*1] 立命館大学糖鎖工学研究センター
[*2] 滋賀医科大学

Yokota, N.[*], Kataoka, Y.[*], Hashii, N., Kawasaki, N. Sawada, H.[*]: **Sperm-specific C-terminal processing of the proteasome PSMA1/α6 subunit**

Biochem. Biophys. Res. Commun., **410**(4), 809-815 (2011)

We previously reported that the ascidian sperm proteasome degrades the egg-coat protein extracellularly during fertilization. In order to explore an extracellular transport signal, we purified the proteasome from ascidian sperm and compared its subunit structure with egg and muscle proteasomes. The results showed that PSMA1/α6 subunit of the sperm proteasome is distinct from egg and muscle proteasomes. LC/MS/MS analysis revealed that the C-terminal 16 residues of sperm α6 subunit are processed. Whereas sperm-specific paralogous genes of α subunits are reported, its sperm-specific C-terminal processing is a newly discovered novel post-translational modification of the proteasome.

Keywords: Proteasome, Sperm, Fertilization

---

[*] 名古屋大学理学部

橋井則貴，川崎ナナ，秦　艶，山口照英：**日局医薬品各条ヘパリンナトリウム確認試験及び純度試験**

医薬品医療機器レギュラトリーサイエンス，**42**，827-835（2011）

現行ヘパリンナトリウム標準品，ヘパリンナトリウム試薬及び類縁物質を用いて，理化学試験用ヘパリンナトリウム標準品の品質に必要な要件とその試験方法を検討した．

Keywords：各条ヘパリンナトリウム，理化学試験用ヘパリンナトリウム標準品，純度試験

Nakazawa, S.[*], Hashii, N., Harazono, A., Kawasaki, N.: **Analysis of oligomeric stability of insulin analogs using hydrogen/deuterium exchange mass spectrometry**

Anal. Biochem., **420**, 61-67 (2012)

Insulin analog products for subcutaneous injection are prepared as solutions in which insulin analog molecules exist in several oligomeric states. Oligomeric stability can affect their onset and duration of action and has been exploited in designing them. To investigate the oligomeric stability of insulin analog products having different pharmacokinetics, we performed hydrogen/deuterium exchange mass spectrometry (HDX/MS), which is a rapid method to analyze dynamic aspects of protein structures. Two rapid-acting analogs (lispro and glulisine) incorporated deuteriums more and faster than recombinant human insulin, whereas a long-acting analog (glargine) and two intermediate-acting preparations (protamine-containing formulations) incorporated them less and more slowly. Kinetic analysis revealed that the number of slowly exchanged hydrogens (D(s)) ($k<0.01$ min(-1)) accounted for the difference in HDX reactivity among analogs. Furthermore, we found correlations between HDX kinetics and pharmacokinetics reported previously. Their maximum serum concentration (C(max)) was linearly correlated with D(s) ($r=0.88$) and the number of maximum exchangeable hydrogens (D(∞)) ($r=0.89$). The maximum drug concentration time (t(max)) was also correlated with reciprocals of D(s) and D(∞) ($r=0.86$ and $r=0.96$, respectively). Here we demonstrate the ability of HDX/MS to evaluate oligomeric stability of insulin analog products.

Keywords: insulin, HDX/MS, oligomeric stability

---

[*] 北海道大学大学院生命科学院

栗林亮佑，村上真紀[*]，益山光一[*]，近澤和彦[*]：**遺伝子組換えFSHの排卵誘発における臨床評価について**

レギュラトリーサイエンス学会，**1**(3)，133-141（2011）

遺伝子組換えヒト卵胞刺激ホルモンを有効成分とするフォリトロピンベータ及びホリトロピンアルファの両品目で承認されている排卵誘発について，新薬の承認審査時のポイントをまとめた．

Keywords：遺伝子組換えFSH，臨床評価，レギュラトリーサイエンス

---

[*] (独)医薬品医療機器総合機構

Hyuga, S.[*], Shiraishi, M.[*], Hyuga, M., Goda, Y., Hanawa, T.[*]: **Ephedrae herba, a major component of maoto, inhibits the HGF-induced motility of human breast cancer MDA-MB-231 cells through suppression of c-Met tyrosine phosphorylation and c-Met-expression**

J. Trad. Med., **28**（3）, 128-138（2011）

We have previously reported that maoto inhibits a serum-induced motility of human breast cancer MDA-MB-231 cells. However, the molecular mechanism by which maoto realizes this inhibition was not elucidated. In this study, we focused on the effects of maoto on hepatocyte growth factor（HGF）-c-Met signaling, because HGF is one of the growth factors in serum and stimulates cell migration through tyrosine phosphorylation of c-Met. A Transwell migration assay demonstrated that maoto extract prevented the HGF-induced motility, and a major component of maoto, Ephedrae herba extract, had the same effect. However, both extracts of maoto that did not contain Ephedrae herba（maotokyomao）and a reference prescription, shikunshito, had no such effect. To confirm the effects of maoto and Ephedrae herba on HGF-c-Met signaling, we examined the effects of these medicines on HGF-induced phosphorylation of c-Met. Both extracts of maoto and Ephedrae herba inhibited c-Met phosphorylation, but neither maotokyomao extract nor shikunshito extract had such effects. Moreover, Ephedrae herba extract directly inhibited the tyrosine-kinase activity of c-Met and suppressed the HGF-induced phosphorylations of Akt, which is a signal molecule downstream of c-Met. We further investigated whether maoto and Ephedrae herba inhibit the expression of c-Met. The c-Met protein and gene expression were reduced after 24 h of the treatment with maoto extract or Ephedrae herba extract. These inhibitory effects of maoto were lost by removal of Ephedrae herba from the prescription, suggesting that the effects were attributable to Ephedrae herba. Taken together, these results suggested that Ephedrae herba, a major component of maoto, inhibits the HGF-induced motility of MDA-MB-231 cells by the suppression of HGF-c-Met-Akt signaling through the inhibition of both c-Met tyrosine phosphorylation and c-Met expression.

Keywords: HGF, c-Met, tyrosine phosphorylation

* 北里大学東洋医学総合研究所

Yamaguchi, T., Arato, T.*: **Quality, safety and efficacy of follow-on biologics in Japan**

Biologicals, **39**（5）, 328-332（2011）

Recently, WHO, EU, Japan and Canada have published guidelines on biosimilar/follow-on biologics. While there seems to be no significant difference in the general concept in these guidelines, the data to be submitted for product approval are partially different. Differences have been noted in the requirements for comparability studies on stability, pre-requisites for reference product, or for the need of comparability exercise for determination of process-related impurities. In Japan, there have been many discussions about the amount and extent of data for approval of follow-on biologics. We try to clarify the scientific background and rational for regulatory pathway of biosimilar/follow-on biologics in Japan in comparison with the guidelines available from WHO, EU and Canada. In this article, we address and discuss the scientific background underlying these differences to facilitate the harmonization of follow-on biologic principles in the guidelines in future.

Keywords: Biosimilar, Follow-on biologics, Biotechnology

* Pharmaceuticals and Medical Devices Agency

Arato, T.*, Yamaguchi, T.: **Experience of reviewing the follow-on biologics including Somatropin and erythropoietin in Japan**

Biologicals, **39**（5）, 289-292（2011）

To share the experience of reviewing clinical data required for the licensing of follow-on biologic products（biosimilar products and similar biotherapeutical products as EU and WHO terminology, respectively）in Japan, the data packages of two follow-on biologics, “Somatropin BS s.c.［Sandoz］（Omnitrope®）” and “Epoetin alfa BS［JCR］”, which have been recently approved in Japan according to the “Guidelines for the Quality, Safety and Efficacy Assurance of Follow-on Biologics” published on March 4th 2009, are described. The clinical data package and indication of Somatropin BS/Omnitrope® were different in each country. In case of Epoetin alfa BS［JCR］, non-clinical and clinical data-package was different from those of erythropoietin biosimilar products approved in EU. Submission of post-marketing surveillance plans for both products was required.

Even though there seem to be differences in data requirements by each national regulatory authority, the accumulation of experience will provide the rationale and consensus on how to design the clinical trials for follow-on biologics.

Keywords: Follow-on biologics, Somatropin, Erythropoietin

* Pharmaceuticals and Medical Devices Agency

Murata, D.*1,2, H. Nomura, K.*1, Dejima, K.*1, Mizuguchi, S.*1, Kawasaki, N., Matsuishi-Nakajima, Y., Ito, S., Gengyo-Ando, K.*3, Kage-Nakadai, E.*3, Mitani, S.*3, Nomura, K.*1,2: **GPI-anchor Synthesis Is Indispensable for the Germline Development of the *Nematode Caenor-***

habditis elegans

Mol. Biol. Cell., **23**(6), 982-995（2012）

Glycosylphosphatidylinositol（GPI）-anchor attachment is one of the most common post-translational protein modifications. Using the nematode Caenorhabditis elegans, we determined that GPI-anchored proteins are present in germline cells and distal tip cells（DTCs）, which are essential for the maintenance of the germline stem cell niche. We identified 24 C. elegans genes involved in GPI-anchor synthesis. Inhibition of various steps of GPI-anchor synthesis by RNAi or gene knockout resulted in abnormal development of oocytes and early embryos, and both lethal and sterile phenotypes were observed. The piga-1 gene（ortholog of human PIGA）codes for the catalytic subunit of the phosphatidylinositol N-acetylglucosaminyltransferase complex, which catalyzes the first step of GPI-anchor synthesis. We isolated piga-1 knockout worms and found that GPI-anchor synthesis is indispensable for the maintenance of mitotic germline cell number. The knockout worms displayed 100% lethality with decreased mitotic germline cells and abnormal eggshell formation. Using cell-specific rescue of the null allele, we showed that expression of piga-1 in somatic gonads and/or in germline is sufficient for normal embryonic development and the maintenance of the germline mitotic cells. These results clearly demonstrate that GPI-anchor synthesis is indispensable for germline formation and for normal development of oocytes and eggs.

Keywords : GPI-anchor, germline development, *C. elegans*

[*1] Department of Biological Sciences, Faculty of Sciences, Kyushu University

[*2] Graduate School of Systems Life Sciences, Kyushu University

[*3] Department of Physiology, Tokyo Women's Medical University School of medicine

Park, S.Y.[*1], Lee, S.H.[*1], Kawasaki, N., Itoh, S., Kang, K.[*1], Hee, Ryu, S.[*1], Hashii, N., Kim, J.M.[*2], Kim, J.Y.[*2], Hoe, Kim, J.[*1] : **α1-3/4 Fucosylation at Asn 241 of β-haptoglobin is a novel marker for colon cancer: A combinatorial approach for development of glycan biomarkers**

Int. J. Cancer, **130**(10), 2366-2376（2012）

Aberrant glycosylation has been observed in many types of cancer, but the mechanism of glycosylation change is still poorly understood. To elucidate relationships between glycosylation and colon cancer progression, we analyzed glycosylation status of β-haptoglobin（β-Hp）obtained from 46 cancer patients, 14 inflammatory bowel disease patients and 38 normal subjects. Aleuria aurantia lectin reactivity with cancer β-Hp was much higher than in the other two study groups. These results were confirmed by lectin blotting and microarray assay using other lectins directed to fucosyl residues. Levels of such glycans were correlated with stage of colon cancer progression. Reactivity with fucosylated glycans was eliminated by treatment with α1-3/4 fucosidase but not α1-6 fucosidase, indicating that enhanced lectin reactivity with the fucose moiety of colon cancer β-Hp is due to Fucα1-3/4GlcNAc. Moreover, site-specific glycan occupancy was determined by sequential LC/MS analysis. Mass spectrometric analysis showed that fucosylation of β-Hp was higher in colon cancer patients than in other subjects. In particular, fucosylation at Asn 241 of β-Hp in sera of colon cancer patients was clearly higher than in the other groups, and the ratio of fucosylated glycopeptides containing Asn 241 decreased greatly after treatment with α1-3/4 fucosidase. In conclusion, the level of α1-3/4 fucosyl epitope at Asn 241 of β-Hp is potentially useful as a novel marker for colon cancer.

Keywords : fucosylation, β-haptoglobin, colon cancer

[*1] Korea Advanced Institute of Science and Technology

[*2] Chungnam National University School of Medicine

Zhu, S.[*1], Bai, Y.J.[*1], Oya, M.[*1], Tanaka, K.[*1], Komatsu, K.[*1], Maruyama, T., Goda, Y., Kawasaki, T.[*2], Fujita, M.[*2], Shibata, T.[*3]: **Genetic and chemical diversity of *Eleutherococcus senticosus* and molecular identification of Siberian ginseng by PCR-RFLP analysis based on chloroplast *trn*K intron sequence**

Food Chemistry, **129**, 1844-1850（2011）

Siberian ginseng（SG）, the rhizome and root of *Eleutherococcus senticosus*, has been used as a tonic and anti-fatigue agent in northeastern Asia from ancient time. In recent years, SG has been becoming fairly popular as dietary supplements and health foods worldwide. In order to establish a convenient and sensitive method for authentication, chloroplast *trn*K intron sequences of 6 *Eleutherococcus* species were determined and compared. Genetic polymorphism, representing by 14 types of *trn*K intron sequence, in *E. senticosus* was observed. However, characteristic nucleotide markers stable within this species enabled clear discrimination of it from other congeners. A PCR-RFLP method was further developed, which was demonstrated to be efficient for authentication of crude drugs as well as health foods. Quantitative evaluation of three main bioactive constituents indicated chemical diversity

in *E. senticosus* collected from northeast China and the results suggested good producing areas of SG. The chemical data clearly revealed that *E. sessiliflorus* was unsuitable to be used as SG.

Keywords: *Eleutherococcus senticosus*, *trn*K intron sequence, PCR-RFLP

[*1] 富山大学和漢医薬学総合研究所
[*2] (株) ウチダ和漢薬
[*3] (独) 医薬基盤研究所薬用植物資源研究センター

小金澤　望[*]，鎌倉浩之，最所和宏，合田幸広，武口裕[*]，水嶋好清[*]，三砂　雄[*]：**平成22年度無承認無許可医薬品の買上げ検査結果について**

札幌市衛研年報，**38**，42-47（2011）

平成22 年度に実施された札幌市保健所の健康食品買上げ検査において，市内において販売されている強壮系健康食品11 検体の分析依頼をうけ，強壮系医薬品成分9種類の同時分析を実施した．分析はフォトダイオードアレイ検出器付きHPLC（HPLC-PDA），定性的確認はLC-MS/MS にて実施した．

この結果，買上げ品のうち1 検体から医薬品成分ヒドロキシホモシルデナフィル，アミノタダラフィル，クロロプレタダラフィルが検出された．

Keywords: 無承認無許可医薬品，強壮系健康食品，買上げ検査

[*] 札幌市衛生研究所

Kumeta, Y., Ito, M.[*]: **Genomic organization of δ-guaiene synthase genes in *Aquilaria* crassna and its possible use for the identification of *Aquilaria* species**

J. Nat. Med., **65**, 508-513（2011）

The resinous portions of *Aquilaria* plants, called agarwood, have been used as medicines and incenses. Agarwood contains a great variety of sesquiterpenes, and a study using cultured cells of *Aquilaria* crassna showed that the production of sesquiterpenes（α-guaiene, α-humulene, and δ-guaiene）was induced by treatment with methyl jasmonate, which led to the cloning of δ-guaiene synthases. In the present study, analyses of genomic organization and Southern blotting of δ-guaiene synthase in *A. crassna* were performed in order to examine the genomic background of δ-guaiene synthases in *Aquilaria* plants. Genomic cloning and sequencing revealed five types of sequence in putative δ-guaiene synthases sharing more than 96% identity in exon regions, and that these enzymes belonged to the class III TPS subfamily with seven exons and six introns. Furthermore, Southern blotting revealed that at least five copies of δ-guaiene synthase existed in *A. crassna*. The hybridization of digested DNA of *A. crassna* and *A. sinensis* with probes made with a δ-guaiene synthase cDNA fragment resulted in different banding patterns for these two species. It may be possible to identify *Aquilaria* species by restriction fragment length polymorphism analyses with δ-guaiene synthase cDNA probes.

Keywords: *Aquilaria*, δ-guaiene synthase, species identification

[*] Graduate School of Pharmaceutical Sciences, Kyoto University

粂田幸恵，丸山卓郎，若菜大悟，鎌倉浩之，合田幸広：**シャタバリ製品の基原種鑑別法の開発と同法を利用した日本市場品の実態調査**

日本食品化学学会誌，**18**（3），163-167（2011）

Shatavari is a famous Ayurveda materia medica used as a tonic for woman, and its health food products have been distributed in Japan. The botanical origin of shatavari is specified as the tuberous root of *Asparagus racemosus* in the Ayurvedic Pharmacopoeia of India. However, several reports have pointed out that *Stemona* plants were sold as shatavari sometimes in markets in Southeast Asia and China because the shape of the tuberous root of *A. racemosus* was very similar to that of *Stemona* plants. Since most *Stemona* plants are rich in alkaloids, the contamination of the plant source species in shatavari products may cause various physical disorders to consumers. In the course of our study for the safety evaluations of health foods made from medicinal plants, we investigated the botanical origin of shatavari products obtained in Japanese markets on the basis of DNA sequence analysis. As a result, botanical origin of all products was revealed to be *Asparagus*. In order to confirm whether these products contained *Stemona* or not, ARMS-PCR method using *Stemona*-specific primers was performed. The application data indicated that none of the examined products contained *Stemona* plants at a concentration of >1%. ARMS-PCR method shown here is expected to be useful for quality control of shatavari products to check contamination of source plants.

Keywords: *Asparagus racemosus*, *Stemona* plants, DNA analysis

Kakigi, Y.[*], Hakamatsuka, T., Goda, Y., Icho, T.[*], Mochizuki, N.[*]: **Investigation of biologically active components in Ginkgo leaf products on the Japanese**

market

Biosci. Biotechnol. Biochem., **75**(4), 777-779 (2011)

As part of a research program to evaluate the quality of Ginkgo leaf products, an analytical method involving hydrolytic reaction and UPLC-UV measurement was developped to examine the flavonol composition, with reference to the German pharmacopoeia. The analytical method developed was then used to investigate the flavonol composition in Ginkgo leaf products available on the Japanese market. The results suggested that some health food products contained approximately equivalent amounts of flavonols to medical products, while some other products contained fairly low amounts of flavonols. Finally, we examined the correlation of the amount of flavonols with terpene lactones in products, quoting our previous report.

Keywords: *Ginkgo biloba*, flavonol glycoside, UPLC-UV

* Research Laboratories for Food Safety Chemistry, Asahi Breweries Ltd.

El-Halawany, A.M.[*1,2], El Dine, R.S.[*1,2], Chung, M.H., Nishihara, T.[*3] and Hattori, M.[*2]: **Screening for estrogenic and antiestrogenic activities of plants growing in Egypt and Thailand**

Pharmacognosy Research, **3**(2), 107-113 (2011)

Background: There is a growing demand for the discovery of new phytoestrogens to be used as a safe and effective hormonal replacement therapy. Materials and Methods: The methanol extracts of 40 plants from the Egyptian and Thailand folk medicines were screened for their estrogen agonist and antagonist activities. The estrogenic and antiestrogenic effects of the tested extracts were carried out using the yeast two-hybrid assay system expressing ERα and ERβ. In addition, all the extracts were subjected to a naringinase treatment and retested for their estrogenic activity. Results: The methanol extracts of *Derris reticulata* and *Dracaena lourieri* showed the most potent estrogenic activity on both estrogen-receptor subtypes, while, the methanol extracts of *Butea monosperma*, *Erythrina fusca*, and *Dalbergia candenatensis* revealed significant estrogenic activity on ERβ only. *Nigella sativa*, *Sophora japonica*, *Artabotrys harmandii*, and *Clitorea hanceana* showed estrogenic effect only after naringinase treatment. The most potent antiestrogenic effect was revealed by *Aframomum melegueta*, *Dalbergia candenatensis*, *Dracena loureiri*, and *Mansonia gagei*.

Keywords: estrogenic activity, leguminoseae, yeast two-hybrid assay

*1 Department of Pharmacognosy, Faculty of Pharmacy, Cairo University

*2 Division of Metabolic Engineering, Institute of Natural Medicine, University of Toyama

*3 Faculty of Pharmaceutical Sciences, Hyogo University of Health Sciences

Tokumoto, H., Shimomura, H., Iida, O.[*], Hakamatsuka, T., Goda, Y.: **Morphological discrimination of powdered senna stem and powdered senna leaf rachis**

Jpn. J. Pharmacog., **65**(2), 114-128 (2011)

As per the Pharmaceutical Affairs Law in Japan, leaflets, leaf rachises, leaf stalks (petioles), and fruits of senna (Alexandrian senna: *Cassia acutifolia* Delile or Tinnevelly senna: *C. angustifolia* Vahl) have been designated as raw materials for exclusive use in pharmaceuticals. In contrast, the stems of senna have been regarded as non-pharmaceuticals, unless their products have shown any "medical effects" or "dosage and administration"; this is attributable to the fact that the content of sennosides — the active constituents of crude drugs — is lower in stems than in the other parts mentioned above. Therefore, the stems of senna are used in health foods. However, health foods are sometimes found to contain sennosides in medicinal quantities; this is thought to be due to the illegal use of leaf rachises rather than stems. Inspectors can find leaf rachises when health foods consist of nonpowdered plant tissues, such as tea bags. However, when the product contains the plant in the powder form, it is extremely difficult to distinguish the types of plant tissues therein. To overcome this problem, we developed a method of distinguishing powdered senna leaf rachises from powdered senna stems by using microscopy. Our key findings are as follows. Epidermal cells in the stomata distributional region are distinct in shape in the case of rachises and stems: the former cells are elliptical, while the latter are polygonal. In addition, the relative length of the longitudinal axis of the epidermal cells differs in these 2 parts of the plant: the epidermal cells from the leaf rachis are longer than the adjacent stomatal cells, whereas the epidermal cells from the stem are usually shorter than the adjacent stomatal cells. These morphological characteristics were observed both in slices and in powdered samples of the plant, and were common to both Alexandrian and Tinnevelly senna. When we tested powdered samples containing a mixture of leaf rachises (5%) and stems (95%), we could identify the leaf rachises. Since the microscopic method does not warrant any expensive

equipment, we believe it could be very useful for on-site inspection.

Keywords: senna, *Cassia acutiforia*, *Cassia angustifolia*

* （独）医薬基盤研究所薬用植物資源研究センター

Hosoe, J., Sugimoto, N., Suematsu, T.[*1], Yamada, Y.[*2], Hayakawa, M.[*2], Katsuhara, T.[*3], Nishimura, H.[*3], Goda, Y.: **Validation studies of qNMR for chemical reagents used as reference standards for quantitative analyses of crude drugs in the Japanese Pharmacopoeia**

Pharm. and Med. Dev. Regulatory Sci., **43**(2), 182-193 (2012)

Quantitative NMR (qNMR) qualifies as an absolute quantification method and is theoretically abele to determine the purity of any compounds with SI-traceability. Therefore, we are trying to introduce the qNMR to the Japanese Pharmacopoeia for the specification of reagents using marker compounds of quantitative analyses of crude drugs. In this study, we performed validation studies of qNMR by using two chemical reagents (magnolol: Mw 266.34; and geniposide: Mw 388.37) in 5 independent laboratories. The weighed amount of each sample was 5 mg ± 10% and each participant prepared 3 sample solutions and the absolute purity of each sample was measured with qNMR by 3 times. The total average (the average of the participant average) ± SD of absolute quantification results on magnolol and geniposide were 98.97±0.19% and 96.09±0.28%, respectively. These data suggested that the variability in each NMR measurement (the average of all the SD of each sample average) and each sample liquid preparation (the average of all the SD of each participant average) were about 0.08% and 0.07% (magnolol), and 0.17% and 0.14% (geniposide), respectively. These data indicate that the the purity of these compounds can be determined by qNMR with an accuracy of two significant digits when the molecular weight of target reagent is around 300 with a weighed amount of about 10 mg.

Keywords: quantitative NMR, validation study, the Japanese Pharmacopoeia

*1 JEOL Ltd.
*2 Wako Pure Chemical Industries, Ltd.
*3 TSUMURA & CO.

松本輝樹[*1]，安食菜穂子，有福和紀[*2]，川原信夫，合田幸広：**雪茶製品の^{1}H NMRメタボローム解析**

日本食品化学学会誌，**18**(1)，43-47(2011)

As a part of development of profiling analysis for raw material in the health foods, sixteen kinds of Setsucha products purchased from the Japanese market were performed principal component analysis (PCA) based on the bucket integration of ^{1}H NMR spectra. The results of metabolome analysis by PCA in ^{1}H NMR spectrum showed a characteristic signal at δ 3.66 due to a sterol derivative. This signal was indicated to be existence of a reference compound to distinguish among Setsucha products. These data was different from the profiling results by HPLC analysis with UV detection and taste-sensing system. However, by limiting analysis range of ^{1}H NMR spectrum into three regions observed in three reference compounds identified by HPLC analysis, the metabolome analysis by PCA confirmed a similar classification result to HPLC profiling.

Keywords: Setsucha, NMR, metabolomics

*1 （独）国立健康・栄養研究所
*2 日本電子(株)

堂井美里[*1,2]，安食菜穂子[*1,3]，伊奈小百合[*1]，吉光見稚代[*1,2]，川原信夫，合田幸広，垣内信子[*4]，御影雅幸[*1]：**漢方薬抽出自動包装機を用いた湯液品質の経時変化(1)　ー大黄甘草湯についてー**

Jpn. J. Pharmacog., **65**(2), 103-107 (2011)

In recent years, decocting machines have come into practical use. These machines can provide one month's packages of decoction at one operation, thus, patients can have their Kampo formula decocted by pharmacist with stable quality. However, it is necessary to clarify the change in quality of decoctions by long storage. Therefore in this report, we preserved Daiokanzoto produced by a decocting machine at 4, 25 or 40˚C and elucidated suitable preservation condition and quality assurance period on the basis of color, taste, and principal compound contents. The color of Daiokanzoto was maintained for 1 week at 4˚C, 2 weeks at 40˚C and 2 months at 25˚C. However, the color of decoction changed after preserving for 2 months at 25˚C, hence we consider 1 month is a reasonable period for preservation in terms of color. Sennoside A content after stored for 6 months at 4˚C and 2 months at 25˚C, glycyrrhizin content, and taste hardly showed any change. Thereby we concluded the packed decoction of Daiokanzoto produced by a decocting machine maintained the color, taste, and principal compound contents for a month at 25˚C, i.e., room temperature.

Keywords: Daiokanzoto, decocting machine

[*1] 金沢大学大学院自然科学研究科
[*2] (株)ウチダ和漢薬
[*3] (株)インテリジェントセンサーテクノロジー
[*4] 九州保健福祉大学薬学部

Anjiki, N.[*1,2], Hosoe, J., Fuchino, H.[*3], Kiuchi, F.[*4], Sekita, S.[*5], Ikezaki, H.[*1], Mikage, M.[*2], Kawahara, N., Goda, Y.: **Evaluation of the taste of crude drug and Kampo formula by a taste-sensing system（4）: taste of Processed Aconite Root**

J. Nat. Med., **65**, 293-300（2011）

It is difficult to describe the taste of Processed Aconite Root（PAR）because PAR contains toxic compounds and tasting of PAR poses some risk to the examiner. Therefore, there is no description of the taste of PAR in the latest Japanese Pharmacopoeia, although the taste of crude drugs has been regulated as a criterion for judgment. In this study, we revealed the objective taste of PAR by using a taste-sensing system. The PAR samples examined were classified into four types by how the samples were processed, PAR1: processed by autoclaving, PAR2-a: processed by autoclaving after rinsing in the salt（sodium chloride）solution, PAR2-h: processed by heating after rinsing in calcium chloride solution, PAR3: processed by treating with hydrated lime after rinsing in the salt（sodium chloride）solution. The most characteristic taste factor of PAR is an aftertaste of cationic bitterness and this was detected in all PAR sample solutions, even at the concentration of 0.1 mg/mL. In addition, anionic bitterness and saltiness were also detected in all of the PAR sample solutions at 1 mg/mL. Furthermore, umami was detected in the PAR1, PAR2-a and PAR3 sample solutions at 1 mg/mL. Detailing the analyses of the above four taste factors on the four types of PAR samples, we found the each type of PAR has their own characteristic taste pattern. On the basis of the results, we proposed a method for discriminating one PAR type from another by using the system.

Keywords: processed aconite root, taste evaluation, discrimination method

---

[*1] Intelligent Sensor Technology, Inc.
[*2] Graduate School of Natural Science and Technology, Kanazawa University
[*3] Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation
[*4] Faculty of Pharmacy, Keio University
[*5] Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University

Uchiyama, N., Kikura-Hanajiri, R., Matsumoto, N.[*], Huang, Z.L.[*], Goda, Y., Urade, Y.[*]: **Effects of synthetic cannabinoids on electroencephalogram power spectra in rats**

Forensic Sci. Int., **215**, 179-183（2012）

Several synthetic cannabinoids have recently been distributed as psychoactive adulterants in many herbal products on the illegal drug market around the world. However, there is little information on pharmacology and toxicology of such compounds. Although Δ^9-tetrahydrocannabinol（Δ^9-THC）, a psychoactive cannabinoid of marijuana, was reported to affect electroencephalograms（EEG）of rats, the effects of synthetic cannabinoids are unknown. We examined the pharmacological activities of three synthetic cannabinoids; cannabicyclohexanol（CCH）, CP-47,497 and JWH-018; by analyzing EEG power spectra and locomotor activity after intraperitoneal administration to rats and compared them with those of Δ^9-THC. The three compounds significantly increased the EEG power in the frequency range of 5.0-6.0 Hz for the first 3 h, while Δ^9-THC decreased the power spectra in the wide range of 7.0-20.0 Hz during the first hour. These results indicate that the effect of the three compounds on EEG is different from that of Δ^9-THC. Additionally, CCH, CP-47,497 and JWH-018 significantly decreased the locomotor activity for 11.5 h, 11 h and 4.5 h, respectively, after administration which was longer than that of Δ^9-THC（3.5 h）. Furthermore, all three compounds significantly reduced the total amounts of locomotor activity during a 3-h, 6-h and 12-h period after injection, whereas no statistical difference was observed for the Δ^9-THC injection. Among the three compounds, CCH and CP-47,497 exerted a longer duration of the change in the EEG power spectra and suppression of the locomotor activity than JWH-018.

Keywords: electroencephalogram, synthetic cannabinoids, locomotor activity

---

[*] (公財)大阪バイオサイエンス研究所

内山奈穂子，花尻(木倉)瑠理，正田卓司，福原　潔，合田幸広：**デザイナードラッグとして検出された合成カンナビノイドの異性体分析について**

薬学雑誌，**131**(7)，1141-1147(2011)

Recently, many psychotropic herbal products, named such as “Spice”, were distributed worldwide via the Internet. In our previous study, several synthetic cannabinoids were identified as adulterants in herbal products being available in Japan due to their expected narcotic effects. Among those, two derivatives of Δ^9-tetrahydrocannabinol（Δ^9-THC）, which is major psychotropic cannabinoid of marijuana, cannabi-

cyclohexanol（CCH, 3-[2-hydroxy-4-(2-methylnonan-2-yl) phenyl]cyclohexan-1-ol）and CP-47,497（3-[2-hydroxy-4-(2-methyloctan-2-yl)phenyl]cyclohexan-1-ol), have been controlled as designated substances（Shitei-Yakubutsu）under the Pharmaceutical Affairs Law since November 2009. CCH was detected together with its trans-form（1-epimer）in many herbal products, and CCH and CP-47,497 have two chiral centers in the structures. However, the pharmaceutical activities of the isomers of CCH have not been reported. This study presents chiral separations of CCH, its trans-form and CP-47,497 in the products using LC-circular dichroism（CD）and LC-MS analyses. The enantiomeric pairs of CCH, its trans-form and CP-47,497 were separated, respectively. Subsequently, the analyses of the herbal products showed that CCH and its trans-form existed as mixtures of enantiomers and the relative ratios of CCH and the trans-form enantiomers ranged from 42/58% to 53/47% and from 33/67% to 52/48%, respectively.

Keywords: synthetic cannabinoids, chiral separation, LC-MS

Uchiyama, N., Kikura-Hanajiri, R., Goda, Y.: **Identification of a novel cannabimimetic phenylacethylindole, cannabipiperidiethanone, as a designer drug in a herbal product and its affinity for cannabinoid CB1 and CB2 receptors**

Chem. Pharm. Bull., **59**(9), 1203-1205（2011）

A new cannabimimetic phenylacetylindole（cannabipiperidiethanone, 1）has been found as an adulterant in a herbal product which contains two other known synthetic cannabinoids, JWH-122 and JWH-081, and which is distributed illegally in Japan. The identification was based on analyses using GC-MS, LC-MS, high-resolution MS and NMR. Accurate mass spectrum measurement showed the protonated molecular ion peak of 1 at *m/z* 377.2233 $[M+H]^+$ and the molecular formula of 1 was $C_{24}H_{29}N_2O_2$. Both mass and NMR spectrometric data revealed that 1 was 2-(2-methoxyphenyl)-1-{1-[(1-methylpiperidin-2-yl)methyl]-1*H*-indol-3-yl}ethanone. Compound 1 has a mixed structure of known cannabimimetic compounds: JWH-250 and AM-2233. Namely, the moiety of phenylacetyl indole and *N*-methylpiperidin-2-yl-methyl correspond to the structure of JWH-250 and AM-2233, respectively. However, no synthetic, chemical or biological information about 1 has been reported. A binding assay of compound 1 to cannabinoid receptors revealed that 1 has affinity for the CB1 and CB2（IC_{50} = 591 nM and 968 nM, respectively）receptors, and shows 2.3- and 9.4-fold lower affinities than those of JWH-250. This is the first report to identify cannabimimetic compound（1）as a designer drug and to show its binding affinity to cannabinoid receptors.

Keywords: synthetic cannabinoid, 2-(2-methoxyphenyl)-1-{1-[(1-methylpiperidin-2-yl)methyl]-1*H*-indol-3-yl}ethanone, designer drug

Okamoto, N.[*1], Yamaguchi, K.[*1], Mizohata, E.[*1], Tokuoka, K.[*1], Uchiyama, N., Sugiyama, S.[*1], Matsumura, H.[*1], Inaka, K.[*2], Urade, Y.[*3], Inoue, T.[*1]: **Structural insight into the stereoselective production of PGF2*α* by old yellow enzyme from *Trypanosoma cruzi***

J. Biochem., **150**(5), 563-568（2011）

Old yellow enzyme（OYE）is an NADPH oxidoreductase capable of reducing a variety of compounds. It contains flavin mononucleotide（FMN）as a prosthetic group. A ternary complex structure of OYE from Trypanosoma cruzi（TcOYE）with FMN and one of the substrates, p-hydroxybenzaldehyde, shows a striking movement around the active site upon binding of the substrate. From a structural comparison of other OYE complexed with 12-oxophytodienoate, we have constructed a complex structure with another substrate, prostaglandin H_2（PGH_2）, to provide a proposed stereoselective reaction mechanism for the reduction of PGH_2 to prostaglandin $F_{2\alpha}$ by TcOYE.

Keywords: old yellow enzyme, prostaglandin synthase, X-ray structure

[*1] 大阪大学大学院工学研究科
[*2]（株）丸和栄養食品
[*3]（公財）大阪バイオサイエンス研究所

Kawamura, M., Kikura-Hanajiri, R., Goda, Y.: **Simple and rapid screening for methamphetamine, 3,4-methylenedioxymethamphetamine（MDMA）and their metabolites in urine using DART（Direct Analysis in Real Time）-TOFMS**

Yakugaku Zasshi, **131**(5), 827-833（2011）

An ionization technique, direct analysis in real time（DART）has recently been developed for the ambient ionization of a variety samples. The DART coupled with time-of-flight mass spectrometry（TOFMS）would be useful as a simple and rapid screening for the targeted compounds in various samples, because it provides the molecular information of these compounds without time-consuming extraction. In this study, we investigated rapid screening methods of illicit drugs and their metabolites, such as methamphetamine（MA）, 3,4-methylenedioxymethamphetamine（MDMA）, ampheta-

mine（AP）and 3,4-methylenedioxyamphetamine（MDA）in human urine using DART-TOFMS. As serious matrix effects caused by urea in urine samples and ionizations of the targeted compounds were greatly suppressed in the DART-TOFMS analyses, simple pretreatment methods to remove the urea from the samples were investigated. When a pipette tip-type solid-phase extraction with a dichloromethane and isopropanol mixed solution as an eluent was used for the pretreatment, the limits of detection（LODs）of 4 compounds added to control urine samples were 0.25 μg/ml. On the other hand, the LODs of these compounds were 0.5 μg/ml by a liquid-liquid extraction using a dichloromethane and hexane mixed solution. In both extractions, the recoveries of 4 compounds from urine samples were over 70% and these extraction methods showed good linearity in the range of 0.5-5 μg/ml by GC-MS analyses. In conclusion, our proposed method using DART-TOFMS could simultaneously detect MA, MDMA and their metabolites in urine at 0.5 μg/ml without time-consuming pretreatment steps. Therefore it would be useful for screening drugs in urine with the molecular information.

Keywords: direct analysis in real time, urine, time-of-flight mass spectrometry

Wada, M.[*], Yamahara, K.[*], Ikeda, R.[*], Kikura-Hanajiri, R., Kuroda, N.[*], Nakashima, K.[*]: **Simultaneous determination of N-benzylpiperazine and 1-(3-trifluoromethylphenyl) piperazine in rat plasma by HPLC-fluorescence detection and its application to monitoring of these drugs**

Biomed. Chromatogr., **26**, 21-25（2012）

An HPLC-fluorescence detection method for simultaneous determination of *N*-benzylpiperazine（BZP）and 1-(3-trifluoromethylphenyl)piperazine（TFMPP）labeled with 4-(4,5-diphenyl-1 *H*-imidazol-2-yl)benzoyl chloride（DIB-Cl）was described. DIB-BZP and -TFMPP were well separated within 13 min without interference of peaks from plasma components. The lower detection limits of BZP and TFMPP at a signal-to-noise ratio of 3 were 0.9 and 4.6 ng/mL, respectively. Precisions of the proposed method for intra- and inter-day assays were less than 4.8 and 9.1% as %RSD（n = 5）. Furthermore, the method could be successfully applied to monitor both compounds in plasma after their sole or co-administration to rats（each dose, 2 mg/kg）. Clearance of TFMPP was significantly different under the conditions（P = 0.047）.

Keywords: benzylpiperazine（BZP）, 1-(3-trifluoromethylphenyl) piperazine（TFMPP）, HPLC-fluorescence detection

---

[*] 長崎大学大学院医歯薬学総合研究科

Uchida, Y.[*1], Hasegawa, J.[*1], Chinnapen, D.[*2], Inoue, T., Okazaki, S.[*3], Kato, R.[*3], Wakatsuki, S.[*3], Misaki, R.[*1], Koike, M.[*4], Uchiyama, Y.[*4], Iemura, S.[*5], Natsume, T.[*5], Kuwahara, R.[*6], Nakagawa, T.[*7], Nishikawa, K.[*8], Mukai, K.[*1], Miyoshi, E.[*6], Taniguchi, N.[*9], Sheff, D.[*10], Lencer, W.[*2], Taguchi, T.[*1] and Arai, H.[*1]: **Intracellular phosphatidylserine is essential for retrograde membrane traffic through endosomes**

Proc. Natl. Acad. Sci. USA., **108**, 15846-15851（2011）

Phosphatidylserine（PS）is a relatively minor constituent of biological membranes. Despite its low abundance, PS in the plasma membrane（PM）plays key roles in various phenomena such as the coagulation cascade, clearance of apoptotic cells, and recruitment of signaling molecules. PS also localizes in endocytic organelles, but how this relates to its cellular functions remains unknown. Here we report that PS is essential for retrograde membrane traffic at recycling endosomes（REs）. PS was most concentrated in REs among intracellular organelles, and evectin-2（evt-2）, a protein of previously unknown function, was targeted to REs by the binding of its pleckstrin homology（PH）domain to PS. X-ray analysis supported the specificity of the binding of PS to the PH domain. Depletion of evt-2 or masking of intracellular PS suppressed membrane traffic from REs to the Golgi. These findings uncover the molecular basis that controls the RE-to-Golgi transport and identify a unique PH domain that specifically recognizes PS but not polyphosphoinositides.

Keywords: phosphatidylserine, recycling endosomes, evectin-2, retrograde membrane traffic

---

[*1] 東京大学大学院薬学系研究科
[*2] Harvard Medical School, America
[*3] 大学共同利用機関法人高エネルギー加速器研究機構
[*4] 順天堂大学院医学研究科
[*5]（独）産業技術総合研究所
[*6] 大阪大学大学院医学系研究科
[*7] 大阪医科大学
[*8] 同志社大学生命医科学部
[*9]（独）理化学研究所
[*10] University of Iowa, America

Imae, R.[*1], Inoue, T., Nakasaki, Y.[*1], Uchida, Y.[*1], Ohba, Y.[*1], Kono, N.[*1], Nakanishi, H.[*2], Sasaki, T.[*2], Mitani, S.[*3] and Arai, H.[*1]: **LYCAT, a homologue of *C. elegans acl-8*, *acl-9* and *acl-10*, determines the fatty acid composition**

of phosphatidylinositol in mice

J. Lipid Res., **53**, 335-347（2012）

Mammalian phosphatidylinositol（PI）has a unique fatty acid composition in that 1-stearoyl-2-arachidonoyl species is predominant. This fatty acid composition is formed through fatty acid remodeling by sequential deacylation and reacylation. We recently identified three *Caenorhabditis elegans* acyltransferases（ACL-8, ACL-9, and ACL-10）that incorporate stearic acid into the sn-1 position of PI. Mammalian LYCAT, which is the closest homolog of ACL-8, ACL-9, and ACL-10, was originally identified as a lysocardiolipin acyltransferase by an in vitro assay and was subsequently reported to possess acyltransferase activity toward various anionic lysophospholipids. However, the in vivo role of mammalian LYCAT in phospholipid fatty acid metabolism has not been well elucidated. In this study, we generated LYCAT-deficient mice and demonstrated that LYCAT determined the fatty acid composition of PI in vivo. LYCAT-deficient mice were outwardly healthy and fertile. In the mice, stearoyl-CoA acyltransferase activity toward the sn-1 position of PI was reduced, and the fatty acid composition of PI, but not those of other major phospholipids, was altered. Furthermore, expression of mouse LYCAT rescued the phenotype of *C. elegans acl-8 acl-9 acl-10* triple mutants. Our data indicate that LYCAT is a determinant of PI molecular species and its function is conserved in *C. elegans* and mammals.

Keywords: phosphatidylinositol, acyltransferase

[*1] 東京大学大学院薬学系研究科
[*2] 秋田大学大学院医学系研究科
[*3] 東京女子医科大学医学部

Sakamoto, T.[*1], Inoue, T., Otomo, Y.[*1], Yokomori, N.[*1], Ohno, M.[*1], Sasaki, T.[*1], Arai, H.[*2] and Nakagawa, Y.[*1]:
Deficiency of cardiolipin synthase causes abnormal mitochondrial function and morphology in germ cells of *Caenorhabditis elegans*

J. Biol. Chem., **287**, 4590-4601（2012）

Cardiolipin（CL）is a major membrane phospholipid specifically localized in mitochondria. At the cellular level, CL has been shown to have a role in mitochondrial energy production, mitochondrial membrane dynamics, and the triggering of apoptosis. However, the in vivo role of CL in multicellular organisms is largely unknown. In this study, by analyzing deletion mutants of a CL synthase gene（*crls-1*）in *Caenorhabditis elegans*, we demonstrated that CL depletion selectively caused abnormal mitochondrial function and morphology in germ cells but not in somatic cell types such as muscle cells. *crls-1* mutants reached adulthood but were sterile with reduced germ cell proliferation and impaired oogenesis. In the gonad of *crls-1* mutants, mitochondrial membrane potential was significantly decreased, and the structure of the mitochondrial cristae was disrupted. Contrary to the abnormalities in the gonad, somatic tissues in crls-1 mutants appeared normal with respect to cell proliferation, mitochondrial function, and mitochondrial morphology. Increased susceptibility to CL depletion in germ cells was also observed in mutants of phosphatidylglycerophosphate synthase, an enzyme responsible for producing phosphatidylglycerol, a precursor phospholipid of CL. We propose that the contribution of CL to mitochondrial function and morphology is different among the cell types in *C. elegans*.

Keywords: Cardiolipin, mitochondria, *C. elegans*

[*1] 北里大学大学院薬学研究科
[*2] 東京大学大学院薬学研究科

Saitoh, Y.[*1], Katane, M.[*1], Kawata, T.[*1], Maeda, K.[*1], Sekine, M.[*1], Furuchi, T.[*1], Kobuna, H.[*2], Sakamoto, T.[*1], Inoue, T., Arai, H.[*2], Nakagawa, Y.[*1] and Homma, H.[*1]:
Spatiotemporal localization of D-amino acid oxidase and D-aspartate oxidases during the development in *Caenorhabditis elegans*

Mol. Cell. Biol., **32**, 1967-1983（2012）

Recent investigations have shown that a variety of D-amino acids are present in living organisms and that they possibly play important roles in physiological functions in the body. D-Amino acid oxidase（DAO）and D-aspartate oxidase（DDO）are degradative enzymes stereospecific for D-amino acids. They have been identified in various organisms, including mammals and nematode *Caenorhabditis elegans*, although the significance of these enzymes and the relevant functions of D-amino acids remain to be elucidated. In this study, we investigated the spatiotemporal localization of *C. elegans* DAO and DDOs（DDO-1, DDO-2 and DDO-3）and measured the levels of several D- and L-amino acids in wild-type *C. elegans* and four mutants in which each gene for the DAO and DDOs has been partially deleted and thereby inactivated. Furthermore, several phenotypes of these mutant strains were characterized. The results reported in this study indicate that *C. elegans* DAO and DDOs are involved in egg-laying events and the early development of *C. elegans*. In particular, DDOs appear to play important roles in the development and maturation of germ cells. This work provides

novel and useful insights into the physiological functions of these enzymes and D-amino acids in multicellular organisms.
Keywords: D-amino acid, D-Amino acid oxidase, *C. elegans*

[*1] 北里大学大学院薬学研究科
[*2] 東京大学大学院薬学研究科

佐藤陽治，黒田拓也：**ヒト多能性幹細胞を使った再生医療・細胞治療における造腫瘍性試験の現状**
医学のあゆみ，**239**，1460-1465（2011）

ヒト胚性幹細胞（ES細胞）やヒト人工多能性幹細胞（iPS細胞）などの，いわゆるヒト多能性幹細胞を原材料として細胞・組織加工製品を製造し，再生医療・細胞治療へ応用しようとする試みが，現在，国内外で非常に活発に進んでいる．ヒト多能性幹細胞は動物体内に移植された際に腫瘍を形成する能力，いわゆる「造腫瘍性」を元来の特性として保持しており，ヒト多能性幹細胞を原材料とした医薬品・医療機器においては，未分化細胞の混入・残留による異所性組織形成や腫瘍形成・がん化を防止すること，すなわち最終製品の造腫瘍性の評価と管理が重要な課題となる．しかしながら，患者に投与する動物又はヒト由来の生細胞を対象にした造腫瘍性試験のガイドラインは今のところ存在しない．本稿ではヒト多能性幹細胞加工製品の開発が精力的に進む中で，その造腫瘍性の評価法の現状と課題について概説する．
Keywords：細胞・組織加工製品，ヒト多能性幹細胞，造腫瘍性

Nishioka, K.[*1], Nishida, M.[*1], Ariyoshi, M.[*1], Jian, Z.[*3], Saiki, S.[*1], Hirano, M.[*2], Nakaya, M.[*1], Sato, Y., Kita, S.[*3], Iwamoto, T.[*3], Hirano, K.[*2], Inoue, R.[*3], Kurose, H.[*1]: **Cilostazol suppresses angiotensin II-induced vasoconstriction *via* protein kinase A-mediated phosphorylation of TRPC6 channel**
Arterioscler. Thromb. Vasc. Biol., **31**, 2278-2286（2011）

Objective－The goal of this study was to determine whether inhibition of transient receptor potential canonical（TRPC）channels underlies attenuation of angiotensin II（Ang II）－induced vasoconstriction by phosphodiesterase（PDE）3 inhibition.

Methods and Results － Pretreatment of rat thoracic aorta with cilostazol, a selective PDE3 inhibitor, suppressed vasoconstriction induced by Ang II but not that induced by KCl. The Ang II－induced contraction was largely dependent on Ca2+ influx via receptor-operated cation channels. Cilostazol specifically suppressed diacylglycerol-activated TRPC channels（TRPC3/TRPC6/TRPC7）through protein kinase A（PKA）－dependent phosphorylation of TRPC channels in HEK293 cells. In contrast, we found that phosphorylation of TRPC6 at Thr69 was essential for the suppression of Ang II－induced Ca2+ influx by PDE3 inhibition in rat aortic smooth muscle cells（RAoSMCs）. Cilostazol specifically induced phosphorylation of endogenous TRPC6 at Thr69. The endogenous TRPC6, but not TRPC3, formed a ternary complex with PDE3 and PKA in RAoSMCs, suggesting the specificity of TRPC6 phosphorylation by PDE3 inhibition. Furthermore, inhibition of PDE3 suppressed the Ang II－induced contraction of reconstituted ring with RAoSMCs, which were abolished by the expression of a phosphorylation-deficient mutant of TRPC6.

Conclusion－PKA-mediated phosphorylation of TRPC6 at Thr69 is essential for the vasorelaxant effects of PDE3 inhibition against the vasoconstrictive actions of Ang II.
Keywords: angiotensin II, phosphodiesterase, transient receptor potential canonical

[*1] 九州大学大学院薬学研究科
[*2] 九州大学大学院医学研究科
[*3] 福岡大学大学院医学部

早川堯夫[*1]，青井貴之[*2]，梅澤明弘[*3]，小澤敬也[*4]，佐藤陽治，澤　芳樹[*5]，松山晃文[*6]，大和雅之[*7]，山中伸弥[*2]：**ヒト幹細胞を用いた細胞・組織加工医薬品等の品質・安全性確保に関する指針整備と主なポイント**
再生医療，**10**，206-210（2011）

平成21年度に，ヒト自己由来細胞・組織加工医薬品等全般に関する指針，いわゆる「ヒト自己親指針」をベースとして，①ヒト（自己）体性幹細胞及び②ヒト（自己）iPS細胞加工医薬品等の品質及び安全性の確保に関するそれぞれの指針案（中間報告）を作成した．また「ヒト同種親指針」をベースとして，③ヒト（同種）体性幹細胞，④ヒトES細胞及び⑤ヒト（同種）iPS細胞に関するそれぞれの指針案（中間報告）を作成した．これら5つの指針案については，さらにさまざまな観点からの論議を経て最終案とされるべきものであったが，いち早く広く関係者に公開し，ことの推移を周知のものとするとともに，コメントを頂く機会とすることは非常に意義があると考え，日本再生医療学会の学会誌に5件の論文として公表した（再生医療，9（1），116-180（2010）3-7）．

平成22年度は，海外の動向，学問・技術の進歩，幹細胞由来製品の実用化に関する議論の深まり，再生医療枠組み検討会でのとくに審査のあり方に関する討論結果などを踏まえて，中間案を精査し，最終案とすることを目指した研究を行った．

Keywords：ヒト幹細胞，品質，安全性

*1 近畿大学薬学総合研究所
*2 京都大学iPS細胞研究所
*3 (独)国立成育医療センター生殖医療研究部
*4 自治医科大学医学部内科学講座血液学部門
*5 大阪大学大学院医学系研究科外科学講座
*6 (財)先端医療振興財団再生医療研究開発部門
*7 東京女子医科大学先端生命医科学研究所

早川堯夫*1，青井貴之*2，梅澤明弘*3，小澤敬也*4，佐藤陽治，澤　芳樹*5，松山晃文*6，大和雅之*7，山中伸弥*2：**ヒト(自己)体性幹細胞加工医薬品等の品質及び安全性の確保に関する指針(案)ー総則，原材料及び製造関連物質，製造工程に関する留意事項についてー**
再生医療，**10**，211-218(2011)

本研究の経緯については，本シリーズ第1報において詳細に述べた．本稿ではそのうちヒト(自己)体性幹細胞加工医薬品等の品質及び安全性の確保に関連の深い事項，特に総則，並びに製造方法のうち原材料及び製造関連物質，製造工程に関する留意事項についてその要約を述べる．

Keywords：ヒト体性幹細胞，品質，安全性

*1 近畿大学薬学総合研究所
*2 京都大学iPS細胞研究所
*3 (独)国立成育医療センター生殖医療研究部
*4 自治医科大学医学部内科学講座血液学部門
*5 大阪大学大学院医学系研究科外科学講座
*6 (財)先端医療振興財団再生医療研究開発部門
*7 東京女子医科大学先端生命医科学研究所

早川堯夫*1，青井貴之*2，梅澤明弘*3，小澤敬也*4，佐藤陽治，澤　芳樹*5，松山晃文*6，大和雅之*7，山中伸弥*2：**ヒト(同種)体性幹細胞加工医薬品等の品質及び安全性の確保に関する指針(案)ー総則，原材料及び製造関連物質，製造工程に関する留意事項についてー**
再生医療，**10**，219-226(2011)

本研究の経緯については，本シリーズ第1報において詳細に述べた．本稿ではそのうちヒト(同種)体性幹細胞加工医薬品等の品質及び安全性の確保に関連の深い事項，特に総則，並びに製造方法のうち原材料及び製造関連物質，製造工程に関する留意事項についてその要約を述べる．

Keywords：ヒト体性幹細胞，品質，安全性

*1 近畿大学薬学総合研究所
*2 京都大学iPS細胞研究所
*3 (独)国立成育医療センター生殖医療研究部
*4 自治医科大学医学部内科学講座血液学部門
*5 大阪大学大学院医学系研究科外科学講座
*6 (財)先端医療振興財団再生医療研究開発部門
*7 東京女子医科大学先端生命医科学研究所

早川堯夫*1，青井貴之*2，梅澤明弘*3，小澤敬也*4，佐藤陽治，澤　芳樹*5，松山晃文*6，大和雅之*7，山中伸弥*2：**ヒト(自己)iPS(様)細胞加工医薬品等の品質及び安全性の確保に関する指針(案)ー総則，原材料及び製造関連物質，製造工程に関する留意事項についてー**
再生医療，**10**，227-237(2011)

本研究の経緯については，本シリーズ第1報において詳細に述べた．本稿ではそのうちヒト(自己)iPS(様)細胞加工医薬品等の品質及び安全性の確保に関連の深い事項，特に総則，並びに製造方法のうち原材料及び製造関連物質，製造工程に関する留意事項についてその要約を述べる．

Keywords：ヒトiPS細胞，品質，安全性

*1 近畿大学薬学総合研究所
*2 京都大学iPS細胞研究所
*3 (独)国立成育医療センター生殖医療研究部
*4 自治医科大学医学部内科学講座血液学部門
*5 大阪大学大学院医学系研究科外科学講座
*6 (財)先端医療振興財団再生医療研究開発部門
*7 東京女子医科大学先端生命医科学研究所

早川堯夫*1，青井貴之*2，梅澤明弘*3，小澤敬也*4，佐藤陽治，澤　芳樹*5，松山晃文*6，大和雅之*7，山中伸弥*2：**ヒト(同種)iPS(様)細胞加工医薬品等の品質及び安全性の確保に関する指針(案)ー総則，原材料及び製造関連物質，製造工程に関する留意事項についてー**
再生医療，**10**，238-248(2011)

本研究の経緯については，本シリーズ第1報において詳細に述べた．本稿ではそのうちヒト(同種)iPS(様)細胞加工医薬品等の品質及び安全性の確保に関連の深い事項，特に総則，並びに製造方法のうち原材料及び製造関連物質，製造工程に関する留意事項についてその要約を述べる．

Keywords：ヒトiPS細胞，品質，安全性

*1 近畿大学薬学総合研究所
*2 京都大学iPS細胞研究所
*3 (独)国立成育医療センター生殖医療研究部
*4 自治医科大学医学部内科学講座血液学部門

[*5] 大阪大学大学院医学系研究科外科学講座
[*6] (財)先端医療振興財団再生医療研究開発部門
[*7] 東京女子医科大学先端生命医科学研究所

早川堯夫[*1]，青井貴之[*2]，梅澤明弘[*3]，小澤敬也[*4]，佐藤陽治，澤　芳樹[*5]，松山晃文[*6]，大和雅之[*7]，山中伸弥[*2]：**ヒトES細胞加工医薬品等の品質及び安全性の確保に関する指針（案）－総則，原材料及び製造関連物質，製造工程に関する留意事項について－**

再生医療，**10**，249-260（2011）

本研究の経緯については，本シリーズ第1報において詳細に述べた．ヒトES細胞加工医薬品等の品質及び安全性の確保に関連の深い事項，特に総則，並びに製造方法のうち原材料及び製造関連物質，製造工程に関する留意事項についてその要約を述べる．

Keywords：ヒトES細胞，品質，安全性

[*1] 近畿大学薬学総合研究所
[*2] 京都大学iPS細胞研究所
[*3] (独)国立成育医療センター生殖医療研究部
[*4] 自治医科大学医学部内科学講座血液学部門
[*5] 大阪大学大学院医学系研究科外科学講座
[*6] (財)先端医療振興財団再生医療研究開発部門
[*7] 東京女子医科大学先端生命医科学研究所

早川堯夫[*1]，青井貴之[*2]，梅澤明弘[*3]，小澤敬也[*4]，佐藤陽治，澤　芳樹[*5]，松山晃文[*6]，大和雅之[*7]，山中伸弥[*2]：**ヒト幹細胞加工医薬品等の品質及び安全性の確保に関する指針（案）－ヒト体性幹細胞，iPS（様）細胞又はES細胞を加工して製造される医薬品等（ヒト幹細胞加工医薬品等）の最終製品の品質管理－**

再生医療，**10**，261-266（2011）

本研究の経緯については，本シリーズ第1報において詳細に述べた．「製造方法のうち原材料及び製造関連物質，製造工程」に関しては，体性幹細胞，iPS細胞，ES細胞のいずれを原材料にするか，あるいは自己由来か，同種由来か，などにより区別して留意事項を明確にすることが望ましいと考え，その内容を本シリーズの第2報から第6報にかけて報告してきた．しかし，最終製品の品質管理のあり方や安定性評価については，由来する細胞に特化した留意事項に重きを置くと云うよりもむしろ，個々の製品そのものに焦点をあてた留意事項として捉えることがより重要である．言い換えれば，由来する細胞に関してはそれぞれに適切に考慮に入れるにしても，由来はともあれ，実際に患者に投与するのは個々の製品であり，事後管理していくのも個々の製品レベルであるので，そのことに焦点をあてた対応をすることが肝要であるということである．

Keywords：ヒト幹細胞，品質管理，安定性評価

[*1] 近畿大学薬学総合研究所
[*2] 京都大学iPS細胞研究所
[*3] (独)国立成育医療センター生殖医療研究部
[*4] 自治医科大学医学部内科学講座血液学部門
[*5] 大阪大学大学院医学系研究科外科学講座
[*6] (財)先端医療振興財団再生医療研究開発部門
[*7] 東京女子医科大学先端生命医科学研究所

早川堯夫[*1]，青井貴之[*2]，梅澤明弘[*3]，小澤敬也[*4]，佐藤陽治，澤　芳樹[*5]，松山晃文[*6]，大和雅之[*7]，山中伸弥[*2]：**ヒト幹細胞加工医薬品等の品質及び安全性の確保に関する指針（案）－ヒト体性幹細胞，iPS（様）細胞又はES細胞を加工して製造される医薬品等（ヒト幹細胞加工医薬品等）の非臨床試験及び臨床試験について－**

再生医療，**10**，267-272（2011）

本研究の経緯については，本シリーズ第1報において詳細に述べた．「製造方法のうち原材料及び製造関連物質，製造工程」に関しては，体性幹細胞，iPS細胞，ES細胞のいずれを原材料にするか，あるいは自己由来か，同種由来か，などにより区別して留意事項を明確にすることが望ましいと考え，その内容を本シリーズの第2報から第6報にかけて報告してきた．一方，「最終製品の品質管理や安定性評価のあり方」については，由来する細胞に特化した留意事項に重きを置くと云うよりもむしろ，最終製品そのものに焦点をあてた留意事項として捉えることがより重要であると考えて第7報で一括して報告した．非臨床試験及び臨床試験についても製品レベルで考慮することであるので，本報で一括して報告する．

Keywords：ヒト幹細胞，非臨床試験，臨床試験

[*1] 近畿大学薬学総合研究所
[*2] 京都大学iPS細胞研究所
[*3] (独)国立成育医療センター生殖医療研究部
[*4] 自治医科大学医学部内科学講座血液学部門
[*5] 大阪大学大学院医学系研究科外科学講座
[*6] (財)先端医療振興財団再生医療研究開発部門
[*7] 東京女子医科大学先端生命医科学研究所

Yasuda, S., Hasegawa, T., Hosono, T., Satoh, M.[*1], Watanabe, K., Ono, K.[*2], Shimizu, S.[*3], Hayakawa, T.[*4], Yamaguchi, T., Suzuki, K., Sato, Y.: **AW551984: a novel regulator of cardiomyogenesis in pluripotent embryonic cells**

Biochem. J., **437**, 345-355（2011）

An understanding of the mechanism that regulates the cardiac differentiation of pluripotent stem cells is necessary for the effective generation and expansion of cardiomyocytes as cell therapy products. In the present study, we have identified genes that modulate the cardiac differentiation of pluripotent embryonic cells. We isolated P19CL6 cell sublines that possess distinct properties in cardiomyogenesis and extracted 24 CMR（cardiomyogenesis-related candidate）genes correlated with cardiomyogenesis using a transcriptome analysis. Knockdown of the CMR genes by RNAi（RNA interference）revealed that 18 genes influence spontaneous contraction or transcript levels of cardiac marker genes in EC（embryonal carcinoma）cells. We also performed knockdown of the CMR genes in mouse ES（embryonic stem）cells and induced in vitro cardiac differentiation. Three CMR genes, AW551984, 2810405K02Rik（RIKEN cDNA 2810405K02 gene）and Cd302（CD302 antigen）, modulated the cardiac differentiation of both EC cells and ES cells. Depletion of AW551984 attenuated the expression of the early cardiac transcription factor Nkx2.5（NK2 transcription factor related locus 5）without affecting transcript levels of pluripotency and early mesoderm marker genes during ES cell differentiation. Activation of Wnt/β-catenin signalling enhanced the expression of both AW551984 and Nkx2.5 in ES cells during embryoid body formation. Our findings indicate that AW551984 is a novel regulator of cardiomyogenesis from pluripotent embryonic cells, which links Wnt/β-catenin signalling to Nkx2.5 expression.

Keywords: cardiac differentiation, embryonic stem cell, Wnt signalling

[*1] 東邦大学薬学部
[*2] 帝京大学薬学部
[*3] 昭和大学薬学部
[*4] 近畿大学薬学総合研究所

Kitajima, N.[*1], Watanabe, K.[*1], Morimoto, S.[*2], Sato, Y., Kiyonaka, S.[*3], Hoshijima, M.[*4], Ikeda, Y.[*5], Nakaya, M.[*1], Ide, T.[*2], Mori, Y.[*3], Kurose, H.[*1], Nishida, M.[*1]: **TRPC3-mediated Ca^{2+} influx contributes to Rac1-mediated production of reactive oxygen species in MLP-deficient mouse hearts**

Biochem. Biophys. Res. Commun., **409**, 108-113（2011）

Dilated cardiomyopathy（DCM）is a myocardial disorder that is characterized by dilation and dysfunction of the left ventricle（LV）. Accumulating evidence has implicated aberrant Ca（2+）signaling and oxidative stress in the progression of DCM, but the molecular details are unknown. In the present study, we report that inhibition of the transient receptor potential canonical 3（TRPC3）channels partially prevents LV dilation and dysfunction in muscle LIM protein-deficient（MLP（-/-））mice, a murine model of DCM. The expression level of TRPC3 and the activity of Ca（2+）/calmodulin-dependent kinase II（CaMKII）were increased in MLP（-/-）mouse hearts. Acitivity of Rac1, a small GTP-binding protein that participates in NADPH oxidase（Nox）activation, and the production of reactive oxygen species（ROS）were also increased in MLP（-/-）mouse hearts. Treatment with pyrazole-3, a TRPC3 selective inhibitor, strongly suppressed the increased activities of CaMKII and Rac1, as well as ROS production. In contrast, activation of TRPC3 by 1-oleoyl-2-acetyl-sn-glycerol（OAG）, or by mechanical stretch, induced ROS production in rat neonatal cardiomyocytes. These results suggest that up-regulation of TRPC3 is responsible for the increase in CaMKII activity and the Nox-mediated ROS production in MLP（-/-）mouse cardiomyocytes, and that inhibition of TRPC3 is an effective therapeutic strategy to prevent the progression of DCM.

Keywords: transient receptor potential channel, dilated cardiomyopathy, reactive oxygen species

[*1] 九州大学大学院薬学研究科
[*2] 九州大学大学院医学研究科
[*3] 京都大学大学院工学研究科
[*4] カリフォルニア大学サンディエゴ校医学部
[*5] 山口大学大学院医学系研究科

Nishida, M.[*1], Ogushi, M.[*1], Suda, R.[*1], Toyotaka, M.[*1], Saiki, S.[*1], Kitajima, N.[*1], Nakaya, M.[*1], Kim, K.M.[*2], Ide, T. [*1], Sato, Y., Inoue, K.[*1], Kurose, H.[*1]: **Heterologous down-regulation of angiotensin type 1 receptors by purinergic P2Y2 receptor stimulation through S-nitrosylation of NF-κB**

Proc. Natl. Acad. Sci. USA., **108**, 6662-6667（2011）

Cross-talk between G protein-coupled receptor（GPCR）signaling pathways serves to fine tune cellular responsiveness by neurohumoral factors. Accumulating evidence has implicated nitric oxide（NO）-based signaling downstream of GPCRs, but the molecular details are unknown. Here, we show that adenosine triphosphate（ATP）decreases angiotensin type 1 receptor（AT（1）R）density through NO-mediated S-nitrosylation of nuclear factor κB（NF-κB）in rat cardiac fibroblasts. Stimulation of purinergic P2Y（2）receptor

by ATP increased expression of inducible NO synthase（iNOS）through activation of nuclear factor of activated T cells, NFATc1 and NFATc3. The ATP-induced iNOS interacted with p65 subunit of NF-κB in the cytosol through flavin-binding domain, which was indispensable for the locally generated NO-mediated S-nitrosylation of p65 at Cys38. β-Arrestins anchored the formation of p65/IκBα/β-arrestins/iNOS quaternary complex. The S-nitrosylated p65 resulted in decreases in NF-κB transcriptional activity and AT（1）R density. In pressure-overloaded mouse hearts, ATP released from cardiomyocytes led to decrease in AT（1）R density through iNOS-mediated S-nitrosylation of p65. These results show a unique regulatory mechanism of heterologous regulation of GPCRs in which cysteine modification of transcriptional factor rather than protein phosphorylation plays essential roles.

Keywords: angiotensin II, phosphodiesterase, transient receptor potential canonical

---

[*1] 九州大学大学院薬学研究科
[*2] 全南大学校（Chonnam National University）薬学部

Luan, Y.[*1], Kogi, M.[*2], Rajaguru, P.[*1], Ren, J., Yamaguchi, T., Suzuki, K. and Suzuki, T.: **Microarray analysis of responsible genes in increased growth rate in the subline of HL60（HL60RG）cells**

Mutation Res., **731**, 20-26（2012）

To gain information of the mechanisms involved in growth acceleration in HL60RG, we performed a genome scan using 10K SNP mapping array. Characteristic genomic alterations in HL60RG cells were identified including uniparental disomy（UPD）of chromosome 1, hemizyous deletion in 10p and 11p. However, no such defects were observed in HL60 cells. Changes in gene expression in HL60RG cells were determined using expression arrays. Candidate genes associated with the rapid growth were identified. TNFRSF1B and TNFRSF8, which are adjacently located on chromosome 1 showed opposing changes in gene expression in HL60RG cells － over-expression of TNFRSF8 and repression of TNFRSF1B. Differences in the DNA methylation status in the transcriptional regulatory regions of both genes between HL60 and HL60RG was detected. In conclusion, alterations in chromosome and gene expression in HL60RG may be associated with growth acceleration.

Keywords: HL60, CGH, methylation

[*1] 中国科学院上海薬物研究所
[*2] 金沢工業大学

Ramadan, A.[*] and Suzuki, T.: **Detection of genotoxicity of phenolic antioxidants, butylated hydroxyanisole and tert-butylhydroquinone in multiple mouse organs by the alkaline Comet assay**

J. American Science, **8**, 722-727（2012）

We tested the genotoxicity of two widely used phenolic antioxidants, butylated hydroxyanisole（BHA）and tert-butylhydroquinone（TBHQ）in multiple mouse organs using the alkaline comet assay. The two compounds induced significant increase in DNA migration in a time dependant manner in specific organs. Extensive DNA damage was observed in stomach cells at 24 h post treatment in treatment groups. In addition to stomach, TBHQ treatment induced significant increase in DNA migration in liver and kidney cells. Evidently, bone marrow cells did not show genotoxicity in response to treatment with TBHQ and BHA. Considering these findings, although TBHQ and BHA are generally considered non-genotoxic, the DNA damage observed in this experiment may be related to their indirect action on DNA via ROS mechanism.

Keywords: BHA, TBHQ, comet assay

---

[*] Ain-Shams University

Uchida, M.[*], Ishii, I.[*], Hirata, K.[*], Yamamoto, F.[*], Tashiro, K.[*], Suzuki, T., Nakayama, Y.[*], Ariyoshi, N.[*] and Kitada, M.[*]: **Degradation of filamin induces contraction of vascular smooth muscle cells in type-I collagen matrix honeycombs**

Cell Physiol Biochem., **27**, 669-680（2011）

Dedifferentiated rabbit vascular smooth muscle cells（SMCs）exhibit similar features to differentiated SMCs when cultured in three-dimensional matrices of type-I collagen called “honeycombs,” but the mechanism is unknown. The role of filamin, an actin-binding protein that links actin filaments in SMCs, was investigated. Full-length filamin was expressed in subconfluent SMCs cultured on plates; however, degradation of filamin, which might be regulated by calpain, was observed in confluent SMCs cultured on plates and in honeycombs. Filamin was detected in the cytoplasm in SMCs cultured in honeycombs, and degraded filamin was mainly detected in the cytoplasmic fraction of these cells. Degradation of filamin in SMCs cultured in honeycombs induces structural weakness of β-non-muscle actin filaments, thereby permitting SMCs in honeycombs to achieve contractility.

Keywords: smooth muscle cells, filamin, honeycombs

* 千葉大学

Matsuoka, A., Kodama, Y., Yoshida, M., Isama, K., Inoue, K., Kawakami, T., Nishikawa, A.: **Toxicological studies of nano-suspensions of silica, silver and zinc oxide**

Proceedings of 24th European Conference on Biomaterials, 87-90（2011）

Nano-suspensions of silica, silver and zinc oxide were subjected to the cytotoxicity test, the chromosomal aberration test, and the 13-week repeated dose test for their safety evaluation. Silver showed the strongest cytotoxicity among the three. Only zinc oxide induced chromosome aberrations. In the *in vivo* test, zinc oxide caused inhibition of the normal body weight increase, increase in the relative lung weight, and pulmonary fibrosis. We propose the three tests as a candidate of a primary screening test battery for safety evaluation of nanomaterials（NMs）.

Keywords: Nano-Suspensions, Silica, Silver, Zinc Oxide

松岡厚子，伊佐間和郎：**生体機能化されたチタン合金の生物学的安全性評価**

日本金属学会分科会シンポジウム「バイオメタルサイエンス研究の最前線」予稿集，21-23（2011）

擬似体液中でアパタイト形成能が高い医用材料は，生体内で骨と直接結合することが期待できる．近年，アパタイト形成能を付与したアルカリ加熱処理チタン合金が製品化され，さらに，より高いアパタイト形成能を付与するためにアルカリ処理したチタン合金へのカルシウム導入も図られている．そこで，我々は，骨組織適合性の高いTi-Zr-Nb合金にアルカリ処理後カルシウム導入のための表面処理を施し，そのアパタイト形成能を評価し，加えて，カルシウム導入したTi-Zr-Nb合金の細胞毒性試験及び骨芽細胞適合性試験を行って生物学的安全性を評価した．

Keywords：骨組織適合性，Ti-Zr-Nb合金，アパタイト形成

Ishizaki, T.[*1,2,3], Tamiya, T.[*1,2], Taniguchi, K.[*1,2], Morita, R.[*1,2], Kato, R., Okamoto, F.[*4], Saeki, K.[*4], Nomura, M.[*4], Nojima, Y.[*3], Yoshimura, A.[*1,2]: **miR126 positively regulates mast cell proliferation and cytokine production through suppressing Spred1**

Genes Cells, 16, 803-814（2011）

The protein known as Spred1（Sprouty-related Ena/VASP homology-1 domain-containing protein）has been identified as a negative regulator of growth factor-induced ERK/mitogen-activated protein kinase activation. Spred1 has also been implicated as the target of microRNA-126（miR126）, a miRNA located within the *Egfl7* gene, and is involved in the regulation of vessel development through its role in regulating VEGF signaling. In this study, we examined the role of miR126 and Spred1 in the hematopoietic system, as miR126 has been shown to be overexpressed in leukemic cells. miR126 levels were down-regulated during mast cell differentiation from bone marrow cells, whereas Spred1 expression was inversely up-regulated. Overexpression of miR126 suppressed Spred1 expression and enhanced ERK activity in primary bone marrow cells and MC9 mast cells, which were associated with elevated FcεRI-mediated cytokine production. To confirm the effect of Spred1 reduction *in vivo*, we generated hematopoietic cell-specific Spred1-conditional knockout mice. These mice showed increased numbers of mast cells, and Spred1-deficient bone marrow-derived mast cells were highly activated by cross-linking of Fcε-R stimulation as well as by IL-3 and SCF stimulation. These results suggest that Spred1 negatively regulates mast cell activation, which is modulated by miR126.

Keywords: negative regulator, microRNA

*1 Keio University
*2 CREST
*3 Gunma University
*4 Kyushu University

Jung, Y.S.[*1], Kato, R., Tsuchiya, T.[*2]: **Biodegradable Polymers Induce CD54 on THP-1 Cells in Skin Sensitization Test**

Int. J. Biomater., 424571-424576（2011）

Currently, nonanimal methods of skin sensitization testing for various chemicals, biodegradable polymers, and biomaterials are being developed in the hope of eliminating the use of animals. The human cell line activation test（h-CLAT）is a skin sensitization assessment that mimics the functions of dendritic cells（DCs）. DCs are specialized antigen-presenting cells, and they interact with T cells and B cells to initiate immune responses. Phenotypic changes in DCs, such as the production of CD86 and CD54 and internalization of MHC class II molecules, have become focal points of the skin sensitization test. In this study, we used h-CLAT to assess the effects of biodegradable polymers. The results showed that several biodegradable polymers increased the expression of CD54, and the relative skin sensitizing abilities of bio-

degradable polymers were PLLG（75 : 25）< PLLC（40 : 60）< PLGA（50 : 50）< PCG（50 : 50）. These results may contribute to the creation of new guidelines for the use of biodegradable polymers in scaffolds or allergenic hazards.
Keywords: skin sensitization test, biodegradable polymers, allergenic hazards

---

[*1] Institute of New Jersey, Robert Wood Johnson Medical School
[*2] Osaka University Hospital

迫田秀行，松岡厚子：**打ち抜き試験による超高分子量ポリエチレンの機械特性評価**

臨床バイオメカニクス，**32**，277-281（2011）

打ち抜き試験は試験片が小さいため応用範囲が広い．しかし，人工関節用超高分子量ポリエチレンの試験を目的としたASTM規格があるが，この規格では治具と試験片の寸法精度の要求が厳しく，実用的ではない．そこで，試験片厚さの影響について検討し，より実用的で簡便な試験法の開発を目指した．平板状の治具に様々な厚さの試験片を固定し，半球状のポンチで打抜いた．その結果，試験片を固定する際に試験片に加わる力が変化すると，試験結果がばらつくことがわかった．これは試験片厚さに応じた適切なスペーサを用いることで克服できることがわかった．試験により得られる力学的パラメータは試験片厚さの影響を受けるが，両者の間には高い相関があり，試験片厚さのばらつきは統計学的な手法により克服できることがわかった．本試験の有用性を確認するため，生体脂質であるスクアレンを含浸させた試料を試験したところ，文献と同様に剛性の低下が確認された．本研究の結果，寸法精度が得られなくても，複数の試験片を使用することにより，超高分子量ポリエチレンの引張特性が評価できることが示された．

Keywords: UHMWPE, tensile property, punch test

中岡竜介：**ISO/TC 150の動向について：SC 7「再生医療機器」の動向を中心に**

PHARMSTAGE, **11**（12）, 1-3（2012）

近年，様々な面で複数の国家間，利害関係者間での共通の「ものさし」としてISOが作成する国際規格が利用されるようになっている．ISO規格自体に強制力はないものの，ISO規格を自国規格に取り入れるケースが多いこと，国際貿易の公正競争にISO規格が寄与していること等から，各業界では関連するISO規格を無視することはできなくなっている．日本国内でも，医療機器の製造販売に係る許認可で利用されている認証基準，承認基準，評価指標及び各種ガイドラインに種々の規格が引用されているため，医療機器におけるISO規格は重要となっている．一方，自国技術がISO規格となれば，その業界の国際市場で主導権を握ることができるため，新規技術に関するISOでの活動は大きな意味をもつ．これらの現状及び重要性について，日本が設立に関わったISO/TC 150/SC 7「再生医療機器」を中心にISO/TC 150「外科用インプラント」の活動状況を紹介しながら説明する．

Keywords：医療機器，国際標準化，再生医療機器

上内洋輝[*1]，佐藤生馬[*2]，鈴木孝司[*3]，植松美幸，中村亮一[*2]，村垣善浩[*3,4]，伊関　洋[*3,4]，正宗　賢[*1]：**タブレットPCを使用した医用画像重畳表示ナビゲーションシステムの開発**

日本コンピュータ外科学会誌，**13**（4），445-452（2011）

手術計画あるいはナビゲーションに対する期待の大きいMRIを使用し，術前MRI画像から生成される病変部の三次元CGモデルを，タブレットPCに搭載されている背面カメラの画像に重畳表示するビデオシースルー式画像重畳表示システムを提案した．特に，タブレットPCに搭載されている背面カメラの画像に映る実世界と術前MRI画像の座標系統合を行うシステムの構築と画像重畳表示位置精度評価について報告した．

Keywords: image overlay system, tablet PC, augmented reality

[*1] 東京大学大学院情報理工学系研究科
[*2] 千葉大学大学院工学研究科
[*3] 東京女子医科大学先端生命医科学研究所
[*4] 東京女子医科大学脳神経外科

Saito, A.[*1], Kono, K., Nomaguchi, M.[*2], Yasutomi, Y[*3], Adachi, A.[*2], Shioda, T.[*4], Akari, H.[*1], Nakayam, E.E.[*4]: **Geographical, genetic and functional diversity of antiretroviral host factor TRIMCyp in cynomolgus macaque（*Macaca fascicularis*）**

J. Gen. Virol., **93**, 594-602（2012）

The antiretroviral factor tripartite motif protein 5（TRIM5）gene-derived isoform（TRIMCyp）has been found in at least three species of Old World monkey: rhesus（*Macaca mulatta*）, pig-tailed（*Macaca nemestrina*）and cynomolgus（*Macaca fascicularis*）macaques. Although the frequency of TRIMCyp has been well studied in rhesus and pig-tailed macaques, the frequency and prevalence of TRIMCyp in cynomolgus macaques remain to be definitively elucidated. Here, the geographical and genetic diversity of TRIM5α/TRIMCyp in cynomolgus macaques was studied in comparison with their anti-lentiviral activity. It was found that the frequency of

TRIMCyp in a population in the Philippines was significantly higher than those in Indonesian and Malaysian populations. Major and minor haplotypes of cynomolgus macaque TRIMCyp with single nucleotide polymorphisms in the cyclophilin A domain were also found. The functional significance of the polymorphism in TRIMCyp was examined, and it was demonstrated that the major haplotype of TRIMCyp suppressed human immunodeficiency virus type 1（HIV-1）but not HIV-2, whilst the minor haplotype of TRIMCyp suppressed HIV-2 but not HIV-1. The major haplotype of TRIMCyp did not restrict a monkey-tropic HIV-1 clone, NL-DT5R, which contains a capsid with the simian immunodeficiency virus-derived loop between α-helices 4 and 5 and the entire vif gene. These results indicate that polymorphisms of TRIMCyp affect its anti-lentiviral activity. Overall, the results of this study will help our understanding of the genetic background of cynomolgus macaque TRIMCyp, as well as the host factors composing species barriers of primate lentiviruses.

Keywords: HIV, TRIMCyp, cynomolgus macaque

[*1] 京都大学霊長類研究所
[*2] 徳島大学大学院ヘルスバイオサイエンス研究部
[*3] (株)医薬基盤研究所霊長類医科学研究センター
[*4] 大阪大学微生物病研究所

Miura, T.[*1], Shinkai, Y.[*1], Jiang, H.Y.[*1], Iwamoto, N.[*1], Sumi, D.[*1], Taguchi, K.[*2], Yamamoto, M.[*2], Jinno, H., Tanaka-Kagawa, T., Cho, A.K.[*3], Kumagai, Y.[*1]: **Initial Response and Cellular Protection through the Keap1/Nrf2 System during the Exposure of Primary Mouse Hepatocytes to 1,2-Naphthoquinone**

Chem. Res. Toxicol., **24**, 559-567（2011）

Quinones are reactive chemical species that cause cellular damage by modifying protein thiols and/or catalyzing the reduction of oxygen to reactive oxygen species, thereby promoting oxidative stress. Transcription factor Nrf2 plays a crucial role in cellular defense against electrophilic modification and oxidative stress. In studies using 1,2-naphthoquinone（1,2-NQ）as a model quinone, we found that Keap1, the negative regulator of Nrf2, was readily arylated at its reactive thiols by 1,2-NQ. Exposure of primary mouse hepatocytes to 1,2-NQ resulted in the activation of Nrf2 and the upregulation of some of Nrf2's downstream genes. This interaction was further investigated in hepatocytes from Nrf2 knockout mice in which the proteins responsible for the metabolism and excretion of 1,2-NQ are minimally expressed. The chemical modification of cellular proteins by 1,2-NQ was enhanced by Nrf2 deletion, resulting in increased toxicity. However, deletion of the negative regulatory protein, Keap1, drastically reduced the covalent binding by 1,2-NQ and its cellular toxicity. Experiments with chemicals that inhibit the biotransformation and extracellular excretion of 1,2-NQ suggest that 1,2-NQ undergoes detoxification and excretion into the extracellular space predominantly by two-electron reduction and subsequent glucuronidation by NAD(P)H:quinone oxidoreductase 1 and uridine 5'-diphosphate-glucuronosyltransferases, followed by multidrug resistance-associated protein-dependent excretion. These findings suggest that the Keap1/Nrf2 system is essential for the prevention of cell damage resulting from exposure to 1,2-NQ.

Keywords: 1,2-Naphthoquinone, Keap1/Nrf2 system

[*1] University of Tsukuba
[*2] Tohoku University
[*3] University of California

Takayama, N.[*], Iwamoto, N.[*], Sumi, D.[*], Shinkai, Y.[*], Tanaka-Kagawa, T., Jinno, H., Kumagai, Y.[*]: **Peroxiredoxin 6 is a molecular target for 1,2-naphthoquinone, an atmospheric electrophile, in human pulmonary epithelial A549 cells**

J. Toxicol. Sci., **36**, 817-821（2011）

1,2-Naphthoquinone（1,2-NQ）is an electrophile found in the atmosphere, which reacts readily with protein nucleophiles to form a stable protein adduct. Peroxiredoxin 6（Prdx6）is predominantly expressed in lung tissue and functions in antioxidant defense by facilitating the repair of damaged cell membranes via reduction of peroxidized phospholipids. In the present study, human A549 pulmonary epithelial cells were exposed to 1,2-NQ to explore whether 1,2-NQ can bind covalently to Prdx6, thereby disrupting its catalytic activity. Two-dimensional SDS/PAGE followed by western blot analysis with a specific antibody against 1,2-NQ showed that Prdx6 was covalently modified by 1,2-NQ. Using purified human Prdx6, it was found that 1,2-NQ bound covalently to Prdx6 through Cys47, Lys144 and Cys91, resulting in a significant reduction in phospholipase A2 activity. These results suggest that arylation of Prdx6 by 1,2-NQ may, at least in part, be involved in the cellular toxicity induced by 1,2-NQ.

Keywords: 1,2-Naphthoquinone, Peroxiredoxin 6, Phospholipase A2

[*] University of Tsukuba

Xu, J.[*1,4], Sagawa, Y.[*2], Futakuchi, M.[*1], Fukamachi, K.[*1], Alexander, D.B.[*1,4], Furukawa, F.[*3], Ikarashi, Y., Uchino, T., Nishimura, T., Morita, A.[*2], Suzui, M.[*1], Tsuda, H.[*4]: **Lack of promoting effect of titanium dioxide particles on ultraviolet B-initiated skin carcinogenesis in rats**

Food Chem. Toxicol., **49**, 1298-1302（2011）

Titanium dioxide（TiO2）is used in sunscreens and cosmetics as an ultraviolet light screen. TiO2 has carcinogenic activity in the rat lung, but its effect on the skin has not been reported. We examined the promoting/carcinogenic effect of nano-size TiO2 particles using a two-stage skin model. c-Ha-ras proto-oncogene transgenic（Hras128）rats, which are sensitive to skin carcinogenesis, and their wild-type siblings were exposed to ultraviolet B radiation on shaved back skin twice weekly for 10 weeks; then the shaved area was painted with a 100 mg/ml TiO2 suspension twice weekly until sacrifice. All rats were killed at week 52 except for female Hras128 rats which were sacrificed at week 16 because of early mammary tumor development. Skin tumors developed in male Hras128 rats and mammary tumors developed in both sexes of Hras128 rats and in wild-type female rats, but tumor incidence was not different from controls. TiO2 particles were detected in the upper stratum corneum but not in the underlying skin tissue layers. TiO2 particles also did not penetrate a human epidermis model in vitro. Our data suggest that TiO2 does not cause skin carcinogenesis, probably due to its inability to penetrate through the epidermis and reach underlying skin structures.

Keywords: titanium dioxide, skin, carcinogenesis

[*1] Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences

[*2] Department of Dermatology, Nagoya City University Graduate School of Medical Sciences

[*3] Daiyukai Institute of Medical Science, Inc.

[*4] Laboratory of Nanotoxicology Project, Nagoya City University

Uchino, T., Takezawa, T.[*], Ikarashi, Y., Nishimura, T.: **Development of an alternative test for skin sensitization using a three-dimensional human skin model consisting of dendritic cells, keratinocytes and fibroblasts**

AATEX., **16**, 1-8（2011）

In order to evaluate water-insoluble chemicals using the skin model which is more similar to real skin and detectable interaction among three kind cells, we established a test method which is a three-dimensional human skin model consisting of normal fibroblasts, normal keratinocytes and normal dendritic cells utilizing a collagen vitrigel membrane（VG-KDF-Skin）. Nine sensitizers and five non-sensitizers were then examined. After 24 hr, the amount of IL-1α and IL-4 release was measured, and then positive/negative outcomes were evaluated（VG-KDF-Skin method）. The accuracy, sensitivity and specificity of positive/negative outcomes of the VG-KDF-Skin method, whose indicator is IL-4 release vs. local lymph node assay（LLNA）, were 93%, 89% and 100%, respectively. The accuracy, sensitivity and specificity of the VG-KDF-Skin method, whose indicator is IL-1α release vs. LLNA, were 50%, 56% and 40%, respectively. In order to study the possibility of applying an established test method to cosmetic products such as milky lotion and cream, two model cosmetic samples containing a typical skin sensitizer［2,4-dinitrochlorobenzene（DNCB）］were made and IL-4 release evaluated. Significant IL-4 release was induced. These results suggest that it is possible the VG-KDF-Skin-method using IL-4 as an indicator for skin sensitization potential would be useful for evaluating the skin sensitization potential of chemicals and cosmetic products.

Keywords: skin sensitization test, 3D human skin model, collagen vitrigel membrane

[*] National Institute of Agrobiological Sciences

Kubota, R., Tahara, M., Shimizu, K., Sugimoto, N., Hirose, A., Nishimura, T.: **Time-dependent variation in the biodistribution of C_{60} in rats determined by liquid chromatography－tandem mass spectrometry**

Toxicol. Lett., **206**, 172-177（2011）

We examined the biodistribution of C_{60} in rats after tail vein administration using LC-MS/MS. C_{60} was detected in various tissues, such as brain, kidneys, liver, lungs, and spleen of rats. On the other hand, no C_{60} was found in blood. The highest C_{60} concentration was observed in the lungs, followed by spleen, liver, kidneys, and brain. These results suggested that C_{60} injected in the tail vein could be filtered by lung capillary vessels and accumulate in the lungs prior to being distributed to other tissues. Moreover, C_{60} not being detected in the blood indicates that clearance of C_{60} from the blood by filtration might effectively occur in the lungs. The time-dependent variation in the biodistribution of C_{60} was evaluated. A time-dependent decrease in C_{60} concentrations was observed in all tissues, except spleen. Moreover, a decreasing trend of C_{60} levels differed among tissues, which could be due to differences in accumulation. These results suggest that

unmodified C_{60} and/or C_{60} metabolites by metabolic enzymes could be excreted into feces and/or urine. In further studies, the metabolic and excretion pathways of C_{60} should be evaluated to understand the toxicokinetics of C_{60}.

Keywords: fullerenes, tissue distribution, LC-MS/MS

田原麻衣子，杉本直樹，大槻　崇，多田敦子，穐山浩，合田幸広，西村哲治：**定量分析値の信頼性確保のためのqNMRを用いた市販試薬の純度決定**

環境化学，**22**，33-41（2012）

In environmental analysis, the commercial reagent and reference material products of analyte compounds are indispensable for chromatography such as GC/MS and LC/MS. However, most of their purities are not certificated traceability to the International System of Units（SI）. Hence the possibility that their obscure purities greatly ruin the reliability of the quantitative value is incontrovertible. In this study, the purities of forty one commercial pesticide reagent products（new or old）were determined by a quantitative analytical method which is traceable to SI using nuclear magnetic resonance（qNMR）. qNMR is a rapid and simple quantitative analysis method and no reference compound of analyte is needed. The purities of ten commercial reagent products among our measured forty one products are different more than 5% to their labeled purities by the manufacturers, and the values were found in 47.9-94.8%. Therefore it consequently seems that the differences between SI traceable purities and labeled purities cause the error of 5.1-50.8% to the quantitative values of analytes. This result represents that qNMR analysis has potential to work as a bridge of SI traceability and the quality control of reagent product using qNMR is greatly important to secure the accuracy of analytical data.

Keywords: quantitative NMR, standard, purity, reliability

鈴木俊成*，小杉有希*，保坂三継*，矢口久美子*，中江　大*，西村哲治，小縣昭夫*：**水環境中の抗インフルエンザウイルス剤の分析法**

東京都健康安全研究センター研究年報，**62**，233-236（2011）

The analytical method for the antiviral medicine oseltamivir（OT）and it's the major active metabolite oseltamivir carboxylate（OC）in the aquatic environment was investigated. OT and OC in the water sample extracted by tandem solid-phase extraction cartridges and were eluted by acetonitrile. The extract was concentrated and analyzed by liquid chromatography with tandem mass spectrometry with reverse-phase octadecylsilyl-silica（ODS）analytical column. The detection limits and recovery rates of OT and OC were 2 and 5 ng/L, and 89% and 61%, respectively. This method could be applied to analysis of drinking water, raw water for water supply, and river water. As for accurate determination of OC, correction by surrogate or standard addition method was necessary.

Keywords: aquatic environment, oseltamivir, oseltamivir carboxylate

---

* 東京都健康安全研究センター

Kawakami, T., Isama, K., Matsuoka, A., Nishimura, T.: **Analysis of phthalic acid diesters, monoesters, and other plasticizers in polyvinyl chloride household products in Japan**

J. Environ. Sci. Health Part A, **46**, 855-864（2011）

The aim of this study was to determine the concentrations of six phthalic acid diesters（PAEs）[di(2-ethylhexyl) phthalate（DEHP）, di-n-butyl phthalate（DBP）, butyl benzyl phthalate（BBP）, diisononyl phthalate（DINP）, di-n-octyl phthalate（DNOP）, and diisodecyl phthalate（DIDP）], two non-phthalic plasticizers [di(2-ethylhexyl) adipate（DEHA）, 2,2,4-trimethyl-1,3-pentanediol diisobutylate（TMPDIB）], and mono 2-ethylhexyl phthalate（MEHP）in polyvinyl chloride（PVC）household products that children often places in their mouths and/or contact with their skin（41 products, 47 samples）in Japan. The detection frequencies of the studied compounds were as follows: DEHP（79%）, DINP-2（13%）, DINP-1（11%）, DBP（8.5%）, DEHA（8.5%）, DIDP（4.3%）, and DNOP（2.1%）. Concentrations of these compounds ranged from 0.021% to 48%. BBP and TMPDIB were not detected in the all samples. Most samples contained DEHP and DINP at high concentrations over 0.1%. High concentrations of PAEs were detected in PVC household products that appear appealing to children and can possibly be licked and chewed by them. Di(2-ethylhexyl) terephtalete, diisononyl 1,2-cyclohexanedicarboxylic acid, acetyl tributyl citrate, and di(2-ethylhexyl) 4-cyclohexene-1,2-dicarboxylate used as substitute plasticizers were also detected in several samples. MEHP was present in 70% of the samples, with concentrations ranging from trace amounts to 140 μg/g. The ratios of MEHP against DEHP were 6.2×10^{-4} to 1.6×10^{-1}%. MEHP in the household products investigated in this study was most probably an impurity in DEHP. The high concentrations of PAEs detected in products that children often place in their mouth reveal the importance of replacing plasticizers in

common household products, and not just children's toys, with safer alternatives.

Keywords: Phthalic acid diester and monoester, polyvinyl chloride, household products

Kawakami, T., Isama, K., Matsuoka, A., Nishimura, T.: **Determination of dimethyl fumarate and other fumaric and maleic acid dieters in desiccants and consumer products in Japan**

J. Health Sci., **57**, 236-244（2011）

Recently, many contact dermatitis cases related to leather furniture and footwear containing dimethyl fumarate（DMF）as an anti-mold agent have been reported in European countries. We investigated the concentrations of DMF and several fumaric and maleic acid diesters in desiccants and household products（footwear and rack）enclosed with a desiccant sachet in Japan. We sorted the product samples by material, and analyzed the product parts that can come into contact with the skin of consumers. Twenty-one desiccant samples and eighteen product samples（seven footwear products and one rack product）were analyzed. DMF was detected in the range of 0.11 to 2.3 mg/kg in two desiccant samples and three product samples（different parts of one product）. The DMF concentrations detected in this study exceeded the value regulated by the EU（0.1 mg/kg）; the concentration of one desiccant sample was exceeded 1.0 mg/kg which showed a strong reaction in the patch tests in a precious study. The notes printed on the sachets of the desiccant samples containing DMF read "mold-proof desiccant" and "do not eat" in one case and merely "do not eat" in the other case. DMF has strong sensitization and irritation activities; hence, it is necessary to analyze more samples to prevent DMF-related contact dermatitis in Japan. Dibutyl maleate（DBM）was detected in the rack product and enclosed desiccant; its concentration ranged from 29 to 720 mg/kg. DBM may be a constituent of the adhesive used for the rack. Further investigation is necessary to verify the cross-reaction of DBM with DMF.

Keywords: dimethyl fumarate, contact dermatitis, mold-proof

伊佐間和郎，河上強志，西村哲治：**小児が誤飲する可能性のある合成樹脂製家庭用品からの有害8元素の溶出**

薬学雑誌，**131**，1135-1140（2011）

Harmful elements are used as stabilizers and colorants in synthetic resin products. Accidental ingestion of harmful elements from such synthetic resins by infants is a dangerous health hazard. The Japanese Food Sanitation Law and the International Standard ISO 8124-3 "Safety of toys-Part 3: Migration of certain elements" control the levels of migrated harmful elements, such as lead or cadmium, from infants toys. However, the levels of migrated harmful elements from household products that are not infants toys are not controlled, since they are not covered by the law or standard. Therefore, we investigated the level of eight harmful elements（antimony, arsenic, barium, cadmium, chromium, lead, mercury and selenium）migrated from household products made of synthetic resin that infants may swallow by mistake. The extraction test of ISO 8124-3: 2010 was executed in 135 products（total 150 specimens）, and the concentration of these elements was measured by inductively coupled plasma mass spectroscopy（ICP-MS）. As a result, 1810 mg/kg and 1660 mg/kg of lead, exceeding the maximum acceptable level of the ISO standard, migrated from two products. In addition, lead and/or chromium at levels more than 1/10 of the maximum acceptable levels of the ISO standard migrated from four products. Household products that infants may swallow by mistake should ideally not release harmful elements such as lead and chromium.

Keywords: household product, harmful element, synthetic resin

齊藤静夏，根本　了，松田りえ子：**LC-MS/MSによる農産物中のピンドン分析法**

食品衛生学雑誌，**52**，237-243（2011）

A sensitive and selective analytical method for the determination of pindone in agricultural products by LC-MS/MS was developed. Pindone was extracted with acetone, and an aliquot of the crude extract was re-extracted with hexane. For lipid-rich samples, the crude extract was further cleaned up by acetonitrile-hexane partitioning. The extract was cleaned up on a tandem graphitized carbon-silica gel column. For brown rice, soybean, and tea, PSA column cleanup was added prior to LC-MS/MS determination. Average recoveries of pindone from brown rice, soybean, potato, spinach, cabbage, apple, orange, tomato, cucumber, and tea fortified at 0.001 mg/kg were 81-93%, and the relative standard deviations were 2-7%. The limit of quantitation（S/N ≥ 10）of the developed method was 0.001 mg/kg for all the tested agricultural products.

Keywords: pindone, rodenticide, LC-MS/MS

齊藤静夏，坂井隆敏，根本　了，松田りえ子：**LC-MS/MSによる畜水産物およびはちみつ中の4-ヒドロ**

キシクマリン系殺鼠剤分析法

食品衛生学雑誌，**52**，244-250（2011）

A sensitive and selective method for the determination of 4-hydroxycoumarin-type rodenticides（warfarin, coumatetralyl, bromadiolone, and brodifacoum）in animal products, fishery products, and honey was developed. 4-Hydroxycoumarin rodenticides were extracted with acidified acetone, and the crude extract was purified by liquid-liquid partitioning followed by PSA column cleanup. Gradient liquid chromatographic separation was performed by using an Inertsil ODS-4 column, with methanol and water containing ammonium acetate as the mobile phase. Detection was carried out on a tandem mass spectrometer with electrospray ionization in the negative mode. Average recoveries from bovine muscle, bovine liver, bovine fat, swine muscle, salmon, eel, freshwater clam, egg, milk, and honey spiked at 0.0005-0.001 mg/kg were in the range of 79-108%, and the relative standard deviations were 2-8%. The limits of quantitations of the developed method were 0.0005 mg/kg for brodifacoum, 0.001 mg/kg for warfarin, coumatetralyl, and bromadiolone.

Keywords: 4-hydroxycoumarin, rodenticide, LC-MS/MS

青柳光敏*，新山和人*，根本　了：**LC/MSによる畜水産物中のクロフェンセットの分析法**

食品衛生学雑誌，**52**，156-160（2011）

畜水産物中のクロフェンセットをLC/MSを用いて分析する方法を検討した．試料にヘキサンを加えて脂肪を溶解した後，含水アセトニトリルでクロフェンセットを抽出し，抽出液をC18ミニカラムで精製した．溶出液を濃縮後，1%炭酸水素ナトリウムを含む10%塩化ナトリウム溶液を加え，酢酸エチルで洗浄後，塩酸で酸性とし，酢酸エチルに転溶した．溶媒を除去後，残留物を水－メタノール（7：3）に溶解してLC/MSで測定した．畜水産物10種類の試料からの回収率は77.8～97.8%（相対標準偏差0.6～5.8%）と良好な結果が得られた．クロフェンセット0.01 mg/kgを添加した各試料のクロマトグラムでは，クロフェンセットのピークはS/N>10であり，また，定量を妨害するピークも認められなかった．

Keywords: clofencet, animal and fishery product, LC-MS

* 北海道立衛生研究所

Nakamura, M.*, Furumi, Y.*, Watanabe, F.*, Mizukoshi, K.*, Taniguchi, M.* and Nemoto, S.: **Determination of Carbendazim, Thiophanate, Thiophanate-methyl and Benomyl Residues in Agricultural Products by Liquid Chromatography-Tandem Mass Spectrometry**

Food Hyg. Saf. Sci., **52**, 148-155（2011）

A simple and reliable liquid chromatographic-tandem mass spectrometric（LC-MS/MS）method was developed for carbendazim（MBC）, thiophanate（TE）, thiophanate-methyl（TM）and benomyl（BM）in agricultural products. These compounds were extracted from agricultural products with methanol after addition of sodium A-ascorbate. BM was hydrolyzed to MBC during the extraction with methanol. TE and TM were cyclized to ethyl 2-benzimidazole carbamate（EBC）and MBC by refluxing at 120˚C for 30 min with copper acetate in 50% acetic acid. MBC and EBC were cleaned up by an n-hexane wash and extraction with ethyl acetate and determined by LC-MS/MS. The mean recoveries from 10 agricultural products were in the range of 75.8−100.0%, and the relative standard deviations of 5 experiments were in the range of 1.5−9.2% at concentrations equal to the maximum residue limits（MRLs）. The calibration curves were made by using commercial MBC and EBC as reference analytical standards without refluxing. The quantification limits were 0.01 mg/kg（as MBC）, which is the uniform limit in the positive list system for agricultural chemical residues in foods in Japan.

Keywords: carbendazim, agricultural products, LC-MS/MS

* Japan Food Research Laboratories

Kojima, H.*1, Takeuchia, S.*1, Tsutsumi, T., Yamaguchi, K.*2, Anezaki, K.*2, Kubo, K.*2,3, Iida, M.*4, Takahashi, T.*1, Kobayashi, S.*1, Jin, K.*1, Nagai, T.*1: **Determination of dioxin concentrations in fish and seafood samples using a highly sensitive reporter cell line, DR-EcoScreen cells**

Chemosphere, **83**, 753-759（2011）

There is a strong need for the development of relatively rapid and low-cost bioassays for the determination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans（PCDD/Fs）, and dioxin-like polychlorinated biphenyls（dl-PCBs）in environmental and food samples. In this study, we applied a reporter gene assay using DR-EcoScreen cells（DR-cell assay）, which is highly sensitive to dioxins, to the determination of PCDD/Fs and dl-PCBs in fish and seafood samples. The PCDD/Fs and dl-PCBs were extracted from homogenated samples（10 g）of 30 fish and shellfish, purified by clean-up procedure using a multilayered silica gel column and an alumina column, and applied to DR-cell assay. Interestingly, the bioanalytical equivalent（BEQ）values obtained from the DR-cell assay［<0.1～5.4 pg BEQ g^{-1} wet weight（ww）］were closely correlated with the toxicity

equivalent（TEQ）values from conventional high-resolution gas chromatography/high-resolution mass spectrometry（HRGC－HRMS）analysis（r^2 = 0.912）, and the slope of regression line was 0.913. Therefore, we multiplied the BEQ values from the DR-cell assay by a conversion coefficient（1.095, the reciprocal of 0.913）to approximate the TEQ values from the HRGC － HRMS analysis. Furthermore, we used this DR-cell assay to perform a prescreening test of PCDD/Fs and dl-PCBs in 16 fish and seafood samples purchased from a supermarket, revealing that a sample from the fatty flesh of a bluefin tuna exceeded 8 pg TEQ g^{-1} ww（the European Union-tolerance limit）. Taken together, these results suggest that the DR-cell assay might be applicable as a rapid and low-cost prescreening method to determine dioxin levels in fish and seafood samples.

Keywords: dioxin, fish, reporter gene assay

[*1] Hokkaido Institute of Public Health
[*2] Hokkaido Institute of Environmental Sciences
[*3] Hokkaido University
[*4] Otsuka Pharmaceutical Company, Ltd.

齊藤静夏，坂井隆敏，根本　了，松田りえ子：**LC-MS/MSによる畜水産物およびはちみつ中のピンドン分析法**

食品衛生学雑誌，**52**，294-298（2011）

A sensitive and selective analytical method for the determination of the rodenticide pindone in animal products, fishery products, and honey by LC-MS/MS was developed. Pindone was extracted with acidified acetone, and the crude extract was purified by liquid-liquid partitioning, followed by silica gel and ODS column chromatography. LC separation was performed on an ODS column with methanol/water containing ammonium acetate as the mobile phase, and detection was carried out using tandem mass spectrometry（MS/MS）with electrospray ionization（ESI）in the negative mode. The average recoveries from fortified bovine muscle, bovine liver, bovine fat, chicken muscle, salmon, eel, freshwater clam, egg, milk, and honey spiked at 0.001 mg/kg were in the range of 76-92%, and the relative standard deviations were 4-8%. The limit of quantitation（S/N≥10）of the developed method was 0.001 mg/kg for all the tested foods.

Keywords: pindone, rodenticide, LC-MS/MS

石原三知代[*]，藤田和弘[*]，伊藤裕信[*]，松田高博[*]，八津川洋一[*]，中村宗知[*]，坂井隆敏，根本　了：**LC-MS/MSによる畜水産物中のベダプロフェンの定量**

食品衛生学雑誌，**52**，304-308（2011）

LC-MS/MSによる畜水産物中のベダフロフェン（VPF）の分析法を開発した．試料中から酸性アセトンでVPFを抽出し，この抽出液に塩化ナトリウム溶液を加えて酢酸エチルに転溶した．精製は弱陰イオン交換カートリッジ（Bond Elut DEA）を用いて行った．測定条件として，分析カラムはC18，移動相はアセトニトリル−0.0025 mol/Lギ酸溶液（3：2），イオン化モードはESIのネガティブモードを用いた．検量線は，0.001～0.1 μg/mLの範囲で良好な直線性を示した．馬筋肉，牛筋肉・肝臓・脂肪，さけ，うなぎ，しじみ，牛乳，鶏卵および蜂蜜（そば蜜）の10試料を用いて添加回収実験を行った結果，平均回収率は72～94%，併行精度（RSD%）は1.1～2.0%の良好な結果が得られた．本法による定量限界は，0.001～0.007 μg/gであった．

Keywords: vedaprofen, livestock product, LC-MS/MS

---

[*] （財）日本食品分析センター

He, G.[*], Tsutsumi, T., Zhao, B.[*], Baston, D.S.[*], Zhao, J.[*], Heath-Pagliuso, S.[*], Denison, M.S.[*]: **Third-generation Ah receptor－responsive luciferase reporter plasmids: Amplification of dioxin-responsive elements dramatically increases CALUX bioassay sensitivity and responsiveness**

Toxicological Sciences, **123**, 511-522（2011）

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin（TCDD, dioxin）and related dioxin-like chemicals are widespread and persistent environmental contaminants that produce diverse toxic and biological effects through their ability to bind to and activate the Ah receptor（AhR）and AhR-dependent gene expression. The chemically activated luciferase expression（CALUX）system is an AhR-responsive recombinant luciferase reporter gene-based cell bioassay that has been used in combination with chemical extraction and cleanup methods for the relatively rapid and inexpensive detection and relative quantitation of dioxin and dioxin-like chemicals in a wide variety of sample matrices. Although the CALUX bioassay has been validated and used extensively for screening purposes, it has some limitations when screening samples with very low levels of dioxin-like chemicals or when there is only a small amount of sample matrix for analysis. Here, we describe the development of third-generation（G3）CALUX plasmids with increased numbers of dioxin-responsive elements, and stable transfection of these new plasmids into mouse hepatoma（Hepa1c1c7）cells has produced novel am-

plified G3 CALUX cell bioassays that respond to TCDD with a dramatically increased magnitude of luciferase induction and significantly lower minimal detection limit than existing CALUX-type cell lines. The new G3 CALUX cell lines provide a highly responsive and sensitive bioassay system for the detection and relative quantitation of very low levels of dioxin-like chemicals in sample extracts.

Keywords: dioxin, CALUX, Ah receptor

* Department of Environmental Toxicology, University of California, Davis

Tsutsumi, T., Todoriki, S.*, Nei, D.*, Ishii, R., Watanabe, T., Matsuda, R.: **Detection of Irradiated Food Using 2-Alkylcyclobutanones as Markers: Verification of the European Committee Standardization Method EN1785 for the Detection of Irradiated Food Containing Lipids**

Food Hyg. Saf. Sci., **52**, 321-329 (2011)

Alkylcyclobutanones (ACBs) are specific radiolytic products in irradiated lipid-containing food and can be used to detect irradiation of foodstuffs. EN1785, a European Committee standardization method, can detect 2-dodecylcyclobutanone (DCB) and 2-tetradecylcyclobutanone (TCB), which are ACBs, using GC/MS, thereby determining if foodstuffs have been irradiated. In this study, the performance of EN1785 as a qualitative test in a single laboratory was evaluated and its applicability to beef, pork, chicken and salmon was verified. In the performance evaluation test, lipids extracted from unirradiated food using the Soxhlet extraction method were used as negative samples; negative samples, to which DCB and TCB were added at 0.05 μg/g lipid (equivalent to the amount generated in food when irradiated at 0.5 kGy or more) were used as positive samples. For each food type examined, 4 negative and 16 positive samples were analyzed by EN1785 to verify the method's ability to detect irradiation. All of the negative samples were determined negative and all of the positive samples were determined positive. Therefore, it was shown that the method was able to detect irradiation in beef, pork chicken and salmon, irradiated at 0.5 kGy or higher. Next, to confirm the detecting ability of verified EN1785, the same types of food examined above, both unirradiated and irradiated (0.5－4 kGy), were analyzed by the method. All of the unirradiated samples were determined negative and all of the irradiated samples were determined positive. In a laboratory different from the one where aforementioned evaluation was conducted, a performance evaluation test was performed to verify EN 1785. Blind coded samples, including unirradiated and irradiated samples, were then analyzed in the laboratory. Ten samples (2 unirradiated and 8 irradiated samples) were analyzed for each type of food and the verified method was found to be 100% accurate. Even after the irradiated foodstuffs were frozen for 6－12 months, it was still possible to determine whether the foodstuffs had been irradiated or not using the EN1785 method.

Keywords: irradiated food, 2-alkylcyclobutanone, GC/MS

* National Food Research Institute, NARO

堤　智昭，石井利華，高附　巧，松田りえ子：**豆類中のシアン化合物分析法の性能評価と豆類中のシアン化合物の実態調査**

食品衛生学雑誌，**52**，370-375（2011）

水蒸気蒸留―ピリジンカルボン酸・ピラゾロン法によるシアン化合物分析法の性能評価を行った．分析の目的は，豆類中のシアン化合物の規格への適合判定とした．水蒸気蒸留液のpH調整が真度に影響することを見いだし，最適なpHを6付近と設定した．シアン化物イオンの定量下限の目標値を5 mg/kgとし，定量下限濃度およびその2倍濃度を5種類の豆に添加して性能評価を行った．真度は78～90%，室内精度はRSD 1.7～6.0% であった．評価した分析法を用いて，国内で流通する豆類50試料中のシアン化合物量を測定した結果は，すべて定量下限である5 mg/kg未満であった．

Keywords: cyanogen, pyridine carbonate-pyrazolone method, bean

上野英二*，大野春香*，渡邉美奈恵*，大島晴美*，三上栄一*，根本　了，松田りえ子：**LC-MSによる畜水産物中のスピノサドの分析**

食品衛生学雑誌，**52**，330-335（2011）

畜水産物中のスピノサドの活性成分てあるスピノシンAおよびスピノシンDを定量するための分析法を検討した．牛筋肉，うなぎ，はちみつなど11種類の試料(5～20 g)に，1 mol/Lリン酸水素二カリウム水溶液を加えて，アセトン-ヘキサンでホモジナイズ抽出し，多孔性ケイソウ土カラムを用いたオンカラム液-液分配法，次いでSAX/PSA連結ミニカラムクロマトグラフィーにより脱脂・精製したのち，ESIポジティブ-SIMモードLC-MSで測定した．回収率は0.01 μg/g添加で76.1～93.8%(RSD≦8.7%)，0.05 μg/g添加で75.1～104.1%(RSD≦8.6%)と良好であった．

Keywords: spinosad, animal and fishery product, LC-MS

* 愛知県衛生研究所

Watanabe, T., Maitani, T.*, Matsuda, R.: **Analysis of trans-Fat Levels in Total Diet and One-Serving Samples Using the Verified GC-Method and Estimation of the Intake in Japan**

Food Hyg. Saf. Sci., **52**, 167-177（2011）

In Japan, discussions on the regulation and labeling of trans-fat（TF）have under way for several years in the Food Safety Commission and the Consumer Affairs Agency. However, administrative measures for TF have not yet been taken, partly because of the insufficiency of scientific data in Japan. To provide data about the TF intake by Japanese, we determined the levels of TF contained in total diet samples and in food samples that were served as individual meals（one-serving samples）. We analyzed 5 groups of total diet samples prepared in 11 regions throughout Japan, and 5 categories of one-serving samples using the GC-method after verifying its performance. The estimated daily intake of TF based on the analytical results of the total diet samples was around 500 mg and no significant difference was observed in the intake of the TF among the 11 surveyed regions. On the other hand, many one-serving samples classified into “hamburger”, “pizza” and “Western food” categories contained more than 500 mg of TF per serving, the standard value in the labeling regulation in the United States. If these one-serving meals are taken to represent one meal out of 3 in a day, the intake of TF can easily be expected to exceed the daily intake estimated through the analysis of the total diet samples.

Keywords: trans-fat, estimation of intake, total diet study

---

* University of Shizuoka

Akiyama, H., Sakata, K., Makiyama, D., Nakamura, K., Teshima, R., Nakashima, A.*1, Ogawa, A.*2, Yamagishi, T.*3, Futo, S.*4, Mano, J.*5, Oguchi, T.*5 and Kitta, K.*5: **Inter-laboratory Validation Study of Individual Kernel Detection Method for Genetically Modified Maize**

J. AOAC. Int., **94**, 1540-1547（2011）

In many countries, the labeling of grains, feed and foodstuff is mandatory if the genetically modified（GM）organism content exceeds a certain level of the approved GM varieties. We previously developed an individual kernel detection system consisting of grinding individual kernels, DNA extraction from the individually ground kernels, GM detection using multiplex real-time PCR, and GM event detection using multiplex qualitative PCR to analyze the precise comingling level and varieties of GM maize in real sample grains. We performed the inter-laboratory study of the DNA extraction with multiple ground samples, multiplex real-time PCR detection and multiplex qualitative PCR detection to evaluate its applicability, practicability and ruggedness for the individual kernel detection system of GM maize. DNA extraction with multiple ground samples, multiplex real-time PCR and multiplex qualitative PCR were evaluated by five laboratories in Japan, and all results from these laboratories were consistent with the expected results in terms of the comingling level and event analysis. Thus, the DNA extraction with multiple ground samples, multiplex real-time PCR and multiplex qualitative PCR for the individual kernel detection system is applicable and practicable in a laboratory to regulate the comingling level of GM maize grain for GM samples, including stacked GM maize.

Keywords: multiplex real-time PCR, genetically modified maize, detection

---

*1 Hiroshima Prefectural Institute of Public Health and Environment

*2 Yokohama Quarantine Station, Center for Inspection of Imported Foods

*3 Kobe Quarantine Station, Center for Inspection of Imported Foods and Infectious Diseases

*4 FASMAC Co., Ltd.

*5 National Food Research Institute

Takabatake, R.*1, Akiyama, H., Sakata, K., Onishi, M.*2, Koiwa, T.*3, Futo, S.*2, Minegishi, Y.*4, Teshima, R., Furui, S.*1 and Kitta, K.*1: **Development and Evaluation of Event-Specific Quantitative PCR Method for Genetically Modified Soybean A2704-12**

Food Hygiene and Safety Science, **52**, 100-107（2011）

A novel real-time PCR-based analytical method was developed for the event-specific quantification of a genetically modified（GM）soybean event; A2704-12. During the plant transformation, DNA fragments derived from pUC19 plasmid were integrated in A2704-12, and the region was found to be A2704-12 specific. The pUC19-derived DNA sequences were used as primers for the specific detection of A2704-12. We first tried to construct the standard plasmid for A2704-12 quantification using pUC19. However, non-specific signals appeared with both qualitative and quantitative PCR analyses using the specific primers with pUC19 as a template, and we then constructed the plasmid using pBR322. The conversion

factor（Cf）which is required to calculate the genetically modified organism（GMO）amount was experimentally determined for two real-time PCR instruments, the ABI PRISM 7900HT and the ABI PRISM 7500. The determined Cf values were both 0.98 for these two instruments. The quantitative method was validated by a blind test in an inter-laboratory collaborative study. The limit of quantitation for the method was estimated to be 0.1%. The trueness and precision were evaluated as the bias and reproducibility of relative standard deviation（RSDR）, and the determined bias and RSDR values for the method were each less than 20%. These results suggest that the developed method would be suitable for practical analyses for the detection and quantification of A2704-12.

Keywords: event-specific, genetically modified（GM）, real-time PCR

[*1] National Food Research Institute
[*2] FASMAC Co., Ltd.
[*3] Food and Agricultural Materials Inspection Center
[*4] Nippon Gene Co., Ltd.

Suzuki, A.[*1,2], Pharm, Nguyen, H.D.[*2], Akiyama, H., Nakamura, K. and Kasahara, Y.[*3]: **Remarkable growth variation in a natural Japanese population of pleurocybella porrigens**

Jpn. J. Food Chem. Safety, **18**, 18-24（2011）

In 2004, an outbreak of serious acute encephalopathy exclusively occurred in patients with chronic kidney diseases after the intake of basidiomycetous wood rotting fungus *Pleurocybella porrigens*. The exact factors that induced encephalopathy by this mushroom remain unknown partly due to its extreme slow growth. We attempted to develop media suitable for vegetative growth of *P. porrigens* for application in various fields. Fifteen isolates of *P. porrigens* collected from rotting conifers, *Cryptomeria japonica* and *Pinus densiflora*, in different geographical areas in Japan were cultivated on potato･dextrose agar（PDA）medium; large variation in growth rate and colony features was observed among these isolates. The five isolates with the best growth rates were then cultured in five kinds of liquid media, potato･dextrose（PD）medium, malt extract･yeast extract（MY）medium, potato extract･carrot extract（PC）medium, Amazake medium, and Ohta's medium at 20˚C in the dark. Dry biomasses of the isolates cultured in the liquid media were determined after 8 weeks of static cultivation. Among the tested liquid media, PD medium was the most suitable for biomass growth, followed by Ohta's, MY, Amazake and PC media. The average biomass growth of the isolates cultured in the synthetic medium（Ohta's medium）was 20-92% of that in PD medium. Remarkably large biomass variation was also observed among the isolates cultured on each liquid medium. Mycelia of this mushroom had abortive lateral branching at high frequency which could be one reason why this mushroom grows very slowly. Moreover, the Japanese population of P. porrigens has large variation in vegetative growth. Taken together, elucidation of the possible association between its chemical constituents and the onset of encephalopathy may be possible by culturing isolates with high growth ability on PD medium as a natural medium and Ohta's medium as a synthetic medium.

Keywords: abortive branching, growth variation, Sugihiratake

[*1] Faculty of Education, Chiba University
[*2] Graduate School of Horticulture, Chiba University
[*3] Yamagata Prefectural Institute of Public Health

Sakai, Y.[*1], Kotoura, S.[*2], Yano, T.[*1], Kurihara, T.[*1], Uchida, K.[*1], Miake, K.[*2], Akiyama, H. and Tanabe, S.[*3]: **Quantification of pork, chicken and beef by using a novel reference molecule**

Biosci. Biotechnol. Biochem., **75**, 1639-1643（2011）

A standard plasmid was constructed as a novel reference molecule for use in real-time quantitative PCR assays to verify the identity of beef, pork, chicken, mutton, and horseflesh. The plasmid contained a target domain of the cytochrome b（cyt b）gene and an artificial DNA sequence. The primers CO-F and CO-R, and the probe CO-P were designed specifically to detect the artificial sequence. In the quantification analysis, the calculated R2 values of the standard curves（103−107 copies per reaction）for the 5 species ranged between 0.998 and 0.999. The constructed plasmid enabled a universal method for measuring the copy number of cyt b DNA in minced meat. This method would be a useful procedure for verifying food labels.

Keywords: real-time PCR, reference molecule, meat

[*1] Oriental Yeast Co., Ltd.
[*2] Central Research Institute, Marudai Food Co., Ltd.
[*3] Graduate School of Biosphere Science, Hiroshima University

Takabatake, R.[*1], Koiwa, T.[*2], Kasahara, M.[*2], Takashima, K.[*1], Futo, S.[*3], Minegishi, Y.[*4], Akiyama, H., Teshima, R.,

Oguchi, T.[*1], Mano, J.[*1], Furui, S.[*1] and Kitta, K.[*1]: **Interlaboratory validation of quantitative duplex real-time PCR method for screening analysis of genetically modified maize**

Food Hygiene and Safety Science, **52**, 265-269（2011）

To reduce the cost and time required for routinely performed genetically modified organism（GMO）test, we developed a duplex quantitative real-time PCR method for screening analysis simultaneously targeting an event-specific segment for GA21 and Cauliflower Mosaic Virus 35S promoter（P35S）segment. To confirm the validity of the method, an interlaboratory collaborative study was conducted. In the collaborative study, conversion factors（Cfs）which are required to calculate GMO amount were first determined for two real-time PCR instruments, the ABI PRISM 7900HT and the ABI PRISM 7500. Then, a blind test was conducted. The limit of quantitation for both GA21 and P35S was estimated to be 0.5% or less. The trueness and precision were evaluated as the bias and reproducibility of relative standard deviation（RSDR）, and the determined bias and RSDR were each less than 25%. We believe the developed method would be useful for the practical screening analysis of GM maize.

Keywords: screening, quantification, genetically modified（GM）

[*1] National Food Research Institute
[*2] Food and Agricultural Materials Inspection Center
[*3] FASMAC Co., Ltd.
[*4] Nippon Gene Co., Ltd.

Mano, J.[*1], Yanaka, Y.[*1], Ikezu, Y.[*1], Onishi, M.[*2], Futo, S.[*2], Minegishi, Y.[*3], Ninomiya, K.[*4], Yotsuyanagi, Y.[*4], Spiegelhalter, F.[*5], Akiyama, H., Teshima, R., Hino, A.[*1], Naito, S.[*1], Koiwa, T.[*1], Takabatake, R.[*1], Furui, S.[*1] and Kitta, K.[*1]: **Practicable group testing method to evaluate weight/weight GMO content in maize grains**

J. Agric. Food Chem., **59**, 6856-6863（2011）

Because of the increasing use of maize hybrids with genetically modified（GM）stacked events, the established and commonly used bulk sample methods for PCR quantification of GM maize in non-GM maize are prone to overestimate the GM organism（GMO）content, compared to the actual weight/weight percentage of GM maize in the grain sample. As an alternative method, we designed and assessed a group testing strategy in which the GMO content is statistically evaluated based on qualitative analyses of multiple small pools, consisting of 20 maize kernels each. This approach enables the GMO content evaluation on a weight/weight basis, irrespective of the presence of stacked-event kernels. To enhance the method's user-friendliness in routine application, we devised an easy-to-use PCR-based qualitative analytical method comprising a sample preparation step in which 20 maize kernels are ground in a lysis buffer and a subsequent PCR assay in which the lysate is directly used as a DNA template. This method was validated in a multilaboratory collaborative trial.

Keywords: Genetically modified organism（GMO）, detection, group testing

[*1] National Food Research Institute
[*2] FASMAC Co., Ltd.
[*3] Nippon Gene Co. Ltd.
[*4] Shimadzu Corporation
[*5] Genescan, Inc.

久保田浩樹，箕川　剛，小関良宏[*]，佐藤恭子，穐山浩：**食品添加物ステアロイル乳酸ナトリウムのLC-MSによる組成分析**

食品衛生学雑誌，**53**，14-18（2012）

国内で流通する食品添加物ステアロイル乳酸ナトリウム（SSL）の成分について，TLC及びLC-MSを用いて質的，量的に解析した．ステアロイル乳酸（SL）及びステアロイルラクトイル乳酸（SLL）の標準試薬は，TLC及びシリカゲルクロマトグラフィーを用いてSSLより単離精製し実験に用いた．SSLの成分は，乳酸が8.4%，ステアリン酸が15%，SLが57%，SLLが13%であった．本解析で得た成分比から求めたSSL中の乳酸量は，JECFAの総乳酸試験で求めた総乳酸量の実測値と近似した．

Keywords: sodium stearoyl lactylate, TLC, LC-MS

[*] 東京農工大学

大槻　崇，川崎洋子，久保田浩樹，並木達也[*1]，飯塚太由[*1]，塩谷典子[*2]，吉井信彦[*2]，小原礼子[*3]，田中麻紀子[*3]，小林　尚[*4]，佐藤恭子，河村葉子：**鮮魚中の一酸化炭素分析法の改良**

食品衛生学雑誌，**52**，130-134（2011）

通知で規定されている鮮魚中の一酸化炭素（CO）分析法のうちA法（通知A法）は，試料を多量に必要とし，また試料気相調製時に鮮魚中のCOの一部が散逸するなどの問題が指摘されている．そこで本研究では，これらの問題点の解消ならびに現在の通知法の改正を目指して，宮崎らの方法を一部変更した分析法（改良法）の適用性を検討した．また，改良法を用いて通知で規制されている

マグロ，ブリ，ハマチおよびティラピア中のCO濃度のバックグラウンド値を調査した．その結果，改良法は，試料気相調製時のCOの散逸抑制，試料量の低減，操作の簡便性の点で通知A法より優れており，鮮魚中のCO分析に適用可能であることが確認された．また4機関共同で実施した各鮮魚中のCO濃度のバックグラウンド値については，改良法が通知A法と比較してCOの回収率が向上することから，特にCO未処理のティラピア中のCO濃度が現在の規制値を上まわることが判明した．従って，改良法を今後新たな鮮魚中のCO分析法として適用する場合には，ティラピアの規制値の変更が必要であると考えられた．

Keywords: carbon monoxide, GC-FID

[*1] (財)食品環境検査協会
[*2] (財)日本食品分析センター
[*3] (財)日本冷凍食品検査協会
[*4] (財)食品開発分析センター SUNATEC

河﨑裕美，髙木繁行，大西有希子，浦嶋幸雄[*1]，関根百合子[*2]，佐藤睦実[*2]，田口信夫[*3]，西岡千鶴[*4]，安永恵[*4]，川原るみ子[*5]，酒井國嘉[*5]，古謝あゆ子[*6]，佐藤恭子，穐山　浩，河村葉子：**マーケットバスケット方式による食品添加物の一日摂取量の推定(2006-2008年度)**

日本食品化学学会誌，**18**，150-162(2011)

甘味料8種類，保存料9種類，着色料14種類，酸化防止剤9種類，防かび剤4種類，製造用剤等3種類について成人(20歳以上)の一日摂取量をマーケットバスケット方式により推定した．推定一日摂取量が最も多かったのはD-ソルビトール(452 mg/人/日)，次いでリン酸化合物(リンとして233 mg/人/日)，D-マンニトール(92 mg/人/日)であった．一日摂取許容量に対する推定一日摂取量の割合(ADI比)はトコフェロール(17.2%)で最も高く，次いでリン酸化合物(対最大耐容一日摂取量比，リンとして6.7%)であったが，その他の食品添加物は1.1%以下であった．

Keywords: market basket method, food additives, daily intake

[*1] 札幌市衛生研究所
[*2] 仙台市衛生研究所
[*3] 東京都健康安全研究センター
[*4] 香川県環境保健研究センター
[*5] 長崎市保健環境試験所
[*6] 沖縄県衛生環境研究所

多田敦子，石附京子，小山朗夫[*1]，深井俊夫[*2]，秋山卓美，山崎　壮，河村葉子：**既存添加物クワ抽出物の成分組成に基づく基原植物の検討**

食品衛生学雑誌，**52**，258-264(2011)

既存添加物クワ抽出物は，天然由来の製造用剤として，平成8年に既存添加物名簿に収載された食品添加物である．既存添加物製品の基原植物の確認は，品質や安全性確保の上から極めて重要であるが，クワ抽出物の基原はクワ科クワ(*Morus bombycis* Koidz.)の根茎の皮と記載されているものの，実際の製品がどのクワ品種の成分組成に一致または類似するのか確認されていなかった．本研究では，数種の国内クワ栽培品種の標準植物の根皮乾燥物から抽出物を調製して成分組成を調べ，既存添加物クワ抽出物として提供された製品および生薬ソウハクヒ製品の成分組成と比較することにより基原植物の検討を行った．その結果，既存添加物クワ抽出物製品の基原は，定義の記載とは異なり，国内でマグワ*M. alba*とされている栽培品種またはその交雑種と推定された．また，*M. alba*が基原と定義されている中国産クワを原料とする生薬ソウハクヒ製品とは成分組成が異なった．また，LC/MSでのピーク面積を説明変数として行った主成分分析の結果でも同様の結果が得られた．なお，本研究実施以後，クワ抽出物は，平成23年に既存添加物名簿から消除される品目の1つと確定された．

Keywords: mulberry bark extract, food additive, *Morus bombycis*

[*1] (独)農業生物資源研究所
[*2] 横浜薬科大学

Mutsuga, M., Sato, K., Hirahara, Y. and Kawamura, Y.: **Analytical methods for SiO_2 and other inorganic oxides in titanium dioxide or certain silicates for food additive specifications**

Food Additives and Contaminants Part A, **28**, 423-427 (2011)

An analytical method has been developed for the detection of SiO_2 and other oxides in titanium dioxide and certain silicates used in food additives using inductively coupled plasma (ICP) atomic emission spectrometry without hydrofluoric acid. SiO_2 and other oxides in titanium dioxide or certain silicates were resolved by alkali fusion with KOH and boric acid and then dissolved in dilute hydrochloric acid as a test solution for ICP. The recovery of SiO_2 and Al_2O_3 added at 0.1 and 1.0%, respectively, in TiO_2 was 88－104%; coefficient of variation was <4%. The limit of determination of SiO_2 and Al_2O_3 was about 0.08%, and the accuracy of the ICP method was better than that of the Joint FAO/WHO Expert Committee

on Food Additives（JECFA）test method. The recovery of SiO_2 and other oxides in silicates was 95－107% with a coefficient of variation of <4%. Using energy dispersive X-ray fluorescence spectrometry（EDX）with fundamental parameter determination, the content of SiO_2 and other oxide in titanium dioxide and silicate showed good agreement with the ICP results. ICP with alkali fusion proved suitable as a test method for SiO_2, Al_2O_3 and other oxides in titanium dioxide and certain silicates, and EDX proves useful for screening such impurities in titanium dioxide and componential analysis of certain silicates.

Keywords: titanium dioxide, silicate, alkali fusion

六鹿元雄，建部千絵，平原嘉親，河村葉子：**洗浄剤中のメタノール試験法**

食品衛生学雑誌，**53**，28-32（2012）

ヘッドスペースGC法を用いた洗浄剤中のメタノール試験法を確立した．試料1 gに内部標準として2-プロパノールを0.4 mg加え，さらに水を加えて20 mLとした．この試験溶液5 mLをヘッドスペース用バイアルに採り，密封した．60℃で30分間加温後，ヘッドスペースガスをGC-FIDで測定した．試料に1 mg/gのメタノールを添加した際の回収率は95.6～100.6%であり，定量限界は0.1 mg/gであった．本法を用い14種の洗浄剤についてメタノール含有量を調査した結果，2検体から検出され，その量は0.13及び0.27 mg/gであった．

Keywords: detergent, methanol, headspace GC-FID

阿部　裕，六鹿元雄，平原嘉親，河村葉子：**ポリ塩化ビニル製品中の6種のフタル酸エステル試験法**

食品衛生学雑誌，**52**，309-313（2011）

ポリ塩化ビニル（PVC）製品中のフタル酸ビス（2-エチルヘキシル）（DEHP），フタル酸ジブチル（DBP），フタル酸ベンジルブチル（BBP），フタル酸ジイソノニル（DINP），フタル酸ジイソデシル（DIDP）及びフタル酸ジ-*n*-オクチル（DNOP）の試験法を検討した．測定にはGC/MSをSIM条件下で用い，定量イオンとしてDBP，BBP及びDEHPには*m/z* 149，DNOP，DINP及びDIDPには*m/z* 279，293及び307を用いることにより分別定量が可能であった．また，添加回収試験により抽出法及び溶解法は試験溶液の調製法としていずれも有用であることが確認された．一方，GC/MS測定には，試料溶液に混入するPVCのマトリックス効果により測定値がばらつくという問題点があり，それを抑制するためには試験溶液の希釈が有効であった．9機関による共同試験を実施したところ，機関内再現性は良好であったが，一部機関では測定値がばらつくことがあった．したがって本法により合否判定を行うことは難しいものの，試料中のフタル酸エステル含有量を明らかとする方法としては十分な実用性を有すると考えられた．

Keywords: polyvinyl chloride（PVC）, phthalate, collaborative study

阿部　裕，山口未来，六鹿元雄，平原嘉親，河村葉子：**ポリ塩化ビニル製玩具中の可塑剤使用実態**

食品衛生学雑誌，**53**，19-27（2012）

我が国で流通するポリ塩化ビニル（PVC）製玩具101検体の可塑剤使用実態を調査した．指定玩具からは，いずれのフタル酸エステルも検出されず使用は認められなかったが，指定玩具以外の半数以上からフタル酸ビス（2-エチルヘキシル），フタル酸ジイソノニル，フタル酸ジイソブチル，フタル酸ジブチル，フタル酸ジイソデシル，フタル酸ベンジルブチルが検出された．また，フタル酸エステルの代替可塑剤として2,2,4-トリメチル-1,3-ペンタンジオールジイソブチレート，*o*-アセチルクエン酸トリブチル，アジピン酸エステル，ジアセチルラウロイルグリセロールなども検出された．さらに構造解析の結果，国内では今までに報告例がないテレフタル酸ジ（2-エチルヘキシル），クエン酸トリブチル，1,2-シクロヘキサンジカルボン酸ジイソノニル及びネオペンチルグリコールエステル類の含有も認められた．このように，指定玩具に使用される可塑剤はフタル酸エステルから代替可塑剤へ移行しており，その種類も増加していることが明らかとなった．

Keywords: polyvinyl chloride（PVC）, toy, plasticizer

Sakano, C.[*1], Morita, Y.[*1,2], Goto, K.[*1], Yokota, Y.[*1], Annaka, H.[*1], Fujita, M.[*1], Kobatake, S.[*1], Ishioka, T.[*1], Hoshino, T.[*1], Boonmar, S.[*1], Pulsrikarn, C.[*3], Nishina, A.[*4], Kozawa, K.[*1], Yamamoto, S. and Kimura, H.[*1,5]：**Prevalence and genotypeof *Salmonella* Choleraesuis in Gunma Prefecture, Japan**

Thai J. of Veteri. Med., **41**, 321-326（2011）

We studied the prevalence of swine salmonellosis and PFGE genotype of isolates in Gunma Prefecture, Japan. Between 2005 and 2008, swine salmonellosis was confirmed in 430 of 2,707,402（0.02%）swine at slaughterhouses. All isolates were identified as deriving from *Salmonella* Choleraesuis, biotype Choleraesuis（negative for H2S production）. We used 30 bacterial strains from 15 farms that had experienced outbreaks in 2006 and 2007. All strains were susceptible to various antibiotics such as cephems（cefotaxime）, fluoroquinolones（norfloxacin and ciprofloxacin）, and fosfomycin. On the other hand, all strains were resistant to tetracycline

（TC）, and 29 of 30（97%）strains were resistant to streptomycin（SM）. The most predominant profiles were those of SM-TC（26 strains）. During *Bln* I digestion, 30 strains showed 6 profiles on PFGE as G1 to G6, and each profile was assigned into 1 of 4 clusters（I to IV）. The most prevalent profile was G1（22 strains）, followed by G3（3 strains）, and G2（2 strains）. Strains showing the same antimicrobial resistance profiles（SM-TC）and the same PFGE profiles（G1）were isolated from 5 of 15 farms（A to E）during the 2006 and 2007 outbreaks. In conclusion, the prevalence of swine salmonellosis caused by SM-TC resistant-*S*. Choleraesuis biotype Choleraesuis is around 0.02%, as determined by infection rate at pig farms between 2005 and 2008 in Gunma prefecture. S. Choleraesuis usually causes systemic infections in swine and humans and antimicrobial treatment is necessary. The antimicrobial susceptibility of *Salmonella* in swine should be surveyed further.

Keywords: antimicrobial resistance, genotyping, *Salmonella*

[*1] Gunma Prefectural Institute of Public Health and Environmental Sciences
[*2] Tokyo Kasei University
[*3] WHO International Salmonella & Shigella Center, National Institute of Health, Thailand
[*4] Yamagata Prefectural Yonezawa Women's Junior College
[*5] National Institute of Infectious Disease

Okada, Y., Okutani, A., Suzuki, H., Asakura, H., Monden, S., Nakama, A.[*1], Maruyama, T.[*2], Igimi, S.: **Antimicrobial susceptibilities of *Listeria monocytogenes* isolated in Japan**

J .Vet. Med. Sci., **73**（12）, 1681-1684（2011）

The antimicrobial susceptibility of 201 *Listeria monocytogenes* isolates from foods, environments, animals and human patients in Japan was determined. All isolates were susceptible to ampicillin, the first choice of drug for listeriosis treatment, chloramphenicol, dihydrostreptomycin, erythromycin, enrofloxacin, gentamicin, kanamycin, lincomycin, nosiheptide, salinomycin, vancomycin, and virginiamycin. A human strain was resistant to oxytetracycline. The Minimum Inhibitory Concentration（MIC）for 50% of the strains and the MIC for 90% of the strains were comparable in all the isolates. This is the first investigation to compare antibiotic resistances between isolates from foods and isolates from human patients in Japan. The result showed that most of the isolates were susceptible to antibiotics used in this study.

Keywords: antibiotics, *Listeria*, resistance

[*1] Tokyo Metropolitan Institute of Public Health
[*2] Japan Food Hygiene Association

Okada, Y., Monden, S., Igimi, S., Yamamoto, S.: **The Occurrence of *Listeria monocytogenes* in Imported Ready-to-Eat Foods in Japan**

J. Vet. Med. Sci., **74**（3）, 373-375（2012）

Quantitative analyses of *Listeria monocytogenes* in imported ready-to-eat（RTE）foods sold at retail stores in Japan were performed. Of the 77 non-cooked meat products, 6 samples（7.8%）tested positive. The levels of contamination of 4 of the samples were below 100 colony-forming units（CFU）/g, which is the microbiological criterion for *L. monocytogenes* in RTE foods as determined by Codex. However, *Listeria* cells at levels of 100 and 400 CFU/g were detected in a salami sample and a raw ham sample, respectively. All of the 70 cheese samples and the 3 samples made from raw ham and cheese showed negative test results. These results suggest that imported RTE foods are potential sources of the causative agent of listeriosis.

Keywords: contamination, imported foods, *Listeria monocytogenes*

Asakura, H., Kawamoto, K.[*], Okada, Y., Kasuga, F., Makino, S.[*], Yamamoto, S., Igimi, S.: **Intrahost passage alters SigB-dependent acid resistance and host cell-associated kinetics of *Listeria monocytogenes***

Infect. Genet. Evol., **12**, 94-101（2012）

We report that an intrahost genome mutation alters bacterial acid resistance and the abilities for replication/invasion in tissue cell culture, though there were no alterations in pulsed-field gel electrophoresis（PFGE）and ribotyping patterns. Genetic and proteomic analyses revealed a link between acid resistance and SigB（RNA polymerase SigmaB subunit）activity. We found a mutation in the *rsbW* locus, whose product controls the regulation of SigB activity, was a key regulator for the above phenotypic conversion during infection. Our study provides new insight into the potential role of intrahost environment in the process of bacterial evolution.

Keywords: *Listeria monocytogenes*, Acid resistance, SigB

[*] Obihiro University of Agriculture and Veterinary Medicine

Asakura, H., Momose, Y., Kasuga, F.: **Enterohemorrhagic**

***Escherichia coli*- Its control from a viewpoint of Food Safety-**

J. Dis. Res., **6**, 426-434（2011）

This review focuses on the bacteriological nature and epidemics of enterohemorrhagic *Escherichia coli*（EHEC）, a global scourge, from the viewpoint of food safety. Many human EHEC infections are linked to eating undercooked food and untreated water. We are still struggling to control this pathogen in the food chain, so we discuss current knowledge on sources of infection and EHEC distribution and survival mechanisms in foreign environments including the food matrix. We also introduce ways to effectively prevent food-borne EHEC infection.

Keywords: enterohemorrhagic *E. coli*（EHEC）, food outbreak, food safety

Asakura, H., Saito, E.[*1], Momose, Y., Ekawa, T., Sawada, M.[*2], Yamamoto, A.[*1], Hasegawa, A.[*3], Iwahori, J.[*4], Tsutsui, T.[*5], Osaka, K.[*6], Matsushita, T.[*3], Kakinuma, M.[*3], Motoyama, K.[*2], Hayama, Y.[*5], Kitamoto, H., Igimi, S., Kasuga, F.: **Prevalence and growth kinetics of Shiga toxin-producing *Escherichia coli*（STEC）in bovine offal products in Japan**

Epidemiol. Infect., **140**, 655-664（2011）

This study examined the prevalence of Shiga toxin producing *E. coli*（STEC）in various types of these foods. PCR screened 229 bovine offal products for the presence of Shiga toxin（*stx*）gene. Thirty-eight（16.6%）samples were *stx* positive, of which 8 were positive for $rfbE_{O157}$ and 3 were positive for wzy_{O26}. Four O157 and one O26 STEC isolates were finally obtained from small-intestine and omasum products. Generic E. coli contaminating in such offal products competitively inhibited the growth of STEC during detection procedures.

Keywords: Shiga toxin-producing *E. coli*（STEC）, bovine offal

[*1] Hyogo Prefectural Institute of Public Health and Consumer Sciences
[*2] Hitachi East Japan Solutions
[*3] Mitsubishi Research Institute
[*4] Kochi Medical School
[*5] National Institute of Animal Health
[*6] Tohoku University

Hayama, Y.[*1], Yamamoto, T.[*2], Kasuga, F. and Tsutsui, T.[*1]: **Simulation model for *Campylobacter* cross-contamination during poultry processing at slaughterhouses**

Zoonoses and Public Health, **58**, 399-406（2011）

食鳥処理場におけるカンピロバクターの交差汚染について，個々のと体に着目したモデルを構築した.

Keywords: *Campylobacter*, cross-contamination, simulation model, poultry processing

[*1]（独）農業・食品産業技術総合研究機構・動物衛生研究所
[*2] 農林水産省消費安全局

Suzuki, H., Ohtsuka, R.[*], Takeda, M.[*]: **Regional Differences in Gene Expression Profiles of Mouse Peyer's Patches**

Res. J. Immunol., **4**, 19-24（2011）

Few studies have reported the regional differences in mouse Peyer's patches. This study aims to determine whether regional differences exist in the immunological activation status and/or immunological functions of mouse Peyer's patches in the normal state. The most proximal Peyer's patches, the most distal Peyer's patches, and the Peyer's patches nearest to the midpoint were obtained from the mouse small intestine. The gene expression levels in the PPs obtained from different regions were compared using the DNA microarray technique. Of the 187 genes that were expressed differently among the Peyer's patches from different regions, 6 genes were related to immune system process. These findings suggest that the regional differences among Peyer's patches in mice in terms of the immunological activation status and/or immunological functions may be subtle.

Keywords: Peyer's patch, mouse, regional difference

[*] The Institute of Environmental Toxicology

Yoshida, T.[*1], Miyasaka, T.[*1], Azegami, Y.[*1], Uchiyama, Y.[*1], Kasahara, H.[*1], Ueda, H.[*1], Ishii, K.[*2] and Noda, M.: **Investigation of epidemiology and HAV genomes regarding three hepatitis A infections that occurred in April－May, 2010**

Jpn. J. Infect. Dis., **64**, 260-261（2011）

Three hepatitis A cases occurred in April to May, 2010 were molecularepidemiologically analyzed to know epidemiological background such as infection route, common source or epidemiological relatedness between them. One Hapatitis A virus（HAV）strain detected from patient A that had traveled through Korea and Taiwan was genotyped as IIIA. Other HAV strain detected from patients B had a history of consumption

of raw bivalve（Raw salt shsort-necked clams）was genotype IIIA. The sequences of the two IIIA HAV were closely related each other. The rest HAV strain detected from patient C that had traveled to the Philippines was genotyped as IA. The sequence of the IA HAV was similar to HAV strains detected in river water samples in the Philippines.

Keywords: Hepatitis A, molecularepidemiology, genotype

---

*1 長野県環境保全研究所
*2 国立感染症研究所

Ishii, K.*, Kiyohara, T.*, Yoshizaki, S.*, Wakita, T.*, Shimada, T.*, Nakamura, N.*, Nakashima, K.*, Tada, Y.* and Noda, M.: **Epidemiological and genetic analyses of a diffuse outbreak of hepatitis A in Japan, 2010**

J. Clin. Virol., **53**, 219-224（2012）

Hepatitis A virus（HAV）is still one of the most common causative agents of acute hepatitis in Japan. Although a relatively small number of annual acute hepatitis A cases（approximately 100-150, 0.78-1.17 per million）were recently reported, a larger number of cases（346, 2.71 per million）were reported in 2010. We investigated the causes of the 2010 HAV resurgence in Japan by using molecular epidemiological and genetic analyses. HAV specimens were obtained from 61 cases from 22 different prefectures. These viral specimens were genotyped by PCR amplification and sequencing of the VP1/2A region of HAV genome. Phylogenetic analysis revealed that 61 HAV strains could be divided into three genotypes: IA（44 cases）, IB（1 case）and IIIA（16 cases）. The IA genotype consisted of two genomic sub-lineages. The sequences of one of the two IA sub-lineages（corresponding to 31 cases）were very similar, 26 of these 31 isolates had 100% identity. The other IA sub-lineage corresponded to strains endemic to Japan. The sequences of Japanese IIIA strains were similar to those of strains that caused a large epidemic in the Republic of Korea from 2007 to 2009. The resurgence of HAV in 2010 can be attributed to importation of two newly emerged HAV genotypes.

Keywords: Hepatitis A, molecularepidemiology, genotype

---

* 国立感染症研究所

Ishii, K.*, Kiyohara, T.*, Yoshizaki, S.*, Shimada, T.*, Nakamura, N.*, Tada, Y.*, Noda, M. and Wakita, T.*: **Epidemiological and genetic analysis of a diffuse outbreak of hepatitis A in Japan, 2010**

Hepatol. Int., **5**, 204-205（2011）

Hepatitis A virus（HAV）is still one of the most common causative agents of acute hepatitis in Japan. Although a relatively small number of annual acute hepatitis A cases（approximately 100-150, 0.78-1.17 per million）were recently reported, a larger number of cases（346, 2.71 per million）were reported in 2010. We investigated the causes of the 2010 HAV resurgence in Japan by using molecular epidemiological and genetic analyses. HAV specimens were obtained from 61 cases from 22 different prefectures. These viral specimens were genotyped by PCR amplification and sequencing of the VP1/2A region of HAV genome. Phylogenetic analysis revealed that 61 HAV strains could be divided into three genotypes: IA（44 cases）, IB（1 case）and IIIA（16 cases）. The IA genotype consisted of two genomic sub-lineages. The sequences of one of the two IA sub-lineages（corresponding to 31 cases）were very similar, 26 of these 31 isolates had 100% identity. The other IA sub-lineage corresponded to strains endemic to Japan. The sequences of Japanese IIIA strains were similar to those of strains that caused a large epidemic in the Republic of Korea from 2007 to 2009. The resurgence of HAV in 2010 can be attributed to importation of two newly emerged HAV genotypes.

Keywords: Hepatitis A, molecularepidemiology, genotype

---

* 国立感染症研究所

田中俊光*1，横井　一*1，水村綾乃*1，小林圭子*1，木原顕子*1，都竹豊茂*1，中台啓二*1，加曽利東子*1，落合弘章*2，大山照雄*2，西村正樹*2，山本一重*2，野田衛：**生シラスが原因食品と疑われる有症苦情事例について-千葉市**

病原微生物検出情報，**32**，363-364（2011）

2011年5月に千葉市内の高等学校2年生（生徒数8クラス327名）が校外学習を実施後，下痢，発熱，腹痛などの食中毒様症状を呈した．調査の結果，患者便からノロウイルス，サポウイルスおよびアストロウイルスが検出され，原因食品として生シラスが疑われたので，その概要を報告した．

Keywords：生シラス，有症苦情事例，ウイルス性食中毒

---

*1 千葉市環境保健研究所
*2 千葉市保健所

野田　衛，上間　匡，片山和彦*1，岡　智一郎*1，山下和予*1，岡部信彦*1，石丸　歩*2，松岡隆介*2，温泉川肇彦*2，研究協力地方衛生研究所：**食品媒介事例を**

中心としたノロウイルス，サポウイルスの塩基配列情報および疫学情報の共有化の取り組み

病原微生物検出情報，**32**，354-355（2011）

我々はノロウイルス（NoV）等の食品媒介性ウイルスによる広域食中毒事例の探知など，食中毒調査の精度向上に資することを目的として，全国で検出されたNoVおよびサポウイルスの塩基配列情報の共有化を試行的に実施している．昨年度までは13の地方衛生研究所（地研）の協力の下に実施していたが，今年度から51の地研に拡大するとともに，疫学情報の共有化を強化した．本報告では2011年5～7月に発生した岩カキを中心とするカキ関連事例から検出されたNoVを中心に，2011年1月以降のNoVの遺伝子型の特徴等について取りまとめた．

Keywords：ウイルス性食中毒，塩基配列情報，共有化

[*1] 国立感染症研究所
[*2] 厚生労働省医薬食品局監視安全課食中毒被害情報管理室

斎藤博之[*1]，東方美保[*2]，岡智一郎[*3]，片山和彦[*3]，田中智之[*4]，野田　衛：**パンソルビン・トラップ法による食品からのウイルス検出法**

病原微生物検出情報，**32**，355-357（2011）

ノロウイルスの食品からの検出は二枚貝を除き困難である．そのため2007年から厚生労働科学研究費補助金（食品の安心・安全確保推進研究事業）による研究の一環として，食品中のウイルスを検出するための実践的手法の開発に関する研究をスタートした．その結果，固形，液状，練り物，油物などの多種・多様な食品からノロウイルス（NoV）に代表される食中毒起因ウイルスを検出することができるパンソルビン・トラップ法（パントラ法）を開発した．ルーチンの食品検査として実施可能な段階に達してきたため，その概要を報告した．

Keywords：食品，ウイルス検出法，ウイルス性食中毒

[*1] 秋田県健康環境センター
[*2] 福井県衛生環境研究センター
[*3] 国立感染症研究所
[*4] 堺市衛生研究所

篠原美千代[*]，富岡恭子[*]，峯岸俊貴[*]，内田和江[*]，鈴木典子[*]，島田慎一[*]，河橋幸恵[*]，岸本　剛[*]，野田衛：**非晶性リン酸カルシウム微粒子を用いた食品からのウイルス検出法**

病原微生物検出情報，**32**，357-358（2011）

ノロウイルスの食品中のウイルス汚染量は一般に微量であること，食品成分がウイルス濃縮や遺伝子増幅反応等を阻害することなどから，食品からのウイルスの検出は極めて困難であり，その検出報告例も少ない．我々は，短時間で簡便に実施でき，かつ特殊な試薬や装置を必要としない食品からのウイルス検出方法の構築を目的として，非晶性リン酸カルシウム（Amorphous calcium phosphate；ACP）微粒子を用いたウイルス濃縮方法（ACP微粒子濃縮法）を検討しているので，これまで得られた結果の概要を報告した．

Keywords：食品，ウイルス検出法，ウイルス性食中毒

[*] 埼玉県衛生研究所

吉澄志磨[*]，後藤明子[*]，石田勢津子[*]，野田　衛：**二枚貝関連の食中毒疑い事例における各種胃腸炎ウイルスの関与-北海道**

病原微生物検出情報，**32**，361-363（2011）

食中毒疑い事例の原因究明において，ウイルス検査の対象は主にノロウイルスであり，その他の胃腸炎ウイルスの食中毒への関与については十分には把握されていない．そこで，二枚貝の喫食がみられた食中毒疑い事例を対象に，胃腸炎ウイルス感染の実態調査を行った．その結果，少なくとも二枚貝関連事例についてはサポウイルス等，他の胃腸炎ウイルスを含めた検索が望ましいと考えられた．また，検討数は少ないが，二枚貝のウイルス汚染状況と喫食者の感染状況が必ずしも相関しないことや，SaVの増殖が混合感染，特にNoV GIIの存在に影響を受ける可能性があることが示された．

Keywords：胃腸炎ウイルス，混合感染，ウイルス性食中毒

[*] 北海道立衛生研究所

溝口嘉範[*1]，木田浩司[*1]，葛谷光隆[*1]，濱野雅子[*1]，藤井理津志[*1]，岸本壽男[*1]，安原広己[*2]，上間　匡，野田衛：**ふき取り検体のノロウイルス検査法の改良**

病原微生物検出情報，**32**，358-359（2011）

近年ノロウイルス（NoV）による食中毒は調理従事者を介する事例が多くを占めている．調理従事者から食品への汚染経路の解明や施設環境等の汚染状況の把握にはふき取り検査が有用であるが，ふきとり検体からのNoV検出法はまだ十分に確立されていない．そこで，ふきとり検体からの簡便，安価，高感度なNoV検出法の確立を目的として，RNA抽出以前の工程に焦点を当て，ポリエチレングリコール（PEG）沈澱におけるBeef extract添加の影響および効果的なふき取り方法等について検討した．

Keywords：ふき取り，ウイルス検査法，ウイルス性食中毒

*1 岡山県環境保健センター
*2 岡山市保健所

吉田徹也*，粕尾しず子*，畔上由佳*，内山友里恵*，笠原ひとみ*，上田ひろみ*，長瀬　博*，藤田　暁*，野田　衛：**ノロウイルスおよびサポウイルスの掃除機内ダスト中の汚染実態調査**

小児科，**52**，1419-1423（2011）

ノロウイルスによる感染症の感染様式の一つに，塵埃感染のあることが知られている．著者らは，結婚式披露宴会場において，この塵埃感染が疑われた事例に遭遇した．その際，感染経路の推定に役立ったのは，掃除機内のダストであり，それを検査したところ患者便由来のノロウイルス株と同一株が検出された．この事例をきっかけとして，一般家庭の掃除機内ダストについて，ノロウイルスおよびサポウイルスの汚染実態調査を実施した．その結果，ノロウイルスは59検体中2検体（3.4%），サポウイルスは59検体中1検体（1.7%）から検出され，その汚染ウイルス量はダスト1gあたり106コピーを超えるものも存在したことから，汚染ダストは重要な感染源の一つになると考えられた．

Keywords：掃除機内ダスト，ノロウイルス，サポウルス

* 長野県環境保全研究所

細見卓司*1，谷脇　妙*1，松本一繁*1，藤戸亜紀*1，鍋島　民*1，下司　勲*1，松本道明*1，今井　淳*1，大野雅子*2，麻岡文代*3，吉澄志磨*4，井手　忍*5，山崎謙治*6，左近直美*6，中田恵子*6，増本久人*7，南　亮仁*7，野田日登美*7，野田　衛，片山和彦*8：**高知県で発生したNorovirus GII/14による食中毒事例と他県事例株との比較**

病原微生物検出情報，**32**，199-201（2011）

2011年2月19日～3月2日の間，高知県の某ホテルに宿泊した大学の野球部員等63名中部員6名が嘔吐，下痢，発熱を主症状とする食中毒症状を呈した．共通食は当該ホテルの食事のみであること，大学野球部員の発症者（NoV GII感染者）と同じ食事を取ったホテル従業員からNoV GIIが検出されたことなどから同ホテルを原因施設とするNoVによる食中毒事例と断定された．検出NoV 8株は，すべてNoV GII/14に分類され，塩基配列は100％一致した．また，大阪府2株（2010年1，2月），佐賀県1株（2010年3月），静岡市1株（2010年4月），北海道1株（2010年10月）の5株の配列とも100％一致した．

Keywords：ノロウイルス，GII/14，ウイルス性食中毒

*1 高知県衛生研究所
*2 高知県安芸保健所
*3 高知県中央東保健所
*4 北海道立衛生研究所
*5 静岡市環境保健研究所
*6 大阪府立公衆衛生研究所
*7 佐賀県衛生薬業センター
*8 国立感染症研究所

植木　洋*1，高橋由理*1，鈴木優子*1，阿部美和*1，佐藤由紀*1，沖村容子*1，高橋達也*2，佐藤　淳*2，豊嶋潤*2，熊谷　祥*2，野田　衛：**2010年度に県内で集団発生した感染性胃腸炎事例から検出されたノロウイルスの遺伝子型－宮城**

病原微生物検出情報，**32**，173-174（2011）

2010年度宮城県内（仙台市を除く）では67事例の感染性胃腸炎の集団発生があった．幼稚園と保育所での集団発生が34事例で全体の半数以上（51％）を占め，次いで小学校での発生が20事例（30％）で，乳幼児や子供での発生が多かった．一方，介護保険施設での発生は6事例（9.0％）に留まった．67事例から検出されたウイルスのうち，NoVが91％を占めた．解析したNoV 34株はすべてGII群で，GII/2が最も多く16株，次いでGII/3が11株で，例年最も多く検出されていたGII/4は3株であった．県内では東日本大地震で被害を受けた多くの住民が避難所で生活している．一部の避難所では断水しており，感染症対策が困難な状況にある．今後，避難所で感染性胃腸炎の流行が拡大しないように早急に対策を行う必要がある．

Keywords：ノロウイルス，感染性胃腸炎，遺伝子型

*1 宮城県保健環境センター
*2 宮城県保健福祉部疾病感染症対策室

Sugiyama, K., Kinoshita, M.*, Kamata, Y., Minai, Y.* and Sugita-Konishi, Y.: **(-)-Epigallocatechin gallate suppresses the cytotoxicity induced by trichothecene mycotoxins in mouse cultural macrophages**

Mycotoxin Res., **27**, 281-285 (2011)

Deoxynivalenol (DON) and HT-2 toxin (HT-2) belong to the trichothecene group of mycotoxins and the occurrence of cereals and foodstuffs with these compounds are serious health problems. The aim of this study was to examine the effect of (−)-epigallocatechin gallate (EGCG), one of the main components in green tea catechins, on DON- or HT-2-induced cytotoxicity in mouse macrophages. EGCG had protective effects against the trichothecene-induced cytotoxicities of both

mycotoxins. Additionally, EGCG suppressed the DON-induced activation of caspase-3/7, which is an indicator of apoptosis. These results indicate that EGCG might be useful in protection against DON- or HT-2-induced cell death, suggesting that EGCG could contribute to reducing the toxicities of trichothecenes.

Keywords: trichothecene, (−)-epigallocatechin gallate, cytotoxicity

---

* 玉川大学

Hara-Kudo, Y. and Takatori, K.: **Contamination level of foodborne pathogens in food associated with the infections**

Epidemiol. Inf., **139**, 1505-1510 (2011)

Intake of a relatively small dose of foodborne pathogens can cause infection. Hence, in this study, an estimation of the infectious dose of the pathogens was obtained by conducting microbiological risk assessments. The contamination levels of foodborne pathogens were analyzed in 17 outbreaks of *Salmonella, Escherichia coli* O157, enterotoxigenic *E. coli*, *Vibrio parahaemolyticus*, and *Campylobacter jejuni* occurring in Japan between 2004 and 2006. The infectious dose was estimated in 14 of the 17 outbreaks with the help of the existing data. In three outbreaks of *Salmonella* infection in which the infection rate was 89−100%, the dose of the ingested pathogens was estimated to be 259,000−14,000,000,000 cfu. In other outbreaks of *Salmonella* infection, the infection rate and dose of the ingested pathogens were 10−66.4% and 81−1,560 cfu or most probable number (MPN), respectively. The ingested *Salmonella* dose is likely to be related to the infection rate; however, the storage conditions should be taken into account when making this determination. In an outbreak of *E. coli* O157 infection, the infection rate and ingestion dose were 100% and 2 to <9 cfu, respectively, while in an outbreak of enterotoxigenic *E. coli* infection, they were 93% and 25−1,000 cfu, respectively. Finally, in an outbreak of *C. jejuni* infection, the infection rate and ingestion dose were 37.5% and 360 MPN, respectively. These results would be particularly valuable for risk assessments.

Keywords: contamination level, infectious dose, foodborne infections

Lee, K.[*1], French, N. P.[*2], Hara-Kudo, Y., Iyoda, S.[*3], Kobayashi, H.[*4], Sugita-Konishi, Y. and Kumagai, S.[*1]: **Multivariate analyses revealed distinctive features between human and cattle isolates of Shigatoxin-producing *Escherichia coli* O157**

J. Clin. Microbiol., **49**, 1495-1500 (2011)

Genotypes of Shiga toxin-producing *Escherichia coli* (STEC) O157 isolated from humans and cattle were analyzed by uni- and multivariable logistic regression, and population structure methods, to gain insight into transmission and the nature of human infection. Eleven genotyping assays, including PCR typing of five virulence factors (stx_1, stx_2, stx_{2c}, *eae*, and *ehxA*) and a lineage-specific polymorphism assay using six markers (LSPA6) were considered in the analyses. The prevalence of stx_1, stx_2, and stx_{2c} was significantly different between human and cattle isolates. However, multivariable regression revealed that only the presence of stx_2 was significantly associated with human isolates after controlling for confounding. LSPA6 typing demonstrated an apparent difference in the distribution of LSPA6 lineages between human and cattle isolates, and a strong association between *stx* genotypes and LSPA6 genotypes. Population genetics tools identified three genetically distinct clusters of STEC O157. Each cluster was characterized by *stx* genotypes and LSPA6 genotypes. The human isolates typically comprised LSPA6 lineage I with $stx_1 + stx_2$ strains and LSPA6 lineage I/II with stx_2 or $stx_2 + stx_{2c}$ strains. In contrast, the cattle isolates comprised LSPA6 lineage II strains with stx_{2c} or $stx_2 + stx_{2c}$ strains in addition to the clusters identified for the human isolates. Our analyses provide new evidence that stx_2 is the most distinctive feature in human isolates compared to cattle isolates in Japan, and only a subset of the genetically diverse population isolated from cattle is involved in human illnesses. Our results may contribute to international comparisons and risk assessments of STEC O157.

Keywords: Shiga toxin-producing *Escherichia coli* O157, *stx* genotype, LSPA6 genotype

---

[*1] University of Tokyo
[*2] Massey University
[*3] National Institute of Infectious Diseases
[*4] National Agriculture and Food Research Organization, National Institute of Animal Health

Hasegawa, A.[*], Hara-Kudo, Y., Kumagai, S.[*]: **Survival of *Salmonella* strains differing in their biofilm-formation capability upon exposure to hydrochloric and acetic acid and to high salt**

J. Vet. Med. Sci., **73**(9), 1163-1168 (2011)

Acidic and osmotic treatments are part of hurdle systems to control pathogens such as *Salmonella* in food. In the current

study, *Salmonella enterica* isolates previously shown to differ in their ability to form biofilms were grown in diluted tryptic soy broth（TSB）(1:5 dilution in distilled water) and subsequently exposed to phosphate-buffered saline（PBS）adjusted to pH 3.0 with HCl, PBS adjusted to pH 3.9 with acetic acid, or rice vinegar diluted 1:15 with distilled water（pH 3.9）. Cells grown in diluted TSB were also exposed to PBS, pH 7.6, containing 5 M NaCl. No differences in survival upon exposure to PBS adjusted to pH 3.0 with HCl or containing high salt were observed between the isolates; however, exposure to acetic acid resulted in lower survival levels of isolates previously shown to be poor biofilm formers. The number（log10 cfu/ml）of surviving cells after 36 hr exposure to acetic acid and rice vinegar were 4.43 ± 0.24 vs. 2.27 ± 0.87（$P<0.05$）, and 5.19 ± 0.12 vs. 2.33 ± 0.93（$P<0.05$）for isolates with a high vs. low biofilm-forming ability. The survival data could be fitted with the Weibull model. The data suggest that the ability of *Salmonella* strains to survive in the presence of acetic acid and rice vinegar parallels their ability to form biofilms. Thus *Salmonella* with a high biofilm-formation capability might be more difficult to kill with acetic acid found in foods or cleaning solutions.

Keywords: Survival, Salmonella, Biofilm

* The University of Tokyo

Nemoto, J.[*1], Ikedo, M.[*1], Kojima, T.[*1], Momoda, T.[*1], Konuma, H.[*2] and Hara-Kudo, Y.: **Development and evaluation of a loop-mediated isothermal amplification assay for rapid and sensitive detection of *Vibrio parahaemolyticus***

J. Food Prot., **74**, 1462-1467（2011）

Loop-mediated isothermal amplification（LAMP）assays targeting the rpoD and toxR gene were developed to detect *Vibrio parahaemolyticus*. All of 78 tested *V. parahaemolyticus* strains yielded positive results within 40 min, with negative results obtained for 69 strains of other organisms even at 60 min. For *V. parahaemolyticus* ATCC 17802 in pure culture, the detection limits of LAMP assays targeting *rpoD* and *toxR* were 3.7 and 450 colony-forming units（CFU）per test, respectively. Due to the performance of higher sensitivity than *toxR*-LAMP, *rpoD*-LAMP had been further evaluated for the ability to detect *V. parahaemolyticus* in seafood samples. The concentration of *V. parahaemolyticus* in short-necked clams spiked with *V. parahaemolyticus* was enumerated by the most probable number（MPN）method combined with the *rpoD*-LAMP assay and the MPN method with culture method using agar medium. The MPN-*rpoD*-LAMP method was advantageous on sensitivity and rapidity compared with the conventional method. These results indicate that the MPN-LAMP assay targeting the *rpoD* gene is a specific, sensitive and rapid method to enumerate *V. parahaemolyticus*.

Keywords: *Vibrio parahaemolyticus*, LAMP, PCR

[*1] Eiken Chemical Company Ltd.
[*2] Tokai University

Arakawa, Y.[*1], Sawada, T.[*1], Takatori, K., Lee, K.[*2] and Hara-Kudo, Y.: **Rapid detection of Shiga toxin-producing *Escherichia coli* in ground beef by an immunochromatography kit in combination with short-term enrichment and treatment for Shiga toxin release**

Biocontrol Sci., **16**, 159-164（2011）

To establish rapid methods to detect Shiga toxin（Stx）-producing *Escherichia coli*（STEC）in ground beef samples by using an immunochromatography kit, results of 8-h enrichment in various types of broth with shaking were compared. In pure culture, Stx was detected in the culture of trypticase soy broth（TSB）at 42˚C and modified EC broth（mEC）at 36˚C from all or most serogroups of O26, O111, O128, O157 and OUT. Ground beef samples inoculated with each serogroup were enriched in TSB at 42˚C, mEC at 36˚C and mEC with novobiocin（NmEC）at 42˚C. Although all conditions led to the successful recovery of each serogroup by the plating method, enrichment in NmEC was relatively superior to the other conditions in the detection of Stx by an immunochromatography kit. These results indicated that the growth of STEC and the release of Stx from cells were different in pure cultures and in culture with ground beef. In addition, polymyxin B treatment for 10 min at 37˚C and homogenizing with glass beads enhanced the detection of Stx. From the results, it was suggested that an immunochromatography kit in a combination with enrichment in NmEC at 42˚C for 8 h, and treatment with polymyxin B or homogenizing would be a rapid method to detect STEC contamination in ground beef.

Keywords: Shiga toxin-producing *Escherichia coli*, immunochromatography kit, detection

[*1] Nippon Veterinary and Life Science University
[*2] Graduate School of Agricultural and Life Sciences, University of Tokyo

小沼ルミ[*1]，瓦田研介[*1]，井上雅史[*2]，宮崎　巖[*1]，飯

田孝彦[*1]，浜野智子[*1]，渡辺麻衣子，工藤由起子：**桐たんすの変色部に生育した糸状菌の分離および同定**
防菌防黴，**39**，205-211（2011）

桐たんすの変色部に生育した糸状菌および桐たんす表面処理液中に存在する糸状菌の分離・同定を行った．異なる環境で使用されていた桐たんすAおよびBの変色部から分離された糸状菌について形態観察及び分子生物学的同定を行ったところ，*Aspergillus penicillioides*が共通して同定された．また，砥粉およびヤシャ液中から好湿性の*Chaetomium*属および*Paecilomyces*属等が分離された．桐たんす内部の湿度は調湿機能によって概ね80% RH以下に保たれていて多くの糸状菌は生育しにくい環境であるが，*A. penicillioides*などの好乾性菌では生育が可能となることが明らかになった．桐たんす内部に発生する変色を予防するためには，適切な防カビ剤によって一般的な糸状菌に加えて好乾性菌の生育抑制が必要であることが明らかとなった．

Keywords: Furniture made from Kiri（*Paulownia tomentosa*）, Fungal contamination, Xerophilic fungi

[*1] （地独）東京都立産業技術研究センター
[*2] （株）相徳

Watanabe, M., Yonezawa, T.[*1], Lee, K.[*2], Kumagai, S.[*2], Sugita-Konishi, Y., Goto, K.[*3], Hara-Kudo, Y.: **Evaluation of genetic markers for identifying isolates of the species of the genus *Fusarium***
J. Sci. Food Agr., **91**, 2500-2504（2011）

Members of the genus *Fusarium* are well-known as one of the most important plant pathogens causing food spoilage and loss worldwide. Moreover, they are associated with human and animal diseases through contaminated foods because they produce mycotoxins. To control fungal hazard of plants, animals and humans, there is a need for a rapid, easy and accurate identification system of *Fusarium* isolates with molecular methods. To specify gene appropriate for identifying isolates of various Fusarium species, we sequenced the 18S rRNA gene（rDNA）, internal transcribed spacer region 1, 5.8S rDNA, 28S rDNA, β-tubulin gene（*β-tub*）, and aminoadipate reductase gene（*lys2*）, and subsequently calculated the nucleotide sequence homology with pairwise comparison of all tested strains and inferred the ratio of the nucleotide substitution rates of each gene. Inter-species nucleotide sequence homology of *β-tub* and *lys2* ranged from 83.5 to 99.4% and 56.5 to 99.0%, respectively. The result indicated that sequence homologies of these genes against reference sequences in database have a high possibility to identify unknown *Fusarium* isolates when it is more than 99.0 %, because these genes had no inter-species pairwise combinations that had 100% homologies. Other markers often showed 100% homology in inter-species pairwise combinations. The nucleotide substitution rate of *lys2* was the highest among the six genes. The *lys2* is the most appropriate genetic marker with high resolution for identifying isolates of the genus *Fusarium* among the six genes we examined in this study.

Keywords: *Fusarium*, aminoadipate reductase gene, phylogenetic species concept, molecular phylogenetic alanalysis

[*1] 復旦大学
[*2] 東京大学大学院
[*3] 三井農林（株）食品総合研究所

Watanabe, M., Tsutsumi, F.[*1], Konuma, R.[*2], Lee, L.[*3], Kawarada, K.[*2], Sugita-Konishi, Y., Kumagai, S.[*3], Takatori, K.[*4], Konuma, H.[*1], Hara-Kudo, Y.: **Quantitative analysis of mycoflora on commercial domestic fruits in Japan**
J. Food Prot., **74**, 1488-1499（2011）

A comprehensive and quantitative analysis of the mycoflora on the surface of the commercial fruit was performed. Nine kinds of fruits grown in Japan were tested. Overall fungal counts on the fruits ranged from 3.1 to 6.5 log cfu/g. The mean percentages of the total yeast counts were higher than that of molds in apples, Japanese pears and strawberries, ranging from 58.5 % to 67.0 %, and were lower than that of molds in the other six fruits, ranging from 9.8 % to 48.3 %. *Cladosporium* was the most frequent genus of fungi and was found in all of the fruits, followed by *Penicillium* found in eight kinds of fruits. The predominant fungal genus with the highest percentage in total fungal count in each fruit was *Acremonium* in cantaloupe melons（47.6 %）, *Aspergillus* in grapes（32.2 %）, *Aureobasidium* in apples（21.3 %）, blueberries（63.6 %）and peaches（33.6 %）, *Cladosporium* in strawberries（38.4 %）, *Cryptococcus* in Japanese pears（37.6 %）, *Penicillium* in mandarins（22.3 %）and *Sporobolomyces* in lemons（26.9 %）. These results demonstrated that the mycoflora on the fruit surface mainly consist of common inhabitants on plant or in the environment of pre- and post-harvest, while the fungi which produce mycotoxins or cause market diseases were not prominent in the mycoflora of the healthy tissues of fruits. This study suggested that it is necessary to handle fruits in consideration of mounts of the fungal contaminants including non-pathogenic fungi on fruits, in order to control the quality of fruits and processed fruit foods.

Keywords: mycoflora, fungal contamination, fruit, enumeration

[*1] 東海大学
[*2] （地独）東京都立産業技術研究センター
[*3] 東京大学大学院
[*4] NPO法人カビ相談センター

Watanabe, M., Yonezawa, T.[*1], Lee, K.[*2], Kumagai, S.[*2], Sugita-Konishi, Y., Goto, K.[*3], Hara-Kudo, Y.: **Molecular phylogeny of the higher and lower taxonomy of the *Fusarium* genus and differences in the evolutionary histories of multiple genes**

BMC Evo. Bio., **11**, 322（2011）

Species of the *Fusarium* genus are important fungi which is associated with health hazards in human and animals. Although many researchers have applied molecular phylogenetic analysis to examine the taxonomy of *Fusarium* species, their phylogenetic relationships remain unclear. We performed phylogenetic analyses based on the nucleotide sequences of the rDNA cluster region（rDNA cluster）, and the β-tubulin gene（*β-tub*）, the elongation factor 1α gene（*EF-1α*）, and the aminoadipate reductase gene（*lys2*）. Although incongruence of the tree topologies between *lys2* and the other genes was detected, all genes supported the classification of *Fusarium* species into 7 major clades, I to VII. To obtain a reliable phylogeny for *Fusarium* species, we excluded the *lys2* sequences from our dataset, and reconstructed a maximum likelihood（ML）tree based on the combined data of the rDNA cluster, *β-tub*, and *EF-1α*. Our ML tree indicated some interesting relationships in the higher and lower taxa of *Fusarium* species and related genera. Moreover, we observed a novel evolutionary history of *lys2*. We suggest that the unique tree topologies of *lys2* are not due to an analytical artifact, but due to differences in the evolutionary history of genomes caused by positive selection of particular lineages. This study showed the reliable species tree of the higher and lower taxonomy in the lineage of the *Fusarium* genus. Our ML tree clearly indicated 7 major clades within the *Fusarium* genus. Furthermore, this study reported differences in the evolutionary history among multiple genes within this genus for the first time.

Keywords: *Fusarium* phylogeny, aminoadipate reductase gene, positive selection

[*1] 復旦大学
[*2] 東京大学大学院
[*3] 三井農林（株）食品総合研究所

Kitadokoro, K.[*1], Nishimura, K.[*1], Kamitani, S.[*2], Fukui-Miyazaki, A.[*2], Toshima, H.[*2], Abe, H.[*2], Kamata, Y., Sugita-Konishi, Y., Yamamoto, S., Karatani, H.[*1], Horiguchi, Y.[*2]: **Crystal structure of *Clostridium perfringens* Enterotoxin Displays Features of *β*-Pore-forming Toxins**

J. Biol. Chem., **286**, 19549-19555（2011）

Clostridium perfringens enterotoxin（CPE）is a cause of food poisoning, and considered a pore-forming toxin, which damages target cells by disrupting the selective permeability of the plasma membrane. However, the pore-forming mechanism and the structural characteristics of the pore are not well documented. Here, we present the structure of CPE determined by X-ray crystallography at 2.0 Å. The overall structure of CPE displays an elongated shape, composed of three distinct domains, I, II, and III. Domain I corresponds to the region that was formerly referred to as C-CPE, which is responsible for binding to the specific receptor claudin. Domains II and III comprise a characteristic module, which resembles those of βpore-forming toxins such as aerolysin, *C. perfringes* ε-toxin, and *Laetiporus sulphureus* hemolytic pore-forming lectin. The module is mainly made up of βstrands each, by which they are distinguished. In addition, domain II has and αhelix and preceding β strand demonstrate an alternating pattern of hydrophobic residues that is characteristic of transmembrane domains forming β barrel-made pores. These structural features imply that CPE is a βpore-forming transmembrane domain is inserted into the membrane upon the buckling of the two long βstrands spanning the module, a mechanism analogous to that of the cholesterol-dependent cytolysins.

Keywords: *Clostridium perfringes* enterotoxin, crystal structure, pore-forming

[*1] Graduate School of Science and Tecnology, Department of Biomolecular Engineering, Kyoto Institute of Tecnology
[*2] Department of Molecular Bacteriology, Research Institute for Microbial Diseas, Osaka University

Wang, L.[*], Wakushima, M.[*], Kamata, Y., Nishikawa, Y.[*]: **Exhaustive isolation of diarrhoeagenic *Escherichia coli* by a colony hybridization method using hydrophobic grid-membrane filters in combination with multiplex real-time PCR**

Lett. Appl. Microbiol., **53**, 264-270（2011）

The present study aimed to develop a colony hybridization method for the exhaustive detection and isolation of diarrhoeagenic Escherichia coli（DEC）from samples containing numerous coliform bacteria. Digoxigenin-labelled DNA probes were designed to detect seven pathotypes of DEC based on type-specific genes. A total of 615 meat, food and faeces samples identified as DEC-positive by multiple real-time PCR for the virulence genes（eae, stx, elt, est, virB, aggR, afaB and astA）were analysed by a colony hybridization method, which involved filtering enrichment cultures through hydrophobic grid-membrane filters. DEC were isolated from 72.5%（446/615）of samples by the colony hybridization method but were only detected in 26.3%（162/615）of samples by a conventional culture method. The hybridization method was particularly effective for isolating low-level contaminants, such as enterotoxigenic and Shiga toxin-producing E. coli, which were isolated from 51.8%（58/112）of samples identified as positive by PCR for the enterotoxin genes, in contrast to only 4.5%（5/112）of samples analysed by the conventional method. The developed colony hybridization system allows for the efficient and simultaneous isolation of all DEC pathotypes.

Keywords: *Escherichia coli*, colony hybridization, multiplex real-time PCR

---

* Graduate School of Human Life Science, Osaka City University

Sakuma, H., Kamata, Y., Sugita-Konishi, Y., Kawakami, H.*: **Method for Determination of Aflatoxin M1 in Cheese and Butter by HPCL Using Immunoaffinity Column**

Food Hyg. Saf. Sci., **52**, 220-225（2011）

A rapid, sensitive convenient method for determination of aflatoxin M_1（AFM_1）in cheese and butter by HPLC was developed and validated. The method employs a safe extraction solution（mixture of acetonitrile, methanol and water）and an immunoaffinity column（IAC）for clean-up. Compared with the widely used method employing chloroform and a Florisil column, the IAC method has a short analytical time and there are no interference peaks. The limits of quantification（LOQ）of the IAC method were 0.12 and 0.14 μg/kg, while those of the Florisil column method were 0.47 and 0.23 μg/kg in cheese and buffer, respectively. The recovery and relative standard deviation（RSD）for cheese（spiked at 0.5 μg/kg）in the IAC method were 92% and 7%, respectively, while for the Florisil column method the corresponding values were 76% and 10%. The recovery and RSD for butter（spiked at 0.5 μg/kg）in the IAC method were 97% and 9%, and those in the Florisil method were 74% and 9%, respectively. In the IAC method, the values of in-house precision（n=2, day=5）of cheese and butter（spiked at 0.5 μg/kg）were 9% and 13%, respectively. The IAC method is superior to the Florisil column method in terms of safety, ease of handling, sensitivity and reliability. A survey of AFM_1 contamination in imported cheese and butter in Japan was conducted by the IAC method. AFM_1 was not detected in 60 samples of cheese and 30 samples of butter.

Keywords: Aflatoxin M_1, HPLC, quantification

---

* Kyoritsu Women's University

Kadota, T.*1,2, Kimura, M.*3, Hirano, S.*1, Tajima, O.*1, Nakajima, T.*4, Kamata, Y., Sugita-Konishi, Y.: **Development of a simultaneous liquid chromatography/- tandem mass spectrometric method for the determination of type B trichothecenes, their derivatives, and precursors in wheat**

Rapid Commun. Mass Spectrom, **25**, 3481-3490（2011）

A method coupling liquid chromatography with tandem mass spectrometry（LC/MS/MS）was developed for the simultaneous quantitative determination of trichothecenes, nivalenol, deoxynivalenol, deoxynivalenol-3-glucoside, fusarenon-X, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, isotrichodermin, calonectrin, 3-deacetylcalonectrin, 15-deacetylcalonectrin, 3,15-diacetylnivalenol, 4,15-diacetylnivalenol, 3,15-diacetyldeoxynivalenol, and 3,4,15-triacetylnivalenol. The analytical parameters of trichothecenes and their derivatives were optimized to enable their highly sensitive detection. Evaluation of clean-up procedures using Multisep #226 and #227 indicated that Multisep #227 was more suitable for their simultaneous detection in wheat. In performance validation studies using the LC/MS/MS method with Multisep #227 cleanup, good recoveries ranging from 84% to 115% with relative standard deviations from 0.4% to 7.2% were measured. The limits of detection and quantification ranged from 0.03 to 1.4 ng•g(-1) and from 0.1 to 4.7 ngng•g(-1), respectively. The effect of matrices using matrix-matched calibration was estimated to range from 80% to 117% after Multisep #227 cleanup. Multisep #227 clean-up procedure with matrix-free standard calibration achieved accurate quantification without having a considerable effect on matrix compounds. Using the developed method, several trichothecene derivatives and precursors were detected in fungally inoculated wheat samples. The developed LC/MS/MS method

is a practical technique that can be used for the quantification of trichothecenes in wheat. This study is the first report of an analytical method used for the simultaneous quantification of major trichothecenes, their derivatives and precursors.

Keywords: Trichothecene mecotoxins, LS/MS, Determination

[*1] Food Safety Assurance Center, Kirin Group Office Co., Ltd.
[*2] United Graduate School of Veterinary Sciences, Gifu University
[*3] Plant & Microbial Metabolic Engineering Research Unit, Discovery Research Institute
[*4] Research Team for Fusarium Head Blight Control, National Agricultural Research Center for Kyushu Okinawa Region

Saka, M.[*], Tada, N.[*], Kamata, Y.: **The annual ovarian cycle of the reeves' pond turtle *Chinemys reevesii* (Reptilia: *Geoemydidae*) Based on Seasonal Variations in the serum vitellogenin level and follicular growth**

Current Herpetology, **30**, 103-110（2011）

To determine the annual ovarian cycle of a multiclutched turtle *Chinemys reevesii*, we quantified vitellogenin（VTG, a yolk-precursor protein）in the serum collected monthly from turtles kept in an outdoor enclosure. We also sacrificed wild adult females（one or two individuals per month）captured from a river site in Kyoto, Japan, and observed oviductal eggs and follicles assigned to five size classes: C1 to C5, in ascending order. The seasonal variation in the serum VTG level showed a sharp peak in late spring and a broad peak during autumn, indicating that vitellogenesis accelerated rapidly in spring, decreased in summer, increased slowly but steadily in autumn, and creased in winter. From May to July, ovulations occurred in succession preceded by the C4-to-C5 growth of follicles, but without substantial growth of C1-C3 follicles. The vernal peak of vitellogenesis would therefore contribute largely to the sequential growth of the follicles prepared for the second and third clutches of the breeding season. In August, when the successive ovulations had been completed, no remarkable growth of C1-C3 follicles was observed anymore, reflecting the ovarian quiescence. Newly-formed C1 follicles appeared in September when C2 and C3 follicles markedly increased in number but C4 and C5 follicles were still absent. In October and November, C4 and C5 follicles were observed again, suggesting that the follicles for the first clutch of the next breeding season reached preovulatory size before hibernation. The production and remarkable growth of follicles occurring from September to November would account for the broad peak of vitellogenesis in autumn. Thus the observed seasonal variations in the serum VTG level and follicular growth were concordant with each other.

Keywords: Vitellogenin, Annual Change, Follicular growth

[*] Division of Aquatic Environment, Kyoyo Prefectural Institute of Public Health and Environment

Hosokawa, M.[*1], Asakawa, H.[*2], Kaido, T.[*3], Sugaya, C.[*3], Tsunoda, M.[*3], Itai, K.[*4], Kodama, Y., Sugita-Konishi, Y., Takata, A.[*5], Yokoyama, K.[*1], Aizawa, Y.[*3]: **Fluoride in drinking water exacerbates glomerulonephritis and induces liver damage in ICR-derived glomerulonephritis mice**

Toxicol. environ. chem., **93**（10）, 2072-2084（2011）

To evaluate the effects of fluoride on the kidney and the liver of ICR-derived glomerulonephritis（ICGN）mice by using laboratory tests and pathological examinations, fluoride was administered to the ICGN mice at 0, 25, 50, 100, and 150 ppm in drinking water for 4 weeks and to the ICR mice, which have normal kidney function at 0 and 150 ppm. The BUN, creatinine, GOT, and GPT in the serum of each mouse were determined. When a mouse died, the sample from the day closest to the death was assigned for the mean. Pathological changes in the kidney were examined after PAS（periodic acid-Schiff）staining. All of the ICGN mice in the 150 ppm group and one of seven in the 100 ppm group died before the end of week 4, but no ICR mice died. For ICGN mice, the mean value of body weight in the 150 ppm group was significantly lower than those in 0 ppm group and other fluoride-administered groups. The mean values of relative liver and kidney weights in the 100 and 150 ppm groups were significantly lower than those in the control. The mean values of BUN, creatinine, and GPT in the 150 ppm group were significantly higher than those in the control. The thickness of the glomerular capillary wall and the increased mesangial matrix in the kidney were prominent in the fluoride-administered ICGN mice. These results suggested that fluoride severely exacerbated glomerulonephritis and tublar-intestitial changes in ICGN mice.

Keywords: fluoride, glomerulonephritis, mouse, blood urea nitrogen, creatinine

[*1] Department of Epidemiology and Environmental Health, Faculty of Medicine, Juntendo University
[*2] Meiji Seika Co., Ltd.
[*3] Department of Preventive Medicine, Kitasato University

School of Medicine, Kitasato
[*4] Department of Hygine and Public Health, School of Medicine, Iwate Medical University
[*5] Department of Preventive Medicine, St. Marianna University School of Medicine

Lee, K.[*], Watanabe, M., Sugita-Konishi, Y., Hara-Kudo, Y. and Kumagai, S.[*]: **_Penicillium camemberti_ and _Penicillium roqueforti_ enhance the growth and survival of Shiga toxin-producing _Escherichia coli_ O157 under mild acidic conditions**

J. Food Sci., **77**, M102-M107（2012）

The effects of secondary starter molds of common mold-ripened cheeses on the Shiga toxin-producing *Escherichia coli*（STEC）O157 were assessed in 3 model systems. In the 1st model, 8 STEC O157 strains were incubated in the spent culture of *Penicillium camemberti* or *Penicillium roqueforti* under mild acidic conditions at 25 ˚C. In the spent cultures of the mold at pH 4.8 to 5.0, the lag times of STEC O157 growth were significantly shorter than those observed in fresh medium. Analyses of the spent culture of *P. camemberti* showed that the causative agents of the growth enhancement were produced by the mold in response to an acidic environment and were not fully inactivated in heat treatment. In the 2nd model, *P. camemberti* and STEC O157 were cocultured in acidified milk at 25 ˚C. The population of STEC O157 reached 108 CFU/mL in the presence of the mold, whereas the population steadily declined in the absence of the mold. Although this growth enhancement was partially attributable to alkalization by the mold, it was observed even when the pH of this model was stabilized. In the 3rd model, 2 STEC O157 strains were incubated in the spent cultures of molds at pH 4.5 at 10 ˚C. In the spent culture, proportions of injured cells were significantly lower and D values were significantly higher than those in control, except one STEC O157 strain in the spent culture of *P. camemberti*. These results showed that the molds could enhance the growth and survival of STEC O157 by changing the environment.

Keywords: microbial interaction, mold-ripened cheese, Shiga toxin-producing *Escherichia coli* O157

---

[*] 東京大学大学院

Jones, J. L.[*1], Hara-Kudo, Y., Krantza, J. A.[*1], Benner, R. A.[*1], Smith, A. B.[*2], Dambaugh, T. R.[*2], Bowers, J. C.[*3] and DePaola, A.[*1]: **Comparison of molecular detection methods for _Vibrio parahaemolyticus_ and _Vibrio vulnificus_**

Food Microbiology, **30**, 105-111（2012）

Vibrios are a global concern for seafood safety and many molecular methods have been developed for their detection. This study compares some available molecular methods for detection of total and pathogenic *Vibrio parahaemolyticus* and *V. vulnificus*, in MPN enrichments from oyster tissue and fish intestine samples. This study employed the Dupont Qualicon BAX® System Real Time PCR assay for detection of *V. parahaemolyticus* and *V. vulnificus*. Multiplex real-time PCR detection of total, *tdh*+, and *trh*+ *V. parahaemolyticus* was conducted on the Cepheid SmartCycler II. Total and *tdh*+ *V. parahaemolyticus* were also detected using LAMP. *V. vulnificus* detection was performed using a real-time PCR method on the SmartCycler and on the AB 7500 Fast. In addition to recommended template preparations, the BAX lysis samples were examined as a suitable template. There was no significant difference in detection of *V. parahaemolyticus* and *V. vulnificus* using the BAX or SmartCycler assays. The AB assay showed no difference from the other methods in detection of *V. vulnificus* unless boiled templates were utilized. There was a significant difference in detection of *tdh*+ *V. parahaemolyticus* between the SmartCycler and LAMP assays unless the SmartCycler assay omitted the total *V. parahaemolyticus* gene target; a similar trend was observed for *trh*+ *V. parahaemolyticus*.

Keywords: *Vibrio vulnificus*, *Vibrio parahaemolyticus*, real-time PCR

[*1] FDA, Division of Seafood Science and Technology
[*2] DuPont Qualicon
[*3] FDA, CFSAN

Hiroi, M.[*1], Yamazaki, F.[*2], Harada, T.[*1], Takahashi, N.[*1], Iida, N.[*1], Noda, Y.[*1], Yagi, M.[*1], Nishio, T.[*1], Kanda, T.[*1], Kawamori, F.[*1], Sugiyama, K.[*1], Masuda, M.[*1], Hara-Kudo, Y., Ohashi, N.[*3]: **Prevalence of extended-spectrum β-lactamase-producing _Escherichia coli_ and _Klebsiella pneumoniae_ in food-producing animals**

J. Vet. Med. Sci., **74**, 189-195（2012）

To evaluate the diversity of extended-spectrum β-lactamases（ESBL）genes among food-producing animals, 48 isolates of ESBL-producing *Escherichia coli* isolates were obtained from rectal samples of broilers, layers, beef cattle and pigs, at the slaughterhouse level. ESBL-carrying *E. coli* were isolated from 60.0% of individual broiler rectal samples, 5.9% of layers, 12.5% of beef cattle and 3% of pigs. One ESBL-producing *Klebsiella pneumoniae* was isolated from a

broiler. The ESBL-positive *E. coli* isolates from broilers harbored various ESBL genes: bla_{SHV-12}, $bla_{CTX-M-2}$, $bla_{CTX-M-14}$, $bla_{CTX-M-15}$ and $bla_{CTX-M-44}$. The plasmid DNAs were analyzed by restriction patterns. Homogeneous band patterns were yielded in those of *K. pneumoniae* and *E. coli* isolates harboring the $bla_{CTX-M-2}$ gene from different farms. No genetic relation between the 2 CTX-M-14 ESBL-producing strains was found by pulsed-field gel electrophoresis, although 2 plasmids in these strains, obtained from different broiler farms, were similar to each other. This study provides evidence that the proliferation of CTX-M-producing *E. coli* is due to the growth of indigenous CTX-M-producing strains and the possible emergence of strains that acquired CTX-M genes by horizontal transfer in different broiler farms. CTX-M-producing coliforms in broilers should be controlled due to the critical importance of cephalosporins and the zoonotic potential of ESBL-producing bacteria.

Keywords: antimicrobial resistance, broiler, *Escherichia coli*

[*1] Shizuoka Institute of Environment and Hygiene
[*2] Shizuoka Prefectural Western Meat Inspection Center
[*3] University of Shizuoka

Mizutani, N., Sugita-Konishi, Y., Omoe, K.[*1], Shinagawa, K.[*1], Kawakami, H.[*2], Kanno, S., Sugiyama, K., Kamata, Y.: **Advantages of immunoglobulin Y for the detection ofStaphylococcal enterotoxin A in a double-antibody-sandwich enzyme-linked immunosorbent assay**
Int. J. Food Sci. Technol., **47**, 155-159（2012）

To determine the amounts of staphylococcal enterotoxin A（SEA）, a novel and sensitive enzyme-linked immunosorbent assay（ELISA）was developed. Protein A, which is produced by Staphylococcus aureus, interferes with the reaction between SEA and anti-SEA immunoglobulin G（IgG）, resulting in a false-positive reaction. Chicken IgY was introduced as a capture antibody in the sandwich ELISA system, since IgY binds less efficiently to protein A. When the anti-SEA IgG antibody was used as the capture and detection antibodies（IgG-IgG ELISA）, the backgroud levels of protein A increased, thus resulting in a false-positive reaction. A 0.01 ng/ml concentration of protein A significantly increased the absorbance value of the blank wells. When the anti-SEA IgY antibody was used as the capture antibody, 1,000 ng/ml of protein A did not affect the absorbance value. The ELISA system using anti-SEA IgY as a capture antibody and anti-SEA IgG as a detection antibody（IgY-IgG ELISA）showed a detection limit of less than 0.25 ng/ml and a creditability of R2=0.98. These findings demonstrate the advantage of chicken IgY for the detection of SEA by means of double antibody sandwich ELISA.

Keywords: Staphylococcal enterotoxin, IgY, ELISA

[*1] Iwate University
[*2] Kyoritsu Woman's University

Kawai, T.[*1], Sekizuka, T.[*2], Yahatac, Y.[*2], Kuroda, M.[*2], Kumeda, Y.[*1], Iijima, Y.[*3], Kamata, Y., Sugita-Konishi, Y., Ohnishi, T.: **Identification of *Kudoa septempunctata* as the causative agent of novel food poisoning outbreaks in Japan by consumption of *Paralichthys olivaceus* in raw**
Clin. Infect. Dis., **54**, 1046-1052（2012）

BACKGROUND: Outbreaks of an unidentified food-borne illness associated with the consumption of raw fish have increased in Japan since 2003. Those affected with this illness develop diarrhea and emesis within 2-20 hours after a meal including raw fish. No known causative agents such as bacteria, viruses, bacterial toxins, or toxic chemicals have been detected in the foods that were ingested. Fortunately, this illness is self-limiting with good prognosis in all cases.

METHODS: We conducted an epidemiological analysis of outbreaks that occurred during 2008 and 2010 and analysed a fish sample from one outbreak by metagenomic DNA sequencing, real-time polymerase chain reaction, and direct microscopic observations. The pathogenicity of a putative risk factor identified by these techniques was assessed using the suckling-mouse test and a house musk shrew emetic assay.

RESULTS: The epidemiological analysis of outbreaks in 24 municipalities involving >1300 subjects implicated an olive flounder（*Paralichthys olivaceus*）as the causative food source. The presence of *Kudoa septempunctata*, a recently-described myxosporean species in *P. olivaceus*, was prevalent in the causative foods. *K. septempunctata* induced watery stools and an elevated fluid accumulation ratio in suckling mice, as well as vomiting in house musk shrews.

CONCLUSIONS: These results identify *K. septempunctata* as the etiological agent of this novel food-borne illness outbreak associated with consumption of raw *P. olivaceus*. This is the first report, to our knowledge, demonstrating the human pathogenicity of *Kudoa* spores.

Keywords: *Kodoa septempunctata*, Flounder, Food poisoning

[*1] 大阪府公衆衛生研究所
[*2] 国立感染症研究所
[*3] 神戸市環境保健研究所

Shoda, T., Fukuhara, K., Goda, Y., Okuda, H.: **Enzyme-assisted synthesis of the glucuronide conjugate of psilocin, an hallucinogenic component of magic mushrooms**
Drug Test Anal., **3**, 594-596（2011）

An enzyme-assisted synthesis of psilocin glucuronide（PCG）, a metabolite excreted in the urine of magic mushroom（MM）users, is described. In the presence of Aroclor 1254 pretreated rat liver microsomes, psilocin and the cofactor UDPGA were incubated for 20 h. Purification by HPLC gave PCG in 19% yield（3.6 mg）. The compound structure was characterized by MS and NMR. The milligram amounts of PCG produced by this method will allow the direct identification and quantification of PCG in the urine of MM users.

Keywords: psilocin, glucuronide, enzyme-assisted synthesis

Ohno, A., Oka, K.*, Sakuma, C.*, Okuda, H., Fukuhara, K.: **Characterization of tea cultivated at four different altitudes using ^{1}H NMR analysis coupled with multivariate statistics**
J. Agric. Food Chem., **59**, 5181-5187（2011）

The taste of black tea differs according to the different areas in which the tea is grown, even for the same species of tea. A combination of（1）H NMR spectroscopy and partial least-squares discriminate analysis（PLS-DA）was used to assess the quality differences of tea leaves from four cultivation areas with different elevations, RAN > 1800 m, UDA = 1200 m, MEDA = 600 m, and YATA < 300 m, in Sri Lanka. As a result of a statistical analysis, PLS-DA showed a separation between high- and low-quality black teas derived from the four different tea cultivation areas. RAN from the highest elevation showed characteristic trends in the levels of theaflavin and theaflavin 3,3'-digallate that were found only in RAN, and the levels of theanine and caffeine were higher, and the levels of thearubigins, especially thearubigin 3,3'-digallate, were lower in RAN than in UDA, MEDA, and YATA. The structures of these components were determined by 1D and 2D NMR analyses. These results demonstrate that this method can be used to evaluate black tea quality according to the chemical composition or metabolites, which are characteristic of the tea leaves cultivated in four regions with different elevations in Sri Lanka.

Keywords: black tea, quality, elevation, multivariate analysis

* 東京薬科大学薬学部

Ieda, N.*, Nakagawa, H.*, Horinouchi, T.*, Peng, T.*, Yang, D.*, Tsumoto, H.*, Suzuki, T.*, Fukuhara, K., Miyata, N.*: **Peroxynitrite generation from a NO-releasing nitrobenzene derivative in response to photoirradiation**
Chem Commun（Camb）., **47**, 6449-6451（2011）

Photocontrollable ONOO(-) generation from a nitrobenzene derivative was demonstrated. The designed compound released NO in response to photoirradiation, and the resulting semiquinone reduced molecular oxygen to generate O(2)•(-); reaction of the two generated ONOO(-), as confirmed with an ONOO(-) fluorescent probe, HKGreen-3.

Keywords: nitric oxide, peroxynitrate, nitrobenzene

* 名古屋市立大学大学院薬学研究科

Fukuhara, K., Ohno, A., Ando, Y.*, Yamoto, T.*, Okuda, H.: **A 1H NMR-based metabolomics approach for mechanistic insight into acetaminophen-induced hepatotoxicity**
Drug Metab. Pharmacokinet., **26**, 399-406（2011）

The widely used analgesic-antipyretic drug acetaminophen（APAP）is known to cause serious liver necrosis at high doses in man and experimental animals. For studies of toxic processes, 1H NMR spectroscopy of biofluids allows monitoring of endogenous metabolite profiles that alter characteristically in response to changes in physiological status. Herein, a 1H NMR metabolomics approach was applied to the investigation of APAP toxicity in rats and the effect of phenobarbital（PB）on APAP-induced hepatotoxicity. Metabolite differences due to hepatotoxicity were observed in 1H NMR spectra of serum and urine, and enhanced APAP hepatotoxicity by pretreatment with PB was clearly shown by a principal components analysis of the spectral data. NMR spectra of APAP-dosed rat urine provided profiles of APAP-related compounds together with endogenous metabolites. By comparison of endogenous and APAP-related metabolite spectra with those from rats pretreated with PB, it was possible to show the importance of oxidative metabolism of APAP to N-acetyl-p-benzoquinone, an essential step in APAP hepatotoxicity.

Keywords: acetaminophene, hepatotoxicity, metabolomics

* 第一三共(株)安全性研究所

Imai, K.*1, Nakanishi, I.*2, Anzai, A.*3, Ozawa, T.*4, Miyata, N.*5, Urano, S.*1, Okuda, H., Nakamura, A.*1, Fukuhara, K.: **Synthesis and enhanced radical scavenging activity of a conformationally constrained epigallocatechin analogue**
Chem. Lett., **40**, 1417-1419（2011）

The freely rotating single bond between the pyrogallol and chroman substructures in epigallocatechin by reaction of EGC with acetone in the presence of trimethylsilyl trifluoromethanesulfonate to prepare the rigidified analog in good yield. The synthesized analog was examined for free radical scavenging activity toward the galvinoxyl radical and was found to be 27-fold more potent than EGC.

Keywords: catechin, antioxidant, oxidative stress

[*1] 芝浦工業大学大学院理工学研究科
[*2] (独)放射線医学総合研究所
[*3] 日本薬科大学
[*4] 横浜薬科大学
[*5] 名古屋市立大学大学院薬学研究科

Ohno, A., Kawanishi, T., Okuda, H., Fukuhara, K.: **A new approach to characterization of insulin derived from different species using 1H-NMR coupled with multivariate analysis**

Chem. Pharm. Bull., **60**, 320-324 (2012)

Most of the active components of polypeptides have a complex molecular structure, large molecular size. Such components may also be structurally heterogeneous. Therefore, development of a method that can confirm the consistency of polypeptides amino-acid sequences for product characterization is desirable. In general, it is extremely difficult to distinguish differences of a few amino acid residues in the 1H-NMR spectrum of polypeptides with molecular weights greater than several thousand. However, we have been able to distinguish between three insulin species differing in one to three amino acid residues using a combination of multivariate statistics and 1H-NMR spectra. These results demonstrate that this methodology could be useful for characterization of polypeptides.

Keywords: insulin, principal component analysis, 1H-NMR

Demizu, Y., Doi, M.[*1], Sato, Y., Tanaka, M.[*2], Okuda, H., Kurihara, M.: **Effect of one D-Leu residue on right-handed helical -L-Leu-Aib- peptides in the crystal state**

J. Pept. Sci., **17**, 420-426 (2011)

Four diastereomeric -Leu-Leu-Aib-Leu-Leu-Aib- peptides, Boc-D-Leu-L-Leu-Aib-L-Leu-L-Leu-Aib-Ome (1), Boc-L-Leu-D-Leu-Aib-L-Leu-L-Leu-Aib-Ome (2), Boc-L-Leu-L-Leu-Aib-D-Leu-L-Leu-Aib-Ome (3), and Boc-L-Leu-L-Leu-Aib-L-Leu-D-Leu-Aib-Ome (4), were synthesized. The crystals of the four hexapeptides were characterized by X-ray crystallographic analysis Two diastereomeric hexapeptides 1 and 2 having D-Leu(1) or D-Leu(2) were folded into right-handed (*P*) 3_{10}-helical structures, while peptide 3 having D-Leu(4) was folded into a turn structure nucleated by type III' and I' β-turns, and peptide 4 having D-Leu(5) was folded into a left-handed (*M*) 3_{10}-helical structure.

Keywords: amino acids, peptide, conformation

[*1] 大阪薬科大学
[*2] 長崎大学薬学部

Demizu, Y., Yamagata, N., Nagoya, S., Sato, Y., Doi, M.[*1], Tanaka, M.[*2], Nagasawa, K.[*3], Okuda, H., Kurihara, M.: **Enantioselective epoxidation of α,β-unsaturated ketones catalyzed by stapled helical peptides**

Tetrahedron, **67**, 6155-6165 (2011)

Stapled helical L-leucine-based heptapeptides were synthesized and used as catalysts for the enantioselective epoxidation of α,β-unsaturated ketones. All *N*-terminal free stapled peptides were successfully used as chiral catalysts. Among them, the use of H-$hS_{3,7}hS$-10 gave epoxide products with high enantioselectivities of up to 99% ee. Furthermore, the dominant conformations of the N-terminal protected stapled peptides $R_{3,7}R$-10 and $hS_{3,7}hS$-10 were investigated by ^{1}H NMR, IR, CD spectra, and X-ray crystallographic analysis. The peptide $R_{3,7}R$-10 formed a right-handed (*P*) α-helix in solution and in the crystalline state, while $hS_{3,7}hS$-10 formed a right-handed (*P*) 3_{10}-helix in solution.

Keywords: stapled peptide, helix, organocatalyst

[*1] 大阪薬科大学
[*2] 長崎大学薬学部
[*3] 東京農工大学大学院

Demizu, Y., Wakana, D., Kamakura, H., Kurihara, M., Okuda, H., Goda, Y.: **Identification of mutdenafil in a dietary supplement and its subsequent synthesis**

Chem. Pharm. Bull., **59**, 1314-1316 (2011)

We isolated a new illegal sildenafil analogue named mutaprodenafil from a dietary supplement for erectile dysfunction (ED) and proposed that it is an aildenafil derivative containing an imidazole moiety. We subsequently synthesized mutaprodenafil from a thioaildenafil and authenticated its structure.

Keywords: aildenafil prodrug, sildenafil analogue, mutaprodenafil

Demizu, Y., Doi, M.[*1], Sato, Y., Tanaka, M.[*2], Okuda, H.,

Kurihara, M.: **Screw-sense control of helical oligopeptides containing equal amounts of L- and D-amino acids**
Chem. Eur. J., **17**, 11107-11109（2011）

The preferred secondary structures of Boc-(L-Leu-D-Leu-Aib)$_n$-OMe containing equal amounts of L-Leu and D-Leu residues were right-handed (*P*) α-helices in both solution and the crystalline state.

Keywords: peptide, screw-sense control, X-ray crystallographic analysis

---

[*1] 大阪薬科大学
[*2] 長崎大学薬学部

Demizu, Y., Takahashi, T., Kaneko, F., Sato, Y., Okuda, H., Ochiai, E.[*], Horie, K.[*], Takagi, K.[*], Kakuda, S.[*], Takimoto-Kamimura, M.[*], Kurihara, M.: **Design, synthesis and X-ray crystallographic study of new nonsecosteroidal vitamin D receptor ligands**
Bioorg. Med. Chem. Lett., **21**, 6104-6107（2011）

We designed and synthesized non-secosteroidal vitamin D receptor（VDR）ligands that formed H-bonds with six amino acid residues（Tyr143, Ser233, Arg270, Ser274, His301, and His393）of the VDR ligand-binding domain. The ligand YR335 exhibited potent transcriptional activity, which was comparable to those of 1α,25-dihydroxyvitamin D3 and YR301. The crystal structure of the complex formed between YR335 and the VDR ligand-binding domain was solved, which revealed that YR335 formed H-bonds with the six amino acid residues mentioned above.

Keywords: vitamin D receptor, non-secosteroidal ligand, X-ray crystallographic analysis

---

[*] 帝人ファーマ(株)

Sugiyama, T.[*1], Imamura, Y.[*2], Demizu, Y., Kurihara, M., Takano, M.[*3], Kittaka, A.[*3]: **β-PNA: Peptide nucleic acid（PNA）with a chiral center at the β-position of the PNA backbone**
Bioorg. Med. Chem. Lett., **21**, 7317-7320（2011）

Peptide nucleic acid（PNA）monomers with a methyl group at the β-position have been synthesized. The modified monomers were incorporated into PNA oligomers using Fmoc chemistry for solid-phase synthesis. Thermal denaturation and circular dichroism（CD）studies have shown that PNA containing the S-form monomers was well suited to form a hybrid duplex with DNA, whose stability was comparable to that of unmodified PNA － DNA duplex, whereas PNA containing the R-form monomers was not.

Keywords: peptide nucleic acid, preorganization, chirality, helicity

---

[*1] 東京大学
[*2] 工学院大学
[*3] 帝京大学薬学部

Demizu, Y., Doi, M.[*1], Kurihara, M., Maruyama, T.[*2], Suemune, H.[*3], Tanaka, M.[*4]: **One-handed helical-screw direction of homopeptide-foldamer exclusively induced by cyclic α-amino acid side-chain chiral centers**
Chem. Eur. J., **18**, 2430-2439（2012）

Chiral cyclic α,α-disubstituted amino acids, (3*S*,4*S*)- and (3*R*,4*R*)-1-amino-3,4-(dialkoxy)cyclopentanecarboxylic acids ((*S*,*S*)- and (*R*,*R*)-Ac$_5$c^{dOR}; R: methyl, methoxymethyl), were synthesized from dimethyl L-(+)- or D-(-)-tartrate, and their homochiral homooligomers were prepared by solution-phase methods. The preferred secondary structure of the (*S*,*S*)-Ac$_5$c^{dOMe} hexapeptide was a left-handed (*M*) 3$_{10}$ helix, whereas those of the (*S*,*S*)-Ac$_5$c^{dOMe} octa- and decapeptides were left-handed (*M*) α helices, both in solution and in the crystal state. The octa- and decapeptides can be well dissolved in pure water and are more α helical in water than in 2,2,2-trifluoroethanol solution. The left-handed (M) helices of the (*S*,*S*)-Ac$_5$c^{dOMe} homochiral homopeptides were exclusively controlled by the side-chain chiral centers, because the cyclic amino acid (*S*,*S*)-Ac$_5$c^{dOMe} does not have an α-carbon chiral center but has side-chain γ-carbon chiral centers.

Keywords: amino acids, chirality, helical structures

[*1] 大阪薬科大学
[*2] 徳島文理大学香川薬学部
[*3] 九州大学薬学部
[*4] 長崎大学薬学部

Demizu, Y., Okuhira, K., Motoi, H., Ohno, A., Shoda, T., Fukuhara, K., Okuda, H., Naito, M., Kurihara, M.: **Design and synthesis of estrogen receptor degradation inducer based on a protein knockdown strategy**
Bioorg. Med. Chem. Lett., **22**, 1793-1796（2012）

We designed and synthesized estrogen receptor（ER）degradation inducers 5, 6, and 7, which crosslink the ER and the cellular inhibitor of apoptosis protein 1（cIAP1）. Compounds 5, 6, and 7 induced cIAP1-mediated ubiquitylation of ERα resulting in its proteasomal degradation.

Keywords: protein knockdown, tamoxifen, ubiquitin-proteasome system

Kuriyama, M.[*1], Takeuchi, T.[*1], Ito, M.[*2], Yamasaki, N.[*2], Yamamura, R.[*1], Demizu, Y., Onomura, O.[*1]: **Monoallylation of 1,2-diols by Pd/Sn bimetallic catalysis**

Chem. Eur. J., **18**, 2477-2480（2012）

The selective monoallylation of 1,2-diols was successfully developed with Pd/Sn bimetallic catalysis in good to excellent yields. This process was carried out with high substrate tolerance under mild conditions. The catalyst system achieved the quite high chemoselectivity even in the presence of a 1:1 mixture of the 1,2-diol and mono-ol.

Keywords: allylation, bimetallic catalysis, diol

[*1] 長崎大学
[*2] (株)ダイセル

Demizu, Y., Sano, K.[*], Terayama, N., Hakamata, W., Sato, Y., Inoue, Y.[*1], Okuda, H., Kurihara, M.: **Solid-phase nucleophilic fluorination**

Synth. Commun., **42**, 1724-1730（2012）

This study demonstrates solid-phase nucleophilic fluorination. Polymer-bound 1-phenoxy-2-sulfonyloxyethane, as a model compound, is converted to a fluorinated com- pound in a short time. Furthermore, this method is applied to synthesize a precursor of 2-deoxy-2-fluoro-D-glucose by solid-phase synthesis using a microwave oven.

Keywords: fluorination, microwave, solid-phase synthesis

[*] 東京薬科大学

Okuhira, K., Ohoka, N., Sai, K., Nishimaki-Mogami, T., Itoh, Y.[*], Ishikawa, M.[*], Hashimoto, Y.[*] and Naito, M.: **Specific degradation of CRABP-II via cIAP1-mediated ubiquitylation induced by hybrid molecules that cross-link cIAP1 and the target protein**

FEBS Lett., **585**, 1147-1152（2011）

Manipulation of protein stability with small molecules is a challenge in the field of drug discovery. Here we show that cellular retinoic acid binding protein-II（CRABP-II）can be specifically degraded by a novel compound, SNIPER-4, consisting of (-)-N-[(2S,3R)-3-amino-2-hydroxy-4-phenylbutyryl]-L-leucine methyl ester and all-trans retinoic acid that are ligands for cellular inhibitor of apoptosis protein 1（cIAP1）and CRABP-II, respectively. Mechanistic analysis revealed that SNIPER-4 induces cIAP1-mediated ubiquitylation of CRABP-II, resulting in the proteasomal degradation. The protein knockdown strategy employing the structure of SNIPER-4 could be applicable to other target proteins.

Keywords: ubiquitin, IAP, protein knockdown

[*] 東京大学分子細胞生物学研究所

Maejima, T.[*], Sugano, T.[*], Yamazaki, H.[*], Yoshinaka, Y.[*], Doi, T.[*], Tanabe, S.[*] and Nishimaki-Mogami, T.: **Pitavastatin increases ABCA1 expression by dual mechanisms: SREBP2-driven transcriptional activation and PPARα-dependent protein stabilization but without activating LXR in rat hepatoma McARH7777 cells**

J. Pharmacol. Sci., **116**, 107-115（2011）

Hepatic ATP-binding cassette transporter A1（ABCA1）plays a key role in high-density lipoprotein（HDL）production by apolipoprotein A-I（ApoA-I）lipidation. 3-Hydroxy-3-methylglutaryl coenzyme A（HMG-CoA）reductase inhibitors, statins, increase ABCA1 mRNA levels in hepatoma cell lines, but their mechanism of action is not yet clear. We investigated how statins increase ABCA1 in rat hepatoma McARH7777 cells. Pitavastatin, atorvastatin, and simvastatin increased total ABCA1 mRNA levels, whereas pravastatin had no effect. Pitavastatin also increased ABCA1 protein. Hepatic ABCA1 expression in rats is regulated by both liver X receptor（LXR）and sterol regulatory element-binding protein（SREBP2）pathways. Pitavastatin repressed peripheral type ABCA1 mRNA levels and its LXR-driven promoter, but activated the liver-type SREBP-driven promoter, and eventually increased total ABCA1 mRNA expression. Furthermore, pitavastatin increased peroxisome proliferator-activated receptor α（PPARα）and its downstream gene expression. Knockdown of PPARα attenuated the increase in ABCA1 protein, indicating that pitavastatin increased ABCA1 protein via PPARα activation, although it repressed LXR activation. Furthermore, the degradation of ABCA1 protein was retarded in pitavastatin-treated cells. These data suggest that pitavastatin increases ABCA1 protein expression by dual mechanisms: SREBP2-mediated mRNA transcription and PPARα-mediated ABCA1 protein stabilization, but not by the PPAR-LXR-ABCA1 pathway.

Keywords: ABCA1, hepatic expression, statin

[*] 興和(株)東京創薬研究所

Inoue, J.[*1], Yamasaki, K.[*1], Ikeuchi, E.[*1], Satoh, S.I.[*1], Fujiwara, Y.[*2], Nishimaki-Mogami, T., Shimizu, M.[*1] and

Sato, R[*1].: **Identification of MIG12 as a mediator for stimulation of lipogenesis by LXR activation**

Mol. Endocrinol., **25**, 995-1005（2011）

Liver X receptor（LXR）α and LXRβ belong to the nuclear receptor superfamily and play central roles in the transcriptional control of lipid metabolism. We describe a novel LXR target, midline-1-interacting G12-like protein（MIG12）, which has been recently identified as an acetyl-coenzyme A carboxylase-binding protein. The binding causes the induction of de novo fatty acid（FA）synthesis through the activation of acetyl-coenzyme A carboxylase（a rate-limiting enzyme for de novo FA synthesis）. Luciferase reporter gene assays using the MIG12 gene promoter revealed the existence of a LXR-responsive element（LXRE）and carbohydrate-responsive element-binding protein（ChREBP）-responsive element named LXRE3 and carbohydrate response element 1, respectively. Deletion and mutation of LXRE3 and carbohydrate response element 1 abolished LXR and ChREBP responsiveness, respectively. Electrophoretic mobility shift assays demonstrated that the LXRα/retinoid X receptor α complex was bound to LXRE3. Treatment with high glucose concentration, which leads ChREBP activation, or LXR activator stimulated MIG12 expression in rat primary hepatocytes, and combined treatment further stimulated MIG12 expression. Furthermore, hepatic expression of MIG12 in mice was induced by refeeding. Overexpression of MIG12 stimulated and knockdown of MIG12 attenuated LXR ligand-stimulated de novo FA synthesis and triacylglycerol accumulation. These results indicate that MIG12 is a mediator for stimulation of lipogenesis by LXR activation in the liver.

Keywords: LXR, lipogenesis, MIG12

[*1] 東京大学農学生命科学研究科
[*2] お茶の水女子大学

Matsumura, T.[*1], Kinoshita, H.[*1], Ishii, N.[*1], Fukuda, K.[*1], Motoshima, H.[*1], Senokuchi, T.[*1], Taketa, K.[*2], Kawasaki, S.[*1], Nishimaki-Mogami, T., Kawada, T.[*1], Nishikawa, T.[*1] and Araki, E.[*1]: **Telmisartan exerts antiatherosclerotic effects by activating peroxisome proliferator-activated receptor-{gamma} in macrophages**

Arterioscler. Thromb. Vasc. Biol., **31**, 1268-1275（2011）

OBJECTIVE: Telmisartan, an angiotensin type I receptor blocker（ARB）, protects against the progression of atherosclerosis. Here, we investigated the molecular basis of the antiatherosclerotic effects of telmisartan in macrophages and apolipoprotein E-deficient mice. METHODS AND RESULTS: In macrophages, telmisartan increased peroxisome proliferator-activated receptor-γ（PPARγ）activity and PPAR ligand-binding activity. In contrast, 3 other ARBs, losartan, valsartan, and olmesartan, did not affect PPARγ activity. Interestingly, high doses of telmisartan activated PPARα in macrophages. Telmisartan induced the mRNA expression of CD36 and ATP-binding cassette transporters A1 and G1（ABCA1/G1）, and these effects were abrogated by PPARγ small interfering RNA. Telmisartan, but not other ARBs, inhibited lipopolysaccharide-induced mRNA expression of monocyte chemoattractant protein-1（MCP-1）and tumor necrosis factor-α, and these effects were abrogated by PPARγ small interfering RNA. Moreover, telmisartan suppressed oxidized low-density lipoprotein-induced macrophage proliferation through PPARγ activation. In apolipoprotein E（-/-）mice, telmisartan increased the mRNA expression of ABCA1 and ABCG1, decreased atherosclerotic lesion size, decreased the number of proliferative macrophages in the lesion, and suppressed MCP-1 and tumor necrosis factor-α mRNA expression in the aorta. CONCLUSION: Telmisartan induced ABCA1/ABCG1 expression and suppressed MCP-1 expression and macrophage proliferation by activating PPARγ. These effects may induce antiatherogenic effects in hypertensive patients.

Keywords: Atherosclerosis, ABCA1, PPARgamma

[*1] 熊本大学医学研究科
[*2] 京都大学農学生命研究科

Ohoka, N., Okuhira, K., Cui, H., Wu, W., Sato, R.[*], Naito, M. and Nishimaki-Mogami, T.: **HNF4α increases liver-specific human ATP-binding cassette transporter A1 expression and cholesterol efflux to apolipoprotein A-I in response to cholesterol depletion**

Arterioscler. Thromb. Vasc. Biol., **32**, 1005-1014（2012）

OBJECTIVE: Hepatic ATP-binding cassette transporter A1（ABCA1）plays the major role in maintaining plasma high-density lipoprotein levels by producing cholesterol-accepting nascent high-density lipoprotein, whereas peripheral ABCA1 is responsible for releasing cellular cholesterol. We previously reported that in rodents, cholesterol depletion reduces ABCA1 expression in peripheral but not hepatic cells by increasing a liver-specific ABCA1 transcript via the sterol regulatory element-binding protein-2 system. However, the regulatory element is not conserved in humans. Here we investigated the mechanism of sterol-regulated human hepatic ABCA1 gene expression. METHODS AND RESULTS: ABCA1 mRNA

variant type L3 is a novel and human-liver-specific transcript accounting for ≈25% of total ABCA1 mRNA in the liver and is induced by cellular cholesterol depletion. Specific knockdown or forced expression revealed that type L3 produces functional ABCA1 protein in cholesterol efflux. We identified a regulatory enhancer element for L3 expression lying within intron 3 of the human ABCA1 gene, to which hepatocyte nuclear factor（HNF）4α binds in response to cholesterol depletion. HNF4α knockdown abolished induction of liver-specific L3 and L2b transcripts（and consequently the liver-type response of ABCA1 expression to cellular cholesterol status）and diminished cholesterol efflux activity. CONCLUSIONS: These findings indicate that HNF4α regulates human hepatic ABCA1 expression in response to cholesterol depletion.

Keywords: ABCA1, HNF4α, liver

* 東京大学農学生命科学研究科

Itoh, Y.*, Kitaguchi, R.*, Ishikawa, M.*, Naito, M. and Hashimoto, Y.*: **Design, synthesis and biological evaluation of nuclear receptor-degradation inducers**

Bioorg. Med. Chem., **19**, 6768-6778（2011）

Compounds that regulate the function(s) of nuclear receptors（NRs）are useful for biological studies and as candidate therapeutic agents. Most such compounds are agonists or antagonists. On the other hand, we have developed specific protein degradation inducers, which we designated as SNIPERs（Specific and Nongenetic IAPs-dependent Protein ERasers）, for selective degradation of target proteins. SNIPERs are hybrid molecules consisting of an appropriate ligand for the protein of interest, coupled to a ligand for inhibitor of apoptosis proteins（IAPs）, which target the bound protein for polyubiquitination and proteasomal degradation. We considered that protein knockdown with SNIPERs would be a promising alternative approach for modulating NR function. In this study, we designed and synthesized degradation inducers targeting retinoic acid receptor（RAR）, estrogen receptor（ER）, and androgen receptor（AR）. These newly synthesized RAR, ER, and AR SNIPERs, 9, 11, and 13, respectively, were confirmed to significantly reduce the levels of the corresponding NRs in live cells.

Keywords: ubiquitin, protein knockdown, nuclear receptor

* 東京大学分子細胞生物学研究所

Itoh, Y.*, Ishikawa, M.*, Kitaguchi, R.*, Sato, S.*, Naito, M. and Hashimoto, Y.*: **Development of target protein-selective degradation inducer for protein knockdown**

Bioorg. Med. Chem., **19**, 3229-3241（2011）

Our previous technique for inducing selective degradation of target proteins with ester-type SNIPER（Specific and Nongenetic Inhibitor-of-apoptosis-proteins（IAPs）-dependent Protein ERaser）degrades both the target proteins and IAPs. Here, we designed a small-molecular amide-type SNIPER to overcome this issue. As proof of concept, we synthesized and biologically evaluated an amide-type SNIPER which induces selective degradation of cellular retinoic acid binding protein II（CRABP-II）, but not IAPs. Such small-molecular, amide-type SNIPERs that induce target protein-selective degradation without affecting IAPs should be effective tools to study the biological roles of target proteins in living cells.

Keywords: ubiquitin, protein knockdown, ATRA

* 東京大学分子細胞生物学研究所

Satoh, R.*1, Nakamura, R., Komatsu, A.*2, Oshima, M.*2, Teshima, R.: **Proteomic analysis of known and candidate rice allergens between non-transgenic and transgenic plants**

Regul. Toxicol. Pharmacol., **59**, 437-444（2011）

Salt-soluble proteins extracted from non-transgenic and transgenic rice were evaluated for the presence of known and potential allergens by proteomic techniques. The salt-soluble proteins were extracted, separated by 1D and 2D electrophoresis, and analyzed by Western blotting. 1D immunoblot analysis with patients' sera revealed few qualitative differences between the IgE-binding proteins of the non-transgenic and transgenic rice. 1D immunoblot with antigen-specific-animal sera revealed no qualitative or quantitative differences in two known allergens, RAG2 and glyoxalase I, between non-transgenic and transgenic rice. Multiple spots containing known and novel IgE-binding proteins were detected among the salt-soluble proteins of non-transgenic rice by 2D immunoblotting. Two globulin-like proteins, a 52 kDa protein and a 63 kDa protein, were identified as novel IgE-binding proteins that are candidates for rice allergens. These globulin-like proteins were homologous to Cupin superfamily allergens. Quantitative analysis of 19, 52, and 63 kDa globulins with protein-specific-animal sera showed no significant differences in the expression of these proteins between the transgenic rice and non-transgenic rice. These results indicate that none of the known or novel endogenous IgE-binding proteins detected in this study appear to be altered by genetic

modification.

Keywords: Rice, Allergen, Proteomics

*1 National Agriculture and Food Research Organization
*2 National Institute of Crop Science

佐藤里絵*，中村里香，手島玲子：**イムノプロテオミクス手法を用いたソバIgE結合タンパク質の網羅的検出**

日本食品化学学会誌，**18**，103-109（2011）

To comprehensive IgE-binding capacities of proteins（allergenome）in buckwheat seeds were examined using immunoproteomic techniques. Salt-soluble proteins were extracted from buckwheat weeds, separated using one- and two-dimensional electrophoresis, and analyzed using western blotting with buckwheat-allergic patients' sera revealed some IgE-binding proteins, and multiple spots containing known and novel IgE-binding proteins were detected using two-dimensional immunoblotting. Some spots were newly identified as 13S globulin protein subunits or isoforms. Some spots that were homologous to vicillin-like proteins indicated the presence of newly identified vicillin-like proteins in buckwheat. These results obtained from an immunoproteomic analysis may contribute not only to construction of a comprehensive IgE-binding protein map of buckwheat, but also the detection of isoforms of IgE-binding proteins in buckwheat variants.

Keywords: Buckwheat, Allergen, Immune-proteomics

* National Agriculture and Food Research Organization

Shindo, T.*1, Kanazawa, Y.*2, Saito Y.*1, Kojima, K.*1, Ohsawa, M.*1, Teshima, R.: **Effective induction of oral anaphyaxis to ovalbumin in mice sensitized by feeding of the antigen with aid of oil emulsion and salicylate**

J. Toxicol. Sci., **37**, 307-315（2012）

It is important to evaluate the ability of novel proteins in food crops and products to elicit potentially harmful immunologic responses, including allergic hypersensitivity. We developed a novel mouse model of food allergy involving an oral challenge of a protein antigen after feeding of the antigen in combination with modulating factors often ingested in daily life, namely, dietary oil emulsion and salicylate. In the model, BALB/c mice were sensitized orally for three weeks with ovalbumin（OVA）in linoleic acid/lecithin emulsion, followed immediately by intraperitoneal injection of sodium salicylate. At the end of the sensitization, the incidence of mice positive for serum OVA-specific IgG1 but not IgE had significantly increased in the combined-sensitization group. After the 3-week sensitization, a single or double oral challenge with OVA effectively and significantly caused severeanaphylaxis, as compared with the groups sensitized with OVA in the emulsion or the vehicle alone. Moderate increase of plasma histamine and intestinal abnormality in histology was found only in the combined-sensitization group. Anaphylaxis symptoms in the sensitized mice were induced more by oral challenge than by intravenous challenge, suggesting a critical role for the mucosal system. This is the first model for successfulinduction of oral anaphylaxis in mice sensitized by feeding of food protein without adjuvant. It will be useful to elucidate the mechanism of food allergy and to detect modulating factors of oral allergy at sensitization using this model, which simulates real life conditions.

Keywords: Anaphylaxis, Dietary oil emulsion, Salicylic acid

*1 Food and Drug Safety Center
*2 Pharmaceuticals and Medical Devices Agency

中村里香，中村亮介，手島玲子：**古代米（赤米・黒米）のアレルゲン発現プロテオミクス解析**

日本食品化学学会誌，**18**，143-149（2011）

The levels and features of the allergenic proteins in six cultivars of red or black rice were compared with those in white rice. The two major allergens of rice, the RAG2 family allergenic protein isoforms and the glyoxalase I protein, were targeted so as to compare their contents in the cultivars using immunoblotting and two-dimensional different gel electrophoresis（2D-DIGE）, as a proteomic analytical methods. The immunoblotting results showed that the amounts of RAG2 isoforms were lower in some cultivars of the red and black rice than those of the white rice, while all the cultivars contained similar levels of glyoxalase I. The results of 2D-DIGE showed that some cultivars contained significantly fewer RAG2 isoforms（including RAG1［RA17］, RAG2［RA14］, and allergenic protein［18 kDa］）than white rice. Similar to the immunoblotting results, the contents of glyoxalase I protein were similar among all the cultivars. The differences in the composition of the allergen proteins among the cultivars may have been dependent not on the color of the bran but on the genetic background of the cultivars based on a pattern analysis. Here, we present the differences in the contents of the isoforms of the allergenic proteins in ancient rice cultivars using the 2D-DIGE method.

Keywords: Allergen, Rice, Proteomics

Kondo, K., Obitsu, S., Teshima, R.: **α-Synuclein aggregation and transmission are enhanced by leucine-rich repeat kinase 2 in human neuroblastoma SH-SY5Y cells**

Biol. Pharm. Bull., **34**, 1078-1083（2011）

Formation of α-Synuclein aggregates is a key step in Parkinson's disease pathogenesis although the etiology remains elusive. α-Synuclein is accumulated in degenerating neurons, leading to the production of filamentous inclusions such as Lewy bodies. However, the in vitro overexpression of α-synuclein alone failed to induce inclusion bodies consisting of phosphorylated α-synuclein. The seeded aggregates-initiated polymerization of α-synuclein and tau has been reported elsewhere. What molecule is an initiator of filamentous inclusions remains to be defined. Here, we report that leucine-rich repeat kinase 2（LRRK2）-cotransfection together with α-synuclein enhance the aggregate formation, phosphorylation, release to extracellular media of α-synuclein, and the cell to-cell transmission into neighboring cells in human neuroblastoma SH-SY5Y cells. In cells transfected with α-synuclein alone, the proteins were distributed in the cytosol and did not form inclusions. On the other hand, the inclusions and phosphorylation of α-synuclein were formed in cells cotransfected with α-synuclein and LRRK2 G2019S mutant together. LRRK2 G2019S-cotransfected PC12 cells also induced the aggregates. Furthermore, the cell-to-cell transmission of α-synuclein and the cell toxicity were also enhanced by either LRRK2 wild type or G2019S mutant, whereas the cell viability was not decreased in cells transfected with α-synuclein alone. These results suggest that overexpression of LRRK2, especially G2019S mutant, whose functions remain unclear, initiate the aggregate formation, release and transmission of α-synuclein, resulting in the propagation of α-synuclein to neighboring cells and reduction of cell viability.

Keywords: α-Synuclein, Leucine-rich repeat kinase-2, Aggregation

Nakamura, K., Akiyama, H., Ohmori, K.[*1], Takahashi, Y.[*2], Takabatake, R.[*3], Kitta, K.[*3], Nakazawa, H.[*2], Kondo, K., Teshima, R.: **Identification and detection method for genetically modified papaya resistant to papaya ringspot virus YK strain**

Biol. Pharm. Bull., **34**, 1648-1651（2011）

Unauthorized genetically modified（GM）papaya（*Carica papaya* LINNAEUS）was detected in a commercially processed product, which included papaya as a major ingredient, in Japan. We identified the transgenic vector construct generated based on resistance to infection with the papaya ringspot virus（PRSV）YK strain. A specific detection method to qualitatively monitor papaya products for contamination with the GM papaya was developed using the real-time polymerase chain reaction.

Keywords: Genetically modified organism, Papaya, Polymerase chain reaction

[*1] Kanagawa Prefactural Institute of Public Health
[*2] Hoshi University
[*3] National Food Research Institute

Ohashi-Suzuki, M.[*1], Yabu, Y.[*1], Ohshima, S.[*1], Nakamura, K., Kido, Y.[*2], Sakamoto, K.[*2], Kita, K.[*2], Ohta, N.[*1], Suzuki, T.[*1]: **Differential kinetic activities of glycerol kinase among African trypanosome species: Phylogenetic and therapeutic implications**

J. Vet. Med. Sci., **73**, 615-621（2011）

African trypanosome species are causative agents for sleeping sickness in humans and nagana disease in cattle. *Trypanosoma brucei* can generate ATP via a reverse reaction with glycerol kinase（GK）when alternative oxidase（AOX）is inhibited; thus, GK is considered to be a crucial target for chemotherapy combined with AOX. However, the energy metabolism systems of African trypanosome species other than *T. brucei* are poorly understood. Thus, GK genes were surveyed from genome databases and cloned by PCR from *T. vivax* and *T. congolense*. Then, recombinant GK proteins（rGK）of *T. vivax*, *T. congolense* and *T. brucei* were expressed and purified. Kinetic analysis of these rGK proteins revealed that the K（m）values of *T. congolense* rGK for ADP and G-3-P substrates were lower than those of *T. vivax* and *T. brucei*. The expression level of GK molecules was highest in *T. congolense* cells and lowest in *T. vivax* cells. Based on these results, effective combination dosages of ascofuranone, a specific inhibitor of AOX, and glycerol, an inhibitor of the GK reverse reaction, were determined by using in vitro-cultured trypanosome cells.

Keywords: Ascofuranone, Glycerol, Glycerol kinase

[*1] Graduate School of Tokyo Medical and Dental University
[*2] University of Tokyo

Nakamura, K., Ohtsuki, T.[*1], Mori, H.[*2], Hoshino, H.[*1], Hoque, A.[*1], Oue, A.[*1], Kanou, F.[*3], Sakagami, H.[*3], Tanamoto, K.[*4], Ushijima, H.[*5], Kawasaki, N., Akiyama, H., Ogawa, H.[*3]: **Novel anti-HIV-1 activity produced by**

conjugating unsulfated dextran with poly L-lysine

Antiviral Res., **94**, 89-97（2012）

A conjugate of poly L-lysine（PLL）with unsulfated dextran produced by reductive amination was found to have remarkable anti-HIV-1 activity against both the macrophage-tropic R5 virus Ba-L and T-cell line tropic X4 virus IIIB strains, although neither PLL nor dextran has such activity. The conjugate is a pseudoproteoglycan（pseudoPG）that simulates the structure of a proteoglycan. Conjugation with dextran was found to produce an antiviral effect in three kinds of assay systems including a human CD4(+) T-cell line, and the pseudoPG synthesized using 10kDa PLL and 10kDa dextran showed EC(50) 4-40 times lower than that of sulfated dextran or heparin against Ba-L and EC(50) equal to that against IIIB, indicating that PLL-dextran（PLL-Dex）was more effective against R5 virus than sulfated polysaccharides. PLL-Dex significantly suppressed a clinically isolated R5 virus from primary peripheral blood mononuclear cells. PLL-Dex interacted with the virus during adsorption to the cell and also decreased virus entry into the cell, suggesting PLL-Dex has multiple preventive mechanisms against HIV-1.

Keywords: Unsulfated glycan, Dextran, poly L-lysine

[*1] Gunma University
[*2] Osaka Prefectural Institute of Public Health
[*3] Ochanomizu University
[*4] Musashino University
[*5] Aino University

Matemu, A. O.[*], Nakamura, K., Kayahara, H.[*], Murasawa, H.[*], Katayama, S.[*], Nakamura, S.[*]: **Enhanced antiviral activity of soybean β-conglycinin-derived peptides by acylation with saturated fatty acids**

J. Food Sci., 76, M299-304（2011）

Peptide mixtures prepared from soybean β-conglycinin（7S-peptides）were acylated with saturated fatty acids of different chain length（6C-18C）in order to improve their antiviral activity against *Feline calicivirus*（FCV）strain F9 which is a typical norovirus surrogate. Among the fatty acids varieties, it was revealed that 7S-peptides acylated with myristic and palmitic acids potently inhibited FCV replication. Myristorylation and palmitoylation of 7S-peptides kept host cells viability at 91.51% and 98.90%, respectively. The infectivity of FCV on Crandell-Reese feline kidney cells was further determined after exposure of initial titer of 10(6.47) TCID(50)/mL. Myristoylated and palmitoylated 7S-peptides significantly（$P < 0.006$）reduced FCV infectivity as compared to native 7S-peptides. Native 7S-peptides showed 25% FCV inhibitory activity while myristoylated and palmitoylated 7S-peptides exhibited 98.59% and 99.98% reduction in FCV infectivity, respectively. Myristoylated and palmitoylated 7S-peptides demonstrated higher anti-FCV activity in a wide range of concentration with complete reduction at 25 μg/mL. Surface hydrophobicity was significantly（$P < 0.05$）increased after attachment of long hydrocarbon fatty acids to 7S-peptides as supported by changes in fluorescence intensity. Enzymatic hydrolysis together with acylation will give an insight into surface and physiological functional lipopeptides derived from soy β-conglycinin.

Keywords: Antiviral activity, *Feline calicivirus*, Myristoylation

---

[*] Shinshu University

Taguchi, H.[*1], Watanabe, S.[*1], Temmei, Y.[*1], Hirao, T.[*1], Akiyama, H, Sakai, S., Adachi, R., Sakata, K., Urisu, A.[*2], Teshima, R.: **Differential detection of shrimp and crab for food labeling using polymerase chain reaction**

J. Agric. Food Chem., **59**, 3510-3519（2011）

Shrimp and crab are well-known as allergenic ingredients. According to Japanese food allergy labeling regulations, shrimp species（including prawns, crayfishes, and lobsters）and crab species must be differentially declared when ≥10 ppm（total protein）of an allergenic ingredient is present. However, the commercial ELISA tests for the detection of crustacean proteins cannot differentiate between shrimp and crab. Therefore, two methods were developed to discriminate shrimp and crab: a shrimp-PCR method with postamplification digestion and a crab-PCR method that specifically amplifies a fragment of the 16S rRNA gene. The sensitivity and specificity of both PCR methods were verified by experiments using DNA extracted from 15 shrimp species, 13 crab species, krill, mysid, mantis shrimp, other food samples（cephalopod, shellfish, and fish）, incurred foods, and commercial food products. Both PCR methods could detect 5 pg of DNA extracted from target species and 50 ng of genomic DNA extracted from incurred foods containing 10 ppm（μg/g）total protein of shrimp or crab. The two PCR methods were considered to be specific enough to separately detect species belonging to shrimp and crab. Although false-positive and false-negative results were obtained from some nontarget crustacean species, the proposed PCR methods, when used in conjunction with ELISA tests, would be a useful tool for confirmation of the validity of food allergy labeling and

management of processed food safety for allergic patients.
Keywords: Food allergy, PCR, Crustaceans

[*1] House Foods Corporation
[*2] Fujita Health University

Watanabe, S.[*1], Taguchi, H.[*1], Temmei, Y.[*1], Hirao, T.[*1], Akiyama, H., Sakai, S., Adachi, R., Urisu, A.[*2], Teshima, R.: **Specific detection of potentially allergenic peach and apple in foods using polymerase chain reaction**
J. Agric. Food Chem., **60**, 2018-2015（2012）

Two PCR methods were developed for specific detection of the trnS-trnG intergenic spacer region of *Prunus persica*（peach）and the internal transcribed spacer region of *Malus domestica*（apple）. The peach PCR amplified a target-size product from the DNA of 6 *P. persica* cultivars including 2 nectarine and 1 flat peach cultivar, but not from those of 36 nontarget species including 6 *Prunus* and 5 other Rosaceae species. The apple PCR amplified a target-size product from the DNA of 5 *M. domestica* cultivars, but not from those of 41 nontarget species including 7 Maloideae and 9 other Rosaceae species. Both methods detected the target DNA from strawberry jam and cookies spiked with peach and apple at a level equivalent to about 10 μg of total soluble proteins of peach or apple per gram of incurred food. The specificity and sensitivity were considered to be sufficient for the detection of trace amounts of peach or apple contamination in processed foods.
Keywords: Food allergy, Peach, Apple

[*1] House Foods Corporation
[*2] Fujita Health University

Sheila Galloway, S.[*1], Lorge, E.[*2], Aardema, M. J.[*3], Eastmond, D.[*4], Fellows, M.[*5], Heflich, R.[*6], Kirkland, D.[*7], Levyh, D. D.[*6], Lynch, A. M.[*8], Marzin, D.[*9], Morita, T., Schuler, M.[*10], Speit, G.[*11]: **Workshop summary: Top concentration for in vitro mammalian cell genotoxicity assays; and report from working group on toxicity measures and top concentration for in vitro cytogenetics assays（chromosome aberrations and micronucleus）**
Mutat. Res., **723**, 77-83（2011）

The selection of maximum concentrations for in vitro mammalian cell genotoxicity assays was reviewed at the 5th International Workshop on Genotoxicity Testing（IWGT）, 2009. Currently, the top concentration recommended when toxicity is not limiting is 10 mM or 5 mg/ml, whichever is lower. The discussion was whether to reduce the limit, and if so whether the 1 mM limit proposed for human pharmaceuticals was appropriate for testing other chemicals. The consensus was that there was reason to consider reducing the 10 mM limit, and many, but not all, attendees favored a reduction to 1 mM.
Keywords: *In vitro tests*, *Chromosome aberrations*, *Upper concentration limit*

[*1] Merck Research Laboratories
[*2] Servier Group
[*3] The Procter and Gamble Co
[*4] University of California
[*5] AstraZeneca
[*6] US Food and Drug Administration
[*7] Kirkland Consulting
[*8] GlaxoSmithKline R&D
[*9] Institut PASTEUR de Lille
[*10] PGRD Pfizer Inc,
[*11] Universitat Ulm

Morita, T., Honma, M., Morikawa, K.: **Effect of reducing the top concentration used in the in vitro chromosomal aberration test in CHL cells on the evaluation of industrial chemical genotoxicity**
Mutat. Res., **741**, 32-56（2012）

A current concern with in vitro mammalian cell genotoxicity testing is the high frequency of false or misleading positive results caused in part by the past use of excessively high test concentrations. A dataset of 249 industrial chemicals used in Japan and tested for genotoxicity was analyzed. After an exhaustive review, we conclude 2 mM or 1 mg/mL, whichever is higher, would be an appropriate top concentration limit for testing industrial chemicals for chromosome damage.
Keywords: *Chromosome aberration test*, *CHL cells*, *Test concentration limit*

Morita, T., Morikawa, K.: **Expert Review for GHS Classification of Chemicals on Health Effects**
Ind. Health, **49**, 559-565（2011）

The Globally Harmonized System of Classification and Labelling of Chemicals（GHS）provides a framework for hazard communication on chemicals using labelling or safety data sheets. However, the GHS does not provide any information on the required level of expertise of the Classifiers, definition of who qualifies as an expert, evaluation methods of

WOE or data quality, and the timing of expert judgment and the need for updating/re-classification as new information becomes available. In this paper, key methods and issues in expert reviews are discussed.

Keywords: *GHS*, *Expert review*, *Weight of evidence*

Nakaoka, S.[*1], Ishizaki, T.[*1], Urushihara, H.[*2], Satoh, T.[*3], Ikeda, S.[*4], Morikawa, K., Nakayama, T.[*1]: **Adherence in performing echocardiography to detect valvulopathy associated with the use of ergot-derived dopamine agonists in patients with Parkinson's disease**

Internal Medicine, **50**, 687-694（2011）

The ergot-derived dopamine agonists, cabergoline and pergolide, are associated with valvulopathy risk. In Japan, product labelings were revised in April 2007 to recommend periodic echocardiography for patients taking them, however, physicians' adherence is unknown. This study assessed changes in echocardiography evaluation of patients with Parkinson's disease（PD）taking cabergoline or pergolide before and after the labeling revision and examined factors related with performance of echocardiography. Medical claim data from January 2005 to December 2008 were used. A total of 222 subjects（C-P group（prescribed either cabergoline or pergolide）, 73; reference group（prescribed other anti-PD drugs）, 149）were assessed. The proportion of C-P patients undergoing echocardiography increased from 4.8% to 27.9% after revision of product labels（$p = 0.001$）, which was higher than those in the reference group following label revisions（11.0%）（$p = 0.014$）. Although echocardiography evaluations increased, more than 70% of PD patients prescribed cabergoline or pergolide did not undergo such assessment despite the product labeling notification.

Keywords: Parkinson's disease, dopamine agonist, safety information

[*1] Department of Health Informatics, Kyoto University School of Public Health

[*2] Department of Pharmacoepidemiology, Kyoto University School of Public Health

[*3] Kitasato Clinical Research Center, Kitasato University School of Medicine

[*4] Department of Pharmaceutical Sciences, School of Pharmacy, International University and Health Welfare

Kubota, K., Kasuga, F., Iwasaki, E.[*1], Inagaki,I.[*2], Sakurai, Y.[*3], Komatsu, M.[*3], Toyofuku, H.[*4], Angulo, F.J.[*5], Scallan, E.[*6], Morikawa, K.: **Estimating the Burden of Acute Gastroenteritis and Foodborne llness Caused by *Campylobacter*, *Salmonella*, and *Vibrio parahaemolyticus* by Using Population-Based Telephone Survey Data, Miyagi Prefecture, Japan, 2005 to 2006**

Journal of Food Protection, **74**（10）, 1592-1598（2011）

Most cases of acute gastroenteritis and foodborne disease are not ascertained by public health surveillance because the ill person does not always seek medical care and submit a stool sample for testing, and the laboratory does not always test for or identify the causative organism. We estimated the total burden of acute gastroenteritis in Miyagi Prefecture, Japan, using data from two 2-week cross-sectional, population-based telephone surveys conducted in 2006 and 2007. To estimate the number of acute gastroenteritis illnesses caused by *Campylobacter*, *Salmonella*, and *Vibrio parahaemolyticus* in Miyagi Prefecture, we determined the number of cases for each pathogen from active laboratory-based surveillance during 2005 to 2006 and adjusted for seeking of medical care and submission of stool specimens by using data from the population-based telephone surveys. Monte Carlo simulation was used to incorporate uncertainty. The prevalence of acute gastroenteritis in the preceding 4 weeks was 3.3%（70 of 2,126）and 3.5%（74 of 2,121）in the winter and summer months, yielding an estimated 44,200 episodes of acute gastroenteritis each year in this region. Among people with acute gastroenteritis, the physician consultation rate was 32.0%, and 10.9% of persons who sought care submitted a stool sample. The estimated numbers of *Campylobacter*-, *Salmonella*-, and *V. parahaemolyticus* — associated episodes of acute gastroenteritis were 1,512, 209, and 100 per 100,000 population per year, respectively, in this region. These estimates are significantly higher than the number of reported cases in surveillance in this region. Cases ascertained from active surveillance were also underrepresented in the present passive surveillance, suggesting that complementary surveillance systems, such as laboratory-based active surveillance in sentinel sites, are needed to monitor food safety in Japan.

Keywords: Burden of foodborne illness, Telephone Survey

[*1] Health and Prevention Policy Institute, Miyagi, Japan

[*2] Tohoku Regional Bureau of Health and Welfare, Miyagi, Japan

[*3] Miyagi Medical Association, Miyagi, Japan

[*4] National Institute of Public Health, Japan

[*5] Centers for Disease Control and Prevention, USA

[*6] Colorado School of Public Health, USA

天沼　宏，窪田邦宏，森川　馨：**O157以外の血清群の志賀毒素産生性大腸菌（STEC）：海外における流行の状況**

日本食品微生物学会雑誌，**29**（1），18-23（2012）

下痢原性大腸菌の1種で志賀毒素を産生する志賀毒素産生性大腸菌（STEC）は，出血性下痢症や溶血性尿毒症症候群などの重篤な症状の原因となる，公衆衛生上，懸念すべき食品・水媒介性の病原性細菌の1つである．STECとしては血清型O157:H7が最も良く知られるが，それ以外にも非常に多種類のO血清群が存在しnon-O157 STECと総称されている．これらのSTECの病原性の強さは様々で，O157と同程度の強さを示すものも知られている．海外でどのO血清群のnon-O157 STECが流行しているかを知ることが基礎，応用の両面で有益な情報を与えると考え，公表されている報告書や原著論文による調査を行った．EU，アイルランド，ドイツ，スイス，米国FoodNet 10州，全米，ミネソタ州，コネチカット州，およびオーストラリアを対象地域とした．2009年以前に対象地域において患者から検出される頻度が高かったnon-O157 STEC O血清群を文献より抽出した．その結果，高頻度で検出されるO血清群の多くは，地域によらず共通していた．すなわち，O26，O103，O145，およびO111は欧米に共通して高頻度で検出され，O26，O111はオーストラリアでも代表的なO血清群であった．一方，地域に特徴的なO血清群もあり，O91は主に欧州で，O121およびO45は主に米国で高頻度に検出されていた．日本でもO26，O103，O145，O111は高頻度に検出されるO血清群に数えられ，さらにO91とO121もこれらに含まれていた．世界各地で高頻度に検出されるO血清群の多くが地域を越えて共通であることから，海外でのnon-O157 STECの流行の状況を把握することは国内での流行の防止対策に重要な情報を提供すると考えられた．

Keywords：non-O157志賀毒素産生性大腸菌（STEC），海外

Maekawa, K., Hamaguchi, T.[*1], Saito, Y., Tatewaki, N., Kurose, K., Kaniwa, N., Eguchi Nakajima, T.[*1], Kato, K.[*1], Yamada, Y.[*1], Shimada, Y.[*1], Yoshida, T.[*1], Kamatani, N.[*2], Ura, T.[*3], Saito, M.[*3], Muro, K.[*3], Fuse, N.[*4], Yoshino, T.[*4], Doi, T.[*4], Otsu, A.[*4], Saijo, N.[*4], Sawada, J., Okuda, H., Matsumura, Y.[*4]: **Genetic Variation and Haplotype Structures of the Glutathione S-transferase Genes GSTA1 and GSTA2 in Japanese Colorectal Cancer Patients**

Drug Metab. Pharmacokinet., **26**, 646-658（2011）

Glutathione S-transferases（GSTs）play a vital role in phase II biotransformation of many chemicals, including anticancer drugs. In this study, to elucidate the haplotype structures of the two closely related alpha-class genes, GSTA1 and GSTA2, we screened genetic variation in 214 Japanese colorectal cancer patients who received oxaliplatin-based chemotherapy. By direct resequencing of the 5'-flanking region, all the exons, and their flanking introns for 107 patients, 29 and 27 variants were identified in GSTA1 and GSTA2, respectively. The known functional single nucleotide polymorphisms（SNPs）, -567T>G, -69C>T, and -52G>A in GSTA1*B, were found at allele frequencies of 0.140. Of four major GSTA2 allelic variants reported previously（GSTA2*A, *B, *C, and *E）, only GSTA2*B（frequencies = 0.154）, *C（0.706）, and *E（0.140）were detected. Following linkage disequilibrium analysis, haplotypes of both genes were separately estimated. Then, rapid genotyping methods for 7 and 6 SNPs tagging common haplotypes of GSTA1 and GSTA2, respectively, were developed using the single-base extension assay, and an additional 107 patients were genotyped. Finally, haplotype combinations of both genes were classified into 3 major types: GSTA1*A-GSTA2*C, GSTA1*A-GSTA2*B, and GSTA1*B-GSTA2*E. These findings would be useful in pharmacogenomic studies on xenobiotics including anticancer drugs.

Keywords: glutathione-S-transferase, haplotype, genotyping

[*1] 国立がん研究センター
[*2] （独）理化学研究所
[*3] 愛知がんセンター
[*4] 国立がんセンター東病院

Hanioka, N.[*], Matsumoto, K.[*], Saito, Y. and Narimatsu, S.[*]: **Influence of *CYP2C8*13* and *CYP2C8*14* alleles on amiodarone N-deethylation**

Basic Clin. Pharmacol. Toxicol., **108**, 359-362（2011）

We expressed two variant CYP2C8 enzymes with amino acid substitutions（CYP2C8.13 and CYP2C8.14）found in a Japanese population as well as wild-type CYP2C8（CYP2C8.1）in yeast cells, and the kinetics of amiodarone N-deethylation were determined. The Km and Vmax values of CYP2C8.14 were significantly high and low compared with the respective values of CYP2C8.1; as a result, the CLint value was markedly lower than that of CYP2C8.1. In contrast, the Km, Vmax and CLint values of CYP2C8.13 were comparable to those of CYP2C8.1. These findings may mean that the genetic polymorphism of the CYP2C8*14 allele influences the clinical response to amiodarone, although there is no information on the relationship between CYP2C8*14 allele and the pharmacokinetics of amiodarone at present.

Keywords: genetic polymorphisms, CYP2C8, amiodarone

* 岡山大学

Okiyama, Y.[*1], Fukuzawa, K.[*2], Yamada, H.[*3], Mochizuki, Y.[*3], Nakano, T., Tanaka, S.[*4]: **Counterpoise-corrected interaction energy analysis based on the fragment molecular orbital scheme**

Chem. Phys. Let., **509**, 67-71（2011）

Basis set superposition error（BSSE）correction with counterpoise（CP）procedure under the environmental electrostatic potential is newly introduced to interfragment interaction energy（IFIE）, which is important for interaction analysis in the fragment molecular orbital method. The CP correction for IFIE is applied to a stacked dimer of base pair and a protein－ligand complex of estrogen receptor and 17b-estradiol with scaled third-order Møller－Plesset perturbation theory. The BSSEs amount to about quarter of IFIE for hydrogen-bonding and electrostatic interactions and half or even more for dispersion interactions. Estimation of IFIE with the CP correction is therefore preferred for the quantitative discussion.

Keywords: FMO, Counterpoise

[*1] 東京大学
[*2] みずほ情報総研（株）
[*3] 立教大学
[*4] 神戸大学

Mochizuki, Y.[*1], Yamashita, K.[*2], Nakano, T., Okiyama, Y.[*3], Fukuzawa, K.[*4], Taguchi, N.[*1] and Tanaka, S.[*5]: **Higher-order correlated calculations based on fragment molecular orbital scheme**

Theor. Chem. Acc., **130**, 515-530（2011）

We have developed a new module for higherorder correlated methods up to coupled-cluster singles and doubles with perturbative triples（CCSD（T））. The matrixmatrix operations through the DGEMM routine were pursued for a number of contractions. This code was then incorporated into the ABINIT-MPX program for the fragment molecular orbital（FMO）calculations. Intra-fragment processings were parallelized with OpenMP in a node-wise fashion, whereas the message passing interface（MPI）was used for the fragment-wise parallelization over nodes. Our new implementation made the FMO-based higher-order calculations applicable to realistic proteins. We have performed several benchmark tests on the Earth Simulator（ES2）, a massively parallel computer. For example, the FMO-CCSD（T）/6-31G job for the HIV-1 protease（198amino acid residues）－ lopinavir complex was completed in9.8 h with 512 processors（or 64 nodes）. Another example was the influenza neuraminidase（386 residues）with oseltamivir calculated at the full fourth-order Møller－Plesset perturbation level（MP4）, of which job timing was 10.3 h with 1024 processors. The applicability of the methods to commodity cluster computers was tested as well.

Keywords: FMO-CC

[*1] 立教大学
[*2] NECソフト（株）
[*3] 東京大学
[*4] みずほ情報総研（株）
[*5] 神戸大学

Okiyama, Y.[*1], Nakano, T., Mochizuki, Y.[*2], Yamashita, K.[*3], Fukuzawa, K.[*4], Tsukamoto, T.[*4], Watanabe, C.[*5], and Tanaka, S.[*5]: **Development of ABINIT-MP（X）program for processing fragment molecular orbital calculations**

Proceeding of International Conference on Modeling and Simulation Technology, 102-104（2011）

The ABINIT-MP（X）program, which processes the fragment molecular orbital（FMO）calculations for large biomolecules, has several advantages in running on massively-parallel computer systems. First, written in Fortran language using standard MPI and SMP techniques with basic mathematical libraries, this program successfully works on desktop workstations, PC clusters and vector computers. Next, electron correlation effects are efficiently taken into account with the direct algorithm and thus large-scale calculations are easily performed with high accuracy including dispersion force that is important for interaction analysis in biomolecules. Further development would make this program more attractive for studying large molecular systems with the FMO method.

Keywords: FMO, ABINIT-MP（X）

[*1] 東京大学
[*2] 立教大学
[*3] NECソフト（株）
[*4] みずほ情報総研（株）
[*5] 神戸大学

Nakano, T., Mochizuki, Y.[*1], Katsumi Y.[*2], Watanabe, C.[*3], Fukuzawa, K.[*4], Segawa, K., Okiyama, Y.[*2], Tsukamoto, T.[*3], Tanaka, S.[*4]: **Development of the four-body corrected fragment molecular orbital（FMO4）method**

Chem. Phys. Lett., **523**, 128-133（2012）

The four-body corrected fragment molecular orbital（FMO4）method was implemented at the secondorder Møller−Plesset perturbation（MP2）level. A series of accuracy tests relative to the previous twobody and three-body treatments were performed. As expected, FMO4 provided better accuracy in total energies in comparison with the reference values by regular MO calculations. A nonconventional fragmentation by separating main and side chains in amino acid residues was examined for Ala-pentamer and Chignolin, where the four-body corrections were shown to be substantial. A large complex of HIV-1 protease（total 198 residues）with lopinavir was calculated as well. Furthermore, this new FMO scheme was successfully applied to adamantane-shaped clusters with three-dimensional bonding framework.

Keywords: FMO4

[*1] 立教大学
[*2] NECソフト(株)
[*3] みずほ情報総研(株)
[*4] 神戸大学

Tsukamoto, T.[*1], Mochizuki, Y.[*2], Watanabe, N.[*1], Fukuzawa, K.[*1], Nakano, T.: **Partial geometry optimization with FMO-MP2 gradient: Application to TrpCage**

Chem. Phys. Lett., **535**, 157-162（2012）

The reliability of protein structure is a critical concern to grasp the insights with respect to residue−residue and residue−ligand interactions by computational methods. In such calculations, the molecular geometries are usually prepared by the optimization of experimental structure with empirical molecular mechanics（MM）parameters. As an alternative to MM methods, we have developed a partial geometry optimization with the fragment molecular orbital（FMO）scheme at the second-order Møller−Plesset perturbation（MP2）level. The TrpCage miniprotein was used as a demonstrative example. The geometries of the central region were partially optimized at the FMO-MP2 and Hartree − Fock（FMO-HF）levels, and the former with the correlation correction showed reasonable agreement with the experimental structure.

Keywords: Partial geometry optimization, FMO-MP2, TrpCage

[*1] みずほ情報総研(株)
[*2] 立教大学

Fujiki, R.[*1], Hashiba, W.[*1], Sekine, H.[*1], Yokoyama, A.[*1], Chikanishi, T.[*1], Ito, S.[*1], Imai, Y.[*1], Kim, J.[*2], He, HH.[*3], Igarashi, K., Kanno, J., Ohtake, F.[*1], Kitagawa, H.[*1], Roeder, RG.[*2], Brown, M.[*3], Kato, S.[*1]: **GlcNAcylation of histone H2B facilitates its monoubiquitination**

Nature, **480**（7378）, 557-560（2011）

Chromatin reorganization is governed by multiple post-translational modifications of chromosomal proteins and DNA. These histone modifications are reversible, dynamic events that can regulate DNA-driven cellular processes. However, the molecular mechanisms that coordinate histone modification patterns remain largely unknown. In metazoans, reversible protein modification by O-linked N-acetylglucosamine（GlcNAc）is catalysed by two enzymes, O-GlcNAc transferase（OGT）and O-GlcNAcase（OGA）. However, the significance of GlcNAcylation in chromatin reorganization remains elusive. Here we report that histone H2B is GlcNAcylated at residue S112 by OGT in vitro and in living cells. Histone GlcNAcylation fluctuated in response to extracellular glucose through the hexosamine biosynthesis pathway（HBP）. H2B S112 GlcNAcylation promotes K120 monoubiquitination, in which the GlcNAc moiety can serve as an anchor for a histone H2B ubiquitin ligase. H2B S112 GlcNAc was localized to euchromatic areas on fly polytene chromosomes. In a genome-wide analysis, H2B S112 GlcNAcylation sites were observed widely distributed over chromosomes including transcribed gene loci, with some sites co-localizing with H2B K120 monoubiquitination. These findings suggest that H2B S112 GlcNAcylation is a histone modification that facilitates H2BK120 monoubiquitination, presumably for transcriptional activation.

Keywords: Chromatin reorganization, Histone modification, GlcNAcylation

[*1] Institute of Molecular and Cellular Biosciences, University of Tokyo
[*2] Laboratory of Biochemistry and Molecular Biology, The Rockefeller University
[*3] Dana-Farber Cancer Institute and Harvard Medical School

Matsukura, H.[*], Aisaki, K., Igarashi, K., Matsushima, Y., Kanno, J., Muramatsu, M.[*], Sudo, K.[*], Sato, N.[*]: **Genistein promotes DNA demethylation of the steroidogenic factor 1（SF-1）promoter in endometrial stromal cells**

Biochem. Biophys. Res. Commun., **412**（2）, 366-372（2011）

It has recently been demonstrated that genistein（GEN）, a phytoestrogen in soy products, is an epigenetic modulator in various types of cells; but its effect on endometrium has not yet been determined. We investigated the effects of GEN on

mouse uterine cells, in vivo and in vitro. Oral administration of GEN for 1 week induced mild proliferation of the endometrium in ovariectomized（OVX）mice, which was accompanied by the induction of steroidogenic factor 1（SF-1）gene expression. GEN administration induced demethylation of multiple CpG sites in the SF-1 promoter; these sites are extensively methylated and thus silenced in normal endometrium. The GEN-mediated promoter demethylation occurred predominantly on the luminal side, as opposed to myometrium side, indicating that the epigenetic change was mainly shown in regenerated cells. Primary cultures of endometrial stromal cell colonies were screened for GEN-mediated alterations of DNA methylation by a high-resolution melting（HRM）method. One out of 20 colony-forming cell clones showed GEN-induced demethylation of SF-1. This clone exhibited a high proliferation capacity with continuous colony formation activity through multiple serial clonings. We propose that only a portion of endometrial cells are capable of receiving epigenetic modulation by GEN.

Keywords: DNA demethylation, Steroidogenic factor 1, Genistein

---

* Department of Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental University

Baba, A.[*1], Ohtake, F.[*1], Okuno, Y.[*1], Yokota, K.[*1], Okada, M.[*1], Imai, Y.[*1], Ni, M.[*2], Meyer, CA.[*2], Igarashi, K., Kanno, J., Brown, M.[*2], Kato, S.[*1]: **PKA-dependent regulation of the histone lysine demethylase complex PHF2-ARID5B**
Nat. Cell Biol., **13**(6), 668-675（2011）

Reversible histone methylation and demethylation are highly regulated processes that are crucial for chromatin reorganization and regulation of gene transcription in response to extracellular conditions. However, the mechanisms that regulate histone-modifying enzymes are largely unknown. Here, we characterized a protein kinase A（PKA）-dependent histone lysine demethylase complex, PHF2-ARID5B. PHF2, a jmjC demethylase, is enzymatically inactive by itself, but becomes an active H3K9Me2 demethylase through PKA-mediated phosphorylation. We found that phosphorylated PHF2 then associates with ARID5B, a DNA-binding protein, and induce demethylation of methylated ARID5B. This modification leads to targeting of the PHF2-ARID5B complex to its target promoters, where it removes the repressive H3K9Me2 mark. These findings suggest that the PHF2-ARID5B complex is a signal-sensing modulator of histone methylation and gene transcription, in which phosphorylation of PHF2 enables subsequent formation of a competent and specific histone demethylase complex.

Keywords: Histone methylation, Protein kinase A, PHF2-ARID5B complex

---

[*1] Institute of Molecular and Cellular Biosciences, University of Tokyo

[*2] Dana-Farber Cancer Institute and Harvard Medical School

Aisaki, K.[*], Tsuboi, I.[*], Harada, T.[*], Oshima, H.[*], Yamashita, A.[*], Hirabayashi, Y., Kanno, J., Inoue, T., Aizawa, S.[*]: **Neopterin, inflammation-associated product, prolongs erythropoiesis suppression in aged SAMP1 mice due to senescent stromal-cell impairment**
Exp. Biol. Med.(*Maywood*), **237**, 279-286（2012）

Anemia induced by inflammation is well known to be more serious in the elderly than in non-elderly adults; however, the reason why this is so remains unclear. Neopterin produced by monocytes during inflammation promotes myelopoiesis but suppresses B-lymphopoiesis and erythropoiesis, by activating stromal cells in mice. Here, age-related changes in the erythropoietic response to neopterin were determined using senescence accelerated mice（SAMP1）with senescence stromal-cell impairment. Intravenous injection of neopterin into young mice（8-12 weeks old）resulted in a decrease in erythroid progenitor cell number in the bone marrow（BM）, concomitant with an increase in myeloid progenitor cell number over one week. Intravenous injection of neopterin into aged mice（30-36 weeks old）resulted in a prolonged decrease in erythroid progenitor cell number in the BM over three weeks and a limited increase in myeloid progenitor cell number over one day. Neopterin treatment induced a decrease in serum erythropoietin concentrations in young mice but not in aged mice. The gene expression of tumor necrosis factor alpha（TNF-alpha）, a negative regulator of erythropoiesis, was up-regulated in the BM of both young and aged mice, and the degree of TNF-alpha up-regulation was the same in both groups. The gene expression of interleukin（IL）-11, a positive regulator of erythropoiesis, was also up-regulated over one day in both young and aged mice. However, IL-11 gene expression remained up-regulated thereafter in young mice, whereas it was rapidly down-regulated in aged mice. These data suggest that prolonged suppression of erythropoiesis in aged mice may be due to a decrease in the production of positive regulators rather than to an increase in the production of negative regulators. Our combined data suggest that age-related impairment of stromal cells induces serious anemia in

the elderly during inflammation.

Keywords: aging, erythropoiesis, neopterin

* Nihon University School of Medicine

Hokari, T.*, Tsuboi, I.*, Harada, T.*, Oshima, H.*, Hirabayashi, Y., Kanno, J., Inoue, T., Aizawa, S.*: **Mast cell development and biostresses: different stromal responses in bone marrow and spleen after treatment of myeloablater, 5-fluorouracil, and inflammatory stressor, lipopolysaccharide**

Biol. Pharm. Bull., **34**, 1533-1541（2011）

Mast-cell-development in the bone-marrow（BM）and the spleen is restrictedly controlled by stromal-cells which produce positive-regulators such as stem cell factor（SCF）, and negative-regulators such as transforming growth factor-beta（TGF-beta）. How the balance between positive- and negative-regulation is achieved or maintained by stromal-cells is not well understood. We intravenously injected 5-fluorouracil（5-FU）and lipopolysaccharide（LPS）into C3H/HeN mice to disrupt mast-cell-development in order to reveal mechanisms of mast-cell-regulation. 5-FU treatment induces a rapid decrease in the number of mast-cell-progenitor（colony-forming unit（CFU）-mast）cells in the BM and spleen, followed by rapid recovery of CFU-mast numbers. Expression of the SCF gene is one-fiftieth the level of that of TGF-beta during the steady-state in BM and spleen. After 5-FU treatment, SCF mRNA levels in the BM markedly increased, approaching TGF-beta mRNA levels, whereas SCF levels in the spleen showed limited oscillations whose increases paralleled those in TGF-beta levels. In contrast, LPS treatment induces a rapid decrease in CFU-mast number in the BM and a rapid increase in of CFU-mast number in the spleen. After LPS treatment, SCF mRNA levels in the BM markedly decreased, whereas SCF levels in the spleen remained unchanged. These results suggest that regulation of mast-cell-development is dominated by negative-signals in the BM and spleen during the steady-state, and, under biostress-conditions such as 5-FU and LPS treatment, the balance between positive- and negative-regulation can be changed in the BM but not in the spleen. The difference in the regulation of mast-cell-development in the BM versus the spleen probably reflects the different roles of tissue-specific stromal-cells.

Keywords: mast cell, stem cell factor, transforming growth factor-beta

* Nihon University School of Medicine

Sato, K., Kuriwaki, J., Takahashi, K., Saito, Y.*1, Oka, J.*1, Otani, Y.*2, Sha, Y.*2, Nakazawa, K., Sekino, Y., Ohwada, T.*2: **Discovery of a tamoxifen-related compound that suppresses glial L-glutamate transport activity without Interaction with estrogen receptors**

ACS Chem. Neurosci., **3**, 105-113（2012）

We recently found that tamoxifen suppresses L-glutamate transport activity of cultured astrocytes. Here, in an attempt to separate the L-glutamate transporter-inhibitory activity from the estrogen receptor-mediated genomic effects, we synthesized several compounds structurally related to tamoxifen. Among them, we identified two compounds, 1（YAK01）and 3（YAK037）, which potently inhibited L-glutamate transporter activity. The inhibitory effect of 1 was found to be mediated through estrogen receptors and the mitogen-activated protein kinase（MAPK）/phosphatidylinositol 3-kinase（PI3K）pathway, though 1 showed greatly reduced transactivation activity compared with that of 17β-estradiol. On the other hand, compound 3 exerted its inhibitory effect through an estrogen receptor-independent and MAPK-independent, but PI3K-dependent pathway, and showed no transactivation activity. Compound 3 may represent a new platform for developing novel L-glutamate transporter inhibitors with higher brain transfer rates and reduced adverse effects.

Keywords: Tamoxifen, astrocyte, L-glutamate transporter

*1 Tokyo University of Science

*2 University of Tokyo

Shuvaev, A.N.*1, Horiuchi, H.*1, Seki, T.*2, Goenawan, H.*1, Irie, T., Iizuka, A.*1, Sakai, N.*2, Hirai, H.*1.: **Mutant PKCγ in Spinocerebellar Ataxia Type 14 Disrupts Synapse Elimination and Long-Term Depression in Purkinje Cells In Vivo**

J. Neurosci., **31**, 14324-14334（2011）

Cerebellar Purkinje cells（PCs）express a large amount of the γ isoform of protein kinase C（PKCγ）and a modest level of PKCα. The PKCγ is involved in the pruning of climbing fiber（CF）synapses from developing PCs, and PKCα plays a critical role in long-term depression（LTD）at parallel fiber（PF）-PC synapses. Moreover, the PKC signaling in PCs negatively modulates the nonselective transient receptor potential cation channel type 3（TRPC3）, the opening of which elicits slow EPSCs at PF-PC synapses. Autosomal dominant spinocerebellar ataxia type 14（SCA14）is caused by mutations in PKCγ. To clarify the pathology of this

disorder, mutant（S119P）PKCγ tagged with GFP was lentivirally expressed in developing and mature mouse PCs in vivo, and the effects were assessed 3 weeks after the injection. Mutant PKCγ-GFP aggregated in PCs without signs of degeneration. Electrophysiology results showed impaired pruning of CF synapses from developing PCs, failure of LTD expression, and increases in slow EPSC amplitude. We also found that mutant PKCγ colocalized with wild-type PKCγ, which suggests that mutant PKCγ acts in a dominant-negative manner on wild-type PKCγ. In contrast, PKCα did not colocalize with mutant PKCγ. The membrane residence time of PKCα after depolarization-induced translocation, however, was significantly decreased when it was present with the mutant PKCγ construct. These results suggest that mutant PKCγ in PCs of SCA14 patients could differentially impair the membrane translocation kinetics of wild-type γ and α PKCs, which would disrupt synapse pruning, synaptic plasticity, and synaptic transmission.

Keywords: Purkinje cells, protein kinase C, long-term depression

---

[*1] Gunma University Graduate School of Medicine
[*2] Hiroshima University

Kanda Y., Hinata T.[*1], Kang, S.W.[*2], Watanabe, Y.[*1]: **Reactive oxygen species mediate adipocyte differentiation in mesenchymal stem cells**

Life Sciences, **89**, 250-258（2011）

AIMS: Mesenchymal stem cells（MSC）have the potential to differentiate into various cell lineages, including adipocytes and osteoblasts. The formation of adipose tissue involves the commitment of MSC to the preadipocyte lineage and the differentiation of preadipocytes into mature adipocytes. In the present study, we investigated the involvement of reactive oxygen species（ROS）in adipocyte differentiation from MSC.

MAIN METHODS: ROS signaling was evaluated by the effects of antioxidant N-acetyl-L-cysteine（NAC）or shRNA against NAD(P)H oxidase in the multipotent mesenchymal stem cell line 10T1/2 cells. Intracellular ROS was measured using an H2DCF dye.

KEY FINDINGS: We found that NAC blocked adipocyte differentiation in MSC. An H2DCF assay revealed that differentiation-inducing agents induced ROS generation. These data suggest that ROS is involved in adipocyte differentiation in MSC. Next, we examined the source of ROS. Knockdown of NAD(P)H oxidase 4（Nox4）by RNA interference inhibited ROS production and adipocyte differentiation by differentiation-inducing agents. Furthermore, treatment with NAC blocked the transcriptional activation of CREB, and the expression of dominant-negative mutants of CREB inhibited adipocyte differentiation.

SIGNIFICANCE: The findings suggest that the increase in the intracellular ROS level via Nox4 mediates adipocyte differentiation through CREB in MSC. This data will provide new insight into the drug development for obesity.

Keywords: adipogenesis, CREB, mesenchymal stem cells, NAD(P)H oxidase

---

[*1] National Defense Medical College
[*2] Ewha Womans University

Tanaka, M.[*], Nagai, T.[*], Usami, M., Hasui, K.[*], Takao, S.[*], Matsuyama, T.[*]: **Phenotypic and functional profiles of CRIg（Z39Ig）-expressing macrophages in the large intestine**

Innate Immun., **18**, 258-267（2011）

Intestinal macrophages（M）play significant roles in maintaining homeostasis by the efficient elimination of foreign particles in the large intestine. However, functional complement receptors have not been fully identified. In this study, we showed that a complement receptor of the Ig superfamily（CRIg, also known as Z39Ig）, a receptor for complement fragments（C3b and iC3b）, was expressed on a subset of intestinal M in murine and human large intestine. When abilities of uptake of antigens of murine CRIg(+) M were examined, intestinal CRIg(+) M displayed less endocytic and similar phagocytic abilities compared to resident peritoneal F4/80(+)CRIg(+) M and F4/80(+)CRIg(+) M. Additionally, we found that a significant portion of C3b-dependent phagocytosis by large intestinal M involves CRIg, emphasizing the importance of efficient mechanisms to eliminate foreign particles in the large intestine. On the other hand, intestinal M from 2,4,6-trinitrobenzene sulfonic acid-treated mice had decreased CRIg expression but increased CD11b expression, implying some contribution to the removal of immune complexes. This study will shed new light on opsonization and phagocytosis by large intestinal M.

Keywords: intestinal macrophage, CRIg, complement fragments

---

[*] 鹿児島大学

Kano, S.[*], Todo, H.[*], Furui, K.[*], Sugie, K.[*], Tokudome, Y.[*],

Hashimoto, F.[*], Kojima, H., Sugibayashi, K.[*]: **Comparison of Several Reconstructed Cultured Human Skin Models by Microscopic Observation: Their Usefulness as an Alternative Membrane for Skin in Drug Permeation Experiments**

Altern. Animal Test. EXperiment, **16**, 51-58（2011）

Several reconstructed cultured human skin models（RSMs）are already utilized as membrane alternatives to human and animal skin in skin corrosive/irritation tests. They are also utilized in skin permeation experiments from the viewpoint of animal welfare; however, different permeation profiles of chemicals were found between RSMs and excised human or animal skin. RSMs and excised human skin were morphologically evaluated by a light microscope and a transmission electron microscope. In the results, the micromorphology of all RSMs differed from that of human skin. In particular, the lamellar layer between corneocytes in RSMs was much narrower than that in the human stratum corneum. The lamella layer affects not only the diffusion and partition properties of chemical compounds in RSMs but also the concentration-distance profile of chemicals in the models. Furthermore, esterase distribution in RSMs was different to that in human skin. This difference would certainly affect the permeation of both parent ester compounds and their metabolites through RSMs. Evaluation of the morphological and enzymatic differences between RSMs and human skin would be helpful to understand the differences in the chemical permeation profiles between RSMs and human skin.

Keywords: reconstructed cultured human skin model, skin permeation, skin morphology, lamella layer

[*] Josai University

Kojima, H., Ando, Y.[*1], Idehara, K.[*2], Kato, M.[*3], Kosaka, T.[*4], Miyaoka, E.[*5], Shinoda, S.[*6], Suzuki, T.[*7], Yamaguchi, Y.[*8], Yoshimura, I.[*5], Yuasa, A.[*9], Watanabe, Y.[*10], Omori, T.[*11]: **Validation Study of the In Vitro Skin Irritation Test with the LabCyte EPI-MODEL24**

Altern Lab Anim., **40**, 1-18（2012）

Based on the United Nation-Globally Harmonised System（UN-GHS）classification for assessing the skin irritation potential of a chemical, 12 irritants and 13 non-irritants described in the ECVAM（European Centre for the Validation of Alternative Methods）performance standards and the statement by ESAC（ECVAM Scientific Advisory Committee）were validated by 7 laboratories. Validation studies were performed using Japanese Reconstructed human Epidermis（RhE）and LabCyte EPI-MODEL24 developed by Japan Tissue Engineering Co., Ltd.（J-TEC）, using an optimized protocol. Cells were exposed to chemicals for 15 min and incubated for 42 h using LabCyte EPI-MODEL24. After that, IL-1_ levels in conditioned medium and tissue viabilities were measured using the MTT assay. The results of the MTT assay using LabCyte EPI-MODEL24 demonstrated high reliability within and between laboratories, and acceptable reliability of the positive control（100%）and accuracy（77.5% overall accuracy, 82.3% overall sensitivity, 72.6% overall specificity）for use as a stand-alone assay to distinguish between skin irritants and non-irritants.

Keywords: Skin irritation, reconstructed human epidermis, validation, in vitro

[*1] Aiken Co., Ltd.
[*2] Daicel Chemical Co.
[*3] Japan Tissue Engineering Co. Ltd.
[*4] The Institute of Environmental Toxicology
[*5] Tokyo University of Science
[*6] Drug Safety Testing Center Co., Ltd.
[*7] Fancl Corp.
[*8] KOBAYASHI Pharmaceutical Co., Ltd.
[*9] FUJIFILM Corporation
[*10] Maruishi Pharmaceutical Co., Ltd.
[*11] Kyoto Univ.

Kobayashi-Yamazaki, C.[*], Shirao, T.[*], Sasagawa, Y.[*], Maruyama, Y.[*], Akita, H.[*], Saji, M.[*], Sekino, Y.: **Lesions of the supramammillary nucleus decrease self-grooming behavior of rats placed in an open field**

The Kitakanto Medical Journal, **61**, 287-292（2011）

Although subcortical regions send numerous efferent fibers to the hippocampus, their involvement in hippocampal functions has not been fully elucidated. The aim of this study was to determine the effect of the supramammillary nucleus（SuM）on the hippocampus. Methods: Neurons within the SuM of rats were destroyed by local injections of an excitotoxin, ibotenic acid, and the effects of the SuM−lesion on behaviors in an open field were investigated. Results: SuM lesions increased distance traveled, movement time and latency to start grooming, while they decreased time spent grooming. SuM lesions had no effect on rearing frequency or immobility time. Conclusion: Prolonged exploration and decrease in the total time spent grooming observed in the SuM −lesioned rats were consistent with the behavioral characteristics of hippocampal − lesioned rats of the previous reports, sug-

gesting that the SuM is involved in the establishment of spatial memory by hippocampus during the initial exploration of a novel environment. ln addition, the reduction of grooming in the SuM−lesioned animal suggests that SuM may be involved in emotion, such as anxiety. The results ofthis study show the involvement ofthe SuM in hippocampal function and in anxiety perceived in a novel environment.

Keywords: supramammillary nucleus, self-grooming behavior

* 群馬大学

Hur, K.[*1], Niwa, T.[*1], Toyoda, T., Tsukamoto, T.[*2], Tatematsu, M.[*2], Yang, H.K.[*3] and Ushijima, T.[*1]: **Insufficient role of cell proliferation in aberrant DNA methylation induction and involvement of specific types of inflammation**

Carcinogenesis, **32**, 35-41（2011）

Chronic inflammation is deeply involved in induction of aberrant DNA methylation, but it is unclear whether any type of persistent inflammation can induce methylation and how induction of cell proliferation is involved. In this study, Mongolian gerbils were treated with five kinds of inflammation inducers［*Helicobacter pylori* with cytotoxin-associated gene A（CagA）, *H. pylori* without CagA, *Helicobacter felis*, 50% ethanol（EtOH）and saturated sodium chloride（NaCl）solution］. Two control groups were treated with a mutagenic carcinogen that induces little inflammation（20 p.p.m. of *N*-methyl-*N*-nitrosourea）and without any treatment. After 20 weeks, chronic inflammation with lymphocyte and macrophage infiltration was prominent in the three *Helicobacter* groups, whereas neutrophil infiltration was mainly observed in the EtOH and NaCl groups. Methylation levels of eight CpG islands significantly increased only in the three *Helicobacter* groups. By Ki-67 staining, cell proliferation was most strongly induced in the NaCl group, demonstrating that induction of cell proliferation is not sufficient for methylation induction. Among the inflammation-related genes, *Il1b*, *Nos2* and *Tnf* showed increased expression specifically in the three Helicobacter groups. In human gastric mucosae infected by *H. pylori*, *NOS2* and *TNF* were also increased. These data showed that inflammation due to infection of the three *Helicobacter* strains has a strong potential to induce methylation, regardless of their CagA statuses, and increased cell proliferation was not sufficient for methylation induction. It was suggested that specific types of inflammation characterized by expression of specific inflammation-related genes, along with increased cell proliferation, are necessary for methylation induction.

Keywords: *Helicobacter pylori*, methylation, inflammation

*1 National Cancer Center Research Institute
*2 Aichi Cancer Center Research Institute
*3 Seoul National University

Fujimoto, H., Woo, G-H., Inoue, K., Takahashi, M., Hirose, M.[*1], Nishikawa, A. and Shibutani, M.[*2]: **Impaired oligodendroglial development by decabromodiphenyl ether in rat offspring after maternal exposure from mid-gestation through lactation**

Reprod. Toxicol., **31**, 86-94（2011）

Pregnant Sprague-Dawley rats were given diet containing decabromodiphenyl ether（DBDE）either at 0, 10, 100, or 1000 ppm from gestation day（GD）10 until day 20 after delivery（PND 20）. No significant alterations were observed in maternal and offspring reproductive parameters. At PND 20, serum triiodothyronine concentrations examined in males were slightly reduced at 1000 ppm（84.2% of the control value）, and incidence of thyroid follicular cell hypertrophy was increased in both sexes with significant difference in males at 1000 ppm. Diffuse liver cell hypertrophy accompanying increased relative liver weight and increased cytoplasmic eosinophilia of the renal proximal tubules were observed in both sexes with significant difference from 10 ppm in males and females, respectively. At postnatal week 11, serum thyroxine concentrations examined in males were slightly reduced at 1000 ppm（85.9% of the control value）, and the incidence of thyroid follicular cell hypertrophy was non-significantly increased from 10 ppm in males. There were reductions in the corpus callosum area and density of 2’,3’-cyclic nucleotide 3’-phosphodiesterase-immunoreactive oligodendrocytes in the cingulate deep cortex in males from 100 ppm. Conversely, NeuN-immunoreactive neuronal distribution in the hippocampal CA1 was unchanged. This suggests that developmental DBDE-exposure caused irreversible white matter hypoplasia targeting oligodendrocytes from 100 ppm, accompanied with developmental hypothyroidism. The lowest-observed-adverse-effect level of DBDE was determined to be 10 ppm（0.7-2.4 mg/kg-body weight-d）.

Keywords: decabromodiphenyl ether, developmental toxicity, maternal exposure

*1 Food Safety Commission
*2 Tokyo University of Agriculture and Technology

Kawamoto, K.[*1,2], Sato, I.[*1], Tsuda, S.[*1], Yoshida, M., Yaegashi, K.[*3], Saito, N.[*3], Liu, W.[*4] and Jin, Y.[*4]: **Ultrasonic-induced tonic convulsion in rats after subchronic exposure to perfluorooctane sulfonate（PFOS）**

J. Toxicol. Sci., **36**, 55-62（2011）

Perfluorooctane sulfonate（PFOS）is one of the persistent organic pollutants distributed widely in the global environment. We have found that a single oral administration of PFOS induced tonic convulsion in mice and rats when a brief ultrasonic stimulus was applied to the animals. The aim of this study is to examine whether the neurotoxicity is caused by subchronic dietary exposure to PFOS. Rats were treated with dietary PFOS at 0, 2, 8, 32 and 128 ppm for 13 weeks. Animals were carefully observed for pharmacotoxic signs and responses to the ultrasonic stimulus applied biweekly. PFOS increased liver weight and decreased food consumption and body weight. PFOS concentrations in the serum, brain, liver and kidney were increased almost proportional to its total dose, although the ratios of PFOS concentrations in tissues to total doses in the group treated with the highest concentration were a little lower. The ranges of relative concentrations in the brain, liver and kidney to serum concentration were 0.13 to 0.24, 2.7 to 6.3 and 0.82 to 1.6, respectively. PFOS alone did not cause any neurotoxic symptoms; however, 5 rats out of 6 showed tonic convulsion in the 6th week when ultrasonic stimulus was applied to the 128 ppm rats with the total PFOS dose of 338 mg/kg. The ultrasonic stimulus did not cause convulsion in the other groups. Histopathological examination including electron microscopic examination could not detect any abnormality in the brain. Because the acute oral dose of PFOS causing the convulsion was 250 mg/kg（Sato et al., 2009）, the convulsion induced by PFOS seemed to depend on its total dose regardless of treatment schedule.

Keywords: PFOS, neurotoxicity, convulsion

[*1] Iwate University
[*2] Gifu University
[*3] Research Institute for Environmental Sciences and Public Health of Iwate Prefecture
[*4] Dalian University

Yoshida, M., Takahashi, M., Inoue, K., Nakae, D.[*] and Nishikawa, A.: **Lack of chronic toxicity and carcinogenicity of dietary administrated catechin mixture in Wistar Hannover GALAS rats**

J. Toxicol. Sci., **36**, 297-311（2011）

Chronic toxicity and carcinogenicity of catechin mixture were examined in Wistar Hannover GALAS rats. Administration was in the diet at concentrations of 0, 0.02, 0.3, 1 or 3%. Slight increases in relative liver weight and centrilobular hypertrophy of hepatocytes associated with induction of CYP3A2 were found at the 3% in males of both studies. However, because there were no signs indicative of hepatotoxicity on serum biochemical and histopathological examinations, the changes observed in the liver were regarded as adaptation, and not adverse effects. The slight depressions of body weights at the 3% in females of the chronic toxicity study and in both sexes of the carcinogenicity study were observed. These decreases were because the diet at the highest concentration was frangible and nominal food consumption may not have reflected the actual food consumption resulting in decrease in caloric intake, rather than toxic effects. Thus it was concluded that catechin mixture had no toxicity. In addition, tumor incidences and types were comparable between treated and control groups. Based on the results, the no observed adverse effect levels estimated from the chronic toxicity study were 3% in both sexes equal to 1922.9 in males and 2525.7 mg/kg/day in females. Catechin mixture has no carcinogenic potential in male and female rats.

Keywords: catechin mixture, chronic toxicity, carcinogenicity

[*] Tokyo Metropolitan Institute of Public Health

Nemoto, K.[*1], Tanaka, T.[*1], Ikeda, A.[*1], Ito, S.[*1], Mizukami, M.[*1], Hikida, T.[*1], Gamou, T.[*2], Habano, W.[*2], Ozawa, S.[*2], Inoue, K., Yoshida, M., Nishikawa, A. and Degawa, M.[*1]: **Super-induced gene expression of the N-methyl-D-aspartate receptor 2C subunit in chemical-induced hypertrophic liver in rats**

J. Toxicol. Sci., **36**, 507-514（2011）

To identify gene expression that can be closely involved in chemical-induced hepatocellular hypertrophy, the hepatic gene expression profile was assessed by cDNA microarray analysis in male F344 rats fed for 3 days, 4 weeks, and 13 weeks a diet containing a hepatocellular hypertrophy inducer, either phenobarbital（500 ppm）, clofibrate（2,500 ppm）, or piperonyl butoxide（20,000 ppm）. The results showed that, in all treatment groups, the increased expressional rate of the Grin2c gene, which encodes the N-methyl-D-aspartate receptor 2C subunit（NR2C）, was the highest among those of all the genes tested, as compared with the corresponding gene expression in rats fed a normal diet. Moreover, real-time RT-PCR analysis showed that the expression levels of the Grin2c gene in rats fed with each chemical clearly increased in a chemical

treatment period-dependent fashion, and that the increased rate was closely correlated with the grade of hypertrophy of hepatocytes rather than with the increased rate in liver weight. These results suggest the possibility that chemical-induced NR2C expression relates to the development of hepatocellular hypertrophy.

Keywords: hepatocellular hypertrophy, non-genotoxic hepatocarcinogen, NMDA receptor

[*1] University of Shizuoka
[*2] Iwate Medical University

Ozawa, S.[*1], Gamou, T.[*1], Habano, W.[*1], Inoue, K., Yoshida, M., Nishikawa, A., Nemoto, K.[*2] and Degawa, M.[*1]: **Altered expression of GADD45 genes during the development of chemical-mediated liver hypertrophy and liver tumor promotion in rats**

J. Toxicol. Sci., **36**, 613-623（2011）

The purpose of our study was to examine the altered gene expression associated with nongenotoxic chemical-mediated liver hypertrophy and successive liver tumor promotion. Five-week-old male rats were fed a basal diet or a diet containing phenobarbital（PB）or clofibrate（CF）for 3 days, 4 weeks, and 13 weeks. Hepatic expression profiling of cell growth- and stress-related genes, as well as those involved in xenobiotic metabolism, was performed by DNA microarray and/or real time quantitative reverse transcription-polymerase chain reaction. The induction of liver hypertrophy and hepatic cytochrome P450（CYP）isoforms（CYP2B1/2B2 for PB and CYP4A1 for CF）by PB and CF were clearly observed at all the treatment periods examined. Genes encoding DNA damage-inducible 45（GADD45）family proteins, in particular GADD45g（GADD45 gamma）were down-regulated by treatment with either PB or CF for 4 and 13 weeks. The chemical-mediated development of liver hypertrophy, induction of hepatic CYPs, and suppression of hepatic GADD45g gene at week 13 disappeared at 4 weeks following cessation of the chemical treatment. Additionally, DNA microarray data indicated that cell cycle-related genes such as cyclins CCNB1 and CCNA2 and cyclin-dependent kinase inhibitor CDKN3 were also down-regulated by treatment with either PB or CF at 13 weeks. Since GADD45 functions as a chemical and radiation stress sensor by interacting with cyclins and cyclin-dependent kinase inhibitors, the decrease in the gene expression of GADD45g mRNA observed in this study, may be associated with nongenotoxic chemical-induced tumor promotion of hepatocarcinogenesis rather than liver hypertrophy.

Keywords: liver tumor promotion, liver hypertrophy, GADD45

[*1] Iwate Medical University
[*2] University of Shizuoka

Hasumura, M., Imai, T.[*1], Cho, Y-M., Ueda, M., Hirose, M.[*2], Nishikawa, A. and Ogawa, K.: **Toxic effects of a horseradish extract and allyl isothiocyanate in the urinary bladder after 13-week administration in drinking water to F344 rats**

J. Toxicol. Sci., **36**, 763-774（2011）

Subchronic toxicity of a horseradish extract（HRE）, consisting mainly of a mixture of allyl isothiocyanate（AITC）and other isothiocyanates, was investigated with administration at concentrations of 0, 0.0125, 0.025 and 0.05% of HRE in drinking water for 13 weeks to male and female F344 rats. For comparison, treatment with 0.0425% of AITC was similarly performed. Body weight gain was reduced in the 0.05% HRE and AITC males as compared to the 0% controls, and the cause was considered at least partly related to decreased water consumption due to the acrid smell of the test substance and decreased food consumption. Serum biochemistry demonstrated increased urea nitrogen in 0.025 and 0.05% HRE and AITC males and 0.0125-0.05% HRE and AITC females, along with decreased total cholesterol in 0.0125-0.05% HRE females. On histopathological assessment, papillary/nodular hyperplasia of bladder mucosa was observed in 0.05% HRE and AITC males and females, in addition to simple mucosal hyperplasia found in all treated groups. Based on the above findings, no-observed-adverse-effect levels（NOAELs）were estimated to be below 0.0125% of HRE for both males and females, corresponding to 9.4 and 8.0 mg/kg body weight/day, respectively, and there appeared to be comparable toxicological properties of HRE to AITC, such as the inductive effect of significant proliferative lesions in the urinary bladder.

Keywords: horseradish extract, bladder toxicity, F344 rats

[*1] National Cancer Center Research Institute
[*2] Food Safety Commission

Shimamoto, K.[*], Hayashi, H.[*], Taniai, E.[*], Morita, R.[*], Imaoka, M.[*], Ishii, Y., Suzuki, K.[*], Shibutani, M.[*] and Mitsumori, K.[*]: **Antioxidant *N*-acetyl-*L*-cysteine（NAC）supplementation reduces reactive oxygen species（ROS）-mediated hepatocellular tumor promotion of indole-3-carbinol（I3C）in rats**

J. Toxicol. Sci., **36**, 775-786（2011）

Indole-3-carbinol（I3C）has a liver tumor promoting activity in rats, and is also known as a cytochrome p450 1A（CYP1A）inducer. The generation of reactive oxygen species（ROS）resulting from CYP1A induction due to I3C, is probably involved in the tumor promotion. To clarify whether ROS generation contributes to I3C's induction of hepatocellular altered foci, partially hepatectomized rats were fed a diet containing 0.5% of I3C for 8 weeks with or without 0.3% *N*-acetyl-*L*-cysteine（NAC）, an antioxidant, in their drinking water after N-diethylnitrosamine（DEN）initiation. Immunohistochemical analysis showed that the glutathione-S-transferase placental form（GST-P）positive foci promoted by I3C were suppressed by the administration of NAC. The mRNAs of members of the phase II nuclear factor, erythroid derived 2, like 2（Nrf2）gene batteries, whose promoter region is called as antioxidant response element（ARE）, were down-regulated in the DEN-I3C-NAC group compared to the DEN-I3C group, but Cyp1a1 was not suppressed in the DEN-I3C-NAC group compared to the DEN-I3C group. There was no marked difference in production of microsomal ROS and genomic 8-hydroxy-2'-deoxygunosine（8-OHdG）as an oxidative DNA marker between the DEN-I3C-NAC and DEN-I3C groups, while mapkapk3 and Myc were decreased by the NAC treatment. These results indicate that oxidative stress plays an important role for I3C's tumor promotion, and NAC suppresses induction of hepatocellular altered foci with suppressed cytoplasmic oxidative stress.

Keywords: indole-3-carbinol, reactive oxygen species, *N*-acetyl-*L*-cysteine

* Tokyo University of Agriculture and Technology

Taketa, Y.[*1], Inomata, A.[*1], Hosokawa, S.[*1], Sonoda, J.[*1], Hayakawa, K.[*1], Nakano, K.[*1], Momozawa, Y.[*1], Yamate, J.[*2], Yoshida, M., Aoki, T.[*1] and Tsukidate, K.[*1]: **Histopathological characteristics of lutela hypertrohy induced by ethylene glycol monomethyl ether with a comparison to normal luteal morphology in rats**

Toxicol. Pathol., **39**, 372-380（2011）

Ethylene glycol monomethyl ether（EGME）is a known reproductive toxicant that induces luteal hypertrophy in rat ovaries. In this study, we characterized the histopathological features of corpora lutea（CL）from EGME-treated rats and compared them with normal CL formation and regression. Normally cycling female Sprague-Dawley rats were treated with 5-bromo-2'-deoxyuridine（BrdU）intraperitoneally on the morning of estrus and their ovaries were examined 1（metestrus）, 4（estrus）, 8（estrus）, or 12（estrus）days later to observe the transition of BrdU-labeled cells within in the CL. CL at each time point of estrus stage were classified into 4 types: Type I（newly formed CL）, Type II（mature CL）, Type III（regressing CL）, and Type IV（residual CL）. CL almost fully regressed within 4 estrus cycles. In contrast, in female rats given EGME orally（30, 100, or 300 mg/kg for 2 or 4 weeks）, luteal cells were hypertrophic with abundant cytoplasm. Although the size of CL varied, all CL in EGME-treated rats had histological features similar to Type II CL, but they were more hypertrophic with less apoptosis. These results suggest that EGME has a luteal hypertrophic effect on all CL phases, including regression.

Keywords: ethylene glycol monomethyl ether, corpora lutea, 5-bromo-2'-deoxyuridine

[*1] Eisai Co. Ltd.

[*2] Osaka Prefecture University

Yoshida, M., Takahashi, M., Inoue, K., Hayashi, S., Maekawa, A.[*] and Nishikawa, A.: **Delayed adverse effects of neonatal exposure to diethylstilbestrol and their dose dependency in female rats**

Toxicol. Pathol., **39**, 823-834（2011）

Neonatal exposure to estrogenic chemicals causes irreversible complex damage to the hypothalamus-pituitary-gonadal axis and reproductive system in females. Some lesions are noted after maturation as delayed adverse effects. We investigated the characteristics and dose dependence of delayed effects using female rats neonatally exposed to diethylstilbestrol（DES）. Female Donryu rats were subcutaneously injected with a single dose of DES of 0（control）, 0.15, 1.5, 15, 150, or 1,500 μg/kg bw after birth. All except the lowest dose had estrogenic activity in a uterotrophic assay. All rats at 1500 μg/kg and some at 150 μg/kg showed abnormal morphologies in the genital tract, indicating they were androgenized before maturation. Although no morphological abnormalities were noted at 15 μg/kg or lower, onset of persistent estrus was significantly accelerated in the 1.5, 15, and 150 μg/kg groups with dose dependency, and the latest onset was from seventeen to twenty-one weeks of age at 1.5 μg/kg. The neonatal exposure to DES increased uterine adenocarcinoma development only at 150 μg/kg, although uterine anomalies were detected at 1,500 μg/kg. These results indicate that neonatal exposure to DES, which exerts estrogenic activity in vivo, induces delayed adverse effects in female rats in a dose-

dependent manner. Early onset of persistent estrus appears to be the most sensitive parameter.

Keywords: delayed adverse effect, neonatal exposure, DES

---

* National Institute of Technology and Evaluation

Iwasaki, Y.*, Hirasawa, T.*, Maruyama, Y.*, Ishii, Y., Ito, R.*, Saito, K.*, Umemura, T., Nishikawa, A. and Nakazawa, H.*: **Effect of interaction between phenolic compounds and copper ion on antioxidant and pro-oxidant activities**

Toxicol. In Vitro, **25**, 1320-1327（2011）

Phenolic compounds are widely used in food and cosmetics to prevent undesirable oxidation. On the other hand, phenolic compounds are also strong reducing agents and under in vitro conditions and in the presence of copper ion, they can act as pro-oxidants. In this study, we conducted electron spin resonance（ESR）measurements for the increase in reactive oxygen species（ROS）in relation to their structure and interaction with transition metals. Moreover, the antioxidant activity was assessed with the 1,1-diphenyl-2-picrylhydrazyl（DPPH）assay, and the pro-oxidant effect of phenolic compounds on DNA damage was assessed by measuring 8-hydroxy-2'-deoxyguanosine（8-OHdG）, which is effectively formed during oxidative damage. In conclusion, ortho-dihydroxyl groups that can chelate with Cu（2+）induce the greatest pro-oxidant activity. Moreover, the interaction between phenolic compounds and copper induced to H（2）O（2）. The obtained results indicated that ROS participated in oxidative DNA damage induced by phenolic compounds in the presence of Cu（2+）.

Keywords: antioxidant, electron spin resonance, DNA damage

---

* Hoshi University

Cho, Y-M., Hasumura, M., Takami, S., Imai, T.*1, Hirose, M.*2, Ogawa, K. and Nishikawa, A.: **A 13-week subchronic toxicity study of hinokitiol administered in the diet to F344 rats**

Food Chem. Toxicol., **49**, 1782-1786（2011）

Myocarditis has been reported in male F344 rats given a diet containing hinokitiol（HT）. A subchronic toxicity study was here performed to re-evaluate toxic effects of HT in both sexes of F344 rats with dietary administration at concentrations of 0%, 0.02%, 0.07% and 0.2% for 13weeks. Significant reduction of body weight gain was noted in 0.2% males and 0.07% and above females. Significant decrease in RBC counts, hemoglobin and hematocrit was detected in 0.07% and 0.2% females. Significant increase in MCV was observed in 0.07% and above males and 0.2% females. In the rats given 0.07% and 0.2%, significant increase in total protein and albumin were detected in males, and in total cholesterol in females. Significant increases in total cholesterol, urea nitrogen and creatinine were also detected in the 0.2% males. Significant increase in relative liver weights was detected in the 0.07% and above males and females. Absolute and relative heart weights were significantly decreased in the 0.07% and above males. Based on the above findings the no-observed-adverse-effect level（NOAEL）of HT for both male and female rats was estimated to be 0.02%, translating into 12.7 and 14.8mg/kg b.w./day, respectively. Myocarditis was not evident in the present study.

Keywords: hinokitiol, F344 rats, subchronic toxicity

---

*1 National Cancer Center Research Institute

*2 Food Safety Commission

Ishii, Y., Suzuki, Y., Hibi, D., Jin, M., Fukuhara, K., Umemura, T. and Nishikawa, A.: **Detection and quantification of specific DNA adducts by liquid chromatography-tandem mass spectrometry in the livers of rats given estragole at the carcinogenic dose**

Chem. Res. Toxicol., **24**, 532-541（2011）

Estragole（ES）is a natural constituent of several herbs and spices that acts as a carcinogen in the livers of rodents. Given that the proximal electrophilic form of ES with a reactive carbocation is generated by cytochrome P450 and a sulfotransferase metabolizing pathway, there is a possibility that the resultant covalent adducts with DNA bases may play a key role in carcinogenesis. The existence of ES-specific deoxyguanosine（dG）and deoxyadenosine（dA）adducts has already been reported with the precise chemical structures of the dG adducts being confirmed. In the present study, we examined ES-specific dA adduct formation using LC-ESI/MS after the reaction of dA with 1'-acetoxy-ES produced by a sulfotransferase metabolic pathway mimic. Although two peaks were observed in the LC-ESI/MS chromatogram, the identification of ES-3'-N（6）-dA as the measurable peak was determined by NMR analysis. To confirm ES-specific dG and dA adduct formation in vivo, an isotope dilution LC-ESI/MS/MS method applicable to in vivo samples for ES-3'-N（6）-dA together with the two major dG adducts, that is, ES-3'-C8-dG and ES-3'-N（2）-dG, was developed using selected ion recording. The limit of quantification was 0.2 fmol on column for ES-3'-C8-dG and ES-3'-N（2）-dG and 0.06 fmol on

column for ES-3'-N(6)-dA, respectively. Using the developing analytical method, we attempted to measure adduct levels in the livers of rats treated with ES at a possible carcinogenic dose (600 mg/kg bw) for 4 weeks. ES-3'-C8-dG, ES-N(2)-dG, and ES-3'-N(6)-dA were detected at levels of 3.5 ± 0.4, 4.8 ± 0.8, and 20.5 ± 1.6/10(6) dG or dA in the livers of ES-treated rats. This quantitative data and newly developed technique for adduct observation in vivo might be helpful for ES hepatocarcinogenesis investigations.

Keywords: DNA adduct, estragole, hepatocarcinogenesis

Kemmochi, S.[*1], Fujimoto, H., Woo, G-H., Hirose, M.[*2], Nishikawa, A., Mitsumori, K.[*1] and Shibutani, M.[*1]: **Preventive effects of calcitriol on the development of capsular invasive carcinomas in a rat two-stage thyroid carcinogenesis model**

J. Vet. Med. Sci., **73**, 655-664 (2011)

We have shown phosphoinositide 3-kinase (PI3K)/Akt signaling activation in thyroid capsular invasive carcinomas (CICs), which are highly induced by promotion with sulfadimethoxine (SDM) in a rat two-stage thyroid carcinogenesis model. To examine the potency of calcitriol, a synthetic vitamin D(3) analog, on the development or progression of CICs, male F344 rats were injected with calcitriol (0.1 μ/kg body weight) three times a week intraperitoneally, during an entire period of SDM-promotion for 13 weeks (Experiment 1) or during the last 2 weeks of a 15-week SDM-promotion (Experiment 2). Initiation with N-bis(2-hydroxypropyl) nitrosamine preceded all treatments. In Experiment 1, long-term calcitriol treatment reduced the multiplicity of CICs, while cell proliferation activity, estimated by Ki-67 cell index in the induced CICs, was unchanged with SDM-promotion alone. Considering the strong dependency of promotion with SDM during the early stages on thyroid-stimulating hormone, the reduced multiplicity in Experiment 1 may be due to the effect on an early stage of neoplastic proliferation. Although the magnitude was mild, cell proliferation activity was decreased in existing CICs after short-term calcitriol treatment in Experiment 2, which was associated with a mild decrease in cyclin-dependent kinase-2-positive cells, cytoplasmic immunolocalization of phosphorylated, inactive, Rb protein and a mild increase in nucleocytoplasmic expression of p27 (kip1). Although the effect was mild at the late stage of SDM-promotion in this hypothyroidism-related thyroid carcinogenesis model, our results suggest that calcitriol targets cell proliferation via inhibition of a molecular cascade downstream of PI3K/Akt signaling, controlling G1/S transition.

Keywords: calcitriol, PI3K/Akt signaling, thyroid carcinogenesis

[*1] Tokyo University of Agriculture and Technology
[*2] Food Safety Commission

Kemmochi, S.[*1], Fujimoto, H., Woo, G-H., Inoue, K., Takahashi, M., Mitsumori, K.[*1], Hirose, M.[*2], Nishikawa, A. and Shibutani, M.[*1]: **Involvement of PTEN/Akt signaling in capsular invasive carcinomas developed in a rat two-stage thyroid carcinogenesis model after promotion with sulfadimethoxine**

J. Cancer Res. Clin. Oncol., **137**, 723-732 (2011)

Rat thyroid follicular cell carcinomas invading into the thyroid capsule are highly produced by promotion with sulfadimethoxine (SDM) in a rat two-stage thyroid carcinogenesis model. In this study, we investigated the participation of phosphoinositide 3-kinase (PI3K) signaling pathway that is associated with malignant phenotypes of many cancers on the development of SDM-induced capsular invasive carcinomas. Thyroid proliferative lesions developed 10 or 15 weeks after promotion with SDM in male F344 rats initiated with N-bis (2-hydroxypropyl) nitrosamine were immunohistochemically analyzed with regard to cellular distribution of phosphatase and tensin homolog (PTEN) and Akt isoforms, as well as their downstream molecules. Increased expression of PI3K signaling molecules was evident in association with the development of lesion stages from the early focal hyperplasia to the late carcinomas. Capsular carcinomas, and the less frequent parenchymal carcinomas, exclusively expressed phosphorylated, inactive PTEN, and active Akt isoforms, as did their downstream molecules. Among the Akt isoforms, enhanced expression of Akt1 was more prominent than that of Akt2 in both capsular and parenchymal carcinomas. Activation of the PI3K pathway through phosphorylation of PTEN promotes the high production of capsular carcinomas as well as the development of less frequent parenchymal carcinomas.

Keywords: thyroid follicular cell carcinomas, PI3K pathway, sulfadimethoxine

[*1] Tokyo University of Agriculture and Technology
[*2] Food Safety Commission

Takami, S., Ogawa, K., Umemura, T., Hibi, D., Ishii, Y., Okamura, T., Tasaki, M., Inoue, T., Suzuki, Y., Jin, M., Cho, Y-M. and Nishikawa, A.: **Uterine carcinosarcoma in a**

2-year-old female Wistar Hannover GALAS rat

J. Toxicol. Pathol., **24**, 63-67（2011）

Carcinosarcomas are rare tumors in humans as well as rats and most commonly occur in the uterus. Recently, we observed a case of incidental carcinosarcoma of the uterus in a female Wistar Hannover GALAS［BrlHan:WIST@Jcl（GALAS）］rat at 2 years of age. Histopathologically, the tumor was characterized by an admixture of malignant epithelial and nonepithelial elements. The carcinomatous components represented a type of endometrial carcinoma, consisting of glandular and solid proliferation of large-sized tumor cells. Prominent mitoses and tumor cell invasion were observed. The sarcomatous components were characterized by multifocal proliferation of severe atypical cells with cartilage matrix and were diagnosed as chondrosarcoma. Transitions between carcinomatous and sarcomatous components were observed, and many tumor cells in the solid lesion showed immunohistochemical reactivity with both cytokeratin and vimentin. Based on these findings, this tumor was diagnosed as a uterine carcinosarcoma. This is the first report of uterine carcinosarcoma in Wistar Hannover GALAS［BrlHan:WIST@Jcl（GALAS）］rats.

Keywords: carcinosarcoma, uterus, Wistar Hannover GALAS rat

Toyoda, T.[*1], Tsukamoto, T.[*1], Cho, Y-M., Onami, S., Takasu, S., Shi, L.[*2], Saito, A.[*1], Matsuo, S., Tatematsu, M.[*3], Nishikawa, A. and Ogawa, K.: **Undifferentiated sarcoma of the salivary gland in a Mongolian gerbil（*Meriones unguiculatus*）**

J. Toxicol. Pathol., **24**, 173-177（2011）

A subcutaneous mass was found in the lower ventral neck region of a 55-week-old male Mongolian gerbil（*Meriones unguiculatus*）. Histopathologically, the mass involved salivary glands and featured diffuse proliferation of pleomorphic neoplastic cells with large necrotic foci. The lesion was well demarcated from the surrounding tissue, although invasive growth to fibrous septa was occasionally observed. The neoplastic cells were mainly arranged in irregular sheets with severe cellular atypia, round to oval nuclei and varying amounts of eosinophilic cytoplasm. Mitotic figures and multinucleated giant cells were frequent. Immunohistochemical analysis revealed that the neoplastic cells were strongly positive for vimentin and S-100 and negative for NSE, cytokeratin, α-SMA, c-kit, factor VIII, CD34, α-1-antitrypsin, lysozyme and MSR-A. Based on the results, the mass was diagnosed as an undifferentiated sarcoma of the salivary gland. To the best of our knowledge, this is the first report of such a tumor in Mongolian gerbils.

Keywords: undifferentiated sarcoma, salivary gland, Mongolian gerbil

[*1] Mie University
[*2] Mitsui Chemical Inc.
[*3] Japan Bioassay Research Center

Pitchakarn, P.[*1], Suzuki, S.[*1], Ogawa, K., Pompimon, W.[*2], Takahashi, S.[*1], Asamoto, M.[*1], Limtrakul, P.[*3] and Shirai, T.[*1]: **Induction of G1 arrest and apoptosis in androgen-dependent human prostate cancer by Kuguacin J, a triterpenoid from *Momordica charantia* leaf**

Cancer Lett., **306**, 142-150（2011）

In this study, we focused on the effects of a bitter melon（*Momordica charantia*）leaf extract（BMLE）and a purified component, Kuguacin J（KuJ）, on androgen-dependent LNCaP human prostate cancer cells. Both treatments exerted growth inhibition through G1 arrest and induction of apoptosis. In addition, KuJ markedly decreased the levels of cyclins（D1 and E）, cyclin-dependent kinases（Cdk2 and Cdk4）and proliferating cell nuclear antigen, and caused an increase in p21 and p27 levels. Its induction of apoptosis was accompanied by an increase in cleavage of caspase-3 and poly（ADP-ribose）polymerase, attributable to augment of Bax/Bcl-2 and Bad/Bcl-xL and reduction of survivin levels. BMLE and KuJ also reduced the expression of androgen receptor（AR）, prostate-specific antigen（PSA）while induced P53 protein level. Down-regulation of p53 by RNA interference indicated that BMLE and KuJ inhibited cell growth partly through p53-dependent cell cycle arrest and apoptotic pathways. Both BMLE and KuJ caused less toxicity in a normal prostate cell line, PNT1A. Our results suggest that BMLE and a purified component, KuJ, from its diethyl ether fraction could be promising candidate new antineoplastic and chemopreventive agents for androgen-dependent prostate cancer and carcinogenesis.

Keywords: prostate cancer, bitter melon, Kuguacin J

[*1] Nagoya City University
[*2] Lampang Rajabhat University
[*3] Chiang Mai University

Shimamoto, K.[*], Dewa, Y.[*], Ishii, Y., Kemmochi, S.[*], Taniai, E.[*], Hayashi, H.[*], Imaoka, M.[*], Morita, R.[*], Kuwata, K.[*], Suzuki, K.[*], Shibutani, M.[*] and Mitsumori, K.[*]: **Indole-**

3-carbinol enhances oxidative stress responses resulting in the induction of preneoplastic liver cell lesions in partially hepatectomized rats initiated with diethylnitrosamine

Toxicology, **283**, 109-117（2011）

The liver tumor-promoting effects of indole-3-carbinol（I3C）, a cytochrome P450（CYP）1A inducer found in cruciferous vegetables, were investigated using a medium-term hepatocarcinogenesis model in rats. Six-week-old male F344 rats received an intraperitoneal injection of N-diethylnitrosamine（DEN）and were fed a diet containing 0（DEN-alone）, 0.25, 0.50 or 1.0% of I3C for 8 weeks from 2 weeks after DEN-initiation. The number and area of liver cell foci positive for glutathione S-transferase placental form（GST-P）significantly increased in the livers of rats given 0.5% I3C or more, compared to those in the DEN-alone group. The number of GST-P positive foci also increased in the 0.25% I3C group. The number of liver cells positive for proliferating cell nuclear antigen（PCNA）significantly increased in all I3C groups compared to that in the DEN-alone group. Real-time RT-PCR analysis showed that I3C increased transcript levels of not only Cyp1a1 but also aryl hydrocarbon receptor（AhR）and/or nuclear factor（erythroid-derived 2）-like 2（Nrf2）gene batteries, such as Cyp1a2, Cyp1b1, Ugt1a6, Nrf2, Nqo1, Gsta5, Gstm2, Ggt1and Gpx2. Reactive oxygen species（ROS）in the microsomal fraction significantly increased in all I3C-treated groups compared to the DEN-alone group, and thiobarbituric acid-reactive substances（TBARS）levels and 8-hydroxy-2'-deoxyguanosine（8-OHdG）content significantly increased in all of the I3C-treated groups and 1.0% I3C group, respectively. These results suggest that I3C is an AhR activator and enhances microsomal ROS production resulting in the upregulation of Nrf2 gene batteries, but the oxidative stress generated overcomes the antioxidant effect of Nrf2-related genes. Such ‘a redox imbalance’ subsequently induces liver tumor-promoting effects by enhancing cellular proliferation in rats.

Keywords: indole-3-carbinol, reactive oxygen species, hepatocarcinogenesis

---

* Tokyo University of Agriculture and Technology

Jin, M., Kijima, A., Suzuki, Y., Hibi, D., Inoue, T., Ishii, Y., Nohmi, T., Nishikawa, A., Ogawa, K. and Umemura, T.: **Comprehensive toxicity study of safrole using a medium-term animal model with *gpt* delta rats**

Toxicology, **290**, 313-322（2011）

In order to investigate a medium-term animal model using reporter gene transgenic rodents in which general toxicity, genotoxicity and carcinogenicity are evaluated, F344 *gpt* delta rats were given a diet containing 0.1% and 0.5%（a carcinogenic dose）safrole for 13 weeks. Serum biochemistry and histopathological examinations revealed overt hepatotoxicity of safrole, in line with previous reports. In the current study, safrole treatment possibly resulted in renal toxicity in male rats. In the in vivo mutation assays, an increase or a tendency to increase of the *gpt* mutant frequencies（MFs）was observed in both sexes at the carcinogenic dose. The number and area of foci of glutathione S-transferase placental form（GST-P）positive hepatocytes, ratio of proliferating cell nuclear antigen（PCNA）-positive hepatocytes and 8-hydroxydeoxyguanosine（8-OHdG）levels in liver DNA were significantly increased in both sexes of the 0.5% group. The overall data suggested that the present model might be a promising candidate for investigating comprehensive toxicities of the agents. In addition, data demonstrating the base modification and cell proliferation due to exposure to safrole could contribute to understanding safrole-induced hepatocarcinogenesis, which imply expanding in application of this model.

Keywords: medium-term animal model, *gpt* delta, safrole

Dewa, Y.*, Nishimura, J.*, Jin, M., Kawai, M.*, Saegusa, Y.*, Kenmochi, S.*, Shimamoto, K.*, Harada, T.*, Shibutani, M.* and Mitsumori, K.*: **Immunohistochemical analyses at the late stage of tumor promotion by oxfendazole in a rat hepatocarcinogenesis model**

Arch. Toxicol., **85**, 155-162（2011）

The present study was performed to characterize immunohistochemically the expression levels of molecules related to not only xenobiotic and antioxidant functions but also cell proliferation and apoptosis in neoplastic lesions induced by the benzimidazole anthelmintic, oxfendazole（OX）, at the late stage of its tumor promotion in a rat hepatocarcinogenesis model. Male F344 rats were initiated with an intraperitoneal injection of 200 mg/kg N-diethylnitrosamine, and 2 weeks later they were fed a diet containing 0%（basal diet）or 0.05% OX for 26 weeks. All animals were subjected to a two-thirds partial hepatectomy at week 3 and killed at week 28. Histopathologically, OX increased the incidence and multiplicity of altered foci（4.0- and 3.6-fold, respectively）and hepatocellular adenomas（HCAs）（3.0- and 5.5-fold, respectively）. OX treatment induced 5.2- and 5.6-fold increases in the number of proliferating cell nuclear antigen（PCNA）-positive cells and single-stranded DNA（ssDNA）

-positive cells in HCAs compared with the surrounding tissue, respectively. Staining for the cell cycle regulators P21 and C/EBP_ and the AhR-regulated CYP1A1 molecules decreased but increased reactivity of the Nrf2-regulated, detoxifing/antioxidant molecules aldo-keto reductase 7（AKR7）and glutathione peroxidase 2（GPX2）were also seen in HCAs compared with the surrounding hepatocytes. These results suggest that dysregulation of cell proliferation and apoptosis and escape from oxidative stress elicited by OX treatment play an important role in OX-induced hepatocarcinogenesis in rats.

Keywords: oxfendazole, oxidative stress, hepatocarcinogenesis

---

* Tokyo University of Agriculture and Technology

Ogawa, B.*, Ohishi, T.*, Wang, L.*, Takahashi, M., Taniai, E.*, Hayashi, H.*, Mitsumori, K.* and Shibutani, M.*: **Disruptive neuronal development by acrylamide in the hippocampal dentate hilus after developmental exposure in rats**

Arch. Toxicol., **85**, 987-994（2011）

To examine whether developmental exposure to acrylamide（AA）impairs neuronal development, pregnant Sprague-Dawley rats were treated with AA at 0, 25, 50 or 100 ppm in drinking water from gestational day 6 until weaning on postnatal day 21. Offspring were immunohistochemically examined at the end of exposure. We investigated the expression of Reelin（a molecule regulating neuronal migration and positioning）in the hilus of the hippocampal dentate gyrus. As a positive control for direct exposure, AA（50 mg/kg body weight）was administered to pups by intraperitoneal injection 3 times per week during the lactation period. As well as pups directly injected with AA, maternally exposed offspring decreased body weight at 100 ppm; increased dose-dependently the number of Reelin-immunoreactive cells（from 25 ppm AA）and glutamic acid decarboxylase 67-immunoreactive cells（from 50 ppm AA）, confirming an increase in γ-aminobutyric acid-ergic interneurons. We also noted decreased apoptosis in the neuroblast-producing subgranular zone of the dentate gyrus of maternally exposed pups at 100 ppm, as well as in directly AA-injected pups. These results suggest that a compensatory regulatory mechanism exists to correct impaired neurogenesis and mismigration caused by maternal exposure to AA during neuronal development. The lowest-observed-adverse-effect level of AA was determined to be 25 ppm（3.72 mg/kg body weight/day）.

Keywords: acrylamide, developmental neurotoxicity, dentate gyrus

---

* Tokyo University of Agriculture and Technology

Takahashi, M., Inoue, K., Koyama, N., Yoshida, M., Irie, K., Morikawa, T., Shibutani, M.*, Honma, M. and Nishikawa, A.: **Life stage-related differences in susceptibility to acrylamide-induced neural and testicular toxicity**

Arch. Toxicol., **85**, 1109-1120（2011）

In order to assess age-dependence of susceptibility to acrylamide（ACR）-induced neural and testicular toxicity, 3- and 7-week-old male SD rats were given ACR at 0, 50, 100, or 200 ppm in the drinking water for 4 weeks, and the nervous and male reproductive systems were examined histopathologically. Testicular genotoxicity was evaluated with the comet assay and the micronucleus（MN）test. Glutathione S-transferase（GST）activity and glutathione（GSH）content in the liver and testis were also measured. In both young and adult animals, neurotoxicity was evident from 100 ppm and increased in proportion to ACR intake per body weight. In the testis, marked degeneration and exfoliation, mainly of spermatids, were observed from 100 ppm limited to young animals. The comet assay revealed ACR to significantly induce DNA damage from 100 ppm in both life stages, while MNs were found only in young rats from 100 ppm. The level of GST activity in the testis of young rats at the end of experiment was significantly lower than that of adult animals, regardless of the ACR treatment. There were no life stage-related differences in GSH contents in the liver and testis. These results suggest that susceptibility to neurotoxicity might not differ between young and adult rats when exposure levels are adjusted for body weight. Regarding testicular toxicity, young animals around puberty proved more susceptible than adult animals, possibly due to their lower level of testicular GST activity than that in adult animals.

Keywords: acrylamide, neurotoxicity, testicular toxicity

---

* Tokyo University of Agriculture and Technology

Morita, R.*1, Shimamoto, K.*1, Ishii, Y., Kuwata, K.*1, Ogawa, B.*1, Imaoka, M.*1, Hayashi, S.*2, Suzuki, K.*1, Shibutani, M.*1 and Mitsumori, K.*1: **Suppressive effect of enzymatically modified isoquercitrin on phenobarbital-induced liver tumor promotion in rats**

Arch. Toxicol., **85**, 1475-1484（2011）

To investigate the effect of enzymatically modified

isoquercitrin（EMIQ）on hepatocellular tumor promotion induced by phenobarbital（PB）, male rats were administered a single intraperitoneal injection of 200 mg/kg N-diethylnitrosamine（DEN）and then fed with a diet containing PB（500 ppm）for 8 weeks, with or without EMIQ（2,000 ppm）in the drinking water. One week after PB administration, rats underwent a two-thirds partial hepatectomy. The PB-induced increase in the number and area of glutathione S-transferase placental form-positive foci and the proliferating cell nuclear antigen-positive ratio was significantly suppressed by EMIQ. Real-time reverse transcription-polymerase chain reaction analysis revealed increases in mRNA expression levels of Cyp2b2 and Mrp2 in the DEN-PB and DEN-PB-EMIQ groups compared with the DEN-alone group, while the level of Mrp2 decreased in the DEN-PB-EMIQ group compared with the DEN-PB group. There were no significant changes in microsomal reactive oxygen species（ROS）production and oxidative stress markers between the DEN-PB and DEN-PB-EMIQ groups. Immunohistochemically, the constitutive active/androstane receptor（CAR）in the DEN-PB group was clearly localized in the nuclei, but its immunoreactive intensity was decreased in the DEN-PB-EMIQ group. These results indicate that EMIQ suppressed the liver tumor-promoting activity of PB by inhibiting nuclear translocation of CAR, and not by suppression of oxidative stress.

Keywords: enzymatically modified isoquercitrin, phenobarbital, reactive oxygen species

---

*1 Tokyo University of Agriculture and Technology
*2 San-Ei Gen F.F.I. Inc.

Taketa, Y., Yoshida, M., Inoue, K., Takahashi, M., Sakamoto, Y., Watanabe, G.*1, Taya, K.*1, Yamate, J.*2 and Nishikawa, A.: **Differential stimulation pathways of progesterone secretion from newly formed corpora lutea in rats treated with ethylene glycol monomethyl ether, sulpiride, or atrazine**

Toxicol. Sci., **121**, 267-278（2011）

Ethylene glycol monomethyl ether（EGME）, sulpiride, and atrazine are known ovarian toxicants, which increase progesterone（P4）secretion and induce luteal cell hypertrophy following repeated administration. The aim of this study was to define the pathways by which these compounds exerted their effects on the ovary and hypothalamic-pituitary-gonadal（HPG）axis. In the ovary, changes in the steroidogenic activity of new and old corpora lutea（CL）were addressed. EGME（300 mg/kg）, sulpiride（100 mg/kg）, or atrazine（300 mg/kg）were orally given daily for four times from proestrus to diestrus in normal cycling rats. Treatment with all chemicals significantly increased serum P4 levels, and EGME as well as sulpiride induced increases in prolactin（PRL）levels. In new CL, at both the gene and the protein levels, all three chemicals upregulated the following steroidogenic factors: scavenger receptor class B type I, steroidogenic acute regulatory protein, P450 cholesterol side-chain cleavage, and 3βhydroxysteroid dehydrogenase（HSD）and downregulated the luteolytic gene, 20α-HSD. Coadministration of EGME and bromocriptine, a D2 agonist, completely inhibited PRL but not P4 secretion. Additionally, steroidogenic factor expression levels were upregulated, and 20α-HSD level was down-regulated in new CL. These results suggest that EGME both directly and indirectly stimulates P4 production in luteal cells, whereas sulpiride elevates P4 through activation of PRL secretion in the pituitary. Atrazine may directly activate new CL by stimulating steroidogenic factor expressions. The present study suggests that multiple pathways mediate the effects of EGME, sulpiride, and atrazine on the HPG axis and luteal P4 production in female rats in vivo.

Keywords: progesterone, ethylene glycol monomethyl ether, sulpiride

---

*1 Eisai Co. Ltd.
*2 Osaka Prefecture University

Hibi, D., Suzuki, Y., Ishii, Y., Jin, M., Watanabe, M., Sugita-Konishi, Y., Yanai, T.*, Nohmi, T., Nishikawa, A. and Umemura, T.: **Site-specific in vivo mutagenicity in the kidney of *gpt* delta rats given a carcinogenic dose of ochratoxin A**

Toxicol. Sci., **122**, 406-414（2011）

Ochratoxin A（OTA）can induce renal tumors that originate from the S3 segment of the proximal tubules in rodents, but the results of conventional mutagenicity tests have caused controversy regarding the role of genotoxic mechanisms in the carcinogenesis. Human exposure to OTA from various foods is unavoidable. Therefore, an understanding of OTA-induced renal carcinogenesis is necessary for accurate estimates of the human risk hazard. In the present study, a 13-week exposure of *gpt* delta rats to OTA at a carcinogenic dose induced karyomegaly and apoptosis at the outer stripe of the outer medulla of the kidney, but failed to affect the reporter gene mutations in DNA extracted from whole kidneys. This site-specificity resulting from the kinetics of specific transporters might be responsible for the negative outcome of in vivo

mutagenicity. The kidney was then macroscopically divided, based on anatomical characteristics, into the cortex, the outer and inner medullae, each of which was histopathologically confirmed. Spi(-) mutant frequencies (MFs), but not *gpt* MFs in the outer medulla after a 4-week exposure to OTA were significantly higher than in controls despite the absence of cortical changes. There were also no changes in 8-hydroxydeoxyguanosine levels in kidney DNA. These results strongly suggest the involvement of a genotoxic mechanism, with the exception of oxidative DNA damage in OTA-induced renal carcinogenesis. In addition, the reporter gene mutation assay using DNA from target sites could be a more powerful tool to investigate in vivo genotoxicities.

Keywords: gpt delta, mutagenicity, ochratoxin A

---

* Gifu University

Iwasaki, Y.*, Nomoto, M.*, Oda, M.*, Mochizuki, K.*, Nakano, Y.*, Ishii, Y., Ito, R.*, Saito, K.*, Umemura, T., Nishikawa, A. and Nakazawa, H.*: **Characterization of nitrated phenolic compounds for their anti-oxidant, pro-oxidant, and nitration activities**

Arch. Biochem. Biophys., **513**, 10-18 (2011)

Coffee is one of the most widely consumed beverages worldwide. Evidence of the health benefits and the important contribution of coffee brew to the intake of anti-oxidants in the diet has increased coffee consumption. Chlorogenic acid (ChA) and caffeic acid (CaA) are the major phenolic compounds in coffee. However, phenolic compounds, which are generally effective anti-oxidants, can become pro-oxidants in the presence of Cu(2+) to induce DNA damage under certain conditions. On the other hand, sodium nitrite (NaNO(2)) is widely used as a food additive to preserve and tinge color on cured meat and fish. It is possible that phenolic compounds react with NaNO(2) under acidic conditions, such as gastric juice. In this study, we identified compounds produced by the reaction between ChA or CaA in coffee and NaNO(2) in artificial gastric juice. The identified phenolic compounds and nitrated phenolic compounds were assessed for their anti-oxidant, pro-oxidant, and nitration activities by performing an in vitro assay. The nitrated phenolic compounds seemed to show increased anti-oxidant activity and decreased pro-oxidant activity. However, one nitrated CaA compound that has a furoxan ring showed the ability to release NO(2)(-) in the neutral condition.

Keywords: chlorogenic acid, caffeic acid, sodium nitrite

---

* Hoshi University

Tatematsu, K.*, Koide, A.*, Hirose, M., Nishikawa, A. and Mori, Y.*: **Effect of cigarette smoke on mutagenic activation of environmental carcinogens by cytochrome P450 2A8 and inactivation by glucuronidation in hamster liver**

Mutagenesis, **26**, 323-330 (2011)

To elucidate the mechanism underlying suppression of N-nitrosobis(2-oxopropyl)amine (BOP)-induced hamster pancreatic carcinogenesis by cigarette smoke (CS), hepatic levels of microsomal cytochrome P450 (CYP) enzymes, mutagenic activation of environmental carcinogens and three types of uridine diphosphate-glucuronyltransferase (UDPGT) and sulphotransferase (ST) activities were assayed in male Syrian golden hamsters and F344 rats exposed to CS. Immunoblot analyses of microsomal CYP proteins revealed induction of constitutive CYP1A2 (2.6-fold increase) and 2A8 (4.0-fold increase) and induction of CYP1A1 and constitutive CYP1A2 (3.9-fold increase) in rats following exposure to CS for 4 weeks using a Hamburg type II smoking machine. CS exposure enhanced mutagenicities of four heterocyclic amines in the presence of liver S9 in both species, whereas the mutagenicities of aflatoxin B(1) (AFB(1)), 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAαC) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) were significantly increased by CS in hamsters but not in rats. However, no CS-induced alterations in the mutagenic activities of other carcinogens, including BOP and other pancreatic carcinogens, were observed in either species. Application of several CYP inhibitors revealed that the mutagenic activities of MeAαC, AFB(1) and NNK in the hamster liver S9 were partly associated with CYP2A8, whereas those of the three pancreatic carcinogens were selectively associated with CYP2B. CS enhanced UDPGT activities towards 4-nitrophenol (4-NP) (1.9- to 2.0-fold) but did not affect those of bilirubin, testosterone UDPGTs and three STs in both species. Together with the previous findings that BOP does not induce tumourigenesis in rats and that the glucuronidation of β-oxypropylnitrosamines is higher in rats than in hamsters, suppression of BOP-induced pancreatic carcinogenesis by CS might be attributed to increased detoxification by 4-NP UDPGT and not decreased CYP2B activation. This is the first demonstration of the induction of CYP2A protein by CS; CYP2A protein polymorphisms have been associated with oral and pulmonary carcinogenesis in smokers.

Keywords: cigarette smoke, mutagenicity, cytochrome P450

* Gifu Pharmaceutical University

Masumura, K., Sakamoto, Y., Ikeda, M., Asami, Y., Tsukamoto, T.[*1], Ikehata, H.[*2], Kuroiwa, Y., Umemura, T., Nishikawa, A., Tatematsu, M.[*1], Ono, T.[*2], Nohmi, T.: **Antigenotoxic effects of p53 on spontaneous and ultraviolet light B-induced deletions in the epidermis of *gpt* delta transgenic mice**

Environ. Mol. Mutagen., **52**, 244-252（2011）

Tumor development in the skin may be a multistep process where multiple genetic alterations occur successively. The p53 is involved in genome stability and thus is referred to as "the guardian of the genome." To better understand the antigenotoxic effects of p53 in ultraviolet light B（UVB）-induced mutagenesis, mutations were measured in the epidermis of UVB-irradiated p53(+/+) and p53(-/-) *gpt* delta mice. In the mouse model, point mutations and deletions are separately identified by the *gpt* and Spi(-) assays, respectively. The mice were exposed to UVB at single doses of 0.5, 1.0, or 2.0 kJ/m^2. The mutant frequencies（MFs）were determined 4 weeks after the irradiation. All doses of UVB irradiation enhanced *gpt* MFs by about 10 times than that of unirradiated mice. There were no significant differences in *gpt* MFs and the mutation spectra between p53(+/+) and p53(-/-) mice. The predominant mutations induced by UVB irradiation were G:C to A:T transitions at dipyrimidines. In contrast, in unirradiated p53(-/-) mice, the frequencies of Spi(-) large deletions of more than 1 kb and complex-type deletions with rearrangements were significantly higher than those of the Spi(-) large deletions in p53(+/+) counterparts. The specific Spi(-) mutation frequency of more than 1 kb deletions and complex types increased in a dose-dependent manner in the p53(+/+) mice. However, no increase of such large deletions was observed in irradiated p53(-/-) mice. These results suggest that the antigenotoxic effects of p53 may be specific to deletions and complex-type mutations induced by double-strand breaks in DNA.

Keywords: UVB, epidermis, p53

[*1] 愛知県がんセンター
[*2] 東北大学大学院

Honma, M. and Hayashi, M.[*]: **Comparison of *in vitro* micronucleus and gene mutation assay results for p53-competent versus p53-deficient human lymphoblastoid cells**

Environ. Mol. Mutagen., **52**, 373-384（2011）

The high frequency of false or irrelevant positive results in *in vitro* mammalian cell genotoxicity tests is a critical concern for regulators. Here, we tested whether such results may be due to the mammalian cells used in the tests being deficient in p53, which is involved in the maintenance of genomic stability. We compared the *in vitro* responses of two human lymphoblastoid cell lines derived from the same progenitor cell-p53-competent（TK6）and p53-deficient（WTK-1）cells-in a micronucleus（MN）test and a thymidine kinase gene（TK）mutation assay. We tested 14 chemicals including three mutagens and 11 clastogens and spindle poisons. The three mutagens evoked clear positive responses in both assays in both cell lines. The responses to the clastogens and spindle poisons, on the other hand, depended on the assay endpoint and/or the cell line. Most of clastogens and spindle poisons were positive in the MN test in both cell lines. In the TK mutation assay, on the other hand, WTK-1 cells but not TK6 cells detected spindle poisons, which may have been due to the disturbance of the spindle checkpoint and lack of apoptosis in the p53-deficient cells. Some chemicals that induced chromosome aberrations in rodent cells were negative in both TK6 and WTK-1 cells, indicating that a species-specific factor rather than p53 status was associated with the response. In conclusion, the p53 status did not seriously influence the MN test results but it did influence the TK mutation assay results.

Keywords: p53, micronucleus, gene mutation

[*] (財)食品農医薬安全性評価センター

Yamamoto, A., Sakamoto, Y., Masumura, K., Honma, M., Nohmi, T.: **Involvement of mismatch repair proteins in adaptive responses induced by *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine against γ-induced genotoxicity in human cells**

Mutat. Res., **713**, 56-63（2011）

As humans are exposed to a variety of chemical agents as well as radiation, health effects of radiation should be evaluated in combination with chemicals. To explore combined genotoxic effects of radiation and chemicals, we examined modulating effects of *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine（MNNG）, a direct-acting methylating agent, against genotoxicity of γ-radiation. Human lymphoblastoid TK6 cells and its mismatch-deficient derivative, i.e., MT1 cells, were treated with MNNG for 24h before they were exposed to γ-irradiation at a dose of 1.0 Gy, and the resulting genotoxicity was examined. In TK6 cells, the pretreatments with MNNG at

low doses suppressed frequencies of the thymidine kinase（TK）gene mutation and micronucleus（MN）formation induced by γ-irradiation and thus the dose responses of TK and MN assays were U-shaped along with the pretreatment doses of MNNG. In contrast, the genotoxic effects of MNNG and γ-irradiation were additive in MT1 cells and the frequencies of TK mutations and MN induction increased along with the doses of MNNG. Apoptosis induced by γ-radiation was suppressed by the pretreatments in TK6 cells, but not in MT1 cells. The expression of p53 was induced and cell cycle was delayed at G2/M phase in TK6, but not in MT1 cells, by the treatments with MNNG. These results suggest that pretreatments of MNNG at low doses suppress genotoxicity of γ-radiation in human cells and also that mismatch repair proteins are involved in the apparent adaptive responses.

Keywords: mismatch repair, γ-radiation, adaptive response

Toyoda-Hokaiwado, N., Yasui, Y.,[*1,2], Muramatsu, M.[*3], Masumura, K., Takamune, M., Yamada, M., Ohta, T.[*3], Tanaka, T.[*1,4], Nohmi, T.: **Chemopreventive effects of silymarin against 1,2-dimethylhydrazine plus dextran sodium sulfate-induced inflammation-associated carcinogenicity and genotoxicity in the colon of *gpt* delta rats**

Carcinogenesis, **32**, 1512-1517（2011）

Silymarin, a natural flavonoid from the seeds of milk thistle, is used for chemoprevention against various cancers in clinical settings and in experimental models. To examine the chemopreventive mechanisms of silymarin against colon cancer, we investigated suppressive effects of silymarin against carcinogenicity and genotoxicity induced by 1,2-dimethylhydrazine（DMH）plus dextran sodium sulfate（DSS）in the colon of F344 *gpt* delta transgenic rats. Male *gpt* delta rats were given a single subcutaneous injection of 40 mg/kg DMH and followed by 1.5% DSS in drinking water for a week. They were fed diets containing silymarin for 4 weeks, starting 1 week before DMH injection and samples were collected at 4, 20 and 32 weeks after the DMH treatment. Silymarin at doses of 100 and 500 ppm. suppressed the tumor formation in a dose-dependent manner and the reduction was statistically significant. In the mutation assays, DMH plus DSS enhanced the *gpt* mutant frequency（MF）in the colon, and the silymarin treatments reduced the MFs by 20%. Silymarin also reduced the genotoxicity of DMH in a dose-dependent manner in bacterial mutation assay with *Salmonella typhimurium* YG7108, a sensitive strain to alkylating agents, and the maximum reduction was >80%. These results suggest that silymarin is chemopreventive against DMH/DSS-induced inflammation-associated colon carcinogenesis and silymarin might act as an antigenotoxic agent, in part.

Keywords: Silymarin, chemoprevention, *gpt* delta transgenic rat

---

[*1] 金沢医科大学
[*2] 酪農学園大学
[*3] 東京薬科大学
[*4] （株）東海細胞研究所

Hakulinen, P., Yamamoto, A., Koyama, N., Kumita, W., Yasui, M., Honma, M.: **Induction of TK mutations in human lymphoblastoid TK6 cells by the rat carcinogen 3-chloro-4-（dichloromethyl）-5-hydroxy-2（5H）-furanone（MX）**

Mutat. Res., **725**, 43-49（2011）

3-Chloro-4-（dichloromethyl）-5-hydroxy-2（5H）-furanone（MX）, a chlorine disinfection by-product in drinking water, is carcinogenic in rats and genotoxic in mammalian cells *in vitro*. In the current study, the mechanism of genotoxicity of MX in human lymphoblastoid TK6 cells was investigated by use of the Comet assay, the micronucleus test, and the thymidine kinase（TK）gene-mutation assay. MX induced a concentration-dependent increase in micronuclei and TK mutations. The lowest effective concentrations in the MN test and the TK gene-mutation assay were 37.5 μM and 25 μM, respectively. In the Comet assay, a slight although not statistically significant increase was observed in the level of DNA damage induced by MX in the concentration range of 25-62.5 μM. Molecular analysis of the TK mutants revealed that MX induced primarily point mutations or other small intragenic mutations（61%）, while most of the remaining TK mutants（32%）were large deletions at the TK locus, leading to the hemizygous-type loss-of-heterozygosity（LOH）mutations. These findings show that aside from inducing point mutations, MX also generates LOH at the TK locus in human cells and may thus cause the inactivation of tumour-suppressor genes by LOH.

Keywords: MX, drinking water, genotoxicity

Toyoda-Hokaiwado, N., Yasui, Y.[*1], Takamune, M., Yamada, M., Muramatsu, M.[*2], Masumura, K., Ohta, T.[*2], Tanaka, T.[*3], Nohmi, T.: **Modulatory Effects of Capsaicin on *N*-diethylnitrosamine（DEN）-induced Mutagenesis in *Salmonella typhimurium* YG7108 and DEN-induced Hepatocarcinogenesis in *gpt* Delta Transgenic Rats**

Genes & Environ., **33**, 160-166（2011）

Capsaicin from the red chili pepper is a prospective chemopreventive agent. To explore the possible antigenotoxic effects of capsaicin on *N*-diethylnitrosamine（DEN）-induced mutagenesis *in vitro*, we conducted bacterial mutation assays with *Salmonella typhimurium* YG7108, a sensitive strain to mutagenic alkylating agents. Capsaicin was not mutagenic either with or without S9 activation. Unexpectedly,it enhanced the mutagenicity of DEN in the presence of S9 activation significantly. To examine whether capsaicin modulates DEN-induced mutagenesis and hepatocarcinogenesis *in vivo*, we took advantage of *gpt* delta rats, transgenic rodents that carry reporter genes for mutations. Female *gpt* delta rats were given drinking water containing 40 ppm DEN for five weeks. They were fed diets containing capsaicin at doses of 0, 100 or 500 ppm for seven weeks, starting one week before the DEN treatment. Samples were collected at weeks 7 and 32, respectively, for mutagenicity and carcinogenicity assays. DEN enhanced *gpt* mutant frequency more than 200 fold in the liver. However, capsaicin displayed no modulating effects on the mutagenesis. Rather, it reduced the number of liver neoplasms, especially liver cell adenomas, in a dose-dependent manner although the reduction in hepatocellular carcinoma was statistically insignificant. These results suggest that chemopreventive effect of capsaicin against DEN-induced hepatocarcinogenesis is slight and that the effect is not due to antimutagenesis.

Keywords: Capsaicin, chemoprevention, *gpt* delta transgenic rat

[*1] 金沢医科大学
[*2] 東京薬科大学
[*3] (株)東海細胞研究所

Kimoto, T.[*1], Chikura, S.[*1], Suzuki, K.[*1], Kobayashi, X.[*1], Itano, Y.[*1], Horibata, K., Honma, M., Dobrovolsky, V.N.[*2], Heflich, R.H.[*2], Miura, D.[*1], Kasahara, Y.[*1]: **Further Development of the Rat Pig-a Mutation Assay: Measuring Rat Pig-a Mutant Bone Marrow Erythroids and a High Throughput Assay for Mutant Peripheral Blood Reticulocytes**

Environ. Mol. Mutagen., **52**, 774-783（2011）

Recent studies indicate that the Pig-a assay is a promising tool for evaluating *in vivo* mutagenicity. We have developed novel rat Pig-a assays that facilitate measuring mutant frequencies in two early arising populations of blood cells, bone marrow erythroids（BMEs）and peripheral blood（PB）reticulocytes（RETs）. In these assays, bone marrow cells of erythroid origin and PB red blood cells（RBCs）were identified using an antibody against rat erythroid-specific marker HIS49. In addition, RETs were selectivity enriched from PB using magnetic separation of cells positive for CD71, a transferrin receptor expressed on the surface of BMEs and RETs, but not on the surface of mature RBCs. With magnetic enrichment, more than 1×10^6 CD71-positive RETs could be evaluated by flow cytometry for Pig-a mutant frequency within 5 to 8 min. CD59-deficient RET and BME frequencies of more than 100×10^{-6} and 80×10^{-6} were detected 1 week after treating rats with 40 mg/kg *N*-ethyl-*N*-nitrosourea; by comparison, the frequency of CD59-deficient total RBCs in these rats was 13.2×10^{-6}. The frequency of spontaneous Pig-a mutant RETs and BMEs was less than 5×10^{-6} and 15×10^{-6}, respectively. Since approximately 98% of nucleated cells in the BME fraction were erythroblasts, it should be possible to use BMEs to determine the spectrum of CD59-deficient Pig-a mutations in cells of erythroid lineage. Conducting concurrent Pig-a assays on RETs and BMEs may be useful for evaluating the *in vivo* mutagenicity of chemicals, especially when prolonged mutant manifestation is not feasible or when the confirmation of mutation induction is necessary.

Keywords: Pig-a assay, *in vivo* genotoxicity, reticulocyte

[*1] Teijin Pharma Ltd.
[*2] FDA/NCTR

Kawamura, Y.[*1], Hayashi, H.[*1], Tajima, O.[*2], Yamada, S.[*2], Takayanagi, T.[*3], Hori, H.[*3], Fujii, W.[*3], Masumura, K., Nohmi, T.: **Evaluation of the genotoxicity of aristolochic acid in the kidney and liver of F344 *gpt* delta transgenic rat using a 28-day repeated-dose protocol: A collaborative study of the *gpt* delta transgenic rat mutation assa**y

Genes & Environ., **34**, 18-24（2012）

Transgenic rat gene-mutation assays can be used to assess genotoxicity of chemicals in target organs for carcinogenicity. However, few studies have been conducted to examine the suitability of the assays in repeat-dose treatment protocols. We treated *gpt* delta rats with aristolochic acid at 0.3 and 1 mg/kg by gavage daily for 28 days, and autopsied the rats 3 days after the final treatment, which is a protocol recommended by the International Workshop on Genotoxicity Testing（IWGT）. Aristolochic acid exists in herbs and some other plants, and is carcinogenic in the kidney, bladder and stomach in rats. The mutant frequency in both the kidney and the liver increased significantly in a dose-dependent manner when the rats were

treated with aristolochic acid. We concluded that the *gpt* delta rat assay is sensitive enough to detect gene mutations induced by aristolochic acid and also that the 28-day repeated-dose protocol is suitable for assessing genotoxicity of chemicals.
Keywords: Aristolochic acid, genotoxicity, *gpt* delta transgenic rat

[*1] Meiji Seika Pharma Co., Ltd.
[*2] Kirin Group Office Co.,Ltd.
[*3] Suntory Business Expert Ltd.

Sui, H.[*1], Ohta, R.[*1], Shiragiku, T.[*2], Akahori, A.[*3], Suzuki, K.[*3], Nakajima, M.[*3], Hayashi, H.[*4], Masumura, K., Nohmi, T.: **Evaluation of *in vivo* mutagenicity by 2,4-diaminotoluene and 2,6-diaminotoluene in liver of F344 *gpt* delta transgenic rat dosed for 28 days: A collaborative study of the *gpt* delta transgenic rat mutation assay**

Genes & Environ., **34**, 25-33（2012）

The transgenic rodent（TGR）assay has been widely used to study *in vivo* gene mutations by chemicals or radiation. The International Workshop on Genotoxicity Testing（IWGT）strongly recommends a repeated-dose regimen for the TGR assay protocol for regulatory safety assessment as follows: a treatment period of 28 days and a sampling time of 3 days following the final treatment. In this study, TGR assays using F344 *gpt* delta transgenic rats were conducted at three laboratories to evaluate the validity of the IWGT protocol, as part of a collaborative study of the transgenic rat mutation assay. Male F344 *gpt* delta transgenic rats were orally treated with 2,4-diaminotoluene（2,4-DAT; hepatic carcinogen in rodents; 10 and 30 mg/kg/day）or 2,6-diaminotoluene（2,6-DAT; non-carcinogen in rodents; 60 mg/kg/day）once daily for 28 days. Rats were euthanized 3 days after the last dosing, and then mutant frequencies（MFs）of the *gpt* gene in the livers were studied. As a result, a significant increase in the MF was observed at 30 mg/kg in the 2,4-DAT-treated group, but not in the 2,6-DAT-treated group. These results indicate that 2,4-DAT induces gene mutation in the liver of *gpt* delta rats, but 2,6-DAT does not. These results also indicate that the F344 *gpt* delta transgenic rat mutation assay can distinguish differences in the *in vivo* mutagenic potential between a hepatic carcinogen and a non-carcinogen. Thus, these results demonstrate that the IWGT protocol for the TGR assays is valid, and show that consistent results are obtained among different laboratories.
Keywords: 2,4-diaminotoluene, 2,6-diaminotoluene, *gpt* delta transgenic rat

[*1] Hatano Research Institute, Food and Drug Safety Center
[*2] Otsuka Pharmaceutical Co., Ltd.
[*3] Biosafety Research Center, Foods, Drugs and Pesticides
[*4] Meiji Seika Kaisha, Ltd.

Kamigaito, T.[*1], Noguchi, T.[*1], Narumi, K.[*2], Takashima, R.[*2], Hamada, S.[*2], Sanada, H.[*3], Hasuko, M., Hayashi, H.[*4], Masumura, K., Nohmi, T.: **Evaluation of the *in vivo* mutagenicity of nickel subsulfide in the lung of F344 *gpt* delta transgenic rats exposed by intratracheal instillation: A collaborative study for the *gpt* delta transgenic rat mutation assay**

Genes & Environ., **34**, 34-44（2012）

This study was conducted to evaluate the effectiveness of a transgenic rat mutation assay using F344 *gpt* delta rats. We investigated the mutagenic potential in the lung of nickel subsulfide（Ni_3S_2）, an insoluble fine-crystalline-metallic compound and a carcinogen in the rodent and human lung. Ni_3S_2 carcinogenicity has been proposed to act via both genotoxic and non-genotoxic mechanisms. Ni_3S_2 was intratracheally instilled into male *gpt* delta rats at doses of 0.5 and 1 mg/animal once a week for four weeks; these doses of Ni_3S_2 are high enough to induce inflammation in the lung. Following a period of 28 and 90 days after the first administration, the *gpt* mutant frequencies（MFs）in lung were determined in four independent laboratories, and Spi^- selection for larger deletion mutations was done in one laboratory. The *gpt* MFs of the rats treated with Ni_3S_2 were not increased: all four laboratories obtained similar results with no statistical differences. The Spi^- MFs were also not increased by exposure to Ni_3S_2. These results indicate that intratracheally instilled Ni_3S_2 is non-mutagenic in the lung of *gpt* delta transgenic rats; however, whether Ni_3S_2 is non-mutagenic in the lung or it induces mutations which are not detectable by transgenic rodent mutation assays requires further investigation.
Keywords: nickel subsulfide, mutagenicity, *gpt* delta transgenic rat

[*1] Japan Bioassay Research Center, Japan Industrial Safety and Health Association
[*2] Mitsubishi Chemical Medience Corporation
[*3] Kaken Pharmaceutical Co., Ltd.
[*4] Meiji Seika Pharma Co., Ltd.

Yatagai, F.[*1], Honma, M., Ukai, A., Ohmori, K.[*2], Suzuki, H.[*3], Shimizu, T.[*3], Takahashi, A.[*4], Ohnishi, T.[*5], Dohmae,

N.[*1], Ishioka, N.[*2]: **Preliminary results of space experiment: Implications for the effects of space radiation and microgravity on survival and mutation induction in human cells**

Advance in Space Research, **49**, 479-485（2012）

In view of the concern for the health of astronauts that may one day journey to Mars or the Moon, we investigated the effect that space radiation and microgravity might have on DNA damage and repair. We sent frozen human lymphoblastoid TK6 cells to the International Space Station where they were maintained under frozen conditions during a 134-day mission（14 November 2008 to 28 March 2009）except for an incubation period of 8 days under 1G or μG conditions in a CO_2 incubator. The incubation period started after 100 days during which the cells had been exposed to 54 mSv of space radiation. The incubated cells were then refrozen, returned to Earth, and compared to ground control samples for the determination of the influence of microgravity on cell survival and mutation induction. The results for both varied from experiment to experiment, yielding a large SD, but the μG sample results differed significantly from the 1G sample results for each of 2 experiments, with the mean ratio of μG to 1G being 0.55 for the concentration of viable cells and 0.59 for the fraction of thymidine kinase deficient（TK−）mutants. Among the mutants, non-loss of zygosity events（point mutations）were less frequent（31%）after μG incubation than after 1G incubation, which might be explained by the influence of μG on cellular metabolic or physiological function. Additional experiments are needed to clarify the effect of μG interferes on DNA repair.

Keywords: International Space Station（ISS）, radiation, microgravity, mutation

---

[*1] RIKEN Institute

[*2] Japan Aerospace Exploration Agency

[*3] Japan Space Forum

[*4] Gunma Univ.

[*5] Nara Med. Univ.

Mekenyan, O.G.[*1], Petkov, P.I.[*1], Kotov, S.V.[*1], Stoeva, S.[*1], Kamenska, V.B.[*1], Dimitrov, S.D.[*1], Honma, M., Hayashi, M.[*2], Benigni, R.[*3], Donner, E.M.[*4], Patlewicz, G.[*4]: **Investigating the relationship between *in vitro* - *in vivo* genotoxicity: Derivation of mechanistic QSAR models for *in vivo* liver genotoxicity and *in vivo* bone marrow micronucleus formation which encompass metabolism**

Chem. Res. Toxicol., **25**, 277-296（2012）

Strategic testing as part of an integrated testing strategy（ITS）to maximize information and avoid the use of animals where possible is fast becoming the norm with the advent of new legislation such as REACH. Genotoxicity is an area where regulatory testing is clearly defined as part of ITS schemes. Under REACH, the specific information requirements depend on the tonnage manufactured or imported. Two types of test systems exist to meet these information requirements, *in vivo* genotoxicity assays, which take into account the whole animal, and *in vitro* assays, which are conducted outside the living mammalian organism using microbial or mammalian cells under appropriate culturing conditions. Clearly, with these different broad experimental categories, results for a given chemical can often differ, which presents challenges in the interpretation as well as in attempting to model the results *in silico*. This study attempted to compare the differences between in vitro and *in vivo* genotoxicity results, to rationalize these differences with plausible hypothesis in concert with available data. Two proof of concept（Q）SAR models were developed, one for *in vivo* genotoxicity effects in liver and a second for *in vivo* micronucleus formation in bone marrow. These “mechanistic models” will be of practical value in testing strategies, and both have been implemented into the TIMES software platform（http://oasis-lmc.org）to help predict the genotoxicity outcome of new untested chemicals.

Keywords:（Q）SAR, micronucleus test, *in vivo* genotoxicity

[*1] Bourgas Univ., Bulgaria

[*2] Biosafety Research Center, Japan

[*3] Istituto Superiore di Sanita’, Italy

[*4] DuPont, USA

Xing, G.[*1,2], Qia, X., Chen, M.[*1,2], Wu, Y.[*1,2], Yao, J.[*1,2], Gong, L.[*1], Nohmi, T., Luan, Y.[*1], Ren, J.[*1]: **Comparison of the mutagenicity of aristolochic acid I and aristolochic acid II in the *gpt* delta transgenic mouse kidney**

Mutat. Res., **743**, 52-58（2012）

Aristolochic acid（AA）is known to be a potent mutagen and carcinogen. Aristolochic acid I（AAI）and aristolochic acid II（AAII）, the two major components of AA, differ from each other by a single methoxy group. However, their individual mutagenic characteristics *in vivo* are unclear. In the present study, we compared their DNA adduct formation and mutagenicities in the *gpt* delta transgenic mouse kidney. The dA-AAI, dG-AAI, dA-AAII and dG-AAII were identified in the kidney two days after intragastric administration of AAI or AAII at 5mg/kg. The concentration of DNA adducts formed

by AAII was approximately 2.5-fold higher than that formed by AAI ($p<0.05$). The mutant frequency induced by AAII was nearly two-fold higher than that induced by AAI ($p<0.05$) following administration of 5mg/kg AAI or AAII, five times per week for six weeks. Investigation of the mutation spectra showed no statistically significant difference between AAI- and AAII-treated mice ($p>0.05$). A:T to T:A transversion was the predominant type of mutation in both treated groups, the GC-associated mutation rates, however, differed between the AAI and AAII treatments. The *in vivo* metabolic pathways of AAI and AAII are different, and this may affect their mutagenicity. In the present study, we measured the levels of AAI and AAII in the kidney and plasma of *gpt* delta transgenic mice at multiple time points after a single intragastric dose of 1 or 5mg/kg of either component. Our results showed that the levels of AAII in both kidney and plasma were considerably higher than those of AAI ($p<0.01$). The present study indicated that AAII showed more carcinogenic risk than AAI *in vivo*, and this may be, at least partly, the result of its increased levels in kidney and plasma.

Keywords: Aristolochic acid, DNA adduct, mutagenicity

[*1] Shanghai Institute of Materia Medica, Chinese Academy of Sciences

[*2] Graduate School of the Chinese Academy of Sciences

Hirose, A., Yamazaki, T.[*1], Sakamoto, T.[*1], Sunaga, K.[*1], Tsuda, T.[*1], Mitsumoto, A.[*2], Kudo, N.[*1] and Kawashima, Y.[*1]: **Clofibric Acid Increases the Formation of Oleic Acid in Endoplasmic Reticulum of the Liver of Rats**

J. of Pharmacological Sciences, **116**, 362-372（2011）

The effects of 2-(4-chlorophenoxy)-2-methylpropionic acid（clofibric acid）on the formation of oleic acid（18:1）from stearic acid（18:0）and utilization of the 18:1 formed for phosphatidylcholine（PC）formation in endoplasmic reticulum in the liver of rats were studied in vivo. [14C]18:0 was intravenously injected into control Wistar male rats and rats that had been fed on a diet containing 0.5%（w/w）clofibric acid for 7 days; and the distribution of radiolabeled fatty acids among subcellular organelles, microsomes, peroxisomes, and mitochondria, was estimated on the basis of correction utilizing the yields from homogenates of marker enzymes for these organelles. The radioactivity was mostly localized in microsomes and the radiolabeled fatty acids present in microsomes were significantly increased by the treatment of rats with clofibric acid. The formation of radiolabeled 18:1 in microsomes markedly increased and incorporations of the formed [14C]18:1 into PC and phosphatidylethanolamine in microsomes were augmented in response to clofibric acid. The [14C]18:1 incorporated into PC was mostly located at the C-2 position, but not the C-1 position, of PC, and the radioactivity in 18:1 at the C-2 position of PC was strikingly increased by clofibric acid. These results obtained from the in vivo experiments directly link the findings that clofibric acid treatment induces microsomal stearoyl-CoA desaturase and 1-acylglycerophosphocholine acyltransferase in the liver and the findings that the treatment with the drug elevated absolute mass and mass proportion of 18:1 at the C-2 position, but not the C-1 position, of PC in the liver together.

Keywords: clofibric acid, oleic acid, endoplasmic reticulum

[*1] 城西大学

[*2] 城西国際大学

Hirata-Koizumi, M., Fujii, S.[*], Ono, A., Hirose, A., Imai, T., Ogawa, K., Ema, M. and Nishikawa, A.: **Evaluation of the reproductive and developmental toxicity of aluminium ammonium sulfate in a two-generation study in rats**

Food Chem. Toxicol., **49**, 1948-1959（2011）

Aluminium ammonium sulfate（AAS）was tested for reproductive/developmental toxicity in a two-generation study. Male and female rats were continuously given AAS in drinking water at 0, 50, 500 or 5000 ppm. Water consumption was decreased in all AAS-treated groups, and the body weight of parental animals transiently decreased in the 5000 ppm group. In either generation, no compound-related changes were found in estrous cyclicity, sperm parameters, copulation, fertility and gestation index, number of implantations and live birth pups, sex ratios of pups or viability during the preweaning period. Male and female F1 pups in the 5000 ppm group showed a lower body weight on postnatal day 21, while there were no differences in the birth weight of F1 and F2 pups between the control and AAS-treated groups. Preweaning body weight gain in F2 males and females indicated a similar decreasing tendency at 5000 ppm. In F1 and F2 weanlings, the weight of the liver, spleen and thymus decreased at 5000 ppm, but no histopathological changes were found in these organs. In F1 females in the 5000 ppm group, vaginal opening was delayed slightly. There were no compound-related changes in male preputial separation or in other developmental landmarks. In behavioral tests conducted for F1 animals at 4 − 6 weeks of age, no compound-related changes were found in

spontaneous locomotor activity and performance in a water-filled multiple T-maze. In conclusion, the NOAEL of AAS for two-generation reproductive/developmental toxicity was considered to be 500 ppm in rats. Considering the aluminium content in the basal diet, the total ingested dose of aluminium from drinking water and food in this 500 ppm group was calculated to be 5.35 mg Al/kg bw/day.

Keywords: Aluminium ammonium sulfate, Food additive, Two-generation reproductive/developmental toxicity

---

* （株）化合物安全性研究所

Hirata-Koizumi, M., Fujii, S.*, Furukawa, M.*, Ono, A. and Hirose, A.: **Repeated dose and reproductive/developmental toxicity of perfluorooctadecanoic acid in rats**

J. Toxicol. Sci., **37**, 63-79（2012）

Male and female rats were given perfluorooctadecanoic acid（PFOdA）by gavage at 40, 200 or 1000 mg/kg/day, and each female was mated with a male in the same dose group after 14-day administration. Males were dosed for 42 days and females were dosed throughout the gestation period until day 5 of lactation. One female given 1000 mg/kg/day was euthanized on day 18 of gestation due to a moribund condition; however, no other treatment-related clinical signs of toxicity were observed. Body weights fell at 1000 mg/kg/day from day 28 through the administration period in males and throughout gestation and lactation in females. Red blood cell count, hemoglobin level and hematocrit were decreased at 200 and 1000 mg/kg/day in males and activated partial thromboplastin time was prolonged at 1000 mg/kg/day in females. Histopathological examination revealed hepatic changes, such as centrilobular hypertrophy and necrosis, in males given 200 and 1000 mg/kg/day and in females given 1000 mg/kg/day. Pancreatic zymogen granule was decreased in both sexes at 1000 mg/kg/day. As for reproductive and developmental toxicity, there were decreases in the number of corpora lutea, implantation, total number of pups born and the number of live pups on postnatal days 0 and 4 at 1000 mg/kg/day. At this dose, birth weights of pups were decreased and postnatal body weight gain was inhibited. Based on these findings, the NOAEL of PFOdA was considered to be 40 mg/kg/day for repeated dose toxicity and 200 mg/kg/day for reproductive/developmental toxicity.

Keywords: perfluorooctadecanoic acid, repeated dose toxicity, reproductive and developmental toxicity

---

* （株）化合物安全性研究所

Fujitani, T.*, Ohyama, K.*, Hirose, A., Nishimura, T., Nakae, D.* and Ogata, A.: **Teratogenicity of multi-wall carbon nanotube（MWCNT）in ICR mice**

J. Toxicol. Sci., **37**, 81-89（2012）

A possible teratogenicity of multi-wall carbon nanotube（MWCNT）was assessed using ICR mice. MWCNTs were suspended in 2% carboxymethyl cellulose and given intraperitoneally or intra-tracheally to pregnant ICR mice on day 9 of the gestation. All fetuses were removed from the uterus on day 18 of the gestation, and were examined for external and skeletal anomalies. In the intraperitoneal study, various types of malformation were observed in all MWCNT-treated groups（2, 3, 4 and 5 mg/kg body weight, intraperitoneal）. In contrast, such malformations were observed in groups given 4 or 5 mg/kg body weight, but not in that treated with 3 mg/kg in the intratracheal study. In either study, the number of litters having fetuses with external malformation and that of litters having fetuses with skeletal malformations were both increased in proportion to the doses of MWCNT. The present results are the first to report that MWCNT possesses the teratogenicity at least under the present experimental conditions. Mechanism（s）to result such malformations is yet unclear and further experiment is necessary.

Keywords: Multi-wall carbon nanotube, Nanomaterial, Teratogenicity

---

* 東京都健康安全研究センター

Uehara, T.*1,2, Minowa, Y.*2, Morikawa, Y.*2, Kondo, C.*1, Maruyama, T.*1, Kato, I.*1, Nakatsu, N.*2, Igarashi, Y.*2, Ono, A., Hayashi, H.*3,4, Mitsumori, K.*3, Yamada, H.*2, Ohno, Y. and Urushidani, T.*5: **Prediction model of potential hepatocarcinogenicity of rat hepatocarcinogens using a large-scale toxicogenomics database**

Toxicol. Appl. Pharmacol., **255**, 297-306（2011）

The present study was performed to develop a robust gene-based prediction model for early assessment of potential hepatocarcinogenicity of chemicals in rats by using our toxicogenomics database, TG-GATEs（Genomics-Assisted Toxicity Evaluation System developed by the Toxicogenomics Project in Japan）. The positive training set consisted of high- or middle-dose groups that received 6 different non-genotoxic hepatocarcinogens during a 28-day period. The negative training set consisted of high- or middle-dose groups of 54 non-carcinogens. Support vector machine combined with wrapper-type gene selection algorithms was used for modeling. Consequently, our best classifier yielded prediction

accuracies for hepatocarcinogenicity of 99% sensitivity and 97% specificity in the training data set, and false positive prediction was almost completely eliminated. Pathway analysis of feature genes revealed that the mitogen-activated protein kinase p38- and phosphatidylinositol-3-kinase-centered interactome and the v-myc myelocytomatosis viral oncogene homolog-centered interactome were the 2 most significant networks. The usefulness and robustness of our predictor were further confirmed in an independent validation data set obtained from the public database. Interestingly, similar positive predictions were obtained in several genotoxic hepatocarcinogens as well as non-genotoxic hepatocarcinogens. These results indicate that the expression profiles of our newly selected candidate biomarker genes might be common characteristics in the early stage of carcinogenesis for both genotoxic and non-genotoxic carcinogens in the rat liver. Our toxicogenomic model might be useful for the prospective screening of hepatocarcinogenicity of compounds and prioritization of compounds for carcinogenicity testing.

Keywords: Hepatocarcinogen, Toxicogenomics, Screening

[*1] 塩野義製薬（株）
[*2] （独）医薬基盤研究所
[*3] 東京農工大学
[*4] 岐阜大学
[*5] 同志社女子大学