Iida-Tanaka, N.[*1], Namekata, N.[*2], Kaneko, M.[*1], Tamura, M.[*2], Kawanishi, T., Nakamura, R.[*3], Shigenobu, K.[*2], and Tanaka, H.[*2]: **Involvement of intracellular Ca^{2+} in the regulatory volume decrease after hyposmotic swelling in MDCK cells**.

J Pharmacol Sci., **104**, 397-401（2007）

We examined the source of Ca^{2+} involved in the volume regulation of Madin-Darby canine kidney（MDCK）cells with confocal microscopy and fluoroprobes. Hyposmosis induced a transient increase in cell volume, as well as cytoplasmic Ca^{2+}, which peaked at 3 to 5 min and gradually decreased to reach the initial value within about 30 min. This late decrease in cell volume, as well as the transient rise in cytoplasmic Ca^{2+}, was reduced in Ca^{2+}-free solution and was abolished by pretreatment with thapsigargin. In conclusion, Ca^{2+} released from the intracellular store contributes to the regulatory volume decrease following hyposmotic swelling in MDCK cells.

Keywords: hyposmosis, cell volume, intracellular Ca^{2+}

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Namekata, I.[*], Fujiki, S.[*], Kawakami, Y.[*], Moriwaki, R.[*], Takeda, K.[*], Kawanishi, T., Takahara, A.[*], Shigenobu, K.[*], and Tanaka, H.[*]: **Intracellular mechanisms and receptor types for endothelin-1-induced positive and negative inotropy in mouse ventricular myocardium**,

Nauny-Schmied Arch Pharmacol **376**, 385-395（2008）

We examined the intracellular mechanisms for endothelin-1-induced positive and negative inotropic components that coexist in the mouse ventricular myocardium using isolated ventricular tissue and myocytes from 4-weekold mice. In the presence of SEA0400, a specific inhibitor of the Na^+-Ca^{2+} exchanger, endothelin-1 produced positive inotropy. Endothelin-1, when applied to cardiomyocytes in the presence of SEA0400, did not change the peak amplitude of the Ca^{2+} transient but increased intracellular pH and Ca^{2+} sensitivity of contractile proteins. On the other hand, in the presence of dimethylamiloride（DMA）, a specific inhibitor of the Na^+-H^+ exchanger, endothelin-1 produced negative inotropy. In cardiomyocytes, in the presence of DMA, endothelin-1 produced a decrease in peak amplitude of the Ca^{2+} transient. In the presence of both DMA and SEA0400, endothelin-1 produced neither positive nor negative inotropy. Positive inotropy was blocked by BQ-123 and negative inotropy by BQ-788. These results suggested that endothelin-1-induced positive inotropy is mediated by ETA receptors, activation of the Na^+-H^+ exchanger and an increase in intracellular pH and Ca^{2+} sensitivity and that the negative inotropy is mediated by ETB receptors, activation of the Na^+-Ca^{2+} exchanger and decrease in Ca^{2+} transient amplitude.

Keywords: Na^+-Ca^{2+} exchange, cardiomyocyte, endothelin

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Iida-Tanaka, N.[*1], Nnamekata, I.[*2], Tamura, M.[*2], Kawamata, Y.[*1], Kawanishi, T. and Tanaka, H.[*2]: **Membrane-Labeled MDCK cells and confocal microscopy for the analyses of cellular volume and Morphology**,

Biol. Pharm. Bull. **31**, 731-734（2008）

A clone of Madin-Darby canine kidney（MDCK）cells whose cell membrane was stably labeled with expressed cyan fluorescent protein（CFP）was established, and changes in their volume and shape induced by hyposmotic stress were analyzed with confocal microscopy. The membrane-targeted CFP was present not only on the cell membrane but also in the endoplasmic reticulum and Golgi apparatus, but was excluded from the mitochondria and cell nucleus. During hyposmosis, the initial swelling and the following regulatory volume decrease could be accurately measured by summation of the cellular volume in every confocal slice crossing the cell at different heights. Changes in the cellular height roughly paralleled the changes in cellular volume when the mean value was compared, but dissociation as much as 30% was observed for individual cells due to changes in cell shape. The present experimental system, which enables direct measurement of cell volume and simultaneous observation of various intracellular phenomena, would be useful for further investigation of cellular volume regulation.

Keywords: confocal microscopy, regulatory volume decrease, hyposmosis

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川西 徹：**バイオ医薬品の日局収載環境整備に関する研究**

医薬品研究，**38**, 381-390（2007）

日局原案記載要領の改訂のために，15局日局原案作成要領についてバイオ医薬品関係の問題点についてアンケート調査を行い，その結果をまとめるとともに，その対応案を検討した．

Keywords: 日本薬局方改正，バイオ医薬品，日局原案作成要領

柘植英哉[*1]，中島辰巳[*1]，大内 正[*1]，青木光夫[*2]，大久保恒夫[*2]，四方田千佳子：**浸透圧測定による機種間差に関する研究（第一報）**

医薬品研究，**39**，251-264（2008）

日本薬局方一般試験法2.47浸透圧測定法の機種間差の問題を取り上げ，アンケート方式による実態調査を行った．また，製薬メーカー33社と浸透圧計メーカー５社の協力の下に，共通試料を用いた共同実験を計画・実施し，機種間差の実態の一部を明らかにした．

Keywords: osmolarity determination, osmometer, intra-apparatus difference

Katori, N., Saito, Y., Nakajima, Y., Yoshitani, T., Kim, S.-R., Fukushima-Uesaka, H., Kaniwa, N., Kamatani, N.[*1], Minami, H.[*2], Yoshida, T.[*2], Yamamoto, N.[*2], Tamura, T.[*2], Saijo, N.[*2], and Sawada, J.: ***CYP2C8* haplotype structures and influence of genetic polymorphisms on pharmacokinetics of paclitaxel in a Japanese population**

International Proceeding; 8th Congress of the European Association for Clinical Pharmacology and Therapeutics, 157-161（2007）.

CYP2C8 is known to metabolize various drugs, including the anti-cancer drug paclitaxel (PTX). Haplotype structures of the *CYP2C8* gene were inferred using 40 genetic variations detected in 437 Japanese subjects. We identified 40 haplotypes that were not and 9 haplotypes that were associated with amino acid changes. The 40 haplotypes were classified into 6 groups. Patients with heterozygous **IG* group haplotypes harboring several intronic variations had a 2.5-fold higher ($p<0.001$) median AUC for C3'-p-OH-PTX, and a 16% higher ($p<0.05$) median AUC for PTX than patients with no **IG* group haplotypes. However, no statistically significant differences were observed for the AUC of 6α-OH-PTX. The *ABCB1* genotype also influenced plasma levels of C3'-p-OH-PTX, but not the levels of 6α-OH-PTX or PTX.

Keywords: paclitaxel, pharmacogenomics, CYP2C8, haplotypes

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Kadoya, S.[*], Izutsu, K., Yonemochi, E.[*], Terada, K.[*], Yomota, C., Kawanishi, T.: **Glass-state amorphous salt solids formed by freeze-drying of amines and hydroxy carboxylic acids: effect of hydrogen-bonding and electrostatic interactions**.

Chem. Pharm. Bull. 56(6)821-826（2008）

We studied effect of molecular interactions on the physical properties of binary freeze-dried solids and frozen aqueous solutions using model chemicals containing various functional groups (amino, carboxyl, hydroxyl). Thermal analysis of frozen solutions containing alkyl diamines and hydroxy di- or tricarboxylic acids showed thermal transitions (T_g': glass transition of maximally freeze-concentrated phase) at temperatures higher than those of the individual solutes. A binary frozen solution containing 80 mM 1,3-diamino-2-hydroxypropane (single-solute T_g'<-60℃) and 120 mM citric acid (single-solute T_g': -55.0℃) made the transition at -30.8℃. The molecular weight of the solutes had smaller effects on the transition temperatures of the frozen mixture component solutions. Lyophilization of some high T_g' mixture frozen solutions (e.g., 1,3-diamino-2-hydroxypropane and citric acid) resulted in cake-structure amorphous solids with glass transition temperatures (T_g) higher than those of the individual components. Networking of intense hydrogen-bondings and electrostatic interactions between the heterogeneous molecules through the multiple functional groups was suggested to reduce the component mobility in the amorphous freeze-concentrated phase and the freeze-dried solids. Controlling the interactions should be a key to optimizing the physical properties of multi-component amorphous freeze-dried pharmaceutical formulations.

Keywords: freeze-drying, glass solid, thermal analysis, molecular interaction

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Ishibashi, M.[*1], Tatsuda, S.[*1], Izutsu, K., Kumeda, K.[*1], Arakawa, T.[*2], Tokunaga, M.[*1]: **A single Gly114Arg mutation stabilizes the hexameric subunit assembly and changes the substrate specificity of halo-archaeal nucleoside diphosphate kinase**.
FEBS Lett., **581**, 4073-4079（2007）

Nucleoside diphosphate kinase from extremely halophilic archaeon（HsNDK）requires above 2 M NaCl concentration for in vitro refolding. Here an attempt was made to isolate mutations that allow HsNDK to refold in low salt media. Such a screening resulted in isolation of an HsNDK mutant, Gly114Arg, which efficiently refolded in the presence of 1 M NaCl. This mutant, unlike the wild type enzyme, was expressed in Escherichia coli as an active form. The residue 114 is in close proximity to Glu155 of the neighboring subunit in the three dimensional hexameric structure of the HsNDK. It is thus possible that the attractive electrostatic interactions occur between Arg114 and Glu155 in the mutant HsNDK, stabilizing the hexameric subunit assembly.
Keywords: halophilic, mutation, subunit assembly

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伊豆津健一, 角谷沙織[*], 四方田千佳子, 川西徹, 吉橋泰生[*], 米持悦生[*], 寺田勝英[*]：**塩基性アミノ酸と有機酸の凍結乾燥によるガラス固体の形成とタンパク質安定化**
低温生物工学会誌, **53**, 117-122（2007）

複数の医薬品添加剤を組み合わせた凍結乾燥や溶融体急冷によるガラス固体形成の機構を明らかにするため，各種のモデル物質を用いて凍結水溶液や固体の物性を検討した．複数のアミノ基を持つ物質と水酸基を持つ有機酸の混合により，凍結濃縮相や乾燥固体のガラス転移温度の顕著な上昇がみられ，異種分子間のイオン結合や水素結合が強固な非晶質固体形成に寄与する事が示唆された．分子間相互作用の制御は高機能と保存安定性を兼ね備えた非晶質製剤の設計に有用と考えられる．
Keywords: formulation, glass solid, freeze-drying

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Abe, Y.[*1], Yoshikawa, T.[*1], Kamada, H.[*1], Shibata, H., Nomura, T.[*2], Minowa, K.[*4], Kayamuro, H.[*1], Katayama, K.[*5], Miyoshi, H.[*6], Mukai, Y.[*2], Yoshioka, Y.[*3], Nakagawa, S.[*2], Tsunoda, S.[*1], Tsutsumi, Y.[*1]: **Simple and highly sensitive assay system for TNFR2-mediated soluble- and transmembrane-TNF activity**.
J Immunol Methods, **335(1-2)**, 71-8（2008）.

Drugs that target tumor necrosis factor-alpha（TNF）are particularly important in the treatment of severe inflammatory progression in rheumatoid arthritis, Crohn's disease and psoriasis. Despite the central role of the TNF/TNF receptor（TNFR）in various disease states, there is a paucity of information concerning TNFR2 signaling. In this study, we have developed a simple and highly sensitive cell-death based assay system for analyzing TNFR2-mediated bioactivity that can be used to screen for TNFR2-selective drugs. Using a lentiviral vector, a chimeric receptor was engineered from the extracellular and transmembrane domain of human TNFR2 and the intracellular domain of mouse Fas and the recombinant protein was then expressed in TNFR1（-/-）R2（-/-）mouse preadipocytes. Our results demonstrate that this chimeric receptor is capable of inducing apoptosis by transmembrane- as well as soluble-TNF stimuli. Moreover, we found that our bioassay based on cell death phenotype had an approximately 80-fold higher sensitivity over existing bioassays. We believe our assay system will be an invaluable research tool for studying TNFR2 and for screening TNFR2-targeted drugs.
Keywords: TNF, TNFR2, Bioassay

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Shibata, H., Yoshioka, Y.[*3], Ohkawa, A.[*2], Minowa, K.[*4], Mukai, Y.[*2], Abe, Y.[*1], Taniai, M.[*5], Nomura, T.[*1], Kayamuro, H.[*1], Nabeshi, H.[*1], Sugita, T.[*1], Imai, S.[*1], Nagano, K.[*1], Yoshikawa, T.[*1], Fujita, T.[*4], Nakagawa, S.[*2], Yamamoto, A.[*4], Ohta, T.[*5], Hayakawa, T.[*7], Mayumi, T.[*6], Vandeenabeele, P.[*8], Aggarwal, BB.[*9], Nakamura, T.[*10], Yamagata, Y.[*10], Tsunoda, S.[*1], Kamada, H.[*1], Tsutsumi, Y.[*1]: **Creation and X-ray structure analysis of the tumor necrosis factor receptor-1-**

selective mutant of a tumor necrosis factor-alpha antagonist.,

J. Biol. Chem., **283**, 998-1007（2008）

Tumor necrosis factor-alpha（TNF）induces inflammatory response predominantly through the TNF receptor-1（TNFR1）. Thus, blocking the binding of TNF to TNFR1 is an important strategy for the treatment of many inflammatory diseases, such as hepatitis and rheumatoid arthritis. In this study, we identified a TNFR1-selective antagonistic mutant TNF from a phage library displaying structural human TNF variants in which each one of the six amino acid residues at the receptor-binding site（amino acids at positions 84-89）was replaced with other amino acids. Consequently, a TNFR1-selective antagonistic mutant TNF（R1antTNF）, containing mutations A84S, V85T, S86T, Y87H, Q88N, and T89Q, was isolated from the library. The R1antTNF did not activate TNFR1-mediated responses, although its affinity for the TNFR1 was almost similar to that of the human wild-type TNF（wtTNF）. Additionally, the R1antTNF neutralized the TNFR1-mediated bioactivity of wtTNF without influencing its TNFR2-mediated bioactivity and inhibited hepatic injury in an experimental hepatitis model. To understand the mechanism underlying the antagonistic activity of R1antTNF, we analyzed this mutant using the surface plasmon resonance spectroscopy and x-ray crystallography. Kinetic association/dissociation parameters of the R1antTNF were higher than those of the wtTNF, indicating very fast bond dissociation. Furthermore, x-ray crystallographic analysis of R1antTNF suggested that the mutation Y87H changed the binding mode from the hydrophobic to the electrostatic interaction, which may be one of the reasons why R1antTNF behaved as an antagonist. Our studies demonstrate the feasibility of generating TNF receptor subtype-specific antagonist by extensive substitution of amino acids of the wild-type ligand protein.

Keywords : TNF, Antagonist, TNFR1

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Sugita, T.[*1], Yoshikawa, T.[*1], Mukai, Y.[*2], Yamanada, N.[*1], Imai, S.[*1], Nagano, K.[*1], Yoshida, Y.[*1], Shibata, H., Yoshioka, Y.[*3], Nakagawa, S.[*2], Kamada, H.[*1], Tsunoda, S.[*1], Tsutsumi, Y.[*1]: **Comparative study on transduction and toxicity of protein transduction domains**.

Br J Pharmacol. **153**, 1143-52（2008）

Protein transduction domains（PTDs）, such as Tat, antennapedia homeoprotein（Antp）, Rev and VP22, have been extensively utilized for intracellular delivery of biologically active macromolecules in vitro and in vivo. There is little known, however, about the relative transduction efficacy, cytotoxicity and internalization mechanism of individual PTDs. EXPERIMENTAL APPROACH: We examined the cargo delivery efficacies of four major PTDs（Tat, Antp, Rev and VP22）and evaluated their toxicities and cell internalizing pathways in various cell lines. KEY RESULTS: The relative order of the transduction efficacy of these PTDs conjugated to fluorescein was Rev>Antp>Tat>VP22, independent of cell type（HeLa, HaCaT, A431, Jurkat, MOLT-4 and HL60 cells）. Antp produced significant toxicity in HeLa and Jurkat cells, and Rev produced significant toxicity in Jurkat cells. Flow cytometric analysis demonstrated that the uptake of PTD-fluorescein conjugate was dose-dependently inhibited by methyl-beta-cyclodextrin, cytochalasin D and amiloride, indicating that all four PTDs were internalized by the macropinocytotic pathway. Accordingly, in cells co-treated with 'Tat-fused' endosome-disruptive HA2 peptides（HA2-Tat）and independent PTD-fluorescent protein conjugates, fluorescence spread throughout the cytosol, indicating that all four PTDs were internalized into the same vesicles as Tat. CONCLUSIONS AND IMPLICATIONS: These findings suggest that macropinocytosis-dependent internalization is a crucial step in PTD-mediated molecular transduction. From the viewpoint of developing effective and safe protein transduction technology, although Tat was the most versatile carrier among the peptides studied, PTDs should be selected based on their individual characteristics.

Keywords: protein transduction domains, Tat, anten-

napedia

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Shibata, H., Kamada, H.*1, Nishibata, K.*2, Yoshioka, Y.*3, Nishibata, T.*2, Abe, Y.*1, Nomura, T.*1, Nabeshi, H.*1, Minowa, K.*4, Mukai, Y.*2, Nakagawa, S.*2, Mayumi, T.*5, Tsunoda, S.*1, Tsutsumi, Y*1.: **Role of amino acid residue 90 in bioactivity and receptor binding capacity of tumor necrosis factor mutants**.
BBA-Proteins and Proteomics., **1774**(8), 1029-1035 (2007).

We have previously produced two bioactive lysine-deficient mutants of TNF-alpha (mutTNF-K90R,-K90P) and found that these mutants have bioactivity superior to wild-type TNF (wtTNF). Because these mutants contained same amino acid except for amino acid 90, it is unclear which amino acid residue is optimal for showing bioactivity. We speculated that this amino acid position was exchangeable, and this amino acid substitution enabled the creation of lysine-deficient mutants with enhanced bioactivity. Therefore, we produced mutTNF-K90R variants (mutTNF-R90X), in which R90 was replaced with other amino acids, to assay their bioactivities and investigated the importance of amino acid position 90. As a result, mutTNF-R90X that replaced R90 with lysine, arginine and proline were bioactive, while other mutants were not bioactive. Moreover, these three mutants showed bioactivity as good as or better than wtTNF. R90 replaced with lysine or arginine had especially superior binding affinities. These results suggest that the amino acid position 90 in TNF-alpha is important for TNF-alpha bioactivity and could be altered to improve its bioactivity to generate a "super-agonist".
Keywords : TNF, Mutant, Phage display

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Nomura, T.*1, Kawamura, M.*1, Shibata, H., Abe, Y.*1, Ohkawa, A.*1, Mukai, Y.*2, Sugita, T.*1, Imai, S.*1, Nagano, K.*1, Okamoto, T., Tsutsumi, Y.*1, Kamada, H.*1, Nakagawa, S.*2, Tsunoda, S.*1: **Creation of novel cell penetrating peptide, using random 18mer peptides library.**,
Pharmazie, **62**, 569-573 (2007)

Cell penetrating peptides (CPPs) have drawn attention as carriers for intracellular drug delivery. It is commonly believed that TAT peptide is the best carrier among the existing CPPs due to its high translocational activity. Despite considerable research, the cellular uptake mechanism of TAT peptide remains unclear. Additionally, the transduction efficiency of TAT peptide is insufficient for use in intracellular therapy. In this study, we attempted to identify novel CPPs from a random 18mer peptide library using a phage display system. To isolate novel CPPs more effectively, PSIF (protein synthesis inhibition factor) was used with the screening system. Consequently, we isolated 7 novel CPPs from the library and determined by flow cytometry and confocal laser microscopy that these CPPs were taken up into cells. Once the cellular uptake pathway of these CPPs has been determined, it may be possible to use them for intracellular therapy.
Keywords: Cell penetrating peptides, Tat, phage display

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Sugita, T.*2, Yoshikawa, T.*1, Mukai, Y.*2, Yamanada, N.*2, Yamato, T*2., Imai, S.*2, Nagano, K.*2, Yoshida, Y.*1, Shibata H., Yoshioka, Y.*3, Nakagawa, S.*2, Kamada, H.*1, Tsunoda, S.*1, Tsutsumi, Y.*2: **Improved cytosolic translocation and tumor-killing activity of Tat-shepherdin conjugates mediated by co-treatment with Tat-fused membrane-disruptive HA2 peptide.**,
Biochem. Biophys. Res. Commun., **363**, 1027-1032 (2007)

Tat peptides are useful carriers for delivering biologic molecules into the cell for both functional analysis of intracellular disease-related proteins and treatment of refractory diseases. Most internalized Tat-fused cargos (Tat-cargos) are trapped within the endosome, however, which limits the biologic function of the cargo. In this study, we demonstrated that Tat-fused HA2 peptide (HA2Tat), an endosome disrupted pep-

tide, enhanced the endosome-escape efficiency of Tat-cargos. In cells treated with a mixture of fluorescein isothiocyanate-labeled Tat and HA2Tat, widespread fluorescence was observed throughout the cytosol. In addition, this HA2Tat-mediated cytosolic delivery technique led to enhanced cytotoxicity of Tat-fused anticancer peptides, specifically shepherdin. Thus, we improved the function of the delivered molecules by co-treating with HA2Tat and propose that this is a useful method for the delivery of therapeutic macromolecules into the cytosol.

Keywords: Tat, HA2, Protein transduction domain; PTD

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Aso, Y., Yoshioka, S., Miyazaki, T., Kawanishi, T., Tanaka, K.*1, Kitamura, S.*1, Takakura, A.*2, Hayashi, T.*2, Muranushi, N.*1: **Miscibility of nifedipine and hydrophilic polymers as measured by ^{1}H-NMR spin-lattice relaxation**.

Chem. Pharm. Bull., **55**, 1227-1231（2007）

The miscibility of a drug with excipients in solid dispersions is considered to be one of the most important factors for preparation of stable amorphous solid dispersions. The purpose of the present study was to elucidate the feasibility of ^{1}H-NMR spin-lattice relaxation measurements to assess the miscibility of a drug with excipients. Solid dispersions of nifedipine with the hydrophilic polymers poly（vinylpyrrolidone）（PVP), hydroxypropylmethylcellulose（HPMC）and α,β-poly（N-5-hydroxypentyl)-L-aspartamide（PHPA）with various weight ratios were prepared by spray drying, and the spin-lattice relaxation decay of the solid dispersions in a laboratory frame（T_1 decay）and in a rotating frame（$T_{1\rho}$ decay）were measured. $T_{1\rho}$ decay of nifedipine-PVP solid dispersions（3:7, 5:5 and 7:3）was describable with a mono-exponential equation, whereas $T_{1\rho}$ decay of nifedipine-PHPA solid dispersions（3:7, 4:6 and 5:5）was describable with a bi-exponential equation. Because a mono-exponential $T_{1\rho}$ decay indicates that the domain sizes of nifedipine and polymer in solid dispersion are less than several nm, it is speculated that nifedipine is miscible with PVP but not miscible with PHPA. All the nifedipine-PVP solid dispersions studied showed a single glass transition temperature（T_g), whereas two glass transitions were observed for the nifedipine-PHPA solid dispersion（3:7), thus supporting the above speculation. For nifedipine-HPMC solid dispersions（3:7 and 5:5), the miscibility of nifedipine and HPMC could not be determined by DSC measurements due to the lack of obviously evident T_g. In contrast, ^{1}H-NMR spin-lattice relaxation measurements showed that nifedipine and HPMC are miscible, since $T_{1\rho}$ decay of the solid dispersions（3:7, 5:5 and 7:3）was describable with a mono-exponential equation. These results indicate that ^{1}H-NMR spin-lattice relaxation measurements are useful for assessing the miscibility of a drug and an excipient in solid dispersions.

Keywords: miscibility, solid dispersion, spin diffusion

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Maitani, Y.*, Aso, Y., Yamada, A.*, Yoshioka, S.: Effect of sugars on storage stability of lyophilized liposome/DNA complexes with high transfection efficiency.

Int. J. Pharm., **356**, 69-75（2008）

Cationic lipid-based gene delivery systems have shown promise in transfecting cells *in vitro* and *in vivo*. However, liposome/DNA complexes tend to form aggregates after preparation. Lyophilization of these systems, therefore, has become of increasing interest. In this study, we investigated the feasibility of preserving complexes as a dried preparation using a modified dehydration rehydration vesicle（DRV）method as a convenient and reliable procedure. We also studied storage stability of a lyophilized novel cationic gene delivery system incorporating sucrose, isomaltose and isomaltotriose. Liposomes were composed of 3β-[*N*-(*N'*,*N'*-dimethylaminoethane)-carbamoyl] cholesterol（DC-Chol）and dioleoylphosphatidylethanolamine（DOPE), plus sucrose, isomaltose or isomaltotriose. Lyophilized liposome/DNA complexes were stored at -20, 25, 40 and 50℃ and their stability was followed for 50 days. Liposome/DNA complexes with sucrose could be stored even at 50℃ without large loss of transfection efficiency. The transfection efficiency of formulations stored at various temperatures indicated that the stabilizing effect of sugars on plasmid DNA was higher in the following order; isomaltotriose<isomaltose<sucrose, which was inverse to

the order of their glass transition temperature (T_g) values. It was concluded that we could prepare novel lyophilized liposome/DNA complexes with high transfection efficiency and stability, which might be concerned that sucrose stabilized plasmid DNA in liposomes by directly interacting with plasmid DNA rather than by vitrifying to a high T_g solid.

Keywords: Cationic liposome, Dehydration rehydration vesicle, Storage stability

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Sakamoto, T., Fujimaki, Y., Hiyama, Y.: **Studies on the influence of uniformity of particle size of powder, tapping and sample replacement for diffusion reflectance quantitative NIR spectrometric analysis**

Pharmazie, **63**, 841-846 (2007)

The extent of deviation factors and the influence of pre-processing of spectra for a quantitative application of reflectance NIR measurement against powder sample were examined. Lactose monohydrate (NGLM), a medical additive was used for this study. Ground lactose monohydrate (GLM) and NGLM were measured by NIR reflectance spectroscopy. In the wave number range from 12000cm-1 to 4000cm-1, the ratios of absorbance values (a.v.) between the wave numbers of GLM and NGLM were almost same and no influence of intensity of absorbance through the measurement range was observed concerning heterogeneity of particle size. Absorbance values of NGLM were decreased with increasing number of tapping without a bit difference of the change of a.v. The several statistical parameters of a.v. from the both samples were estimated. The relative standard deviation (RSD) and 95% confidence intervals (CIs) of a.v. on successive measurements at a fixed position in GLM and NGLM vials were almost the same. However, the RSD and 95% confidence of absorbance value of NGLM were larger than these GLM, i.e., RSD: 0.66% for NGLM, 0.42% for GLM. The 95% of CI of NGLM was ten times larger than that of GLM in five replacement positions. The two kinds of baseline corrections, the SNV and MSC processing were examined to evaluate the extent of influence against a.v. The 95% CI calculated from a.v. by the SNV pre-processing showed a wider range compared with that from no pre-processing and MSC pre-processing. These results suggest that the statistical confidence of a.v. would also change by pre-processing. It is important to consider the statistical confidence of a.v. for precise quantitative application of the reflectance NIR spectroscopy.

Keywords: NIR, Diffusion reflectance, Deviation factor, Quantitative analysis

Yamaguchi, T., Uchida, E.: **Regulatory Aspects of Oncolytic Virus Products**

CCDT Journal, **7**, 203-208 (2007)

Many types of oncolytic viruses, wild-type virus, attenuated viruses and genetically-modified viruses, have been developed as an innovative cancer therapy. The strategies, nature, and technologies of oncolytic virus products are different from the conventional gene therapy products or cancer therapy products. From the regulatory aspects to ensure the safety, efficacy and quality of oncolytic viruses, there are several major points during the development, manufacturing, characterization, non-clinical study and clinical study of oncolytic viruses. The major issues include 1) virus design (wild-type, attenuated, and genetically engineered strains), 2) poof of concept in development of oncolytic virus products, 3) selectivity of oncolytic virus replication and targeting to cancer cells, 4) relevant animal models in non-clinical studies, 5) clinical safety, 6) evaluation of virus shedding. Until now, the accumulation of the information about oncolytic viruses is not enough, it may require the unique approach to ensure the safety and the development of new technology to characterize oncolytic viruses.

Keywords : gene therapy, oncolytic virus, cancer

Mizuguchi, H.[*1], Funakoshi, N.[*1], Hosono, T., Sakurai, F.[*1], Kawabata, K.[*1], Yamaguchi, T., Hayakawa, T.[*2]: **Rapid construction of small interfering RNA-expressing adenovirus vectors on the basis of direct cloning of short hairpin RNA-coding DNAs**

Hum. Gene Ther., **18**, 74-80 (2007)

In the conventional method for constructing an adenoviral (Ad) vector expressing small interfering RNA (siRNA), short hairpin RNA (shRNA)-coding oligonucleotides are introduced downstream of a polymerase III (or polymerase II)-based promoter cloned into a shuttle plasmid. An siRNA expression cassette, which is cloned into the shuttle plasmid, is

then introduced into the E1 deletion region of the Ad vector plasmid by in vitro ligation or homologous recombination in Escherichia coli, and the linearized plasmid is transfected into 293 cells, generating an Ad vector expressing siRNA. Therefore, two-step plasmid manipulation is required. In this study, we developed a method by which shRNA-coding oligonucleotides can be introduced directly into the Ad vector plasmid. To do this, we constructed a new vector plasmid into which the human U6 promoter sequence was cloned in advance. Unique restriction enzyme sites were introduced at the transcription start site of the U6 promoter sequence in the vector plasmid. Luciferase and p53 genes were efficiently knocked down by Ad vectors generated by the new method and expressing siRNA against the target gene. This method should be useful for RNA interference-based experiments, and should make it easy to construct an siRNA-expressing Ad vector library for functional screening.

Keywords: siRNA, gene therapy, shRNA

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Hashii, N., Kawasaki, N., Matsuishi, Y., Toyoda, M.[*], Katagiri, Y.[*], Itoh, S., Harazono, A., Umezawa, A.[*], Yamaguchi T.: **Study on the quality control of cell therapy product: Determination of N-glycolylneuraminic acid incorporated into human cells by nano-flow liquid chromatography/Furrier transformation ion cyclotron resonance mass spectrometry**

J. Chromatogr. A, **1160**, 263-269（2007）

N-Glycolylneuraminic acid（NeuGc）, an acidic nine-carbon sugar, is produced in several animals, such as cattle and mice. Since human cells cannot synthesize NeuGc, it is considered to be immunogenic in humans. Recently, NeuGc contamination was reported in human embryonic stem cells cultured with xenogeneic serum and cells, suggesting that possibly NeuGc may harm the efficacy and safety of cell therapy products. Sialic acids have been determined by derivatization with 1,2-diamino-4,5-methylenedioxybenzene（DMB）followed by liquid chromatography/mass spectrometry（LC/MS）and liquid chromatography/tandem mass spectrometry（LC/MS/MS）; however, the limited availability of cell therapy products requires more sensitive and specific methods for the quality test. Here we studied the use of nano-flow liquid chromatography/Fourier transformation ion cyclotron resonance mass spectrometry（nanoLC/FTMS）and nanoLC/MS/MS for NeuGc-specific determination at a low femtomole level. Using our method, we found NeuGc contamination of the human cell line（HL-60RG cells）cultured with human serum. Our method needs only 2.5x103 cells for one injection and would be applicable to the determination of NeuGc in cell therapy products.

Keywords: *N*-glycolylneuraminic acid, nano-flow liquid chromatography, Fourier transformation ion cyclotron mass spectrometry

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Kizuka, Y.[*], Kobayashi, K.[*], Kakuda, S.[*], Nakajima Y., Kawasaki N., Oka S.[*]: **Laminin-1 is a novel carrier glycoprotein of non-sulfated HNK-1 epitope in mouse kidney**.

Glycobiology, **18**, 331-338（2008）

The HNK-1 epitope has a unique structure comprising the sulfated trisaccharide（HSO(3)-3GlcAβ1-3Galβ4-4GlcNAc）, and two glucuronyltransferases（GlcAT-P and GlcAT-S）are key enzymes for its biosynthesis. However, the different functional roles of these enzymes in its biosynthesis remain unclear. Recently, we reported that a nonsulfated form of this epitope, which is biosynthesized by GlcAT-S but not by GlcAT-P, is expressed on two metalloproteases in mouse kidney. In this study, we found that a novel glycoprotein carrying the nonsulfated HNK-1 epitope in mouse kidney was enriched in the nuclear fraction. The protein was affinity-purified and identified as laminin-1, and we also confirmed the N-linked oligosaccharide structure including nonsulfated HNK-1 epitope derived from laminin-1 by mass spectrometry. Curiously, immunofluorescence staining of kidney sections revealed that laminin-1 appeared not to be colocalized with the nonsulfated HNK-1 epitope. However, proteinase treatment strengthened the signals of both laminin-1 and the nonsulfated HNK-1 epitope, resulting in overlapping of them. These results indicate that the nonsulfated HNK-1 epitope on laminin-1 is usually embedded and masked in the robust basement membrane in tight association with other proteins. To

clarify the associated proteins and the functional role of the carbohydrate epitope, we investigated the interaction between laminin-1 and alpha-dystroglycan through their glycans in mouse kidney using the overlay assay technique. We obtained evidence that glucuronic acid as well as sialic acid inhibited this interaction, suggesting that the nonsulfated HNK-1 epitope on laminin-1 may regulate its binding and play a role in maintenance of the proper structure in the kidney basal laminin.

Keywords: nonsulfated HNK-1, laminin, glucuronyltransferase

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Ito, Y.*, Watanabe, T.*, Nagatomo, S.*, Seki, T.*, Niimi, S. Ariga, T.*: **Annexin A3-expressing cellular phenotypes emerge from necrotic lesion in the pericentral area in 2-acetylaminofluoren/carbon tetrachloride-treated rat livers**

Biosci. Biotechnol. Biochem., **71**, 3082-3089 (2007)

Recently we found a small hepatocyte-specific protein, annexin A3 (AnxA3), in fractionated adult rat hepatocytes. Here we describe the results of an *in vivo* demonstration of AnxA3-expressing cellular phenotypes in the liver with 2-acetylaminofluoren (2-AAF)/carbon tetrachloride (CCl_4)-injury. In association with an elevation of alanine amino transferase (ALT) and aspartic acid amino transferase (AST) activities, hepatic AnxA3 mRNA increased markedly. AnxA3-positive cells were detected in clustered cells present in or emerging from the pericentral region. These albumin-expressed cells were histologically similar to cells expressing CD34, a hematopoietic cell marker protein. The number of clusters decreased in the days following CCl_4 treatment, and annexin-negative, but albumin-positive, oval cells appeared. We concluded that the agent-induced liver defect initially recruits bone marrow-derived cells, and that it promotes differentiation of these cells into AnxA3-positive cells, followed by emergence of the oval cells, which might have a role in the restitution of the damaged liver.

Keywords: small hepatocyte, oval cell, annexin A3

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Mukai, N.*, Akahhori, T.*, Komaki, M.*, Li, Q.*, Kanayasu-Toyoda, T., Ishii-Watabe, A., Kobayashi, A.*, Yamaguchi, T., Abe, M.*, Amagasa, T.*, Morita, I.*: **A comparison of the tube forming potentials of early and late endothelial progenitor cells**.

Exp. Cell Res. **314**, 430-440 (2008)

The identification of circulating endothelial progenitor cells (EPCs) has revolutionized approaches to cell-based therapy for injured and ischemic tissues. However, the mechanisms by which EPCs promote the formation of new vessels remain unclear. In this study, we obtained early EPCs from human peripheral blood and late EPCs from umbilical cord blood. Human umbilical vascular endothelial cells (HUVECs) were also used. Cells were evaluated for their tube-forming potential using our novel in vitro assay system. Cells were seeded linearly along a 60 μm wide path generated by photolithographic methods. After cells had established a linear pattern on the substrate, they were transferred onto Matrigel. Late EPCs formed tubular structures similar to those of HUVECs, whereas early EPCs randomly migrated and failed to form tubular structures. Moreover, late EPCs participate in tubule formation with HUVECs. Interestingly, late EPCs in Matrigel migrated toward pre-existing tubular structures constructed by HUVECs, after which they were incorporated into the tubules. In contrast, early EPCs promote sprouting of HUVECs from tubular structures. The phenomena were also observed in the in vivo model. These observations suggest that early EPCs cause the disorganization of pre-existing vessels, whereas late EPCs constitute and orchestrate vascular tube formation.

Keywords: early endothelial progenitor cells, late endothelial progenitor cells, tube-forming activity

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Kanayasu-Toyoda, T., Ishii-Watabe, A., Suzuki, T., Oshizawa, T., Yamaguchi, T.: **A new role of thrombopoietin enhancing ex vivo expansion of endothelial precursor cells derived from AC133 positive cells**.

J. Biol. Chem. **282**, 33507-14 (2007)

We previously reported that $CD31^{bright}$ cells, which were sorted from cultured $AC133^+$ cells of adult peripheral blood cells, differentiated more efficiently into endothelial cells than $CD31^+$ cells or $CD31^-$ cells, suggesting that $CD31^{bright}$ cells may be endothelial

precursor cells. In this study, we found that CD31^{bright} cells have a strong ability to release cytokines. The mixture of vascular endothelial growth factor (VEGF), thrombopoietin (TPO), and stem cell factor stimulated ex vivo expansion of the total cell number from cultured AC133^{+} cells of adult peripheral blood cells and cord blood cells, resulting in incrementation of the adhesion cells, in which endothelial nitric oxide synthase and kinase insert domain-containing receptor were positive. Moreover, the mixture of VEGF and TPO increased the CD31^{bright} cell population when compared with VEGF alone or the mixture of VEGF and stem cell factor. These data suggest that TPO is an important growth factor that can promote endothelial precursor cells expansion ex vivo.

Keywords: endothelial precursor cells, thrombopoietin, AC133

Ishii-Watabe, A., Kanayasu-Toyoda, T., Suzuki, T., Kobayashi, T., Yamaguchi, T., Kawanishi, T.: **Influences of the recombinant artificial cell adhesive proteins on the behavior of human umbilical vein endothelial cells in serum-free culture**.
Biologicals. **35**, 247-257 (2007)

To improve the safety of cellular therapy products, it is necessary to establish a serum-free cell culture method that can exclude animal-derived materials in order to avoid contamination with transmissible agents. It would be optimal if the proteins necessary to a serum-free culture could be provided as recombinant proteins. In this study, the influences of recombinant artificial cell adhesive proteins on the behavior of human umbilical vein endothelial cells (HUVECs) in serum-free culture were examined in comparison with the influence of plasma fibronectin (FN). The recombinant proteins used were Pronectin F (PF), Pronectin F PLUS (PFP), Pronectin L (PL), Retronectin (RN), and Attachin (AN). HUVECs adhered more efficiently on PF or PFP than on FN. No cells adhered on PL. Regarding the VEGF or bFGF-induced cell growth, the cells on PF and PFP proliferated at a similar rate to the cells on FN. RN and AN were less effective in supporting cell growth. Since cell adhesion on PF and PFP induced phosphorylation of focal adhesion kinase, they are thought to activate integrin-mediated intracellular signaling. The cells cultured on PF or PFP were able to produce prostaglandin I2 or tissue-plasminogen activator in response to thrombin. However, thrombin caused detachment of the cells from PF but not from PFP or FN, meaning that the cells were able to adhere more tightly on PFP or FN than on PF. These data indicate that PFP could be applicable as a substitute for plasma FN.

Keywords: endothelial cells, adhesion protein, fibronectin

Sahin, F. P.[*1], Yamashita, H.[*1], Guo, Y.[*1], Terasaka, K.[*1], Kondo, T.[*2], Yamamoto, Y.[*3], Shimada, H.[*3], Fujita, M.[*4], Kawasaki, T.[*4], Sakai, E.[*5], Tanaka, T.[*5], Goda, Y., Mizukami, H.[*1]: **DNA authentication of Plantago Herb based on nucleotide sequences of 18S-28S rRNA internal transcribed spacer region**
Biol. Pharm. Bull., **30**, 1265-1270 (2007)

Internal transcribed spacer (ITS) regions of nuclear ribosomal RNA gene were amplified from 23 plant- and herbarium specimens belonging to eight *Plantago species* (*P. asiatica, P. depressa, P. major, P. erosa, P. hostifolia, P. camtschatica, P. virginica and P. lanceolata*). Sequence comparison indicated that these Plantago species could be identified based on the sequence type of the ITS locus. Sequence analysis of the ITS regions amplified from the crude drug Plantago Herb obtained in the markets indicated that all the drugs from Japan were derived from *P. asiatica* whereas the samples obtained in China were originated from various *Plantago* species including *P. asiatica*, *P. depressa*, *P. major* and *P. erosa*.

Keywords: Plantago Herb, DNA authentication, ribosomal DNA

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Itokazu, N., Ogihara, Y.[*1], Satake, M.[*2], Hanawa, T.[*3], Muranishi, A.[*3], Hirai, T.[*4], Mikami, M.[*5], Nakamura, T.[*6], Okubo, T.[*7], Matsumoto, R.[*7], Nishikawa, T.[*8], Kitayama, H.[*8], Goda, Y.: **Actual Use Research, a new method for evaluating the effectiveness of OTC Kampo drugs and its application to Kamishoyosan formulation**
J. Trad. Med., **24**, 104-114 (2007)

Actual Use Research（AUR）is a new pharmacist-centered research system to evaluate usefulness of OTC Kampo medicine. The system uses a commercially available OTC drug. First, after an explanatory meeting of the AUR system, a pharmacist（or pharmacy）is contracted by the AUR implementation committee. The pharmacist invites the customers of the pharmacy to participate in AUR. After the consent and answering several questions from the pharmacist, the AUR participant purchased the test OTC drug and begins to keep a daily use record of dosage and time of intake, the condition of disease and use of other drugs. After a predetermined number of days, or when the symptoms of the disease disappear, the participant returns to the pharmacy, and submits the daily record to the pharmacist, and answers a questionnaire evaluating the usefulness of the drug as well as some questions from the pharmacist. The participant then receives a gratuity. Independent from the participant evaluation, the pharmacist evaluates the usefulness of the drug on the basis of the information obtained by the interview with the participant at the second meeting. Then, the pharmacist submits the daily record and the questionnaire from the participant and also his/her evaluation to the AUR implementation committee. In this report, we describe the first trial of AUR using the commercially available OTC "Kamishoyosan"（a Kampo formula used to treat women with painful tension to shoulders, who tire easily, suffer from fear, neuro-psychic disturbance, have a tendency towards constipation, sensitivity to cold, and/or dysmenorrheal and/or oligomenorrhea）.

Keywords: OTC Kampo formulations, Kamishoyosan, Actual Use Research

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酒井信夫，川口基一郎[*]，鎌倉浩之，川原信夫，合田幸広：**セイヨウサンザシ（*Crataegus oxythacantha* L.）葉の主成分並びに同植物葉及び市販セイヨウサンザシ葉製品の分析**

日本食品化学学会誌, **14**, 56-62（2007）

我が国において，セイヨウサンザシ（ホーソーン，*Crataegus oxyacantha* L.）葉は「専ら医薬品として使用される成分本質（原材料）リスト」に収載されている．専ら医薬品として使用される成分本質（原材料）の二次代謝物を同定するための一連の研究において，我々は，セイヨウサンザシ葉のメタノール抽出物から３つの主化合物（A～C）と２つの副次化合物（D，E）を単離した．それらの成分はNMR及 びLC-MS分析によって，3-O-caffeoylquinic acid（A），vitexin 2"-α-L-rhamnoside（B），vitexin 2"-(4-O-acetyl-α-L-rhamnoside）（C），9-O-β-D-glucopyranosyl-4, 9-dihydroxy-3-methoxypropiophenone（D）及び４-O-β-D-glucopyranosyl-p-coumaric acid（E）と同定した．更に，ドラーゲンドルフ試薬で発色させたセイヨウサンザシ葉抽出液の薄層クロマトグラフィー分析において，アルカロイドと推定されるスポットは検出されなかった．次に，我々はヨーロッパ市場より入手した市販ホーソーン葉製品のHPLC分析を行った．その結果，製品中に含まれる主化合物はセイヨウサンザシ葉のメタノール抽出物と同一であり，HPLCプロファイルはセイヨウサンザシ葉のプロファイルと類似していた．これらの結果から，ヨーロッパのホーソーン葉製品に用いられている植物種は*C. oxyacantha* L.の近縁種であることが示唆された．

Keywords: *Crataegus oxythacantha* leaves, hawthorn leaves, phenylpropanoid

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Totsuka, Y.[*], Nishigaki, R.[*], Takamura-Enya, T.[*], Kawahara, N., Sugimura, T.[*], Wakabayashi, K.[*]: **Analysis of the major RNA adduct derived from aminophenylnorharman, a novel endogenous mutagen and carcinogen**

Genes and Environment, **29**(**2**), 54-62 (2007)

9-(4'-Aminophenyl)-9*H*-pyrido［3,4-*b*］indole (aminophenylnorharman, APNH), a novel endogenous mutagenic/carcinogenic heterocyclic amine, is known to be a reaction product of 9*H*-pyrido［3,4-*b*］indole (norharman) and aniline. The major APNH-DNA adduct has been reported to be 2'-deoxyguanosin-8-yl-aminophenylnorharman (dGuo-C8-APIMH). However, RIMA adducts may also be important. We here demonstrated formation of APIMH-RIMA adducts and con-

ducted a structural analysis using various spectrometric approaches. When a reaction mixture of an ultimate mutagenic form of APIMH, *N*-acetoxy-APIMH, and guanosine (Guo) was subjected to LC-ESI/MS analysis, one peak, with a similar UV spectrum to dGuo-C8-APIMH, exhibited a molecular ion peak at m/z 541 along with a fragment peak at m/z 409, consistent with loss of a ribose moiety. From ^{1}H-NMR analysis, its chemical structure was concluded to be N4-(guanosin-8-yl)-9- (4'-aminophenyl)-9*H*-pyrido [3,4-*b*] indole (Guo-C8-APIMH). The same adduct was yielded in yeast tRNA incubated with *N*-acetoxy-APNH. Digestion of tRNA treated with *N*-acetoxy-APNH resulted in the appearance of one adduct spot visualized by the ^{32}P-postlabeling method, corresponding to Guo-C8-APNHH. No spot was seen with tRIMA alone. Additional analysis of in vivo adduct formation in the livers of rats administered APNH at a concentration of 100 mg/kg revealed that several adduct spots, including one corresponded to Guo-C8-APIMH, were observed. The total adduct levels of APNH-RNA were 28 ±13.3 (mean±SD) adducts per 10^{6} nucleotides. Comparisons demonstrated six times higher levels of total APNH-RNA than total APNH-DNA adducts in the same rat liver samples. These results indicate that APNH-RNA might be a useful biomarker for exposure to APNH.

Keywords: aminophenylnorharman (APNH), N-acetoxy-APNH , 32P-postlabeling analysis

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Otsuki, T.[*1], Kaneko, N.[*1], Kayano, T.[*2], Kowithayakorn, T.[*3], Kawahara, N., Goda, Y., Ishibashi, M.[*1]: **Cell growth and cell cycle inhibitory activities of 20-epidiosgenyl saponin from *Calamus insignis***
Heterocycles, **74**, 931-936 (2007)

A new 20 epi-diosgenyl saponin (1) was isolated from the stems of *Calamus insignis* (Palmae) by bioassay guided purification. The chemical structure of 1 was established on the basis of spectroscopic analysis and chemical means. Compound 1 showed cell growth inhibitory activity against HeLa cells (IC50; 5.1 μM) and exhibited a cell cycle inhibitory effect at the G2/M stage at the concentration of 2.9 μM by flow cytometric analysis.

Keywords: 20 epi-diosgenyl saponin, *Calamus insignis*

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Kawahara, N., Anjiki, N., Goda, Y.: Studies on chemical components and HPLC profile analysis of Setsucha products
Jpn. J. Food Chem., **14(2)**, 63-69 (2007)

In the course of our studies on basically research for raw material of health foods, we performed purification and characterization of major components, and HPLC profile analysis of sixteen kinds of Setsucha products purchased from the Japanese market. The raw material of Setsucha products is lichen distributed in highlands of the Southwest part of China. Five major components (1-5) were isolated from the methanol extract, and also three major components (6-8) were obtained from the hot water extract of Setsucha product. These components were identified as decarboxythamnolic acid (1), thamnolic acid (2), squamatic acid (3), baeomycesic acid (4), 2-hydroxy-6-methoxy-3-(methoxycarbonyl)-4-methylbenzoic acid (5), 2-hydroxy-4-methoxy-6-methylisophthalic acid (6), 2-hydroxy-6-methoxy-4-methylbenzoic acid (7) and 2-hydroxy-4-methoxy-6-methylbenzoic acid (8), respectively, by NMR and FAB-MS. A new compound (6) was newly purified and characterized from naturally occurring materials on the basis of the spectral evidence. Meanwhile, three compounds (1-3) were obtained from almost all of Setsucha products by HPLC profile analysis. It is of interest that the existence ratios of the three compounds in five products were quite different from those in the other eleven products.

Keywords: Setsucha, health foods, profile analysis

Morita, H.[*1], Enomoto, M.[*1], Hirasawa, Y.[*1], Iizuka, T.[*1], Ogawa, K.[*2], Kawahara, N., Goda, Y., Matsumoto, T.[*3], Takeya, K.[*3]: **Cyclonatsudamine A, a new vasodilator cyclic peptide from *Citrus natsudaidai***
Bioorganic and Medicinal Chemistry Letters, **17(19)**, 5410-5413 (2007)

A new cyclic heptapeptide, cyclonatsudamine A (1), *cyclo* (-Gly-Tyr-Leu-Leu-Pro-Pro-Ser-), has been isolated from the peels of *Citrus natsudaidai* & the structure was elucidated by 2D NMR analysis and chemical degradation. Cyclonatsudamine A (1) relaxed

norepinephrine-induced contractions of rat aorta, which may be mediated through the increased release of NO from endothelial cells.
Keywords: cyclic heptapeptide, cyclonatsudamine A, *Citrus natsudaidai*

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Hirasawa, Y.[*], Izawa, E.[*], Matsuno, Y.[*], Kawahara, N., Goda, Y., Morita, H.[*]: **Taxodistines A and B, abietane-type diterpenes from *Taxodium distichum***
Bioorganic & Medicinal Chemistry Letters, **17(21)**, 5868-5871 (2007)

Two new abietane-type diterpenes, taxodistines A (1) and B (2), have been isolated by the guidance of inhibitory effect of tubulin polymerization from the fruits of *Taxodium distichum* and the structures were elucidated by using 2D NMR data. Taxodistine B (2) showed inhibition of tubulin polymerization.
Keywords: abietane-type diterpene, taxodistine A, taxodistine B

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Matsuno, Y.[*1], Okamoto, M.[*1], Hirasawa, Y.[*1], Kawahara, N., Goda, Y., Shiro, M.[*2], Morita, H.[*1]: **Pordamacrines A and B, alkaloids from *Daphniphyllum macropodum***
J Nat Prod. **70(9)**, 1516-1518 (2007)

The new *Daphniphyllum* alkaloids, pordamacrines A (1) and B (2), have been isolated from the leaves of *Daphniphyllum macropodum*, and their structures were elucidated on the basis of interpretation of spectroscopic data and by the singlecrystal X-ray diffraction analysis of 2. Pordamacrines A (1) and B (2) exhibited moderate vasorelaxant effects on the rat aorta.
Keywords: *Daphniphyllum macropodum*, pordamacrines A, pordamacrines B

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Anjiki, N., Kawahara, N., Goda, Y.: **Studies on the taste profile analysis of Setsucha products by a taste-sensing system**
Jpn. J. Food Chem., **14(3)**, 121-127 (2007)

The aim of our study is to examine for the relationship between chemical components and taste in the raw materials derived from natural sources. Previously, we reported three major compounds, decarboxythamnolic acid (1), thamnolic acid (2) and squamatic acid (3) as the main components in water extract of Setsucha products and their existence ratio varied among Setsucha products. In this study, we investigated the characteristic taste factors obtained from the profile analysis of Setsucha products by using a taste-sensing system. Then, we examined the correlation between the taste and the amount of major components included in the water extract. As a result, the water extracts showed the large value of the taste intensity in bitterness and aftertaste of bitterness, while these values varied among Setsucha products. In addition, strong correlations were observed between the intensity of bitterness and aftertaste of bitterness and the relative amount of 1 and 2, while strong inverse correlations were observed between the intensity of these taste factors and the relative amount of 3. These suggest that the results of the profiling analysis by the taste-sensing system considerably account for the component combination in water extract of Setsucha products.
Keywords: Setsucha, profile analysis, taste-sensing system

Kawahara, N., Anjiki, N., Kim, I-H., Mikage, M.[*], Goda, Y.: **Studies on relationship between color and content of sulfur dioxides in crude drugs obtained from the Japanese market**
Jpn. J. Food Chem., **14(3)**, 140-144 (2007)

Sulfur dioxides and sulfites are registered in "The Japan's Specifications and Standards for Food Additives" mainly used as bleach and anti-oxidants, and Food Sanitation Law prohibits the use to sesame, legumes and vegetables. In China, sulfur fumigation is performed for the purpose of bleaching, drying, insecticide and antibacterial to some crude drugs. Recently, it has been reported that large quantities of sulfur dioxides are detected from sulfur fumigated crude drugs. In the course of our study of the survey of impurity in herbal materials, we analyzed the content of sulfur dioxides for 31 kinds of crude drugs (5 companies, 151 crude drugs) purchased from the Japanese market. In this study, be aimed for development of a new simple

method for the measurement of sulfur dioxides, we investigated correlation between the color value obtained by a hand-held spectrophotometer and the sulfur dioxides content in 19 kinds of crude drugs. As the results, the good correlation between the color index L^* value and the sulfur dioxide content and the good inverse correlation between the color index C^* value and the content were observed in four powdered crude drugs (Pueraria Root, Gastrodia Tuber, Lilium Bulb and Moutan Bark). Therefore, the color index L^* and C^* values may be suitable as the screening index of sulfur dioxide content in the these powdered crude drugs.

Keywords: sulfur dioxides, $L^*a^*b^*$ color system, crude drugs

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Kuroyanagi, M.[*1], Ikeda, R.[*1], Gao, H-Y.[*1], Muto, N.[*1], Otaki, K.[*1], Sano, T.[*2], Kawahara, N., Nakane, T.[*3]: **Neurite outgrowth-promoting active constituents of the Japanese cypress (*Chamaecyparis obtusa*)**

Chem. Pharm. Bull., **56(1)**, 60-63 (2008)

In the screening of biologically active constituents from woody plants, the methanol extract of leaves of *Chamaecyparis obtusa* showed potent neurite outgrowth-promoting activity in neuronal PC12 cells. The ethyl acetate-soluble fraction of the methanol extract showed potent activity and was separated by means of various chromatographic methods to give the two new compounds 1 and 2, as well as 11 known lignan and sesquiterpene derivatives. The structures of the new compounds were determined to be 9-*O*-acetyldihydrosesamin (1) and 9-*O*-(11-hydroxyeudesman-4-yl) dihydrosesamin (2), respectively, in NMR studies including 2D-NMR experiments. Of the 13 compounds, the known compound hinokinin (5) and the new compound 2 showed potent neurite outgrowth-promoting activity in PC 12 cells.

Keywords: *Chamaecyparis obtuse*, sesquiterpene-lignan conjugate, neurite outgrowth-promoting activity

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Fuchino, H.[*1], Sekita, S.[*2], Mori, K.[*2], Kawahara, N., Satake, M.[*3], Kiuchi, F.[*1]: **A new leishmanicidal saponin from *Brunfelsia grandiflora***

Chem. Pharm. Bull., **56(1)**, 93-96 (2008)

A new furostan-type saponin (1) was isolated from the methanolic extract of *Brunfelsia grandiflor*a leaves, together with four known compounds. The chemical structure of 1 was determined by spectroscopic analysis and chemical reaction to be 26-*O*-β-D-glucopyranosyl 22α-methoxyfurost-3β,26-diol 3-*O*-β-D-xylopyranosyl (1→3)-{β-D-glucopyranosyl(1→2)}-β-D-glucopyranosyl (1→4)-β-D-glucopyranoside. Compound 1 showed potent leishmanicidal activity *in vitro* against *Leishmania major*.

Keywords: *leishmania, Brunfelsia grandiflora*, chiricsan-ango

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Kumasaka, K.[*], Kawahara, N., Doi, K.[*], Kojima, T.[*], Goda, Y.: **Determination of *R*-xanthoanthrafil, a phosphodiesterase-5 inhibitor, in a dietary supplement promoted for sexual enhancement**

Chem. Pharm. Bull., **55(2)**, 227-230 (2008)

We describe here the first case of the finding of xanthoanthrafil, a phosphodiesterase-5 inhibitor, in a dietary supplement. A methanol extract of the supplement product was first analyzed by TLC and HPLC. The results indicated that the extract contained an unknown compound. The molecular weight of the compound was 389 and the accurate mass showed its elemental composition to be $C_{19}H_{23}N_3O_6$. Combined with this data, NMR analysis revealed the planar structure of the unknown compound to be *N*-(3,4-dimethoxybenzyl)-2-(1-hydroxypropan-2-ylamino)-5-nitrobenzamide. The *R*-configuration of this compound had been synthesized as a phosphodiesterase-5 inhibitor, formerly reported as FR226807 by Fujisawa Pharmaceutical Co., Ltd. The absolute configuration of the isolated compound was estimated to have R-configuration by its optical rotation. Considering its general properties, this compound is renamed as (*R*)-xanthoanthrafil with the agreement of Astellas Pharma Inc. which is the successor of Fujisawa Pharmaceutical Co., Ltd. Quantitative analysis revealed that the content of (*R*)-xanthoanthrafil in the product was about 31 mg/capsule.

Keywords: xanthoanthrafil, liquid chromatography-mass spectrometry, NMR

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Kuroyanagi, M.[*1], Ishii, H.[*1], Kawahara, N., Sugimoto, H.[*2], Yamada, H.[*2], Okihara, K.[*2], Shirota, O.[*3]: **Flavonoid glycosides and limonoids from Citrus molasses**

J. Nat. Med., **62**, 107-111 (2008)

Molasses of tangerine orange (*Citrus unshiu Markovich*) is obtained as a waste product in the course of tangerine orange juice production. This molasses is expected to be a useful source of organic compounds such as flavonoids and limonoids. To elucidate a use for this molasses waste, we isolated and identified its organic constituents. Two new flavanonol glycosides were isolated from tangerine orange molasses, along with several flavonoids such as hesperidine, narirutin, eriodictyol, 3',4',5,6,7,8-hexamethoxy-3-*O*-β-D-glucopyranosyloxyflavone, and 3',4',5,6,7,8-hexamethoxy-3-β-D-[4-*O*-(3-hydroxy-3-methylglutaloyl)]-glucopyranosyloxyflavone, and limonoids such as limonin, nomilin, and cyclic peptide, citrusin III. The structures of the new flavanonol glycosides were determined as (2*R*,3*R*)-7-*O*-(6-*O*-α-L-rahmnopyranosyl-β-D-glucopyranosyl) -aromadendrin and 7-*O*-(6-*O*-α-L-rahmnopyranosyl-β-D-glucopyranosyl)-3,3',5,7-tetrahydroxy-4'-methoxyflavanone by means of spectral analyses using ^{1}H-NMR, ^{13}C-NMR, and 2D-NMR. Of these compounds, flavanone glycoside, hesperidin and narirutin were isolated as the main constituents. Thus, molasses is a promising source of flavonoid glycosides.

Keywords: tangerine orange molasses, flavanonol glycoside, limonoid

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Ushijima, M.[*1], Komoto, N.[*2], Sugizono, Y.[*2], Mizuno, I.[*1], Sumihiro, M.[*1], Ichikawa, M.[*1], Hayama, M.[*1], Kawahara, N., Nakane, T.[*3], Shirota, O.[*4], Sekita, S.[*4], Kuroyanagi, M.[*2]: **Triterpene glycosides from the roots of *Codonopsis lanceolata***

Chem. Pharm. Bull., **56(3)**, 308-314 (2008)

In the course of the development of new designer foods using the roots of *Codonopsis lanceolata*, we found that hot-water extracts of *C. lanceolata* recovered decreased testosterone levels in the blood and accelerated the restoration of reproductive dysfunction induced by hyperthermic treatment in male mice. Thus we studied the constituents of the polar fraction of the roots of *C. lanceolata* and identified six new triterpene saponins, lancemasides B (2), C (3), D (4), E (5), F (6), and G (7), along with the known saponin lancemasaide A (1) and phenylpropanoid glycosides 8-10. The structures of the new compounds 2-7 were determined by means of spectral data including 2D-NMR studies and chemical reactions to be oleanan-type bisdesmoside with sugars at C-3 and C-28. Compounds 2-6 have echinocystic acid as an aglycone, and compound 7 has asterogenic acid as an aglycone. Identification of the sugars and determination of their D,L-chiralities were carried out by application of the exciton chirality method to the per-*O*-*p*-bromobenzoylmethyl sugar derived from saponins.

Keywords: *Codonopsis lanceolata*, triterpene saponin, exciton chirality method

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佐藤正幸*，姉帯正樹*，鎌倉浩之，合田幸広：**生薬中の残留有機リン系農薬の分析（第２報）**

医薬品研究, **36**, 203-222 (2008)

A method was developed for simultaneous determination of 28 organophosphorus pesticides in Angelicae Radix, Atractylodis Lanceae Rhizoma, Atractylodis Rhizoma, Bupleuri Radix, Cimicifugae Rhizoma, Coicis Semen, Ephedrae Herba, Foeniculi Fructus, Ginseng Radix, Magnoliae Flos, Menthae Herba, Paeoniae Radix, Puerariae Radix and Zingiberis Rhizoma. The pesticides were extracted with aqueous acetonitrile. The extract was cleaned up on C18 mini-column and concentrated. After addition of sodium chloride to the concentrated aqueous solution, the pesticides were re-extracted with *n*-hexane. In the case of Bupleuri Radix, a little quantity of methanol was added to *n*-hexane because of preventing emulsification. The extract was washed with water and dried over anhydrous sodium sulfate. The extracts of Atractylodis Lanceae Rhizoma and

Atractylodis Rhizoma were further cleaned up on Diol mini-column and Silica gel mini-column. The extracts of the other crude drugs were further cleaned up on Silica gel mini-column. In the case of Bupleuri Radix, the pesticides were eluted with a mixture of acetone and *n*-hexane after washing with *n*-hexane. The analysis was performed by gas chromatography with FPD detection. The recoveries of organophosphorus pesticides added at the concentrations of 0.4 μg/g to the crude drugs, except for Cimicifugae Rhizoma and Paeoniae Radix, were mostly in the range of 70～120%（peak area method）. The recoveries of methidathion, phosmet, edifenphos, phosalone and pyridaphenthion added to Paeoniae Radix were greater than 120%. The recoveries of chlorpyrifos, ethion and leptophos added to Cimicifugae Rhizoma were 51%, 35% and 22%, respectively. This is most likely due to reactions with components of the crude drug during moistening for 1 hour. The detection limits were 0.01～0.06 ppm.

The established method was applied to 51 samples in 15 kinds of crude drugs. Five kinds of organophosphorus pesticides were detected in 8 samples of 4 kinds of crude drugs in the range of trace －0.22 ppm.

Keywords: crude drugs, organophosporus pesticide residues, GC-FPD

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石原島栄二*，角野文代*，世取山守*，鎌倉浩之，合田幸広：**強壮・強精など男性機能回復を暗示する健康食品からの無承認無許可医薬品成分の検出事例について**
栃木県環境保健センター年報，**12**，48-52（2007）

男性機能回復を暗示する無承認無許可医薬品のLC/MS分析において，ヒドロキシホンデナフィルを検出した．UVスペクトル，質量スペクトル及びNMRスペクトル等の解析により構造解析を行いヒドロキシホンデナフィルと同定した．

Keywords: ヒドロキシホンデナフィル，ホンデナフィル，LC/MS，

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Maruyama T., Sugimoto N., Kuroyanagi M.[*1], Kim I. H., Kamakura H., Kawasaki T.[*2], Fujita M.[*2], Shimada H.[*3], Yamamoto Y.[*3], Tada A., Yamazaki T., Goda Y.：**Authentication and chemical study of Isodonis Herba and Isodonis extracts**
Chem. Pharm. Bull., **55**(**11**), 1626-1630（2007）

Isodonis Herba is used as a Japanese dietary supplement and folk medicine. The extract of the herb（Isodonis extract）is also used as a food additive whose major compound is enmein（**1**）. Here we compared internal transcribed spacer sequences of nuclear ribosomal DNA from Isodonis Herba available on the Japanese and Chinese crude drug markets, and found that the former derived from *Isodon japonicus* and *Isodon trichocarpus*, while the latter derived from distinct species such as *Isodon eriocalyx*. The liquid chromatography/mass spectrometry profiles of Isodonis Herba were classified into four chemotypes（A to D）according to the ratio of the major constituents. Types B and C contained **1** and oridonin（**2**）as major components, respectively. An intermediate（or mixed）form of types B and C in various ratios was designed type A. Type D contained eriocalyxin B（**3**）as its major component. Japanese herba were types A-C, while Chinese herba were types C and D. The commercial Isodonis extract products tested were classified as type D, suggesting that they originated from Chinese Herba. Understanding the relationship between extract constituents and DNA profiles is important for the official specification of dietary supplements and food additives of plant origin.

Keywords: Isodonis, LC/MS, internal transcribed spacer

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Maruyama T., Kamakura H., Kikura-Hanajiri R., Goda Y.: **Authentication and ultra performance liquid chromatography（UPLC）/MS analysis of magic mint, *Salvia divinorum* and its related plants**
Yakugaku Zasshi, **128**(**1**), 179-183（2008）

Ultra performance liquid chromatography（UPLC）/mass spectrometry（MS）analysis was performed to investigate whether commercial *Salvia* cultivars available in the Japanese market contain salvinorin A（**1**）, which is an hallucinogen present in magic mint（*Salvia divinorum*）prior to the regulation of S. *divinorum* by the Japanese Pharmaceutical Affairs Law. In addition, a previously reported method to authenticate *S. divinorum*, utilizing an amplification

refractory mutation system（ARMS）was applied to the same samples to estimate the method's accuracy. As a result of the UPLC/MS analysis, it was clear that none of the tested cultivars possessed **1** while *S. divinorum* leaves and its processed products "concentrated salvia" contained **1** in the range from 0.19% to 0.58%. Furthermore, the ARMS method could clearly distinguish *S. divinorum* from the tested cultivars. In conclusion, the authentication method is considered to be useful for the practical regulation of *S. divinorum* due to its simplicity and accuracy.

Keywords: *Salvia divinorum*, ultra performance liquid chromatography（UPLC）/MS, amplification refractory mutation system（ARMS）

玉那覇康二*，佐久川さつき*，合田幸広，丸山卓郎：**沖縄県に生息する幻覚性きのこの実態調査について**

沖縄県衛生環境研究所報, **41**, 77-83（2007）

平成14年６月６日からサイロシビン又はサイロシンを含有する幻覚性きのこ類は，麻薬及び向精神薬取締法に規定する麻薬原料植物として指定され規制することになった．過去に沖縄県において幻覚性きのこによる食中毒の発生や不法採取が行われていたため，県内の牛舎，牧草地の実態調査を行った．また，採取したきのこのDNA 鑑定を行うとともに，麻薬成分であるサイロシビン又はサイロシンの分析を行った結果，採取されたきのこはサイロシビン，サイロシン含有種であり，同成分を含有する事が確認された．実態調査の項目を数値化し，採取した幻覚性きのこの成分分析をもとに乱用されやすい地域を，危害度地図（マップ）として作成した．実態調査で得られた知見を，行政機関において乱用防止対策，監視活動に活用した．

Keywords: psilocin，psilocybin，マジックマッシュルーム

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Kikura-Hanajiri, R., Kawamura, M., Saisho, K., Kodama, Y., Goda, Y.: **The disposition into hair of a new designer drug, methylone and its related compounds**

J. Chromatogra. B., **855(2)**, 121-126（2007）

The disposition into hair of methylone and other new designer drugs, methcathinone and MBDB, was studied with the animal model. Moreover, the incorporation rates of these drugs were compared with those of their related eight compounds previously studied in order to evaluate their incorporation tendency into hair and the usefulness of hair specimens for the retrospective confirmation of the use of these drugs. When the ratio of hair concentration to AUC in plasma（[Hair]/AUC）was represented as an index of the incorporation rate of drugs into hair, the [Hair]/AUC of methylone was 14 times higher than that of methcathinone. It might support earlier findings that the methylenedioxy group on the benzene ring leads to considerably higher incorporation rates. However, [Hair]/AUC of methylone was five-sevenths times lower in comparison with that of MDMA. This suggested that the beta-carbonyl group leads to lower incorporation rates. Although methylone has both groups in its structure, the positive effect of the methylenedioxy group may be stronger than the negative effect of the beta-carbonyl group. On the other hand, the [Hair]/AUC of MBDB, which has methylenedioxyphenyl-2-butanamine structure, was higher than that of MDMA while that of methcathinone, having beta-ketone in its structure, was extremely low. In conclusion, as with MA and MDMA, the incorporation tendency of methylone and MBDB（except for methcathinone）into hair is relatively high, and a hair sample would be a good specimen for the confirmation of retrospective use of these drugs.

Keywords: methylone, hair analysis, GC-MS

Matsumoto, T., Urano, Y.*, Makino, Y.*, Kikura-Hanajiri, R., Kawahara, N., Goda, Y., Nagano*, T.: **Evaluation of characteristic deuterium distributions of ephedrines and methamphetamines by NMR spectroscopy for drug profiling**

Anal Chem., **80(4)**, 1176-81（2008）

A method for quantitative analysis of the deuterium contents（D/H）at the phenyl, methine, benzyl, *N*-methyl and methyl groups of *l*-ephedrine/HCl, *d*-pseudoephedrine/HCl and methamphetamine/HCl by 2H NMR spectroscopy was established. Comparison of the 5 position-specific D/H values of *l*-ephedrine/HCl and *d*-pseudoephedrine/HCl prepared by three methods（chemical synthesis, semichemical synthesis, and biosynthesis）showed that chemically synthesized ephedrines and semisynthetic ephedrines have highly specific distributions of deuterium at the methine position and at the benzyl position, compared with the other positions. The classification of several metham-

phetamine samples seized in Japan in terms of the D/H values at these two positions clearly showed that the methamphetamine samples had been synthesized from ephedrines extracted from Ephedra plants or semisynthetic ephedrines but not from synthetic ephedrine. This isotope ratio analysis method should be useful to trace the origins of seized methamphetamine in Southeast Asia.

Keywords: ^{2}H NMR, ephedrine, methamphetamine

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Uchiyama, N., Kim, I. H, Kikura-Hanajiri, R., Kawahara, N., Konishi, T.*, Goda, Y.: **HPLC separation of naringin, neohesperidin and the C-2 epimers in commercial samples and herbal medicines**

J. Pharm. Biomed. Anal., **46**, 864-869 (2008)

Flavanone glycosides, such as naringin and neohesperidin, are distributed in some *Citrus* species and have a chiral center in the C-2 position of the flavanone moiety. Naringin and neohesperidin (2*S*-form) were separated from the corresponding C-2 epimers (2R-*epi*-form) by normal phase HPLC using a polysaccaride-derived chiral stationary phases (CSPs), CHIRALPAK® IB. The analyses of commercial samples of naringin revealed that the relative ratios of naringin to the C-2 epimer were 29-89%. In the case of a commercial sample of neohesperidin, the relative ratio of the neohesperidin (2*S*-form) is 84%. The HPLC application to *Citrus* species used as crude drugs in Japan (Kijitsu, Kikoku and Tohi) showed that the relative ratios of naringin to the C-2 epimer were 75-93% in Kijitsu, 74-79% in Kikoku and 54-64% in Tohi. However, there is a quite small ratio of the (2*R*)-*epi*-neohesperidin in *Citrus*. This result suggested that the averages of relative ratio of (2*S*)-naringin in *Citrus* species reduced according to the maturity of fruits (Kijitsu<Kikoku<Tohi). Since the relative ratios of (2*S*)-naringin of dry extracts of 5 Kampo formulations (including Kijitsu or Kikoku) decreased to 42-54%, the conversion from naringin to the (2*R*)-epimer might be enhanced during the decoction process of the formulations.

Keywords: naringin, neohesperidin, diastereomeric separation

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Konishi, T.*1, Kondo, S.*2, Uchiyama, N.: **Larvicidal activities of sesquiterpenes from *Inula helenium* (Compositae) against *Aedes albopictus* (Diptera: Culicidae) and *Paratanytarsus grimmii* (Diptera: Chironomidae)**

Appl. Entomol. Zool., **43**, 77-81 (2008)

The larvicidal activities of three sesquiterpenes, alantolactone, isoalantolactone and dihydroisoalantolactone, isolated from the roots of *Inula helenium* (Compositae) against 3rd and 4th instars of *Aedes albopictus* (Diptera: Culicidae) and *Paratanytarsus grimmii* (Diptera: Chironomidae), were examined. The two sesquiterpenes, alantolactone and isoalantolactone, showed LC_{50} values of 2.7 μg/ml and 11.9 μg/ml for *A. albopictus*, and 5.1 μg/ml and 4.1 μg/ml for *P. grimmii* within 48 h, respectively. Alantolactone was significantly more toxic than isoalantolactone against *A. albopictus*; however, dihydroisoalantolactone did not entirely show lethal effects against the larvae of both species at a concentration of 1,000 μg/ml.

Keywords: *Inula helenium*, larvicidal activity, sesquiterpenes

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Sugiyama, S.*1, Tokuoka, K.*2, Uchiyama, N., Okamoto, N.*2, Okano, Y.*2, Matsumura, H.*2, Inaka K.*1, Urade, Y.*3, Inoue, T.*2: **Preparation, crystallization and preliminary crystallographic analysis of old yellow enzyme from *Trypanosoma cruzi***

Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun., **63**, 896-898 (2007)

Old yellow enzyme (OYE) is an NADPH oxidoreductase that contains a flavin mononucleotide as a prosthetic group. The OYE from Trypanosoma cruzi, which produces prostaglandin F (2alpha), a potent mediator of various physiological and pathological processes, from prostaglandin H2. The protein was recombinantly expressed and purified from Escherichia coli and was crystallized using the hanging-drop vapour-diffusion method. The crystal belongs to the

monoclinic space group P2(1), with unit-cell parameters a = 56.3, b = 78.8, c = 78.8 A, beta = 93.4 degrees and two molecules per asymmetric unit. The crystals were suitable for X-ray crystallographic studies and diffracted to 1.70 A resolution. A Patterson search method is in progress using the structure of OYE from Pseudomonas putida as a starting model.

Keywords: old yellow enzyme, NADPH oxidoreductases

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Uchida, E., Kogi, M.[*1], Oshizawa, T., Furuta, B., Satoh K.[*2], Iwata, A.[*2], Murata M.[*3], Hikata, M.[*3], Yamaguchi, T.: **Optimization of the virus concentration method using polyethyleneimine-conjugated magnetic beads and its application to the detection of human hepatitis A, B and C viruses**

J. Virol. Methods, **143**, 95-103 (2007)

To enhance the sensitivity of virus detection by PCR and RT-PCR, we previously developed a novel virus concentration method using polyethyleneimine (PEI) -conjugated magnetic beads. However, several viruses could not be concentrated by this method. In this paper, we optimized the conditions of virus concentration to concentrate a wide range of viruses more efficiently. The PEI-beads adsorbed viruses more efficiently than other cationic polymers, and optimum virus concentration was obtained under a weak acidic condition. Mass-spectrometric analysis revealed that several serum proteins, such as complement type 3, complement type 4, and IgM, were co-adsorbed by the PEI-beads, suggesting that PEI-beads may adsorb viruses not only by direct adsorption, but also via immune complex formation. This hypothesis was confirmed by the result that poliovirus, which PEI-beads could not adsorb directly, could be concentrated by PEI-beads via immune complex formation. On the other hand, hepatitis A and C viruses were directly adsorbed by PEI-beads almost completely. Like poliovirus, hepatitis B virus (HBV) was efficiently concentrated by the addition of anti-HBV IgM. In conclusion, virus concentration using PEI-beads is a useful method to concentrate a wide range of viruses and can be used to sensitively detect human hepatitis viruses, which are clinically important for the viral safety of biologicals.

Keywords: polyethyleneimine, virus concentration, hepatitis virus

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Yokoyama, U.[*1], Sato, Y., Akaike, T.[*1], Ishida, S., Sawada, J., Nagao, T., Quan, H.[*1], Jin, M.[*1], Iwamoto, M.[*1], Yokota, S.[*1], Ishikawa, Y.[*1*2] and Minamisawa, S.[*1*3]: **Maternal vitamin A alters gene profiles and structural maturation of the rat ductus arteriosus**

Physiol. Genomics, **31**, 139-157 (2007)

Retinoic acid (RA), a metabolite of vitamin A, has been proposed to regulate vascular remodeling and reactivity of the ductus arteriosus (DA). Using rat Affymetrix GeneChips, we found that a considerable number of genes in DA varied their expression levels in accordance with developmental mode: namely, preterm-, term-, and postnatal-dominant clusters. Among a total of 8,740 probe sets, maternal vitamin A administration (MVA) changed the expression levels of 91 genes (116 probe sets) >2.5-fold. About half of preterm- and term-dominant genes responded to MVA, whereas only 5% of postnatal-dominant genes responded to MVA, indicating that fetal-dominant genes were susceptible to RA signals. The expression levels of 51 genes in MVA-treated DA at preterm were similar to the expression levels in nontreated DA at term, indicating that the global gene profile at preterm resembled that of the control animal at term. We observed neointima formation in MVA-treated DA at preterm in accordance with upregulation of fibronectin and hyaluronic acid, whereas it was rarely observed in nontreated DA at preterm. Five fetal cardiac myofibrillar genes were also upregulated in MVA-treated in vivo DA, whereas they were developmentally downregulated in nontreated DA. The present study indicates that MVA-mediated alteration in gene profile was associated with early structural maturation of DA, although MVA-mediated maturation may differ from normal vascular remodeling of DA.

Keywords: vitamin A, ductus arteriosus, gene expression

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Suzuki, T., Takeshita, K., Saeki, K*., Kadoi, M.*, Hayashi, M., Sofuni, T.: **Clastogenicity of quinoline and monofluorinated quinolines in Chinese hamster lung cells**.

J. Health Sci., **53**, 325-328 (2007)

Quinoline and four monofluorinated derivatives of quinoline (FQ's) were tested for their clastogenicity in a Chinese hamster lung (CHL) cell line using chromosomal aberration (CA) and micronucleus (MN) tests. Quinoline and all the fluoroquinolines, 3-, 5-, 6-, and 8-FQ, induced CA in the presence of the metabolic activation system. However, the clastogenic property was altered by fluorine-substitution. 3-FQ showed reduced cytotoxicity and clastogenicity. It was positive only at a higher dose than the other compounds. 6-FQ was as cytotoxic and clastogenic as quinoline when tested in the lower dose range (less than 0.075 mg/ml). 5-FQ and 8-FQ were only moderately clastogenic in the CA test although their toxicity was similar to that of quinoline. The MN test showed almost the same tendency in clastogenicity as the CA test, except that 8-FQ showed a negative result. These results demonstrate that fluorine-substitution can modify the clastogenicity of quinoline, probably through interference of the metabolic activation.

Keywords: quinoline, chromosomal aberration, micronucleus test

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Haghighi, K.*1, Chen, G.*1, Sato, Y., Fan, G.C.*1, He, S.*1, Kolokathis, F.*2, Pater, L.*1, Paraskevaidis, I.*2, Jones, W.K.*1, Dorn, G.W. II*1, Kremastinos, D.T.*2 and Kranias, E.G.*1, 3: **A human phospholamban promoter polymorphism in dilated cardiomyopathy alters transcriptional regulation by glucocorticoids**

Hum. Mutat. **29**, 640-647 (2008)

Depressed calcium handling by the sarcoplasmic reticulum (SR) Ca-ATPase and its regulator phospholamban (PLN) is a key characteristic of human and experimental heart failure. Accumulating evidence indicates that increases in the relative levels of PLN to Ca-ATPase in failing hearts and resulting inhibition of Ca sequestration during diastole, impairs contractility. Here, we identified a genetic variant in the PLN promoter region, which increases its expression and may serve as a genetic modifier in dilated cardiomyopathy (DCM). The variant AF177763.1: g.203A>C (at position -36bp relative to the PLN transcriptional start site) was found only in the heterozygous form in 1 out of 296 normal subjects and in 22 out of 381 cardiomyopathy patients (heart failure at age of 18-44 years, ejection fraction=22+/−9%). In vitro analysis, using luciferase as a reporter gene in rat neonatal cardiomyocytes, indicated that the PLN-variant increased activity by 24% compared to the wild type. Furthermore, the g.203A>C substitution altered the specific sequence of the steroid receptor for the glucocorticoid nuclear receptor (GR)/transcription factor in the PLN promoter, resulting in enhanced binding to the mutated DNA site. These findings suggest that the g.203A>C genetic variant in the human PLN promoter may contribute to depressed contractility and accelerate functional deterioration in heart failure.

Keywords: cardiomyopathy, polymorphism, glucocorticoid

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Hakura, A.*1, Kadoi, M.*2, Suzuki, T., Saeki, K*2: **Clastogenicity of quinoline derivatives in the liver micronucleus assay using rats and mice**

J. Health Sci., **53**, 470-474 (2007)

Induction of micronucleated liver cells (MN-liver cells) was examined with the hepatocarcinogenic quinoline and its fluorinated derivatives, 3-fluoroquinoline (3-FQ) and 5-fluoroquinoline (5-FQ), using non-hepatectomized rats and mice. Male F344 rats or ICR mice were given each test chemical at a daily dose of 0.5 mmol/kg for three consecutive days by *i.p.* injection, and sacrificed at six or eleven days after the final treatment. The data may suggest that the induction frequencies of MN-liver cells by the quinoline derivatives correlate with the magnitudes of both their medium-term carcinogenicity and bacterial mutagenicity. Thus, the potently hepatocarcinogenic/mutagenic 5-FQ caused significantly higher levels of induction of MN-liver cells than the vehicle in both rats and mice. The non-hepatocarcinogenic/non- mutagenic 3-FQ showed no

appreciable differences in MN-liver cell induction from the control group in rats and mice. Quinoline showed a slight and statistically insignificant increase of MN-liver cells in mice, but there was not such increase in rats. These findings may suggest the utility of the micronucleus test using hepatocytes from non-hepatectomized animals, although its sensitivity may be low as compared with hepatectomized animals.

Keywords: micronucleus test, quinolines, fluoro-quinolines

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Nishida, M.[*1], Onohara, N.[*1], Sato, Y., Suda, R.[*1], Ogushi, M.[*1], Tanabe, S., Inoue, R.[*2], Mori, Y.[*3], and Kurose, H.[*1]: **$G\alpha_{12/13}$-mediated up-regulation of TRPC6 negatively regulates endothelin-1-induced cardiac myofibroblast formation and collagen synthesis through nuclear factor of activated T cells activation**

J. Biol. Chem., **282**, 23117-23128 (2007)

Sustained elevation of $[Ca^{2+}]_i$ has been implicated in many cellular events. We previously reported that α subunits of G_{12} family G proteins ($G\alpha_{12/13}$) participate in sustained Ca^{2+} influx required for the activation of nuclear factor of activated T cells (NFAT), a Ca^{2+}-responsive transcriptional factor, in rat neonatal cardiac fibroblasts. Here, we demonstrate that $G\alpha_{12/13}$-mediated up-regulation of canonical transient receptor potential 6 (TRPC6) channels participates in sustained Ca^{2+} influx and NFAT activation by endothelin (ET)-1 treatment. Expression of constitutively active $G\alpha_{12}$ or $G\alpha_{13}$ increased the expression of TRPC6 proteins and basal Ca^{2+} influx activity. The treatment with ET-1 increased TRPC6 protein levels through $G\alpha_{12/13}$, reactive oxygen species, and c-Jun N-terminal kinase (JNK)-dependent pathways. NFAT is activated by sustained increase in $[Ca^{2+}]_i$ through up-regulated TRPC6. A $G\alpha_{12/13}$-inhibitory polypeptide derived from the regulator of the G-protein signaling domain of p115-Rho guanine nucleotide exchange factor and a JNK inhibitor, SP600125, suppressed the ET-1-induced increase in expression of marker proteins of myofibroblast formation through a $G\alpha_{12/13}$-reactive oxygen species-JNK pathway. The ET-1-induced myofibroblast formation was suppressed by overexpression of TRPC6 and CA NFAT, whereas it was enhanced by TRPC6 small interfering RNAs and cyclosporine A. These results suggest two opposite roles of $G\alpha_{12/13}$ in cardiac fibroblasts. First, $G\alpha_{12/13}$ mediate ET-1-induced myofibroblast formation. Second, $G\alpha_{12/13}$ mediate TRPC6 up-regulation and NFAT activation that negatively regulates ET-1-induced myofibroblast formation. Furthermore, TRPC6 mediates hypertrophic responses in cardiac myocytes but suppresses fibrotic responses in cardiac fibroblasts. Thus, TRPC6 mediates opposite responses in cardiac myocytes and fibroblasts.

Keywords: endothelin, cardiac remodeling, TRP channel

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Sanda, T.[*1], Okamoto, T.[*1], Uchida, Y.[*1], Nakagawa, H.[*2], Iida, S.[*1], Kayukawa, S.[*1], Suzuki, T.[*2], Oshizawa, T., Suzuki, T., Miyata, N.[*2] and Ueda, R.[*1]: **Proteome analyses of the growth inhibitory effects of NCH-51, a novel histone deacetylase inhibitor, on lymphoid malignant cells**

Leukemia, **21**, 2344-2353 (2007)

Recent reports showing successful inhibition of cancer and leukemia cell growth using histone deacetylase inhibitor (HDACi) compounds have highlighted the potential use of HDACi as anti-cancer agents. However, high incidence of toxicity and low stability *in vivo* were observed with hydroxamic acid-based HDACi such as suberoylanilide hydroxamic acid (SAHA), thus limiting its clinical applicability. In this study, we found that a novel non-hydroxamate HDACi NCH-51 could inhibit the cell growth of a variety of lymphoid malignant cells through apoptosis induction, more effectively than SAHA. Activation of caspase-3, -8 and -9, but not -7 was detected after the treatment with NCH-51. Gene expression profiles showed that NCH-51 and SAHA similarly upregulated *p21* and downregulated anti-apoptotic molecules including *survivin, bcl-w* and *c-FLIP*. Proteome analysis using two-dimensional electrophoresis revealed that NCH-51 upregulated anti-oxidant molecules including peroxiredoxin 1 and 2 and glutathione *S*-transferase at the protein level. Interestingly, NCH-51 induced reactive oxygen species (ROS) after 8 h whereas SAHA continuously declined ROS. Pretreatment with an antioxidant, *N*-acetyl-L-cysteine, abolished the cytotoxicity of NCH-51. These findings suggest

that NCH-51 exhibits cytotoxicity by sustaining ROS at the higher level greater than SAHA. This study indicates the therapeutic efficacy of NCH-51 and novel insights for anti-HDAC therapy.

Keywords: histone deacetylase, reactive oxygen species, peroxiredoxin

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Watanabe, T.[*1], Tobe, K.[*1], Nakachi, Y.[*2], Kondoh, Y.[*2], Nakajima, M.[*3], Hamada, S.[*4], Namiki, C.[*5], Suzuki, T., Maeda, S.[*1], Tadakuma, A.[*1], Sakurai, M.[*1], Arai, Y.[*1], Hyogo, A.[*6], Hoshino, M.[*1], Tashiro, T.[*1], Ito, H.[*1], Inazumi, H.[*1], Sakaki, Y.[*7], Tashiro, H.[*2], Furihata, C.[*1]:

Differential gene expression induced by two genotoxic *N*-nitroso carcinogens, phenobarbital and ethanol in mouse liver examined with oligonucleotide microarray and quantitative real-time PCR

Genes and Environment, **29**, 115-127（2007）

It is known that genotoxic *N*-nitroso carcinogens induce DNA damage in mouse liver within a few hours and induce mutations within 28 days after their administration. However, related-gene expression changes at these time points in liver were not fully elucidated. Differential gene expression induced by two genotoxic *N*-nitroso carcinogens in mouse liver was examined 4 h and 28 days after their administration with in-house oligonucleotide microarray（268 genes）and quantitative real-time PCR, and compared to that of a non-genotoxic carcinogen and a non-carcinogenic toxin. Diethylnitrosamine（DEN, 80 mg/kg bw）, dipropylnitrosamine（DPN, 250 mg/kg bw）, phenobarbital sodium（30 mg/kg bw）and ethanol（1000 mg/kg bw）were injected intraperitoneally into groups of male 9-week-old B6C3F1 mice and liver was dissected after 4 h and 28 days. mRNA from pooled livers was reverse-transcribed to cDNA, and Cy3- and Cy5-labeled cDNA was competitively hybridized with in-house made microarray, scanned and analyzed; additionally, quantitative real-time PCR was performed for selected genes. Differential gene expression between two genotoxic *N*-nitroso carcinogens and phenobarbital and ethanol was observed in 11 genes 4 h after administration, including seven tumor suppressor *p53* target genes, *viz. c-Jun, Ccng1, Mdm2, p21, Bax, Hsp27* and *Snk*; the other genes were *Mbd1, Hmox-1, Ccnf* and *Rad52*. However, only some degree of differential gene expression of *p21, Ccng1* and *Snk* was observed 28 days after administration; no other differentially- expressed genes were evident. The present results suggest that DEN and DPN induce differential gene expression in *p53* target genes in liver within a few hours after administration and that these acute responses remained only partially in liver after 28 days.

Keywords: oligonucleotide microarray, toxicogenomics, genotoxic carcinogens

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Kumada, H.[*], Haishima, Y., Watanabe, K.[*], Hasegawa, C., Tsuchiya, T., Tanamoto, K. and Umemoto, T.[*]:

Biological properties the native and synthetic lipid A of Porphyromonas gingivalis lipopolysaccharide

Oral Micobiol. Immunol., **23**, 60-69（2008）

A pentaacyl and diphosphoryl lipid A molecule found in the lipid A isolated from Porphyromonas gingivalis lipopolysaccharide（LPS）was chemically synthesized, and its characteristics were evaluated to reconfirm its interesting bioactivities including low endotoxicity and activity against LPS-unresponsive C3H/HeJ mouse cells. The synthesized P. gingivalis lipid A（synthetic Pg-LA）exhibited strong activities almost equivalent to those of Escherichia coli-type synthetic lipid A（compound 506）in all assays on LPS-responsive mice, and cells. LPS and native lipid A of P. gingivalis displayed overall endotoxic activities, but its potency was reduced in comparison to the synthetic analogs. In the assays using C3H/HeJ mouse cells, the LPS and native lipid A significantly stimulated splenocytes to cause mitosis, and peritoneal macrophages to induce tumor necrosis factor-alpha and interleukin-6 production. However, synthetic Pg-LA and compound 506 showed no activity on the LPS-unresponsive cells. Inhibition assays using some inhibitors including anti-human Toll-like receptor 2（TLR2）and TLR4/MD-2 complex monoclonal antibodies showed that the biological

activity of synthetic Pg-LA was mediated only through the TLR4 signaling pathway, which might act as a receptor for LPS, whereas TLR2, possibly together with CD14, was associated with the signaling cascade for LPS and native lipid A of P. gingivalis, in addition to the TLR4 pathway. These results suggested that the moderated and reduced biological activity of P. gingivalis LPS and native lipid A, including their activity on C3H/HeJ mouse cells via the TLR2-mediated pathway, may be mediated by bioactive contaminants or low acylated molecules present in the native preparations having multiple lipid A moieties.

Keywords: lipopolysaccharide, lipid A, P. gingivalis

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Takahashi, Y.[*1], Kumada, H.[*1], Hamada, N.[*1], Haishima, Y., Ozono, S.[*1], Isaka, M.[*2], Yasuda, Y.[*2], Tochikubo, K.[*2] and Umemoto, T.[*1]: **Induction of immune responses and prevention of alveolar bone loss by intranasal administration of mice with Porphyromonas gingivalis fimbriae and recombinant cholera toxin B subunit**

Oral Microbiol. Immunol., **22**, 374-380 (2007)

Adult periodontitis is initiated by specific periodontal pathogens represented by Porphyromonas gingivalis; however, an effective measure for preventing the disease has not yet been established. In this study, the effectiveness of a vaccine composed of fimbriae of P. gingivalis and recombinant cholera toxin B subunit (rCTB) was evaluated using BALB/c mice. Fimbriae and rCTB were co-administered intranasally to BALB/c mice on days 0, 14, 21, and 28. On day 35, mice were sacrificed to determine immunoglobulin levels in serum, saliva, and nasal and lung extracts by enzyme-linked immunosorbent assay. The prevention effect of the vaccine on P. gingivalis-induced periodontitis in mice was evaluated by measuring alveolar bone loss. The rCTB significantly increased serum immunoglobulin (Ig) A levels when mice were administered with a minimal amount (0.5 mg) of the fimbrial antigen. The adjuvant effect on serum IgG production was indistinct because the minimal amount of the antigen still induced a large amount of IgG. In contrast to systemic responses, a fimbria-specific secretory IgA response was strongly induced by co-administration of rCTB and 0.5 mg fimbriae; the same amount of the antigen alone scarcely induced a response. Histopathological examination revealed IgA-positive plasma cells in the nasal mucosal tissue but no observable mast cells in the area. In addition, nasal administration of the fimbrial vaccine significantly protected the mice from P. gingivalis-mediated alveolar bone loss. Nasal vaccination with a combination of fimbriae and rCTB can be an effective means of preventing P. gingivalis-mediated periodontitis.

Keywords: P. gingivalis fimbriae, rCTB, vaccine

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Matsuoka, A., Isama, K., Tanimura, S.[*1], Kohno, M.[*1], Yamori, T.[*2]: **A Novel Candidate Compound with Urethane Structure for Anticancer Drug Development**

Current Drug Discovery Tech., **4**, 69-76 (2007)

Diethyl-4,4'-methylenebis (*N*-phenylcarbamate) (MDU) is a urethane compound that we originally synthesized to investigate how polyurethane is hydrolyzed. We tested MDU for cytotoxicity in two Chinese hamster cell lines and a human cancer cell line. MDU showed the strongest cytotoxicity in all the cell lines with an IC_{50} of around 0.1 μg/ml. We further investigated MDU for its ability to induce chromosome aberrations (CA) and micronuclei (MN) in CHL cells. MDU induced around 100% polyploidy cells at 0.5 μg/ml after 24- and 48-h treatment in the CA test and a significantly increased frequency of MN, polynuclear and mtotic cells in the MN test, suggesting that it may induce numerical CAs. MDU's ability to induce mitotic arrest in CHL cells was greater than that of taxol and colchicine. Based on a COMPARE analysis using JFCR39, a panel of cancer cell lines, we predicted MDU to be a tubulin inhibitor. We confirmed this possibility in nerve growth factor-stimulated PC12 cells as well as in HT1080 cells. MDU is simpler in structure than existing anticancer drugs taxol and vincristine and can be synthesized relatively easily. Here we offer MDU as a potential new type of anticancer drug, stable even at room temperature, and inexpensive.

Keywords: polyploidy, diethyl-4,4'-methylenebis (*N*-phenylcarbamate), tubulin inhibitor

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Ito T., Sawada R., Fujiwara Y.*, Seyama Y.*, and Tsuchiya T.: **FGF-2 suppresses cellular senescence of human mesenchymal stem cells by down-regulation of TGF-beta2**

Biochem Biophys Res Commun., **359**(**1**), 108-114（2007）

Human mesenchymal stem cells（hMSCs）are able to both self-replicate and differentiate into a variety of cell types. Fibroblast growth factor-2（FGF-2）stimulates the growth of hMSCs in vitro, but its mechanisms have not been clarified yet. In this study, we investigated whether cellular senescence was involved in the stimulation of hMSCs growth by FGF-2 and the expression levels of transforming growth factor-β1 and -β2（TGF-βs）. Because hMSCs were induced cellular senescence due to long-term culture, FGF-2 decreased the percentage of senescent cells and suppressed G1 cell growth arrest through the suppression of $p21^{Cip1}$, p53, and $p16^{INK4a}$ mRNA expression levels. Furthermore, the levels of TGF-βs mRNA expression in hMSCs were increased by long-term culture, but FGF-2 suppressed the increase of TGF-β2 mRNA expression due to long-term culture. These results suggest that FGF-2 suppresses the hMSCs cellular senescence dependent on the length of culture through down-regulation of TGF-β2 expression.

Keywords: Human mesenchymal stem cells, FGF-2, TGF-β

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Ito T., Sawada R., Fujiwara Y.*, and Tsuchiya T.: **FGF-2 increases osteogenic and chondrogenic differentiation potentials of human mesenchymal stem cells by inactivation of TGF-β signaling**

Cytotechnology, **56**, 1-7（2008）

Human mesenchymal stem cells（hMSCs）are able to self-replicate and differentiate into a variety of cell types including osteoblasts, chondrocytes, adipocytes, endothelial cells, and muscle cells. It was reported that fibroblast growth factor-2（FGF-2）increased the growth rate and multidifferentiation potentials of hMSCs. In this study, we investigated the genes involved in the promotion of osteogenic and chondrogenic differentiation potentials of hMSCs in the presence of FGF-2. hMSCs were maintained in the medium with FGF-2. hMSCs were harvested for the study of osteogenic or chondrogenic differentiation potential after 15 days' culture. To investigate osteogenic differentiation, the protein levels of alkaline phosphatase（ALP）and the mRNA expression levels of osteocalcin were measured after the induction of osteogenic differentiation. Moreover, the investigation for chondrogenic differentiation was performed by measuring the mRNA expression levels of type II and type X collagens after the induction of chondrogenic differentiation. The expression levels of ALP, type II collagen, and type X collagen of hMSCs cultured with FGF-2 were significantly higher than control. These results suggested that FGF-2 increased osteogenic and chondrogenic differentiation potentials of hMSCs. Furthermore, microarray analysis was performed after 15 days' culture in the medium with FGF-2. We found that the overall insulin-like growth factor-I（IGF-I）and transforming growth factor-β（TGF-β）signaling pathways were inactivated by FGF-2. These results suggested that the inactivation of IGF-I and TGF-β signaling promotes osteogenic and chondrogenic differentiation potential of hMSCs in the presence of FGF-2.

Keywords: Human mesenchymal stem cells, FGF-2, TGF-β

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Yamada, T., Jung, D.-Y., Sawada, R., Matsuoka, A., Nakaoka, R., Tsuchiya, T.: **Effects intracerebral microinjection and intraperitoneal injection of［60］fullerene on brain functions differ in rats**

J. Nanosci. Nanotechnol., **8**, 1-9（2008）

Fullerenes are condensed ring aromatic compounds with extend π systems and have unique cage structures. Fullerenes are used for medical devices such as carbon nanotubes and are utilized for medical devices, because fullerenes are become vary form materials and are suitable for drug delivery system. Recently, tube like shape-materials are used for re-nuerogenesis study and we expect that fullerenes and carbon nanotubes are potential materials, which are novel medical devices targeting on brain. However information of effects of fullerenes on brain function are few; thus we examined the effect of［60］fulerene on the central nerve system in this study. In the V79 colony assay, IC_{50} of［60］

fullerene in V79 cells was 1620 μg/ml. In vivo study, the 0.25 mg/kg B.W. of C_{60} was injected into rat brain lateral ventricle or cavitas abdominalis. The brain injection of C_{60} increased locomote behavior of the rats in the day 1 and day 30 after the injection. The intraperitoneal injection of [60] fullerene did not change locomote behavior of the rats acutely, however decreased it in the day 30. The brain injection of [60] fullerene affected monoamine concentration of rat brain, especially serotonin turnover rates were increased in hypothalamus, cerebral cortex, striatum and hippocampus and dopamine turnover rates were increased in hypothalamus, cerebral cortex and striatum. The intraperitoneal injection of [60] fullerene decreased just dopamine turnover rate in the hippocampus. These results suggested brain injection of [60] fullerene led different effects on the central nerve system comparing with intraperitoneal injection of that. In conclusion, it was suggested that fullerene did not pass blood-brain barrier. The brain injection of [60] fullerene affected neurotransmission widely in the brain and the monoamines dysbolism might relate locomte activity.

Keywords: [60] fullerene, neurotransmitter, intracerebral injection

Zhao, D.*, Sakoda, H., Sawyer, W. G.*, Banks, S. A.* and Fregly, B. J.*: **Predicting knee replacement damage in a simulator machine using a computational model with a consistent wear factor**

Journal of Biomechanical Engineering, **130**, 011004 (2008)

Wear of ultrahigh molecular weight polyethylene remains a primary factor limiting the longevity of total knee replacements (TKRs). However, wear testing on a simulator machine is time consuming and expensive, making it impractical for iterative design purposes. The objectives of this paper were first, to evaluate whether a computational model using a wear factor consistent with the TKR material pair can predict accurate TKR damage measured in a simulator machine, and second, to investigate how choice of surface evolution method (fixed or variable step) and material model (linear or nonlinear) affect the prediction. Our results indicate that accurate TKR damage predictions can be made with a computational model using a constant wear factor obtained from pin-on-plate tests for the same material pair, and furthermore, that surface evolution method matters only during the initial "break in" period of the simulation.

Keywords: computer simulation, wear prediction, total knee replacement

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Teramura, S.*1, Sakoda, H., Terao, T.*1, Endo, M. M.*2, Fujiwara, K.*3 and Tomita, N.*1: **Reduction of wear volume from ultrahigh molecular weight polyethylene knee components by the addition of vitamin E.**

Journal of Orthopaedic Science, **26**, 460-464 (2008)

Wear performance and debris-size distribution of vitamin E (DL-a tocopherol, VE)-added ultrahigh molecular weight polyethylene (UHMWPE) was evaluated using a knee-simulator test. VE was mixed with GUR 1050 UHMWPE powder at 0.3 wt%, and the tibial components of the knee joint were made by direct compression molding. The VE-added UHMWPE showed consistently lower wear volume throughout the test.

Keywords: UHMWPE, vitamin E (DL-a tocopherol), wear

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Brems, H.*1, Chmara, M.*1, 2, Sahbatou, M.*3, Denayer, E.*1, Taniguchi, K.*4, Kato, R., Somers, R.*1, 5, Messiaen, L.*6, Schepper, S.D.*7, Fryns, J-P.*1, Cools, J.*1, 5, Marynen, P.*1, 5, Thomas, G.*3, 8, Yoshimura, A.*4 & Legius, E.*1: **Germline loss-of-function mutations in SPRED1 cause a neurofibromatosis 1-like phenotype**

Nature Genet., **39**, 1120-1126 (2007)

We report germline loss-of-function mutations in SPRED1 in a newly identified autosomal dominant human disorder. SPRED1 is a member of the SPROUTY/SPRED family of proteins that act as negative regulators of RAS->RAF interaction and mitogen-activated protein kinase (MAPK) signaling. The clinical features of the reported disorder resemble those of neurofibromatosis type 1 and consist of multiple café-au-lait spots, axillary freckling and macrocephaly. Melanocytes from a café-au-lait spot showed, in addition to the germline SPRED1 mutation, an acquired

somatic mutation in the wild-type SPRED1 allele, indicating that complete SPRED1 inactivation is needed to generate a café-au-lait spot in this syndrome. This disorder is yet another member of the recently characterized group of phenotypically overlapping syndromes caused by mutations in the genes encoding key components of the RAS-MAPK pathway. To our knowledge, this is the first report of mutations in the SPRY (SPROUTY)/SPRED family of genes in human disease.

Keywords; Ras/MAPK, negative regulator, germline mutation

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Taniguchi, K.*1, Kohno, R.*1, Ayada, T.*1, Kato, R., Ichiyama, K.*1, Morisada, T.*2, Oike, Y.*2, Yonemitsu, Y.*1, 3, Maehara, Y.*1 and Yoshimura, A.*1: **Spreds are essential for embryonic lymphangiogenesis by regulating vascular endothelial growth factor receptor 3 signaling**

Mol Cell Biol., **27**, 4541- 4550 (2007)

Spred/Sprouty family proteins negatively regulate growth factor-induced ERK activation. Although the individual physiological roles of Spred-1 and Spred-2 have been investigated using gene-disrupted mice, the overlapping functions of Spred-1 and Spred-2 have not been clarified. Here, we demonstrate that the deletion of both Spred-1 and Spred-2 resulted in embryonic lethality at embryonic days 12.5 to 15.5 with marked subcutaneous hemorrhage, edema, and dilated lymphatic vessels filled with erythrocytes. This phenotype resembled that of Syk (-/-) and SLP-76 (-/-) mice with defects in the separation of lymphatic vessels from blood vessels. The number of LYVE-1-positive lymphatic vessels and lymphatic endothelial cells increased markedly in Spred-1/2-deficient embryos compared with WT embryos, while the number of blood vessels was not different. Ex vivo colony assay revealed that Spred-1/2 suppressed lymphatic endothelial cell proliferation and/or differentiation. In cultured cells, the overexpression of Spred-1 or Spred-2 strongly suppressed vascular endothelial growth factor-C (VEGF-C)/VEGF receptor (VEGFR)-3-mediated ERK activation, while Spred-1/2-deficient cells were extremely sensitive to VEGFR-3 signaling. These data suggest that Spreds play an important role in lymphatic vessel development by negatively regulating VEGF-C/VEGFR-3 signaling.

Keywords; negative regulation, VEGFR-3, lymphangiogenesis

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Wakitani, S.*1, Nawata, M.*2, Kawaguchi, A.*1, Okabe, T.*3, Takaoka, K.*1, Tsuchiya, T., Nakaoka, R., Masuda, H.*4, Miyazaki. K.*4: **Serum keratan sulfate is a promising marker of early articular cartilage breakdown**.

Rheumatology, **46**, 1652-1656 (2007)

To find serum markers that may serve as indices for an early diagnosis of degeneration or damage of the articular cartilage. Twenty-four healthy volunteers, 19 individuals with knee trauma (KT) and 31 with knee osteoarthritis (OA) were evaluated. KT patients were divided into a group (n=5) with an injury <2 months old (recent KT) and a group (n=14) with that >2 months old (old KT). Articular cartilage damage was assessed using either arthroscopy or direct observation. Serum concentrations of hyaluronic acid (HA), cartilage proteoglycan aggrecan turnover epitope (CS846) and cartilage oligomeric protein (COMP) were measured using enzyme-linked immunosorbent assay kits and those of keratan sulfate (KS) and chondroitin-6-sulfate (C6S) using high-performance liquid chromatography. Serum KS in the recent KT group (2095±594 ng/ml) was significantly higher than that in the old KT group (1373±418 ng/ml; P=0.021), and serum COMP in the recent KT group (1572±182 ng/ml) showed a tendency that was higher than that in the old KT group (1350±250 ng/ml; P=0.079). Serum KS in OA patients with Kellgren and Lawrence (KL) grades 0 and I (1456±334 ng/ml) showed a tendency that was higher than that in OA patients with KL grades II, III and IV (1248±220 ng/ml; P=0.084). The

serum concentration of KS correlated with the damage of the articular cartilage and it was significantly increased even at an early stage after the injury.
Keywords: Keratan sulfate, Glycosaminoglycan, Cartilage injury

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Ishikawa, I., Sawada, R.[*1], Higurashi, E.[*2], Sanada, S.[*1] and Chino, D.[*1]: **Integrated micro-displacement sensor that measures tilting angle and linear movement of an external mirror**
Sensors and Actuators A: Physical, **8**, 269-275 (2007)

An integrated optical micro-displacement sensor was developed that uses the beam diverging from a vertical cavity surface emitting laser (VCSEL). The sensor consists of a VCSEL (1.5 mm × 1.5 mm × 1.2 mm) that is surrounded by three photodiodes and can measure the linear distance traveled and the tilting angle of an external mirror. The resolution is 20 nm for a measurement range up to 0.4 mm, or less than 40 nm for a wider measurement range of 1.8 mm. The full range of the measurable tilting angle is 5. This sensor can be incorporated into devices with micro-mirrors, such as integrated scanning microscopes.
Keywords: MEMS, optical lever, displacement and rotation detection

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高橋淳子[*1, 2]，久保田佳子[*1]，小島幸一[*1]，栗田綱義[*2]，渡辺　実[*2]，青木信道[*2]，大沢高温[*2]，菅原英治[*2]，田幡憲一[*2]，佐久間豊夫[*2]，松本秀章[*2]，矢根五三美[*2]，佐藤　望[*3]，田中（相原）真紀，香川（田中）聡子，神野透人，高鳥浩介：**各種浴場施設内における消毒副生成物の暴露評価**
ビルと環境，**117**，27-32（2007）

公衆浴場におけるレジオネラ属菌対策として塩素消毒を実施する環境下における消毒副生成物の暴露評価を行い，一般家庭内の浴室との比較も行った．その結果，特定建築物施設内の温泉（掛け流し浴槽施設）においては問題はなかったが，公衆浴場である銭湯（循環式浴槽施設）においては，浴槽水から消毒副生成物が高濃度検出され，それらの物質がさらに浴室内に気化し浴室内の空気中に高濃度のトリハロメタン類が検出される結果となった．また，浴槽入浴を主とする日本の一般家庭内浴室も欧米のシャワー入浴に匹敵するトリハロメタン類のパターンと同様に曝露されている可能性が今回の研究より明らかとなった．
Keywords: 暴露評価，消毒副生成物，浴場施設

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Hanioka, N.[*1], Yamamoto, M.[*1], Iwabu, H.[*1], Jinno, H., Tanaka-Kagawa, T., Naito, S.[*2], Shimizu, T.[*3], Masuda, K.[*1], Katsu , T.[*1], Narimatsu, S.[*1]: **Functional characterization of human and cynomolgus monkey cytochrome P450 2E1 enzymes**.
Life Sci., **81**, 1436-1445 (2007)

Cytochrome P450 2E1 (CYP2E1) is an enzyme of major toxicological interest because it metabolizes various drugs, precarcinogens and solvents to reactive metabolites. In this study, human and cynomolgus monkey CYP2E1 cDNAs (humCYP2E1 and monCYP2E1, respectively) were cloned, and the corresponding proteins were heterologously expressed in yeast cells to identify the functions of primate CYP2E1s. The enzymatic properties of CYP2E1 proteins were characterized by kinetic analysis of chlorzoxazone 6-hydroxylation and 4-nitrophenol 2-hydroxylation. humCYP2E1 and monCYP2E1 enzymes showed 94.3% identity in their amino acid sequences. The functional CYP content in yeast cell microsomes expressing humCYP2E1 was 38.4 pmol/mg protein. The level of monCYP2E1 was 42.7% of that of humCYP2E1, although no significant differences were statistically observed. The K_m values of microsomes from human livers and yeast cells expressing humCYP2E1 for CYP2E1-dependent oxidation were 822 and 627 μM for chlorzoxazone 6-hydroxylation, and 422 and 514 μM for 4-nitrophenol 2-hydroxylation, respectively. The K_m values of microsomes from cynomolgus monkey livers and yeast cells expressing monCYP2E1 were not significantly different from those of humans in any enzyme source. V_{max} and V_{max}/K_m values of human liver microsomes for CYP2E1-dependent oxidation were 909 pmol/min/mg protein and 1250 nl/min/mg protein for chlorzoxazone 6-hydroxylation, and 1250 pmol/min/mg

protein and 2990 nl /min/mg protein for 4-nitrophenol 2-hydroxylation, respec tively. The kinetic parameter values of cynomolgus monkey livers were comparable to or lower than those of human liver microsomes (49.5-102%). In yeast cell microsomes expressing humCYP2E1, V_{max} and V_{max}/K_m values for CYP2E1-dependent oxidation on the basis of CYP holoprotein level were 170 pmol/min/pmol CYP and 272 nl/min/pmol CYP for chlorzoxazone 6-hydroxylation, and 139 pmol/min/pmol CYP and 277 nl/min/pmol CYP for 4-nitrophenol 2-hydroxylation, respectively, and the kinetic parameters of monCYP2E1 exhibited similar values. These findings suggest that human and cynomolgus monkey CYP2E1 enzymes have high homology in their amino acid sequences, and that their enzymatic properties are considerably similar. The information gained in this study should help with in vivo extrapolation and to assess the toxicity of xenobiotics.

Keywords: cytochrome P450 2E1, cynomolgus monkey, human

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Hanioka, N.*1, Takeda, Y.*1, Tanaka-Kagawa, T., Hayashi, K.*1, Jinno, H., Narimatsu, S.*1: **Interaction of bisphenol a with human UDP-glucuronosyltransferase 1A6 enzyme**.

Environ. Toxicol., **23**, 407-412 (2008)

The effects of bisphenol A (BPA) on UDP-glucuronosyltransferase 1A6 (UGT1A6) activities in microsomes from human livers and yeast cells expressing human UGT1A6 (humUGT1A6) were investigated. Serotonin (5-HT) and 4-methylumbelliferone (4-MU) were used as the substrates for UGT1A6. BPA dose-dependently inhibited 5-HT and 4-MU glucuronidation activities in both enzyme sources. The IC_{50} values of BPA for 5-HT and 4-MU glucuronidation activities were 156 and 163 μM for liver microsomes, and 84.6 and 80.3 μM for yeast cell microsomes expressing humUGT1A6, respectively. The inhibitory pattern of BPA for 5-HT and 4-MU glucuronidation activities in human liver microsomes exhibited a mixture of competitive and noncompetitive components, with K_i values of 84.9 and 72.3 μM, respectively. In yeast cell microsomes expressing humUGT1A6, 5-HT glucuronidation activities were noncompetitively inhibited by BPA (K_i value, 65.5 μM), whereas the inhibition of 4-MU glucuronidation activities by BPA exhibited the mixed type (K_i value, 42.5 μM). These results suggest that BPA interacts with human UGT1A6 enzyme, and that the interaction may contribute to the toxicity, such as hormone disruption and reproductive effects, of BPA.

Keywords: UDP-glucuronosyltransferase 1A6, bisphenol, human

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Omori, T.*1, Ikarashi, Y., Kanazawa, Y.*2, Idehara, K.*3, Kojima, H., Sozu, T.*4, Arima, K.*5, Goto, H.*6, Hanada, T.*7, Inoda, T.*8, Kosaka, T.*9, Maki, E.*10, Morimoto, T.*11, Shinoda, S.*12, Shinoda, N.*13, Takeyoshi, M.*14, Tanaka, M.*15, Uratani, M.*16, Usami, M.*17, Yamanaka, A.*18, Yoneda, T.*19, Yoshimura, I.*20, Yuasa, A.*21: **Validation studies on an alternative endpoint for the local lymph node assay (LLNA-DA): Importance of study management**.

AATEX, **14**, Special Issue, 429-432 (2008)

We conducted 2 validation studies for a modified version of the local lymph node assay (LLNA), which was designated as the LLNA-DA. A total of 17 laboratories tested the validity of the assay by using 14 chemicals. Here, in addition the experimental protocol, we prepared the study protocols, describing the study purpose, role of the participants, etc. Technology transfer was conducted by the developer of the assay. Prior to the studies, preliminary tests were conducted using only a positive control chemical to determine whether the experimental protocol prescribed for the assay was appropriate. A formatted data file was developed for data management. Fortunately, the results of these studies revealed small interlaboratory variations, and we believe that one of the factors that contributed to the successful results was the development of strategies and tools for study management at the planning stage. However, issues related to the management of validation studies have rarely been discussed. Strategies or tools developed for study management should be easily accessible and should be shared with researchers intending to conduct validation studies in the future.

Keywords: interlaboratory validation study, study management, data quality

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Uchino, T., Takezawa, T.*, Ikarashi, Y. and Tokunaga, H.: **Construction of a three-dimensional human skin model consisting of keratinocytes, dendritic cells and fibroblasts and application of this model for alternative animal testing of immune-sensitizing compounds**

J. Soc. Cosmet. Chem. Jpn., **41**, 246-253 (2007)

In order to establish in vitro evaluation of the sensitization of human skin, we attempted to make a three-dimensional human skin model consisting of three different cells, dendritic cells, keratinocytes and fibroblasts. The viability of the cells in the human skin model was observed after staining with hematoxylin and eosin. After 11-14-day incubation (horny layer was initially observed), the three-dimensional human skin model was used for experiments. Due to 2,4-dinitrochlorobenzene (DNCB) under a non-cytotoxic dose, the keratinocytes and dendritic cells in the human skin model significantly induced IL-4 release into the incubating medium and dendritic cells induced CD86 expression. On the other hand, with sodium dodecyl sulfate (SDS; non-sensitizer), the keratinocytes and dendritic cells did not significantly induce IL-4 release and the dendritic cells did not induce CD86 expression. The results suggested that this three-dimensional human skin model with dendritic cells could be applied as an alternative to animal testing of immune-sensitizing compounds.

Keywords: three-dimensional human skin model, CD86, dendritic cells

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Falandysz, J.*1, Kunito, T.*1, Kubota, R., Brzostowski, A.*1, Justyna, M.A.*1, Falandysz, J.J.*1, and Tanabe, S*1: **Selected elements of Poison Pax *Paxillus involutus*.**

J. Environ. Sci. Health, Pt. A, **42(8)**, 1161-1168 (2007)

Concentrations of Ag, Al, Ba, Ca, Cd, Co, Cu, Cr, Cs, Fe, Ga, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Rb, Se, Sb, Sr, V, Tl and Zn have been determined in the whole fruiting bodies as well as separately in caps and stalks of Poison Pax collected from three geographically distant sites across Poland. The elements were determined using ICP-MS, ICP-OES, HG-AAS and CV-AAS, respectively. Based on arithmetic mean and median values for Poison Pax specimens from the Le'zno site the elements such as Ag, Co, Cr, Cs, Mn, Mo, K, Pb, Rb, Sb, Se, V and Tl occur at similar concentration both in the caps and stalks, while for Cd, Cu, Hg, Mg and Zn around two-fold greater concentrations were noted in caps than stalks (cap/stalk concentration quotient >1). Cs, Cd, Ni and Rb occurred at much greater concentration in specimens collected from the Kłodzka Hollow in the Sudety Mountains when compared to the lowland site (Mann Whitney U-test), and slightly greater values were noted also for Cr, Mo and Rb, while for Ca, Co, Mg and Mn were smaller The results provide useful environmental and biological baseline level of information for metallic elements of Poison Pax.

Keywords: Fungi, heavy metals, metallic elements, metalloids, mineral composition, mushrooms, pollution.

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Falandysz, J.[*1], Kunito, T.[*2], Kubota, R., Lipka, K.[*1], Mazur, A.[*1], Falandysz, J.J.[*1], and Tanabe, S.[*2]: **Selected elements in fly agric *Amanita muscaria*.**
J. Environ. Sci. Health, Pt. A, **42(11)**, 1615-1623(2007)

Concentrations of Ag, Al, Ba, Ca, Cd, Co, Cu, Cr, Cs, Fe, Ga, Hg, K, Mg, Mn, Mo, Na, Pb, Rb, Se, Sb, Sr, V, Tl and Zn have been determined in the whole fruiting bodies, as well as separately in caps and stalks, of .y agaric collected from three geographically distant sites in northern part of Poland. The elements were determined using ICP-MS, ICP-OES, HG-AAS and CV-AAS, respectively. For elements such as Al, Ba, Cr, Fe, Ga, Mo, Mn, Pb, Sb, Sr, Tl, and V concentrations were similar in the caps and stalks, respectively, and for K, Zn, Ag, Ca, Cd, Cu, Hg, Mg, Rb and Se were greater in the caps, while for Co, Cs and Na in the stalks. For Ag, Al, Ba, Ca, Cd, Co, Cr, Cs, Fe, Ga, Hg, Mn, Mo, Pb, Rb, Sb, Sr, Tl and V concentration in the caps showed spatial variations ($P < 0.05$), while for Cu, K, Mg, Na, Se and Zn was independent of the site. The elements such as K with median or mean in the caps between 37,000 and 43,000 μg/g・dm and Mg with 920 and 1,100 μg/g dm were most abundant. Next, within median values range from approximately 100 to 500 μg/g dm were such as Ca, Fe and Al, and in descending order they followed by Rb (10015400 μg/g dm); V, Na, Zn (5015200 μg/g dm); Cu, Mn (101550 μg/g dm); Cd (101520 μg/g dm); Se (5 μg/g dm); Ba (< 1153); Cr, Ag, Pb, Sr (< 1152 μg/g dm); Cs, Co, Hg (< 1151 μg/g dm); Ga (< 0.5), Sb, Mo and Tl (< 0.1 μg/g dm).
Keywords: Fungi, heavy metals, metallic elements, metalloids, mineral composition, mushrooms, pollution.

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Falandysz, J.[*1], Kunito, T.[*2], Kubota, R., Bielawski, L.[*1], Mazur, A.[*1], Falandysz, J.J.[*1], and Tanabe, S.[*2]: **Selected elements in Brown Birch Scaber Stalk *Leccinum scabrum*.**
J. Environ. Sci. Health, Pt. A, **42(14)**, 2081-2088(2007)

A survey of 26 metallic elements and metalloids such as Ag, Al, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, Hg, K, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sb, Se, Sr, Tl, V and Zn was carried out using ICP-MS, ICP-OES, HG-AAS and CV-AAS in the caps and stalks of edible mushroom Brown Birch Scaber Stalk collected from two lowland and one mountain sites in Poland. Ag, Al, Cd, Cr, Cs, Cu, Fe, Hg, K, Mg, Mo, Pb, Rb, Se, V and Zn occurred in greater concentration in the caps than stalks of Brown Birch Scaber Stalk, and opposite situation was for Tl and Na. Brown Birch Scaber Stalk collected from the site in Sudety Mountains did contain Al, Ba, Cs, Fe, Ga, Ni, Pb, Sr and V in significantly greater concentration when compared to specimens collected from the lowland sites, and what imply on significance of geological origin and/or soil substrate pollution impacting on mineral composition of this mushroom species. The results provide useful environmental and nutritional baseline level information on mineral composition of Brown Birch Scaber Stalk from unpolluted sites.
Keywords: Mushroom, Fungi, heavy metals, metalloid, wild food

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Falandysz, J.[*1], Kunito, T.[*2], Kubota, R., Gucia M.[*1], Mazur, A.[*1], Falandysz, J.J.[*1], and Tanabe, S.[*2]: **Some mineral constituents of Parasol Mushroom**.
J. Environ. Sci. Health, Pt. B, **43**, 187-192 (2008)

This article reports background concentrations of Ag, Ba, Cd, Co, Cr, Cs, Cu, Ga, Hg, Mn, Mo, Pb, Rb, Sb, Sr, Se, Tl, V and Zn in caps and stalks of *M. procera* collected from four spatially distant sites across Poland. The elements were determined using inductively coupled plasma-mass spectrometry (ICP-MS), hydride generation atomic absorption spectrometry (HG-AAS) or a cold vapor atomic absorption spectrometry (CV-AAS). Copper, zinc, rubidium, selenium, chromium and cobalt were the most abundant amongst elements determined in this mushroom. Some elements (Cu, Zn, Rb, Se, Pb, Hg, Cd, Mo) occurred at greater concentrations in the caps than stalks of *M. procera* and some (Ag, Ba, Sr, V, Tl) dominated in the stalks, while for some other this proportion was similar or varied (Mn, Cr, Co, Ga, Sb, Cs) depending on the sampling site. For elements such as copper, zinc, rubidium as well as

selenium some spatial similarity in distribution and/or concentration values both in caps and stalks was noted. Cadmium and lead content in caps of *M. procera* was usually below the European Union tolerance limit value of 2.0 and 3.0 μg/g dw set for cultivated mushrooms, respectively. These two toxic metals have been found in elevated concentration in *M. procera* from unpolluted stands outside of Poland as reported by some authors, which implies the possibility of relatively high background levels in this species.

Keywords: Environment; food; fungi; heavy metals; mineral elements; nutrition; wild food.

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Tahara, M., Kubota, R., Nakazawa, H. *[*1], Tokunaga, H., Nishimura, T.: **The behaviour and cholinesterase inhibitory activity of fenthion and its products by light and chlorination**.

J. Wat. Supply: Res. Technol. − AQUA, **57(3)**, 143-151 (2008)

We established a method for quantitative analysis of fenthion (MPP) and its related compounds in water samples, using solid-phase extraction and liquid chromatography/mass spectrometry. With this method, the values of the limit of quantification ranged from 0.2 to 100 ng l^{-1}. Using this method, we examined the fate of MPP in water and the products produced by light irradiation and chlorination. MPP decreased gradually and reached 50% of the initial concentration after 48 hours in water. In particular, MPP-sulfoxide was formed. With light irradiation, MPP decomposed immediately into MPP-sulfoxide, O,O-Dimethyl S-[3-methyl-4-(methylthio)phenyl]phosphorothioate and other compounds. With chlorination, MPP decomposed into MPP-sulfoxide, MPP-sulfone, and their oxons. The concentration of oxons increased in a time-dependent manner. In their effects on organisms, MPP, MPP-sulfoxide and MPP-sulfone showed weak inhibitory activity to cholinesterase, whereas their oxons showed strong activity. It is feared that MPP and its products exist in environmental water and are produced by the disinfection treatment process. Comprehensive evaluation of the toxicity of MPP and its related compounds is important in order to understand the effects of MPP on ecosystems and human health.

Keywords: ChE activity; chlorination; light irradiation; MPP; oxidized products; water

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Agusa, T.[*1], Takagi, K.[*1], Kubota, R., Anan, Y.[*1], Iwata, H.[*1], and. Tanabe, S.[*1]: **Specific accumulation of arsenic compounds in green turtles (*Chelonia mydas*) and harksbill turtles (*Eretmochelys imbricate*) from Ishigaki Island, Japan**.

Environ. Pollut., **153**, 127-136 (2008)

Concentrations of total arsenic (As) and individual compounds were determined in green and hawksbill turtles from Ishigaki Island, Japan. In both species, total As concentrations were highest in muscle among the tissues. Arsenobetaine was a major compound in most tissues of both turtles. High concentrations of trimethylarsine oxide were detected in hawksbill turtles. A significant negative correlation between standard carapace length (SCL), an indicator of age, and total As levels in green turtles was found. In contrast, the levels increased with SCL of hawksbill turtles. Shifts in feeding habitats with growth may account for such a growth-dependent accumulation of As. Although concentrations of As in marine sponges, the major food of hawksbill turtles are not high compared to those in algae eaten by green turtles, As concentrations in hawksbill turtles were higher than those in green turtles, indicating that hawksbill turtles may have a specific accumulation mechanism for As.

Keywords: Arsenic; Arsenic compounds; Hawksbill turtle; Green turtle; Muscle; Size-dependent accumulation

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越川富比古[*]，松島昌子[*]，廣庭隆行[*]，宮原　誠：**食品照射検知のLAL/GNB法の測定条件の検討**

防菌防黴, **36**, 213-221 (2008)

グラム陰性菌数（GNB）とエンドトキシン量（EU）を指標に照射有無を検知するLAL/GNB法について検討した．総菌数及びグラム陰性菌数測定での培養温度と培養期間は30℃で72時間，菌数評価では30～300個のコロニー数を採用して評価するのが適切であった．エンドトキシン測定ではLAL試薬の感度0.03EU/ml，試料希釈液

185μl，試料液及びLAL試薬液は90μlの系を用いた．

試料液の希釈にマイクロプレートを使用し，ゲル化の反応に試験管（12.5×75mm）を用いるとゲル化の判定が容易にできた．鶏肉，豚肉及び牛肉のミンチ肉を試料として照射有無の判定基準を検討した結果，｛$\log_{10}$(EU)－$\log_{10}$(GNB)｝＝A値とした場合，A値が＋を示した場合は照射されていた．A値が－を示した場合は総菌数とグラム陰性菌数を加味して評価することが必要であった．総菌数やグラム陰性菌数が10^3個以上でA値が－の場合は，照射されていなかった．一方，総菌数やグラム陰性菌数が10^3個以下（あるいはグラム陰性菌数が検出されない場合）でA値が－の場合は，照射された可能性が高いと判定された．

殆んどグラム陰性菌が検出されない香辛料の照射検知にはLAL/GNB法は適用できないことが分かった．

Keywords: 微生物学，照射食品検知法，LAL/GNB法

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Sakai, T., Hitomi, T.[*1], Sugaya, K.[*2], Kai, S.[*3], Murayama, M. and Maitani, T.: **Determination Method for Ractopamine in Swine and Cattle Tissues Using LC/MS**.

J. Food Hyg. Soc. Japan, **48**, 144-147（2007）

Simple and reliable methods using LC/MS have been developed for the determination of the β-agonist ractopamine in swine and cattle tissues. Ractopamine was extracted with ethyl acetate from muscle and liver, and the ethyl acetate layer was evaporated to dryness. The residue was purified by partition with acetonitorile/*n*-hexane. In the case of fat, ractopamine was extracted and purified by partition with acetonitrile/*n*-hexane. These resulting acetonitrile solutions were evaporated to dryness. The residue was dissolved in methanol, and subjected to LC/MS system. The LC separation was performed on a Wakosil-Ⅱ 3 C18HG column（150 × 3 mm i.d.）with an isocratic mode of 0.05% trifluoroacetic acid-acetonitrile（80:20）as a mobile phase at a flow rate of 0.4 mL/min. The MS detection was performed on the selected ion recording（SIR）mode, $[M+H]^+$ ion of ractopamine（m/z 302）produced by the electrospray ionization（ESI）was detected. The mean recoveries of the drug from swine muscle（0.01 μg/g fortified）, fat（0.01 μg/g fortified）and liver（0.04 μg/g fortified）were 99.7%, 99.5% and 100.8%, and those from cattle samples were 108.3%, 97.0% and 109.4%, respectively. The relative standard deviations（RSDs）were ranged from 0.1% to 9.5%. The limit of quantification（LOQ）of the drug was 1 ng/g.

Keywords: ractopamine; β-agonist; swine; cattle; LC/MS

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Yoshida I.[*], Isagawa S.[*], Kibune N.[*], Nagaoka M. H., Maitani T.: **Rapid and improved determination of furan in baby foods and infant formulas by headspace GC/MS**.

Journal of the Food Hygienic Society of Japan, **48**, 83-89（2007）

Furan is a 5-membered ring compound with high volatility. The U.S. Food and Drug Administration（FDA）has recently published a report on the occurrence of furan in a large number of thermally processed foods. However, the FDA's analytical method, using standard curve addition, is not suitable for high-throughput routine laboratory operations. We developed a rapid and improved method for determination of furan in foods by headspace GC/MS. Quantification was achieved by using an internal standard of d4-furan and an external calibration curve of furan normalized against the internal standard. The incubation temperature for equilibration was set at 60℃ to avoid the formation of furan during analysis. The levels of furan in baby foods and infant formulas were determined with this method. Validation data showed good precision and accuracy. The LOD and LOQ were 0.2-0.5 ng/g and 0.5-2 ng/g for various food matrixes, respectively. The level of furan detected was in the range of 1.4 to 90 ng/g in baby foods and in the range of non-detectable to 36 ng/g in infant formulas.

Keywords: furan, headspace-GC/MS, baby food

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Kitajima, A.[*1], Kashirajima, T.[*2], Minamizawa, T.[*1], Sato, H.[*3], Iwaki, K.[*3], Ueda, T.[*4], Kimura, Y.[*4], Toyo'oka, T.[*5], Maitani, T., Matsuda, R., Hayashi, Y.: **Baseline Noise and Measurement Uncertainty in Liquid**

Chromatography

Anal. Sci., **23**, 1077-1080 （2007）

HPLCのUV吸収及びγ線検出器のベースラインノイズの確率論的性質を調べる理論（FUMI理論）により推定されるノイズパラメータの信頼性について検討した．信頼性は主としてベースラインノイズに含まれるホワイトノイズとMarkov過程の比率，推定に使用したポイント数，推定するピークの幅により変動することが示された．

Keywords: baseline noise, FUMI theory, measurement uncertainty

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Choi,D.H.[*], Katakura, Y.[*], Matsuda, R., Hayashi, Y., Ninomiya, K.[*], Shioya, S.[*]: **Simulation Model for Predicting Limit of Detection and Range of Quantitation of Competitive Enzyme-Linked Immunosorbent Assay**

J. Biosci, Bioeng., **5**, 427-431 (2007)

競合ELISA法の検出限界（LOD）と定量範囲（ROQ）を，検量線と精度プロファイルを記述するモデルから決定した．抗原－抗体複合体濃度及び酵素標識抗原－抗体複合体濃度変化を記述する微分方程式をRunge-Kutta法で解いて検量線を得た．精度プロファイルは個々の測定操作に伴う誤差により記述した．低いLODと十分な発色の両者を満足する適切な酵素標識抗原濃度範囲は狭く，経験法則と一致した．

Keywords: competitive enzyme-linked immunosorbent assay, mathematical model, precision profile, apparent rate constant

[*] 大阪大学

小倉　哲[*1]，藤平弘樹[*2]，西井重明[*3]，岩木和夫[*4]，松田りえ子，林　譲：**抗原固相化競合免疫測定法の精度予測**

分析化学，**56**，921-926 (2007)

抗原固相化競合免疫測定法のひとつであるダイオキシン類分析キット“ダイオクイッカー®”の新規精度予測手法による精度予測を行った．同キットからの実測値を用いて求めた精度プロファイルと精度予測式により求めた精度プロファイルは広い濃度範囲でよく一致することが分かった．また，予測したプロファイルから求めた検出下限値，定量下限値も従来法より求めた値とほぼ一致した．

Keywords: ELISA, dioxins, precision, detection limit, quantitation limit

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Kobari, T.[*1], Kondo, S.[*2], Tanaka, H.[*2], Ijuin, K.[*3], Takeuchi, H.[*4], Sato, H.[*5], Iwaki, K.[*5], Ishii, F.[*6], Matsuda, R., Hayashi, Y., and Yajima, T.[*7]: **Geographical Pattern of Influenza Propagation in Tokyo and Its Neighborhood in Three Seasons**

J. Health Sci., **53**, 722-729 (2007)

薬局におけるタミフルの販売量からインフルエンザの伝播速度を求める方法を，東京近郊及び栃木県，福島県の３シーズンのデータに適用した．インフルエンザは東京都心から近郊に向かって伝播し，この傾向は３年間変化しなかったが，栃木県及び福島県では一定した伝播方向は認められなかった．

Keywords: pharmacy, influenza, anti-influenza drug

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Kobari, T.[*1], Takeuchi, H.[*2], Iwaki, K.[*3], Ishii, F.[*4], Tsubaki, H.[*5], Matsuda, R., Hayashi, Y., and Yajima, T.[*6]: **Factor Analysis of Drug Supply Time Series at Pharmacies**

J. Health Sci., **54**, 107-111 (2008)

薬局における薬剤販売量の変動の確率論的性質を因子分析により検討した．31薬剤から，慢性疾患薬，感冒薬，インフルエンザ用薬，アレルギー用薬に関連づけられる４因子が抽出された．同様の結果は他の薬局からも得られた．

Keywords: factor analysis, pharmacy, drug sale, precsription drug

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Nagaoka, M.H., Yamazaki, T., Nishimura, T., Maitani, T.: **Antigenic evaluation of natural food additives using popliteal lymph node assay（PLNA）**
Japanese Journal of Food Chemistry, **14**, 51-55（2007）

The mouse popliteal lymph node assay（PLNA）has been applied as an immunotoxicological test to predict the allergenicity of chemicals. In our previous reports, we suggested that the antigenicity by PLNA depends on the chemical structures of dyes, rather than on the retention period in the footpads. Herein, we applied this test to evaluate the antigenicity of the natural food colors with anthraquinone structure, cochineal extract and lac color.

The cochineal extract increased the PLN cellularity index. Neither the highly purified cochineal extract nor carminic acid increased the PLN cellularity index. These results are consistent with the reports identifying proteins in cochineal extract as antigens. Lac color, laccaic acid A, and laccaic acid C showed stronger antigenicity by PLNA than laccaic acid B, suggesting that lac color itself has strong antigenicity.

Our results suggest that PLNA is a useful method for evaluating the degree of refinement of products, such as natural food colors with antigenicity.
Keywords: popliteal lymph node assay, PLNA, cochineal extract, lac color, natural food colors

Nagaoka, M.H., Hanaoka, K.[*1], Usui, M.[*1], Nishimura, T.[*2], Maitani, T.: **Nitric acid-based partial-digestion method for selective determination of inorganic arsenic in hijiki and application to soaked hijiki.**
J. Food Hyg. Soc. Japan, **49**, 88-94（2008）

Because there is a great difference between the toxicity of inorganic arsenic（As）and organic As in food, the JECFA has set a PTWI value for inorganic As（iAs）rather than for total As. The difference in As toxicity makes it necessary to extract iAs completely from food samples for toxicological analysis, but complete extraction of As from most foods including seaweed has not been achieved to date. We developed a partial-digestion method that uses nitric acid as a solvent in order to extract almost all arsenicals from the solid matrix of hijiki（Hizikia fusiforme, a brown alga）samples. In this method, organic As species were not converted into iAs. HPLC/ICP-MS was then used to determine the concentration of iAs. Total As was measured by hydride generation-atomic absorption spectrometry. The adopted conditions for 0.1 g of ground fine powder sample were: 2 mL of 0.3 mol/L nitric acid; heating, 80°C for 1 hr. Intra-laboratory validation of the method showed good precision and accuracy. The repeatability and intermediate precision for iAs were 1.5% and 1.5%, respectively. The LOD and LOQ for iAs were 0.14 and 0.46 mg/kg dry weight, respectively. Recovery studies performed by spiking 0.5 mg/kg dry weight as the LOQ level and by spiking 3 mg/kg dry weight as the iAs concentration of an unspiked hijiki sample showed good accuracy. The method was applied to hijiki samples after a water soaking process and a water soaking and simmering process. The results suggested that the As concentration in hijiki after both processes was lower than that before the treatments and that the water soaking and simmering process reduced the iAs concentration much more effectively than the water soaking process.
Keywords: arsenic; inorganic arsenic, hijiki, HPLC/ICP-MS, partial digestion, nitric acid, soaking

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Nagaoka, M.H., Nishimura, T.*, Matsuda, R., Maitani, T.: **Evaluation of a nitric acid-based partial-digestion method for selective determination of inorganic arsenic in rice.**
J. Food Hyg. Soc. Japan, **49**, 95-99（2008）

Arsenic（As）uptake in human occurs via the food chain mainly. The Joint FAO/WHO Expert Committee on Food Additives has established the provisional tolerable weekly intake level for As as an inorganic As（iAs）value, because iAs in food is much more toxic than organic As. In this study, we studied an acid based partial-digestion method for the complete extraction of arsenicals from rice. HPLC/ICP-MS was used to determine the concentration of iAs selectively. The conditions adopted to extract arsenicals from a 0.5 g of finely ground rice sample were addition of 2 mL sof 0.15 mol/L nitric acid and heating at 80°C for 2 hr. The

LOD and LOQ for iAs were 0.0024 and 0.0079 mg/kg dry weight, respectively. Recovery studies showed good accuracy. When the method was applied to ten short-grain brown rice samples, the iAs concentrations were 0.108-0.227 mg/kg dry weight and the total As concentrations were 0.118-0.260 mg/kg dry weight. Although dimethylarsinic acid was also detected in most samples, the percentage of iAs content in total As content was 62.2-96.3%. Thus, iAs was the principal As species in the short-grain brown rice samples tested.

Keywords: arsenic, inorganic arsenic, rice, HPLC/ICP-MS, partial digestion, nitric acid

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食品衛生学雑誌，**48(6)**，170-178（2007）

安全性未審査遺伝子組換えコメ（LLRice601系統）の流通を監視するためには，信頼性のある分析法が必要とされる．そこで本研究では，コメを対象としたDNA抽出法および解析方法を含む検知技術を開発し，その妥当性について検証した．その結果，安定した量のDNAを抽出可能な抽出法および測定結果を安定して得ることが可能なreal-time PCR法が開発された．共同試験の結果，LLRice601系統由来のDNAを0.1%含有した試料の陽性率は100%であった．また，全試料を通じて得られた内在性遺伝子の測定値（Ct値）および，0.1%試料について得られたLLRiceコンストラクト特異的DNA配列の測定値（Ct値）を統計的に解析した結果，有意差は認められず，本方法の妥当性が確認された．0.1%未満の試料については検出率がばらついたため，検出下限は0.1%付近であると考えられた．また，測定結果の判定については，一義的には40未満のCt値が得られるか否かをもって行うことが妥当であることが蛍光強度の解析により示唆された．

Keywords: genetically modified organisms, LL601rice, PCR, DNA extraction method, detection method

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食品衛生学雑誌，**49(1)**，45-50（2007）

安全性未審査の遺伝子組換えトウモロコシCBH351の検知法において，DNA回収が困難なトウモロコシ加工食品からのイオン交換タイプキットを用いたDNA抽出精製法を検討した．コーンフレークおよびジャンボコーンについては，粉砕試料4 gを採取し，KNG－Gtip法を用いてDNAの抽出精製を行うことにより，現行法である厚労通知法またはJASハンドブック法に比べDNA試料原液の濃度は増大し，Zein遺伝子の検出率も向上した．

Keywords: corn-processed food, corn flake, corn snack, DNA extraction, DNA purification, ion-exchange resin

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Ohmori, K.[*], Tsuchiya, H.[*], Watanabe, T., Akiyama, H., Maitani, T., Yamada, T.[*], Hirayama, K.[*], Satoh, S.[*]: **A DNA Extraction Method Using a Sillica-base Resin Type Kit for the Detection of Genetically Modified Papaya**

J. Food Hyg. Soc. Japan, **49(2)**, 63-69 (2008)

Genetically modified (GM) papaya has not yet been approved for importation into, orcultivation in the European Union (EU) and Japan. A DNA extraction method using the Qiagen Dneasy Plant Mini Kit (PM method) and a metho using a buffer containing cetyltrimethyl ammonium bromide (CTAB method) ,have been adopted as the official Japanese methods for detecting GM foods. However, the amounts of DNA extracted from papaya by these methods are very low. Therefore, we investigate an extraction method to obtain a high yield of DNA from raw or freese-dried fresh papaya using the Promega Wizard DNA Clean-Up Resin System (WRC). The incubation for the extraction was carried out at 58℃ without proteinase K for 15 min. The extract was applied to a mini-column, then the column wa washed with 80% isopropyl alchol, and genomi DNA adsorbed on the column was eluted with TE buffer. The WRC method gave a higher yield of genomic DNA, and was simpler and faster than thte PM method or CTAB method. In addition, it could be used to extract genomic DNA from fresh papaya at various stages of ripness. Based on these results, we propose that the present method using WCR is the most practical and useful way to extract genomic DNA for the purpose of detecting GM papaya.

Keywords: genetically modified（GM）papaya, qualitative PCR, DNA extraction, silica base resin type kit, Promega Wizard DNA Clean-Up Resin System

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Sugimoto, N., Koike, R.*, Furusho, N., Tanno, M.*, Yomota, C., Sato, K., Yamazaki, T., Tanamoto, K.：**Quantitative nuclear magnetic resonance spectroscopic determination of the oxyethylene group contents of polysorbates**

Food Add. Contam., **24**, 799-806（2007）

Guidelines for the oxyethylene group（EO）contents of polysorbates are set by the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives. However, the classical titration method for EO determination is difficult and time-consuming. Here, we showed that quantitative ^{1}H-nuclear magnetic resonance spectroscopy could rapidly and simply determine the EO contents of polysorbates. The EO signals were identified through comparisons with sorbitan monolaurate and poly（ethylene glycol）distearate. Potassium hydrogen phthalate was used as an internal standard. The EO contents were estimated from the ratio of the signal intensities of EO to the internal standard. Two nuclear magnetic resonance systems were used to validate the proposed method. The EO contents of commercial polysorbates 20, 60, 65, and 80 were determined to be within the recommended limits using this technique. Our approach thus represents an additional or alternative method of determining the EO contents of polysorbates.

Keywords: oxyethylene, polysorbate, quantitative nuclear magnetic resonance

* Kao Corporation

Uekusa, Y.*, Sugimoto, N., Yun, Y.-S.*, Sato, K., Kunugi, A.*, Yamazaki, T., Tanamoto, K.: **Neocrocin A: a novel crocetin glycoside with a unique system for binding sugars isolated from gardenia yellow**

Chem. Pharm., Bull., **55**, 1643-1646（2007）

A novel crocetin glycosyl ester, neocrocin A（2）, was isolated from gardenia yellow. The structure of 2 was elucidated as that of an all-*trans*-crocetin β-D-gentiobiosyl β-D-glucopyranosyl-(1 → 6)-D-2-deoxy-glucopyranos-2-yl diester based on chemical and spectral data. The findings provide evidence that the binding system of crocetin glycosides is not limited to the anomeric position.

Keywords: *Gardenia jasminoides*, gardenia yellow, crocetin glycoside

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杉本直樹，多田敦子，山崎　壮，棚元憲一：**天然保存料カワラヨモギ抽出物の有効成分の確認**

食品衛生学雑誌, **48**, 106-111（2007）

天然保存料であるカワラヨモギ抽出物の成分組成を明らかとする目的で，本抽出物製品についてGC/MS分析を行い，さらに，主要な５成分については，単離精製し，NMRで化学構造を確認した結果，capillin，capillene，caryophyllene oxide，α-curcummene，methyleugenolであることを明らかとした．次に，ハロー試験法により，*E. coli, S. cerevisiae*および*A. niger*に対する抗菌活性について検討した結果，本抽出物製品の活性本体がcapillinであることが確認された．HPLCにより，本抽出物製品中のcapillinおよびcapilleneの定量を行った結果，今回分析した製品中にはそれぞれ17.9mg/ml，36.1 mg/ml含有されることを明らかとした．

Keywords: *Artemisia capillaris*, rumput roman extract, natural food preservative

多田敦子，増田愛乃*1，杉本直樹，山形一雄*1，山崎　壮，棚元憲一：**既存添加物エステル系ガムベースの成分分析**

食品衛生学雑誌, **48**, 179-185（2007）

天然由来のエステル系ガムベース10品目（ウルシロウ，カルナウバロウ，カンデリラロウ，コメヌカロウ，シェラックロウ，ホホバロウ，ミツロウ，モクロウ，モンタンロウおよびラノリン）の成分分析を行い，含有成分組成の差異を比較検討した．TLC分析の結果，含有脂質成分組成の概要が把握でき，いくつかの品目では，その特徴的なTLCパターンにより，他品目との区別が可能であった．しかし，TLCパターンの類似した品目間では相互の区別ができなかったため，さらに，GC/MSにより構成脂肪酸およびアルコールを分析した．その結果，構成脂肪酸およびアルコールの種類やピーク強度比が品目ごとに特徴的であり，TLCパターンが似ている品目同士も，脂肪酸組成分析とアルコール組成分析を組み合わせることで，相互に区別できることが示唆された．今回得られた結果は，エステル系ガムベース製品の種類の推定・判別を行う上で有用な情報であると考え

られる．
Keywords: gum base, wax, GC/MS

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島村智子*1，松浦理太郎*1，徳田貴志*1，杉本直樹，山崎　壮，松藤　寛*2，松井利郎*3，松本　清*3，受田浩之*1：**酸化防止剤力価評価のための各種抗酸化活性測定法の共同試験**

食品科学工学会誌, **54**, 482-487（2007）

近年，日本では，酸化防止剤の抗酸化活性に基づく新しい品質基準が求められている．特に，食品添加物の抗酸化活性を評価するために，新たに公定法を設定する必要がある．そこで抗酸化活性測定法の公定法化の可能性を評価するため，分光学的な測定法であり，かつ高い簡便性を有する３種類の従来法（DPPH法，ABTS法，WST-1法）の室間再現精度を，３ヶ所の研究室による共同試験において調べた．DPPH法，ならびにABTS法では本研究で使用した９種類の酸化防止剤全てについて，そのTEAC（Trolox等価活性）を求めることが可能であった．その一方，WST-1法では，α-トコフェロールとδ-トコフェロールを除く７種類の酸化防止剤のSOSA（SOD等価活性）を求めることができた．また，共同試験から求めたHorRat値から，DPPH法とABTS法は比較的高い精度を示すことが明らかとなった．それに対して，WST-1法の測定精度は，先の２法と比較して低かった．この測定精度の差は，競合法であるWST-1法と非競合法であるDPPH法，ならびにABTS法との測定原理の違いから生じていると推察された．以上の結果から，DPPH法，ならびにABTS法は公定法化を目的とした，分析法の妥当性確認試験の候補となり得るものと考えられた．
Keywords: antioxidant, food additive, antioxidative activity

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杉本直樹，多田敦子，黒柳正典*1，米田祐子*2，尹　永淑*2，功刀　彰*2，佐藤恭子，山崎　壮，棚元憲一：**グレープフルーツ種子抽出物および配合製品中の合成殺菌剤の調査**

食品衛生学雑誌, **49**, 56-62（2008）

グレープフルーツ種子抽出物（grapefruit seed extract: GSE）は既存添加物名簿に収載されている天然添加物である．最近，GSEが食中毒の原因ウイルスとして重要なノロウイルスに対する不活化効果を有することが報告されて以来，食品業界で注目されている．一方，海外において，GSE中に合成殺菌剤である塩化ベンゼトニウム（BZT-Cl）または塩化ベンザルコニウム（BZK-Cl）が検出されることが報告されている．そこで，我々は，我が国に流通しているGSE製品の実態を早急に確認するため，食品添加物（６社13製品），化粧品配合剤（10社16製品），GSE配合健康食品（４社５製品）および除菌・消臭スプレー（７社７製品）中のベンゼトニウム（BZT）およびベンザルコニウム（BZK）の存否についてNMRおよびLC/MSにより調査した．その結果，41製品中38製品よりBZT（食品添加物からBZT-Cl換算で最高39.1%）またはBZK（食品添加物からBZK-Cl換算で最高13.9%）が検出されたことから，我が国に流通するGSE製品の多くがBZTまたはBZKを含有している可能性が高いと考えられた．
Keywords: grapefruit seed extract, benzethonium chloride, benzalkonium chloride

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六鹿元雄，河村葉子，棚元憲一：**ポリ乳酸の基本的性状の検討**

日本食品化学学会誌, **14**, 87-92（2007）

８種類のポリ乳酸ペレットとそれを成形したシートについて基本的性状を調べた．ペレットおよびシートの重量平均分子量は120000～160000，クロロホルムに対する相対粘度は2.8～4.1，シートのD-乳酸含有率はND（<1.0%）～11.3％であった．重量平均分子量と相対粘度には相関が見られたが，D-乳酸含有率が高いシートは相関直線からはずれていた．シート材質中の遊離ラクチド量は169～1610 μg/gであった．また，材質中の金属含有量ではすべてのシートからスズが3.4～31.8 μg/g検出されたが，他の金属は検出されなかった．そのため，重合触媒としてスズ化合物を使用していると推定された．しかし，いずれのシートからもスズの溶出はみられなかった．過マンガン酸カリウム消費量試験および蒸発残留物試験を行ったところ，いずれも特に問題となる量の溶出はみられなかった．しかし，主な溶出物と考えられる乳酸とラクチドは蒸発残留物試験では測定できなかったことから，今後はこれらを測定対象とした溶出試験を検討する必要がある．
Keywords: polylactide, D-lactic acid, free lactide

河村葉子，山口未来，六鹿元雄，薗部博則*，宮本真一*，棚元憲一：**ピューター製品中のアンチモンおよ**

び鉛の分析

日本食品化学学会誌, **15**, 1-5（2008）

スズ合金であるピューター製品中のアンチモン（Sb）と鉛（Pb）の分析法を検討し，市販品の調査を行った．試料は塩酸－硝酸（3:1）混液に溶解し，水と0.1 mol/L硝酸で希釈した．それをスズ添加標準溶液を用いた検量線法または標準添加法により，ICP-MS，ICP-AESおよびフレームレス原子吸光で測定した．回収率は95.6～114.0％と良好であった．ICP-MSの場合には，スズとアンチモンの吸着を防止するために，試験溶液は適切な濃度に希釈し，オートサンプラーのプローブやチューブは十分に洗浄しなければならない．蛍光X線分析も良好な結果が得られた．市販ピューター製品はアンチモンを0.6～4.0％，鉛をnd～0.09％含有していた．これらは日本の食品衛生法の規格値より低かった．アンチモンと鉛は４％酢酸に対して60℃ 30分で5～73 ng/mlおよび2～30 ng/ml溶出したが，水には溶出しなかった．

Keywords: pewterware, antimony, lead

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六鹿元雄，河村葉子，棚元憲一：**瓶詰食品キャップシーリング中のセミカルバジドの分析**

日本食品化学学会誌, **15**, 23-27（2008）

欧州における瓶詰食品の調査において，いくつかの食品からセミカルバジド（SEM）が検出された．これらは金属キャップのシーリングに添加された発泡剤アゾジカルボンアミド（ADC）の分解物である．本報ではキャップシーリング中のSEMおよび，同じくADCの分解物であるヒドラゾジカルボンアミド（HDC）の分析法を確立し，国内で流通している瓶詰食品のキャップシーリング中の含有量を測定した．その結果，市販瓶詰食品のキャップシーリング92検体のうち，SEMは55検体から0.1～2.3 μg/g（0.9±0.6 μg/g），HDCは58検体から5.6～269 μg/g（113±73 μg/g）検出された．一方，32検体からはいずれも検出されなかった．また，目視によるキャップシーリングの発泡とSEMおよびHDCの検出はほぼ一致していた．キャップの種類別では，プレスオンツイストキャップとスクリューキャップのほぼすべてからSEMまたはHDCが検出されたのに対し，ラグキャップで検出されたのは約1/3程度であった．以上の結果から，国内で流通しているキャップ，特にプレスオンツイストキャップとスクリューキャップのシーリング発泡剤としてADCが汎用されていることが確認された．

Keywords: sealing gasket, semicarbazide, hydrazodicarbonamide

Vijay Chandra JHA[*1], Yukio MORITA[*2], Mermagya DHAKAL[*1], Bishunu BESNET[*1], Teruo SATO[*3], Akira NAGAI[*2], Masahiko KATO[*2], Kunihisa KOZAWA[*2], Shigeki YAMAMOTO and Hirokazu KIMURA[*1, 5]: **Isolation of *Mycobacterium* spp. from milking buffaloes and cattle in Nepal**

J. Vet. Med. Sci. **69**, 819-825（2007）

In Nepal, mycobacterial isolates obtained from the milk and feces of buffaloes and cattle that were positive for the single intradermal cervical tuberculin（SICT）tests were genetically identified. A total of 36 mycobacterial strains were isolated from 39% of the buffaloes（14 of 36）and 34% of the cattle（11 of 32）. Of the 36 strains, 13 were identified as M. bovis, and these strains were isolated from 17% of the buffaloes（6 of 36）and 16% of the cattle（5 of 32）. M. bovis was isolated from both the milk and feces of one buffalo and one cattle, the milk alone of three buffaloes and three cattle, and the feces alone of two buffaloes and one cattle. These results suggest that milking buffaloes and cattle infected with M. bovis exist in Nepal. The remaining 23 strains were atypical mycobacteria. A program for the elimination of bovine tuberculosis should be implemented as soon as possible, and the public health education and proper hygienic practices may be required.

Keywords: buffalo, cattle, isolation, mycobacteria, Nepal.

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Noda, M., Fukuda, S[*1]. and Nishio, O.[*2]: **Statistical analysis of attack rate in norovirus foodborne outbreaks**,

Int. J. Food Microbiol., **122**, 216-220（2008）

Norovirus（NoV）, which causes foodborne gastroenteritis outbreaks, is one of the important viruses in public health. We statistically analyzed the attack rate in foodborne outbreaks caused by NoV. The attack rate in 95 oyster-associated outbreaks was significantly higher than that in 195 food handler-associated outbreaks（P=0.007）. The difference in the number of NoV genotypes implicated is considered to be an important factor for this difference. The attack rate in 20 outbreaks associated only with GII/3 was higher than that in 143 other outbreaks（P=0.247）, while the

attack rate in 27 outbreaks associated only with GII/4 was lower than that in 136 other outbreaks (P=0.004), suggesting that GII/4 NoVs cause asymptomatic infection more frequently than do other NoV genotypes. Our results suggest that differences in implicated foods, susceptibility of the host to NoV infection, and pathogenicity of NoVs may influence the attack rate in NoV foodborne outbreaks.

Keywords: attack rate, norovirus, statistical analysis

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野田　衛，伊藤文明*，山本美和子*，磯野裕之*，池田義文*，松本　勝*：**2006年非流行期に広島市でノロウイルス集団事例の発生要因分析**

広島市衛生研究所年報, **26**, 35-40（2007）

2006年５月から10月の間に，集団感染症，食中毒等の疑いでウイルス学的検査を行った23事例中20事例からノロウイルス（NV）が検出された．８月を除く５月～７月（各３事例），９月（４事例），10月（７事例）の非流行期に継続的に発生がみられた．検出NV の遺伝子群は全てGIIで，17事例から検出されたNVについて遺伝子型を決定した結果，GII/4が15 事例，GII/2とGII/9が各１事例に関与した．これらの事例の発生要因について，各事例の発生状況や疫学調査，検出NV遺伝子の系統樹解析に加え，感染症発生動向調査に基づく感染性胃腸炎報告数，病原体検出情報等を基に分析した．その結果，散発性感染性胃腸炎患者の多発に由来する小児から大人への感染機会の増加，感染力が強く，不顕性感染を起こしやすい特徴をもつGII/4の流行，不顕性感染の食品取扱者からの食品二次汚染による食中毒の発生，回復患者による新たな集団感染の発生などが関与している可能性が考えられた．

Keywords: GII. 4 , norovirus, outbreaks

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* 広島市衛生研究所

伊藤文明*，山本美和子*，野田　衛，池田義文*，松本勝*：**Human Metapneumovirusの検出法の開発と検出状況**

広島市衛生研究所年報, **26**, 41-44（2007）

我々は，Human Metapneumovirus（h MPV）の検出にReal-Time PCRを用い，広島市域における流行状況の把握を目的として検査を行なった． h MPV の検出状況は，391検体中41検体（10.5%）から h MPV 遺伝子が検出された．月別にみると，１月から８月まで検出され，３月が最も多く46検体中17検体（37.0%）であった．臨床診断名別に見ると，検出率が最も高いのは，RSウイルス感染症で50％（1/2），百日咳で45.4％（5/11），その他の呼吸器系感染症が12.5％（25/200）であった．今回の調査で広島市域においても３月をピークとして検出されており，毎年同時期に流行している可能性もあり，今後とも継続していく必要があると考えられた．

Keywords: human metapneumovirus, real-time PCR, respiratory infection

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藤尾公輔*，清水晃*，松村浩介*，河野潤一*，北川　浩*，五十君靜信：**市販食肉，ヒト，豚および鶏から分離された黄色ブドウ球菌の薬剤感受性**

日本食品微生物学会雑誌, **24**, 100-106（2007）

近年，ほとんどの抗生物質が効かないバンコマイシン耐性腸球菌が輸入鶏肉から検出され，公衆衛生上の問題となっている．黄色ブドウ球菌についても古くから薬剤耐性化しやすい菌として知られ，メチシリン耐性黄色ブドウ球菌（MRSA）が鶏肉および食肉加工品から分離されているが，食肉類におけるMRSAを含めた薬剤耐性黄色ブドウ球菌の汚染状況に関する報告は極めて少なく，その実態はほとんど明らかにされていない．

本研究では，市販の食肉類から分離された黄色ブドウ球菌の薬剤耐性を調べた．また，健康成人および家畜・家禽の保菌している薬剤耐性菌が食肉類の汚染にどの程度反映しているか知るために，健康人，豚および鶏由来株の薬剤耐性についても検討し報告した．

Keywords: *Staphylococcus aureus*, antimicrobial resistance, meat

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* 神戸大学

清水　晃*，市場智子*，河野潤一*，五十君靜信：**調理済み食品における黄色ブドウ球菌の汚染実態**

食品衛生学雑誌, **48**, J341-344（2007）

これまで，ブドウ球菌食中毒の防止対策の基礎的資料を得るために，畜産食品や水産食品における黄色ブドウ球菌の汚染実態調査を行ってきた．本稿では，最近急激に消費量が増加しているReady-to-eat食品（加熱しないでそのまま食べる食品，調理済み食品）の黄色ブドウ球菌汚染実態について，これまでに得られた成績を紹介した．

Keywords: *Staphylococcus aureus*, ready-to-eat, contamination

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* 神戸大学

Kim T.W*., Igimi S., Kajikawa A., Kim H.Y*.: **Display of heterologous proteins on the surface of *Lactococcus lactis* using the H and W domain of PrtB from *Lactobacillus delbrueckii* subsp. *bulgaricus* as an anchoring matrix.**

J Appl Microbiol., **104**, 1636-1643（2008）

The aim of this study was to develop a cell-surface display system for foreign antigens on the surface of a Lactococcus lactis strain using an H and W domain of PrtB from Lactobacillus delbrueckii subsp. bulgaricus as an anchoring matrix. To construct a cell-surface display pACL1 vector, a derivative of pSECE1 vector, we amplified the H and W domain of the cell-surface proteinase Prt B from Lact. bulgaricus using specific primers and then cloned it into a site downstream of the secretion signal sequence in the pSECE1 vector. The new system was evaluated by the expression and display of the FliC protein of Salmonella enterica serovar Enteritidis as a reporter gene（pALC1: FliC）. A pALC1 vector using the H and W domain of PrtB from Lact. bulgaricus as an anchoring matrix can be used to successfully display the FliC protein on the surface of L. lactis. This novel way of displaying heterologous proteins on the cell surface of L. lactis using the PrtB anchor domain should prove useful for the delivery of antigens and other proteins.

Keywords: *Lactococcus lactis*, vector, recombinant

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Asakura, H., Morita-Ishihara, T*., Yamamoto, S., and Igimi, S.: **Genetic characterization of thermal tolerance in *Enterobacter sakazakii*.**

Microbiol. Immunol., **51**, 671-677（2007）

Enterobacter sakazakii is an opportunistic pathogen that causes meningitis and necrotizing enterocolitis in neonates. Here we characterized the thermal tolerance of *E. sakazakii* isolates obtained from powdered infant formula and other food products in Japan. Isolates were categorized into three classes according to their thermal tolerance, and differential gene expression analysis showed that the heat-resistant clones expressed a higher level of infB（which encodes a translation initiation factor）, than did the heat-sensitive isolates. Gene expression and DNA polymorphism analyses suggested that this gene target might be useful to unequivocally detect and identify heat-resistant clones, permitting epidemiological surveillance for this pathogen.

Keywords: *Enterobacter sakazakii*, thermal tolerance, gene

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* 国立感染研

Asakura, H., Yamasaki, M*., Yamamoto, S., and Igimi, S.: **Deletion of peb4 gene impairs cell adhesion and biofilm formation in *Campylobacter jejuni*.**

FEMS Microbiology Letters., **275**, 278-285（2007）

Campylobacter jejuni is a microaerophilic bacterium that causes diarrhea in humans. The first step in establishing an infection is adherence to a host cell, which involves two major cell-binding proteins, Peb1A（CBF1）and Peb4（CBF2）. Because the functional role of Peb4 on the cell adhesion remains unclear compared with that of Peb1A, a *C. jejuni* peb4 deletion mutant was constructed and cell adherence and ability to colonize mouse intestine were studied. The result showed that adherence of the peb4 mutant strain to INT407 cells was 1-2% that of the wild-type strain. Mouse challenge experiments showed a reduced level and duration of intestinal colonization by the mutant compared with the wild-type strain. In addition, fewer peb4 mutant cells than wild-type cells responded to stress by forming a biofilm. Proteomic analysis revealed that the expression levels of proteins involved in various adhesion, transport, and motility functions, which are required for biofilm formation by the pathogen, were lower in the peb4 mutant than in the wild-type strain. A Peb4 homolog has prolyl cis/trans-isomerase activity, suggesting that the loss of this activity in the mutant strain may be responsible for the repression of these proteins.

Keywords: *Campylobacter jejuni*, cell-binding protein, mutant

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* 微生物化学研究会

Asakura, H., Panutdaporn, N*., Kawamoto, K*., Igimi, S., Yamamoto, S. and Makino, S.I*.: **Proteomic Characterization of Enterohemorrhagic *Escherichia coli* O157: H7 in the Oxidation-Induced Viable but Non-Culturable State.**

Microbiol. Immunol., **51**, 875-881（2007）

Enterohemorrhagic *Escherichia coli* (EHEC) O157 strain F2, a food isolate of an outbreak, is resistant to oxidative stress, but has increased stress-sensitivity after passage through mice. The stress-sensitive variant of F2 (designated MP37) has decreased culturability, but retains membrane integrity under stress conditions, indicating that the cells enter a viable but non-culturable (VBNC) state. Proteomic analyses revealed that MP37 in the VBNC state had decreased levels of some oxidation-responsive factors (AhpCF, AceF), but it markedly increased levels of outer membrane protein W (OmpW). Because F2 expressed higher levels of some ribosome-associated proteins (RaiA, S6, Bcp) than MP37, the effect of animal passage on the induction of the VBNC state in the EHEC O157 cells might be due to ribosomal activity.

Keywords: EHEC, oxidative stress, VBNC

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* 帯広畜産大学

Sugita-Konishi, Y. Niimi, S. and Sugiyama, K.: **An inter-laboratory study to validate quantitative and qualitative immunoassay kits for screening test of aflatoxin B_1 in corn**

Mycotoxins, **57**, 75-80 (2007)

To validate commercial kit for screening of the presence of AFB_1 in corn, an inter-laboratory study was conducted to evaluate three quantitative and two qualitative immunoassay kits designed for the detection of aflatoxins. Four laboratories performed a screen for the presence of aflatoxin B_1 (AFB_1) in corn. As for the quantitative kits, repeatability relative standard deviation (RSD_r) and reproducibility relative standard deviation (RSD_R) were estimated. One laboratory evaluated the lot variation of each quantitative kit. All kits used in this study showed that the RSD_r and RSD_R were less than 23.3% and 35.7%, respectively, in spiked or naturally contaminated corn samples. As for the qualitative kits, neither false positive nor false negative results were found in corn samples (blank, spiked or naturally contaminated samples) in any laboratories. The RSDs in the lot variation of the same quantitative kit was less than 46.5% in both two brands. The results appeared to indicate that all kits tested in this study could be validated for the screening of the presence of AFB_1 in corn, and were also available for the first step of the detection of AFB_1 at levels of more than 5 ng/g.

Keywords: An inter-laboratory study, immunoassay kits, aflatoxin, corn

Sugita-Konishi, Y.: **Toxicity and control of trichothecene mycotoxins**

Mycotoxins, **58**, 23-28 (2008)

Trichothecenes, such as deoxynevalenol (DON), nivalenol (NIV) and T-2 toxin are typical immunotoxic mycotoxins. Contamination of trichothecene mycotoxins in wheat is a serious world-wide problem affecting human health. In Japan, in particular, it has been reported that relatively high levels of DON and NIV are frequently found in domestic wheat. I have extensively conducted studies on the toxicity and control of trichothecene mycotoxins in order to assess their risk, and this paper is the summary of those findings.

Keywords: Trichthecens, toxicity, reduction, analytical method

Takahashi, M., Shibutani, M., Sugita-Konishi, Y., Aihara, M., Inoue, K., Woo, G-H., Fujimoto, H. and Hirose, M.: **A 90-day subchronic toxicity study of nivalenol, trichothecene mycotoxin, in F344 rats**

Food Chem. Toxicol., **46**, 125-135 (2008)

A subchronic toxicity study of nivalenol (NIV) was conducted in male and female F344 rats fed diet containing 0, 6.25, 25 or 100 ppm concentration for 90 days. Suppression of body weight and loose stool were observed at 100 ppm in both sexes from the start of the experiment, and the body weight suppression was also observed at 25 ppm in males from week 6. At necropsy, many organs reduced the absolute weight at 100 ppm in both sexes mostly due to the reduction in the body growth, among them reduction of relative thymus weight also being evident in females. Hematologically, decrease of white blood cell counts was found at 100 ppm in males and from 6.25 ppm in females. In addition, decreases of platelet counts in both sexes, red blood cell counts in males, and hemoglobin concentration in female were detected at 100 ppm. Histopathologically, treatment-related changes were predominantly observed in the hematopoietic and immune organs as well as the anterior pituitary in both sexes and female reproductive system at 100 ppm, such as thymic atrophy, hypocellularity in the

bone marrow, diffuse hypertrophy of basophilic cells in the anterior pituitary, and increase of the ovarian atretic follicles. Based on the hematological data, the no-observed-adverse-effect level of NIV was determined to be less than 6.25 ppm (0.4 mg/kg body weight/day for both males and females).

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Keywords: Subchronic toxicity, nivalenol, mycotoxin, trichothecene, F344 rats

Kubosaki, A., Aihara, M., Park, B-J. Sugiura, Y.[*1], Shibutani, M., Hirose, M. Suzuki, Y.[*2], Takatori, K. and Sugita-Konishi, Y.: **Immunotoxicity of Nivalenol after Subchronic Dietary Exposure to Rats**

Food Chem. Toxicol., **46**, 253-258 (2008)

Immunobiological effects of nivalenol (NIV), a trichothecene mycotoxin produced by *Fusarium nivale*, were examined after 90-day dietary exposure to male F344 rats at doses of 0, 6.25, 25 and 100 ppm. Although the 90-day dietary study was performed in both sexes, we focused on male in this study because there was possibility that NIV caused the hormone unbalance in female. With regards to the serum immunoglobulin levels, a slight increase of IgM was observed only at 100 ppm (26% increase), while levels of IgG and IgA did not fluctuate at any dose. Flow cytometric analysis of splenic cells revealed a decrease CD3/B220 ratio and an elevated $CD4^+$helper/$CD8^+$cytotoxic T lymphocyte ratio at 25 ppm and100 ppm, respectively. Furthermore, increases of natural killer (NK) activity of splenic lymphocytes against YAC-1 target cells were observed at all doses, while the magnitude of changes was similar between 25 and 100 ppm. At 100 ppm, the reduction of the NKR-$P1A^+$ splenic cell counts, which is an indicator of NK cells in the spleen, was apparent. In summary, NIV affected immune functions in rats after a 90-day dietary exposure, the effects being apparent from 25 ppm judging from the decrease of splenic T cell population, while the increase of NK activity was apparent from 6.25 ppm.

Keywords: Nivalenol, Subchronic exposure, Immunotoxicity, Flow cytometry, Natural killer activity, F344 rats.

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Mino, Y.[*1], Amano, F. [*1], Yoshioka, T.[*2] and Konishi, Y.: **Determination of Organotins in Human Breast Milk by Gas Chromatography with Flame Photometric Detection**

J. Health Science, **54**, 224-228 (2008)

An analytical method for the quantitative determination of monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT) and triphenyltin (TPhT) compounds in human breast milk is described. After the addition of surrogates (deuterium derivatives), milk samples were extracted with hexane-diethyl ether (4:6) in the presence of HCl and NaCl. Each extract was purified by cation exchange chromatography and treated with Grignard reagent to yield ethyl derivatives, which were determined by gas chromatography (GC) with flame photometric detection operated in the tin mode (610nm) . These organotinchlorides, spiked to milk at 12.5, 25, and 50ng/ml (ppb), were recoverd within a range of 85 to 105%. Detection limits were 1.3ng/ml for DBT, TBT, and TPhT, and 2.5ng/ml for MBT. This analytical method was used to determine organotins in about 70 breast milk samples obtained from mothers who had given birth within the previous week. DBT dichloride levels varied from undetectable to 9.5ng/ml in human milk from mothers who habitually ate fish, however, the other organotins were not detectable. No significant difference was observed in DBT contents between mothers who ate fish more than twice a week and those who ate fish less than once a week. Thus, since the levels of organotin even in the milk of mothers who liked to eat fish were very low, human breast milk should be considered safe for feeding infants, at least concerning with regard to the possible transmission of organotin compounds.

Keywords: human breast milk, tributyltin, dibutyltin, monobutyltin, organotins, gas chromatography/ flame photometric detector

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Dong, K.[*1], Sugita-Konishi, Y., Yu, J[3], Tulayakul, P.[*1], and Kumagai, S.[*1]: **The effects of subcutaneous administration of T-2 toxin on liver drug metabolizing enzymes in piglets**

Toxicol. Environ. Chemistry, **90**, 401-413 (2008)

T-2 toxin is one of trichothecenes, which are a structurally diverse group of toxic secondary metabolites produced by *Fusarium* and related species of fungi. The toxin usually contaminates cereal grains throughout the world. Although the pig is increasingly being used in pharmacological and toxicological studies, there is not enough information about the effects of T-2 toxin on drug metabolizing enzymes in pugs. The purpose of this study is to investigate the effects of subcutaneous administration of T-2 toxin on the activities of hepatic Phase Ⅰ and Phase Ⅱ metabolizing enzymes in piglet liver. Piglets were administrated 0.3mg T-2 toxin/Kg BW dissolved in DMSO by single subcutaneous (s.c.) injection. Control animals received only vehicle (DMSO). The activities of Phase Ⅰ and Phase Ⅱ enzymes were determined at 24 and 48h after the last s.c. injection. The activities of cytochrome P-450 (CYP) 1A2 and 2E1 increased slightly at 24 and 48h ($P < 0.05$). The CYP3A4 activity increased at 24 ($P < 0.01$), and tended to decrease at 48h, but not significantly ($P > 0.05$). The glutathione S-transferase (GST) activity towards cumene hydroperoxide increased slightly at 24h ($P < 0.05$), but decreased slightly at 48h ($P < 0.05$). No significant changes were observed in the glutathione S-transferase activity toward 1-Chloro-2,4-dinitrobenzene (CDNB) either at 24 or 48h. Western blot analyses of the liver fractions revealed increased levels of CYP1A2, 2E1, 3A4, GSTα, GST M1-1 at 24h, and that of CYP2E1 at 48h. The results suggest that T-2 toxin causes modulation of Phase Ⅰ and Phase Ⅱ drug metabolizing enzymes in piglets.

Keywords: T-2 toxin, glutathione S-transferase (GST), cytochrome P-450 (CYP), drug metabolizing enzymes, western blotting assays, piglets

[*1] Laboratory of Veterinary Public Health, Graduate School of Agriculture and Life Sciences,

Ohnishi T., Muroi M., Tanamoto K.: **The lipopolysaccharide-recognition mechanism in cells expressing TLR 4 and CD14 but lacking MD-2.**

FEMS Immunol. Med. Microbiol., **51**, 84-91 (2007)

When TLR4 and CD14 were transiently expressed in HEK293 cells, cell-surface TLR4 expression was observed. Membrane CD14-TLR4 complexes were formed in these cells in response to LPS stimulation even in the absence of MD-2, although NF-κB-dependent reporter activity was not induced. NF-κB activation was observed when these cells were stimulated with LPS followed by soluble MD-2 in this order, even when excess LPS was removed after the CD14-TLR4 complex formation by washing cells prior to sMD-2 addition. From these results, we propose an additional LPS-recognition mechanism. In cells expressing TLR4 and CD14 but lacking MD-2, LPS is first transferred to membrane CD14 with the aid of LPS binding protein, which leads to the formation of the TLR4-CD14 complex. Then, the binding of soluble MD-2 to this complex triggers the transmembrane signal transduction. Cells expressing TLR4 and CD14 but lacking MD-2, such as airway epithelial cells, may be activated in response to LPS by this mechanism.

Keywords: Toll-like receptor, NF-κB, lipopolysaccharide

Yokota S.[*1], Ohnishi T., Muroi M., Tanamoto K., Fujii N.[*1], Amano K.[*1]: **Highly-purified *Helicobacter pylori* LPS preparations induce weak inflammatory reactions and utilize Toll-like receptor 2 complex but not Toll-like receptor 4 complex.**

FEMS Immunol. Med. Microbiol., **51**, 140-148 (2007)

We found that the LPS-low-responder stomach cancer cell line MKN28, which expresses TLR4 at extremely low levels, showed similar levels of IL-8 induction by *H. pylori* or *E. coli* LPS preparations. Weak IL-8 induction by *H. pylori* LPS was suppressed by a dominant negative mutant of TLR2 but not of TLR4. Luciferase reporter analysis indicated that co-transfection of TLR2-TLR1 or TLR2-TLR6 was required for the activation induced by *H. pylori* LPS. In conclusion, *H. pylori* LPS significantly induces an inflammatory reaction via the receptor complex containing TLR2-TLR1 or TLR2-TLR6 but not that containing TLR4. The TLR2-TLR1 complex was preferentially recognized by *H. pylori* LPS over the TLR2-TLR6 complex.

Keywords: Toll-like receptor, *Helicobacter pylori*, lipopolysaccharide

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*2 秋田大

Muroi M, Tanamoto K.: **TRAF6 distinctively mediates MyD88- and IRAK-1-induced activation of NF-κB.**

J. Leuk. Biol., **83**, 702-707（2008）

We found in this study that a dominant-negative mutant of TRAF6, lacking the N-terminal RING and zinc-finger domain, did not inhibit IRAK-1-induced activation of NF-κB in HEK293 cells, although the TRAF6 mutant strongly suppressed the MyD88-induced activation. The dominant-negative mutant of TRAF6 did not affect the IRAK-1-induced activation, regardless of the expression level of IRAK-1. In contrast, siRNA silencing of TRAF6 expression inhibited MyD88- induced and IRAK-1-induced activation, and supplementation with the TRAF6 dominant-negative mutant did not restore the IRAK-1-induced activation. Expression of IRAK-1, but not MyD88, induced the oligomerization of TRAF6, and IRAK-1 and the TRAF6 dominant-negative mutant were associated with TRAF6. These results indicate that TRAF6 is involved but with different mechanisms in MyD88-induced and IRAK-induced activation of NF-κB and suggest that TRAF6 uses a distinctive mechanism to activate NF-κB depending on signals.

Keywords: Toll-like receptor, TRAF6, IRAK-1

Ohnishi T., Yoshida T., Igarashi A., Muroi M., Tanamoto K.: **Effects of possible endocrine disruptors on MyD88- independent TLR4 signaling.**

FEMS Immunol. Med. Microbiol., **52**, 293-295（2008）

To evaluate the influence of endocrine disrupting chemicals（EDCs）on the innate immune function of macrophages, we investigated the effects of 37 possible EDCs on LPS-induced activation of the IFN-β promoter. Alachlor, atrazine, benomyl, bisphenol A, carbaryl, diethyl phthalate, dipropyl phthalate, kelthane, kepone, malathion, methoxychlor, octachlorostyrene, pentachlorophenol, nonyl phenol, *p*-octylphenol, simazine and ziram all inhibited the activation. Kepone and ziram showed strong inhibitory effects. Aldicarb, amitrole, benzophenone, butyl benzyl phthalate, 2,4-dichlorophenoxy acetic acid, dibutyl phthalate, 2,4-dichlorophenol, dicyclohexyl phthalate, diethylhexyl adipate, diethylhexyl phthalate, dihexyl phthalate, di-*n*-pentyl phthalate, methomyl, metribuzin, nitrofen, 4-nitrotoluene, permethrin, trifluralin, 2,4,5-trichlorophenoxy- acetic acid and vinclozolin had no significant effects at 100 μM. These results suggest that endocrine disruptors may influence the development of infectious diseases.

Keywords: macrophages, endocrine disrupting chemicals, Toll-like receptor

宮原美知子，宮原　誠：**塩漬け野菜保存での腸管出血性大腸菌O157の生残性**

防菌防黴誌，**35**，779-783（2007）

腸管出血性大腸菌O157: H7はヒト，特に若い子供や高齢者にひどい病気を引き起こす．塩漬け野菜（浅漬け）での大腸菌O157: H7汚染により，食中毒や患者の死までも引き起こされた．大腸菌O157: H7の塩水あるいは塩漬け野菜中での生残を検討した．冷蔵あるいは冷凍中において大腸菌O157: H7の大きな菌数減少はみられなかった．しかし，電子線照射（0.534, 1,097と2.639 kGy）によってはっきりとした大腸菌O157: H7の菌数減少がみられた．生菌数と大腸菌群数についても計測し，大腸菌O157:H７の菌数変化と比較を行った．

Keywords: Salted vegetable/*E. coli* O157: H7/Electron-beam irradiation

Matsutani, S.: **Possible presence and role of the promoter sequence for eukaryotic RNA polymerase III in bacteria**

Genetica, **131**, 127-134（2007）

The bacterial repetitive sequence IS1, is a translocatable DNA segment. The internal region of IS1 acts as a *cis*-element to stimulate RNA synthesis from the upstream promoter. The product of the bacterial *artA* gene works with this *cis*-element to stimulate transcription. Eukaryotic genes for small RNAs and short interspersed repetitive elements（SINEs）have internal promoters, transcribed by RNA polymerase III（RNAP III）. RNAP III requires the multisubunit protein factor TFIIIC in transcription initiation. TFIIIC contains the B-block binding subunit which recognizes the internal promoter. Here, I report that the eukaryotic RNAP III promoter-like sequence was found in the *cis*-element of bacterial IS1. Mutations in the *cis*-element which affect transcription were present in the RNAP III promoter-like sequence. The RNAP III promoter sequence of Alu, which is a human SINE, was cloned into *Escherichia coli*, and was shown to stimulate bacterial transcription like the *cis*-element of IS1. Furthermore, the primary structures of ArtA protein

and B-block binding subunits were compared. The amino acid sequence of ArtA appeared to be similar to the N- and C-terminal regions conserved in many B-block binding subunits. Prokaryotes and eukaryotes have been thought to have inherent transcription machineries. The results shown here, however, suggest a new aspect of the evolution of the RNAP III transcription machinery.

Keywords: evolution, RNA polymerase III, transcription initiation

Hara-Kudo, Y., Niizuma, J.[*1], Goto, I.[*2], Iizuka, S.[*1], Kamakura, K.[*2], Kaji, Y.[*1], Suzuki, S[*2]. and Takatori, K.: **Surveillance of Shiga toxin-producing *Escherichia coli* in Beef with Effective Procedures, Independent of Serotype**.

Foodborne Pathogens and Disease, **5**, 98-104（2008）

To detect various serotypes of Shiga toxin-producing *Escherichia coli*（STEC）in food, methods independent of serotyping are needed. We established procedures to isolate STEC using a rapid and sensitive loop mediated isothermal amplification（LAMP）assay targeting the Shiga toxin（ST）gene and a method of plating onto media for the selection of *E. coli*, LAMP assay positive dilutions were plated onto selective media. After incubation, suspension of a colony or some colonies was tested in LAMP assay. Positive suspension was diluted and plated onto selective media. The procedure was repeated. Finally, LAMP positive colony was confirmed as STEC and serotype. As a result of surveillance in beef in 2005-2007, 11 of 720 samples（1.5 %）tested positive for ST gene by LAMP assay. Serotype O8, O128 and O-untypable STEC were isolated from the samples by the newly established procedure. It was demonstrated that the procedure was effective to detect STEC independent of serotype.

Keywords: Shiga toxin-producing *Escherichia coli*; detection

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Hara-Kudo, Y., Konishi, N[*1]., Otsuka, K.[*2], Hiramatsu, R.[*3], Tanaka, H.[*4], Konuma, H.[*5] and Takatori, K.：**Detection of Verotoxigenic *Escherichia coli* O157 and O26 in food by plating methods and LAMP method: A collaborative study**.

Int. J. Food. Microbiol., **122**/1-2, 156-161（2008）

In order to establish a rapid and sensitive method for the detection of Verotoxigenic *Escherichia coli* O157 and O26, a collaborative study was conducted focusing on a comparison of the efficiency of loop-mediated amplification（LAMP）assay targeting the Verocytotoxin（also called Shiga toxin）gene, utilizing a direct plating method and a plating method with immunomagnetic separation（IMS-plating method）using various agar media. In combination with enrichment with the modified EC supplemented with novobiocin, *E. coli* O157 was detected in most samples of ground beef and alfalfa sprouts by LAMP assay, the direct plating method and the IMS-plating method. *E. coli* O26 was detected in approximately 100% of the food samples by LAMP assay. However, the IMS-plating and direct plating methods recovered 80 and 50% in ground beef samples, respectively. As a result, it was demonstrated the LAMP assay is superior to the IMS-plating method. Based on these results, it appears LAMP assay is effective as a screening assay to detect *E. coli* O157 and O26 from positive samples.

Keywords: Verotoxigenic *Escherichia coli*, O157, O26, detection, collaborative study

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Yoneyama, N.[*1], Hara-Kudo, Y. and Kumagai, S.[*1]: **Effects of Heat-degraded Sugars on Survival and Growth of *Vibrio parahaemolyticus* and Other Bacteria**.

J. Food Prot., **70**, 373-377（2007）

We studied the effects of autoclaved（121℃, 15 min）sugar solutions on the survival and growth of Vibrio parahaemolyticus and other bacteria. The growth and survival of V. parahaemolyticus in Luria-Bertani media and phosphate buffer, respectively, were inhibited by the addition of D-glucose autoclaved in pH 8.0 phosphate buffer. The bactericidal effect of autoclaved D-glucose was very small when autoclaved in pH 7.0 phosphate buffer, but larger effects were observed when autoclaved in the buffer at an alkaline pH. The autoclaving of D-glucose in CH3COONa, NaHCO3, and Na2HPO4

solutions at pH 7.6 to 8.5 also generated bactericidal effects, but it was not the case when D-glucose was autoclaved in Na2SO4, (NH4)2SO4, or NH4Cl solution at pH 8.0. The same effects as autoclaved D-glucose were observed in autoclaved lactose, D-fructose, and D-ribose. The bactericidal effects of autoclaved D-glucose were also noted in Salmonella Enteritidis, Listeria monocytogenes, and E. coli strains, but the effects were smaller than those seen in V. parahaemolyticus and V. vulnificus. The growth of V. parahaemolyticus in clam extracts was also inhibited by the addition of autoclaved D-glucose, indicating that heat-treated reduced sugars can exert bactericidal effects in foods.
Keywords: Heat-degraded, sugars, survival, growth, *Vibrio parahaemolyticus*

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Goto, M., Takahashi, H., Segawa, Y., Hayashidani, H., Takatori, K., Hara-Kudo, Y.: **Real-time PCR method for quantification of *Staphylococcus aureus* in milk**.
J. Food Prot., **70**, 90-96（2007）

A reproducible real-time PCR method targeting the putative transcriptional regulator gene of *Staphylococcus aureus* was developed to quantify this microorganism in milk samples. Based on the partial sequences of this gene determined from S. aureus strains, we designed specific primers and probe for use in the quantitative PCR assay. These specificities were confirmed using 25 strains of *S. aureus* and 35 strains of other bacteria. Real-time PCR assay using serial 10-fold dilutions of purified DNA and pure culture was conducted. It was possible to construct standard curves with a high correlation coefficient ($r^2 = 0.99$) in the range of 50 ng to 50 fg for purified DNA and 10^7 to 10^1 cfu/ml for pure culture. The constructed standard curve for milk samples was similar to that of pure culture and quantification of *S. aureus* in the range of 10^7 to 10^1 cfu/ml was possible. Moreover, we examined the effect of heat treatment as pasteurization of milk on the quantification by the real-time PCR method. The quantification was affected after heat treatment at 63℃ for 30 min (low-temperature long-time method) but not at 72℃ for 15 sec (high-temperature short-time method). The results indicate that the real-time PCR method developed in this study is effective for monitoring *S. aureus* contamination in milk because of its high specificity and sensitivity.
Keywords: *Staphylococcus aureus*, real-time PCR, quantification, milk

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Otomo, Y., Abe, K., Odagiri, K., Shiroto, A., Takatori, K. and Hara-Kudo, Y.: **Detection of *Salmonella* in spent hens and eggs associated with foodborne infections**.
Avian Diseases., **51**, 578-583（2007）

About 16,000 spent hens from 23 farms in the North area of Japan in 1996, 1997, 1998 and 1999 were purchased to isolate *Salmonella* in two poultry processing plants. *Salmonella* was detected in 12 of 23 farms (52.2 %). In particular, the serotypes Enteritidis and Infantis were detected in four and three farms, respectively. The prevalence rates in the hens'ceca, immature eggs and the yolk of mature eggs in oviducts were 14 %, 7.2% and 6.8%, respectively. A total of 23 serotypes were detected. Although major serotypes were Enteritidis, Corvallis, Typhimurium and Infantis strains were isolated in larger numbers than other serotypes, although untypable was most major of the strains. In the same area during 1992 to 1996, *Salmonella* was detected in eggs associated with four outbreaks of Salmonella enterica, serovar Enteritidis infection and one outbreak of *Salmonella* enterica serovar Infantis infection. The ratio of contamination was appeoximately 1%, and the level was estimated to be 93 MPN/100 g in one outbreak. In farms that produced the eggs associated with all of the five outbreaks of *Salmonella*, the serotype Enteritidis or Infantis was isolated from hens. Farms where *Salmonella* was not detected were not related to any of the outbreaks.
Keywords: *Salmonella*, Enteritidis, spent hens, laying farm, egg

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Goto, M., Hayashidani, H.*1, Takatori, K., Hara-Kudo,

Y. : **Rapid detection of enterotoxigenic *Staphylococcus aureus* harboring genes for four classical enterotoxins, SEA, SEB, SEC and SED, by loop-mediated isothermal amplification assay**.

Lett. Appl. Microbiol., **45**, 100-107（2007）

Aims: The aim of this study was to develop a loop-mediated isothermal amplification（LAMP）assay targeting the genes for the four classical enterotoxins SEA, SEB, SEC and SED in *Staphylococcus aureus*.

Methods and Results: Specific primers were designed which target each specific sequence of the enterotoxin genes. With 30 strains of *S. aureus*, the results of the LAMP assay to each enterotoxin SEA to SED completely accorded with the results of PCR assay. Enterotoxin production, determined by a reverse passive latex agglutination assay,（was）strongly correlated with the（possession）presence of the corresponding genes. Amplification was not observed when 14 strains of non-enterotoxigenic *S. aureus* and 20 strains consisting of 19 bacterial species other than *S. aureus* were tested. In addition, the sensitivity of the LAMP assay was generally higher than that of conventional PCR and it（could）rapidly detected enterotoxigenic *S. aureus* strains within 60 minutes.

Conclusions: The LAMP assay developed in this study is（are）rapid, specific and sensitive for the detection of enterotoxigenic *S. aureus*.

Significance and Impact of the Study: The method is suitable for clinical diagnosis and food safety applications.

Keywords: *Staphylococcus aureus*, enterotoxin, LAMP, rapid, detection

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永島江美子[*1]，小田雄一郎[*2]，小澤一弘[*3]，仁科徳啓[*3]，工藤由起子，小沼博隆[*2]：***Vibrio vulnificus*の清水港湾内における分布**.

日本食品微生物学会, **24**, 189-193（2007）

全国的な規模の*V. vulnificus*に関する海水汚染調査が実施された報告は見られず，日本近海における本菌の分布は不明な点が多い．そこで海水浴，釣りなど住民が海水と接する機会が多い清水港の海水，カキおよび泥（海泥，川泥）を対象に*V. vulnificus*の汚染実態を調査した．その結果，*V. vulnificus*の検体別の汚染菌数は，海水で10 MPN/100 mlに満たないことが多かった．カキでは30～150 MPN/100 g，泥は100 MPN/100 gを超えるものが多かった．清水港湾における*V. vulnificus*の検出率は，泥から94.4％，カキから75.0％および海水から51.6％であった．このことから清水港湾内の海水における*V. vulnificus*は，腸炎ビブリオの生態と同様に海水よりも泥・カキで多く検出された．今後は有機物が多い海泥，川泥，カキならびに底生動物などを採取し，それらが*V. vulnificus*の増殖の場になることを明らかにする必要があると考えられる．

Keywords: *Vibrio vulnificus*, seawater, oyster, sea mud

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Kikuchi, Y., Kakeya, T., Nakajima, O., Sakai, A., Ikeda, K.[*1], Yamaguchi, N.[*1], Yamazaki, T., Tanamoto, K., Matsuda, H.[*2], Sawada, J. and Takatori, K: **Hypoxia induces expression of a GPI-anchorless splice variant of the prion protein**

FEBS J., **275**, 2965-2976（2008）

ヒトグリオーマ細胞株T98はPRNPエクソン２内にスプライス変異を生じてPrPのGPIアンカーシグナルが欠損したmRNAを発現し，ヒト脳及び各種ヒト臓器で発現していることを確認した．スプライス変異型mRNAのORFはPrPのN端側１-217残基と共通で，新たに13残基がC端に結合した230残基の蛋白質をコードし，イムノブロット法でT98G細胞がGPI欠損型プリオン蛋白質（$GPI^{-}PrPSV$）を産生すること確認した．スプライス変異は酸素濃度で調節されており，通常の酸素濃度下では発現量が低く，低酸素状態下で誘導された．

Keywords: alternative splicing, Creudzfeldt-Jakob disease, GPI anchor, hypoxia, prion

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Tada, N.[*], Nakao, A.[*], Saka, M.[*], Kamata, Y: **Vitellogenin, a biomarker for environmental estrogenic pollution of Reeves'pond turtles: analysis of similarity for its amino acid sequence and cognate mRNA expression after exposure to estrogen**

J. Vet. Med. Sci., **70**, 227-234（2008）

Vitellogenin（VTG）, a biomarker for environmental estrogenic pollution, can be detected in the bloodstream of oviparous animals before morphological and

functional abnormalities appear due to exposure to environmental estrogens. Reports observing VTG in turtles have been limited. We therefore cloned and sequenced a partial cDNA of VTG in Reeves'pond turtle, *Chinemys reevesii*. The cloned cDNA fragment possessed the start codon and 2,229 bp, encoding 743 amino acid residues. A sequence of deduced amino acid from the cDNA did not contain a high serine content, such as that which exists in phosvitin. Two N-glycosylation sites were found in the sequence. The sequence was compared to those of two birds (chicken and herring gull), one amphibian (xenopus), and five fishes (carp, zebrafish, eel, haddock, and red seabream). The *C. reevesii* VTG was similar to that of herring gull (78%, value of positives), chicken (76%), Xenopus (69%), eel (63%), red seabream (62%), haddock (62%), carp (62%), and zebrafish (61%). The phylogenetic tree showed that *C. reevesii* VTG existed between the amphibian and birds, and it was present far from fish VTGs. A reverse transcription-polymerase chain reaction method was employed to detect the mRNA expression of the *C. reevesii* VTG. The VTG mRNA expression (292 bp) was proven in the total RNA extraction from the liver of the juvenile turtles which were treated with estradiol-17β. The information herein would be useful for ecotoxicological studies using fresh-water turtles and these findings are expected to contribute positively towards wildlife conservation.

Turtle vitellogenin, Cloning, mRNA expresssion

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Rumiko Shimazawa[*1], Naomi Nagai[*2], Satoshi Toyoshima[*2], and Haruhiro Okuda: **Present State of New Chiral Drug Development and Review in Japan**

J. Health Sci., **54**, 23-29 (2008)

The current situation of chiral drug development in Japan was investigated. The trend in the Japanese pharmaceutical development is increasingly moving towards the development of single isomers rather than racemates. The development of single-enantiomer drugs was made possible by the current technologies of asymmetric synthesis and chiral separation, and encouraged by the guidelines on the development of chiral drugs worldwide. Japan has not issued specific guidelines on the development of chiral drugs, however, the chiral drug development approached in Japan were essentially consistent with the approaches recommended by the US and EU guidelines.

Keywords: chiral drugs, single-enantiomer drugs, racemic drugs

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Fukuhara, K., Nakanishi, I.[*1], Matsuoka, A., Matsumura, T.[*2], Honda, S.[*3], Hayashi, M.[*3], Ozawa, T.[*4], Miyata, N.[*5], Saito, S.[*2], Ikota, N.[*6], Okuda, H: **Effect of methyl substitution on antioxidative property and genotoxicity of resveratrol**

Chem. Res. Toxicol., **21**, 282-287 (2008)

Resveratrol (*trans*-3,4',5-trihydroxystilbene) is a natural phytoalexin with various biological activities including inhibition of lipid peroxidation and free radical scavenging properties. In addition to its beneficial effects, resveratrol also has significant genotoxicity that leads to a high frequency of chromosome aberration together with micronucleus and sister chromatid exchanges. In order to enhance the radical scavenging activities and to reduce the genotoxicity of resveratrol, we designed 4'-methyl resveratrol analogues where a methyl group was introduced at the ortho position relative to the 4'-hydroxy group, which is responsible for both antioxidative activities and genotoxicity of resveratrol. These synthesized methyl analogues of resveratrol showed increased antioxidative activities against galvinoxyl radical as an oxyl radical species. Furthermore, the methyl analogues also surprisingly showed reduced in vitro genotoxicities, suggesting methyl substitution may improve resveratrol efficacy.

Keywords: resveratrol, antioxidant, genotoxicity

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Nakanishi, I.[*1], Shimada, T.[*2], Ohkubo K.[*3], Shimizu, T.[*2], Urano, S.[*2], Okuda, H., Miyata, N.[*4], Ozawa, T.[*5], Anzai, K.[*1], Fukuzumi, S.[*3], Ikota, N.[*6], Fukuhara, K.:

Involvement of electron transfer in the radical-scavenging reaction of resveratrol

Chem. Lett., **36**, 1276-1277（2007）

Resveratrol（3,4',5-trihydroxy-trans-stilbene）efficiently scavenges an oxygen radical via an electron transfer from resveratrol to the radical in deaerated acetonitrile, which is significantly accelerated by the presence of magnesium ion. The mechanistic information obtained in this study suggests that the introduction of electron-donating group, such as methyl and methoxy groups, may stabilize the intermediate radical cation, resulting in the enhancement of the antioxidative abilities of resveratrol.

Keywords: resveratrol, antioxidant, radical

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Manda, S.*1, Nakanishi, I.*1, Ohkubo, K.*2, Uto, U.*3, Kawashima, T., Fukuhara, K., Okuda H., Hori, H.*3, Ozawa, T.*4, Ikota, N.*5, Fukuzumi, S.*2 and Anzai K.*1: **Enhanced Radical-Scavenging Activity of Naturally-Oriented Artepillin C Derivatives**

Chem. Commun., 626-628（2008）

Artepilin C［3-｛4-hydroxy-3,5-bis(3-methyl-2-butenyl) phenyl｝-2(E)-propenoic acid］, a major component of Brazilian propolis, has been reported to show antioxidative activity alongside other important biological activities. We reported herein the synthesis of five naturally-oriented artepillin C derivatives and their enhanced scavenging activity toward cumylperoxyl radical（$PhCMe_2COO\cdot$）. $PhCMe_2COO\cdot$, which is less reactive than alkoxy radical, which is known to follow the same pattern of relative reactivity with a variety of substrates. The structure-activities relationship is also discussed based on the results obtained in this study, providing a valuable insight into the development of antioxidants stronger than the naturally occurring ones.

Keywords: artepillin C, propolis, antioxidant

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Takuya Matsumoto*, Yasuteru Urano*, Takuji Shoda, Hirotatsu Kojima*, and Tetsuo Nagano*: **A Thiol-Reactive Fluorescence Probe Based on Donor-Excited Photoinduced Electron Transfer: Key Role of Ortho Substitution**

Org. Lett., **9**, 3375 -3377（2007）

We designed and synthesized a novel thiol-reactive fluorescence probe based on the BODIPY fluorophore. The fluorescence of this probe is strongly quenched by donor-excited photoinduced electron transfer（d-PeT）from BODIPY to maleimide, but after reaction with thiol, the fluorescence of BODIPY is restored, affording a 350-fold intensity increase.

Keywords: thiol, fluorescence, donor-photoinduced electron transfer

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Tanaka, M.*, Demizu, Y.*, Nagano, M.*, Hama, M.*, Yoshida, Y.*, Kurihara, M., Suemune, H.*: **Lipase-Catalyzed Kinetic Resolution of Cyclic trans-1,2-Diols Bearing a Diester Moiety: Synthetic Application to Chiral Seven-Membered Ring α,α-Disubstituted α-Amino Acid**

J. Org. Chem., **72**, 7750 -7756（2007）

Chiral cycloalkane-trans-1,2-diols（±）-3 and（±）-8 having a diester moiety have been prepared from dimethyl dialkenylmalonate using olefin metathesis by Grubbs catalyst, followed by epoxidation and acidic hydrolysis. Kinetic resolution of racemic cyclopentane-trans-1,2-diol（±）-3 by lipase-catalyzed transesterification afforded an optically active monoacetate（−）-5 of 95% ee in 46% yield and the recovered diol（−）-3 of 92% ee in 51% yield, and that of cycloheptane-trans-1,2-diol（±）-8 gave a monoacetate（+）-10 of 95% ee in 51% yield and the diol（−）-8 of >99% ee in 43% yield, respectively. The enantiomer selectivity of racemic cyclic trans-1,2-diols bearing a diester moiety by lipases（Amano PS and Amano AK）was opposite to that of the reported simple racemic cycloalkane-trans-1,2-diols. To explain the lipase-catalyzed enantiomer selectivity, computer modeling of lipase-substrate complexes was performed. Furthermore, the optically active diester（−）- 8 could be eff-

iciently converted into an optically active seven-membered-ring ,-disubstituted amino acid (4R,5R)-(−)-15.
Keywords: lipase, asymmetric synthesis

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Satoh, T.[*1], Cowieson, N.[*2], Hakamata, W., Ideo, H.[*3], Fukushima, K.[*3], Kurihara, M., Kato, R.[*1], Yamashita, K.[*3], Wakatsuki, S.[*1]: **Structural basis for recognition of high-mannose type glycoproteins by mammalian transport lectin VIP36**

J. Biol. Chem., **282**, 28246-28255 (2007)

VIP36 functions as a transport lectin for trafficking certain high mannose type glycoproteins in the secretory pathway. Here we report the crystal structure of VIP36 exoplasmic/luminal domain comprising a carbohydrate recognition domain and a stalk domain. The structures of VIP36 in complex with Ca^{2+} and mannosyl ligands are also described. The carbohydrate recognition domain is composed of a 17-stranded antiparallel β-sandwich and binds one Ca^{2+} adjoining the carbohydrate-binding site. The structure reveals that a coordinated Ca^{2+} ion orients the side chains of Asp131, Asn166, and His190 for carbohydrate binding. This result explains the Ca^{2+}-dependent carbohydrate binding of this protein. The Man-α-1,2-Man-α-1,2-Man, which corresponds to the D1 arm of high mannose type glycan, is recognized by eight residues through extensive hydrogen bonds. The complex structures reveal the structural basis for high mannose type glycoprotein recognition by VIP36 in a Ca^{2+}-dependent and D1 arm-specific manner.
Keywords: lectin, VIP36, glycoprotein

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Kurihara, M., Sato, Y., Hakamata, W., Okuda, H., Demizu, Y.[*1], Nagano, M.[*1], Kawabe, N.[*1], Doi, M.[*2], Tanaka, M.[*1], Suemune, H.[*1]: **Computational Study on Conformation of Oligopeptides Containing Chiral Cyclic α,α-Disubstituted α-Amino Acids**

Peptides 2006, 546-547 (2007)

Conformational search calculations of oligopeptides 1, 2, containing chiral cyclic α,α-disubstituted amino acids, have performed using the Monte Carlo method of MacroModel (ver. 8.1, Schröinger, Inc.). When AMBER* force field was used, the global minimum energy conformation of peptide 1 was a left-handed α-helix, which was more stable than a left-handed 3_{10}-helix by 4.2 kcal/mol. The results were in agreement with its X-ray structure, which showed a left-handed α-helix
Keywords: α,α-disubstituted α-amino acid, conformational search, 3_{10}-helix

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Tanaka, M.[*1], Nagano, M.[*1], Demizu, Y.[*1], Anan, K.[*1], Kurihara, M., Doi, M.[*2], Suemune, H.[*1]: **Side-chain chiral centers of amino acids and helical-screw handedness of their peptides**

Peptides 2006, 268-269 (2007)

We designed and synthesized an optically active bicyclic α,α-disubstituted α-amino acid; (R,R)-Ab(5,6=)c, in which the α-carbon atom is not a chiral center but the asymmetric centers exist at the side-chain bicyclic skeleton. The amino acid (R,R)-Ab(5,6=)c was synthesized from (S,S)-cyclohex-4-ene-1,2-dicarboxylic acid 1.
Keywords: α,α-disubstituted α-amino acid, helical-screw

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Nagano, M.[*1], Tanaka, M.[*1], Demizu, Y.[*1], Kurihara, M., Doi, M.[*2], Suemune, H.[*1]: **Secondary Structure of Heteropeptides Using Chiral Cyclic α,α-Disubstituted Amino Acids**

Peptides 2006, 476-477 (2007)

The chiral cyclic amino acid was incorporated into Aib sequence by solution-phase methods; the (*S*,*S*)-Ac(5)c(dOM) was introduced to the N-terminal, to the C-terminal, and at the center position of Aib peptides. Conformational analysis by using the ^{1}H NMR, FT-IR, and X-ray crystallographic analysis revealed that dominant conformation of the Aib peptides containing a chiral cyclic (*S*,*S*)-Ac(5)c(dOM) was 3(10)-helix both in solution, and in the solid state.
Keywords: 3(10)-helix, X-ray crystallographic analysis

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Sugiyama, T.[*1], Imamura, Y.[*2], Kurihara, M., Kittaka, A.[*3]: **Recognition of longer duplex DNA by cooperative strand invasion**

Nucleic Acids Symp Ser, **51**, 269-270 (2007)

Peptide nucleic acid is a synthetic DNA mimic in which the sugar-phosphate backbone has been replaced by a peptide backbone. A remarkable feature of PNA is its ability to recognize sequences within duplex DNA by strand invasion. We have previously demonstrated that a PNA targeting six bases within duplex DNA cooperatively binds to 12 base-pair site by strand invasion. We here report an successful extension of the target site size to 18 base pairs without the expense of specificity.

Keywords: peptide nucleic acid, duplex DNA

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Hakamata, W., Sato, Y., Okuda, H., Honzawa, S.[*1], Saito, N.[*1], Kishimoto, S.[*1], Yamamoto, A.[*1], Sugiura, T.[*1], Kittaka, A.[*1], Kurihara, M.: **(2S, 2'R)-Analogue of LG190178 is a major active isomer**

Bioorg. Med. Chem. Lett., **18**, 120-123 (2008)

Vitamin D receptor (VDR) ligands are therapeutic agents for the treatment of psoriasis, osteoporosis, and secondary hyperparathyroidism. VDR ligands also show immense potential as therapeutic agents for autoimmune diseases and cancers of the skin, prostate, colon, and breast as well as leukemia. LG190178 is a novel non-secosteroidal ligand for VDR. We synthesized and evaluated stereoisomers of LG190178 and found that only an (2S, 2'R)-analogue of LG190178 (YR301) had strong activity.

Keywords: vitamin D receptor, non-secosteroidal ligand

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Honzawa, S.[*1], Yamamoto, Y.[*1], Yamashita, A.[*1], Sugiura, T.[*1], Kurihara, M., Arai, M. A.[*1], Kato, S.[*2], Kittaka, A.[*1]: **The 2α-(3-hydroxypropyl) group as an active motif in vitamin D3 analogues as agonists of the mutant vitamin D receptor (Arg274Leu)**

Bioorg. Med. Chem., **16**, 3002-3024 (2008)

We designed and synthesized 1- and 1β-hydroxymethyl-2-(3-hydroxypropyl)-25-hydroxyvitamin D_3 (2a,b) and related analogues 2- (3-hydroxypropyl)-25-hydroxyvitamin D_3 (3), Posner's analogues of 1- and 1β-hydroxymethyl-25-hydroxyvitamin D_3 (4a,b), as well as 2-(3-hydroxypropyl)-1,25-dihydroxyvitamin D_3 (5), to confirm the effect of the 1-hydroxy group and/or 2-(3-hydroxypropyl) group of vitamin D_3 analogues with the modified A-ring moiety on the mutant vitamin D receptor, VDR(Arg274Leu). The 2-(3-hydroxypropyl) group showed better effect on enhancement of the transcriptional activity through the mutant VDR than the 1- and 1β-hydroxymethyl groups.

Keywords: mutant vitamin D receptor

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Kurihara, M., Sato, Y., Kaneko, F., Okuda, H., Nagano, M.[*1], Demizu, Y.[*1], Doi, M.[*2], Tanaka, M.[*1], Suemune, H.[*1]: **Computational Study on Secondary Structure of Oligopeptides Containing α,α-Disubstituted α-Amino Acids**

Peptide Science 2007, 137-138 (2008)

Computational simulation of the conformation of oligopeptides presents an interesting challenge to predict the conformation for the design of functionalized and bioactive molecules. Here we report computational study on conformation of oligopeptides containing cyclic α,α-disubstituted α-amino acids with side-chain chiral centers and also conformational search using various force fields and evaluation by MO calculations.

Keywords: α,α-disubstituted α-amino acid, computational simulation, conformational search

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Tamehiro, N., Shigemoto-Mogami, Y., Kakeya, T., Okuhira, K., Suzuki, K., Sato, R.[*], Nagao, T., Nishimaki-Mogami, T.: **Sterol responsive element-binding protein-2- and liver X receptor-driven dual promoter regulation of hepatic ABCA1 gene expression: mechanism underlying the unique response to cellular cholesterol status**

J. Biol. Chem., **282**, 21090-21099 (2007)

ABC transporter A1 (ABCA1) mediates and rate-limits biogenesis of high density lipoprotein (HDL), and hepatic ABCA1 plays a major role in regulating

plasma HDL levels. HDL generation is also responsible for release of cellular cholesterol. In peripheral cells ABCA1 is up-regulated by the liver X receptor (LXR) system when cell cholesterol increases. However, cholesterol feeding has failed to show a significant increase in hepatic ABCA1 gene expression, and its expression is up-regulated by statins (3-hydroy-3-methylglutaryl-CoA reductase inhibitors), suggesting distinct regulation. In this study we investigated the mechanism of regulation of the rat hepatic ABCA1 gene and identified two major ABCA1 transcripts and two corresponding promoter regions. Compactin activated the novel liver-type promoter in rat hepatoma McARH7777 cells by binding the sterol regulatory element-binding protein-2 (SREBP-2). In contrast, compactin repressed the previously identified peripheral-type promoter in an LXR-responsive element-dependent but not E-box-dependent manner. Thus, compactin increased the liver-type transcript and decreased the peripheral-type transcript. The same two transcripts were also dominant in human and mouse livers, whereas the intestine contains only the peripheral-type transcript. Treatment of rats with pravastatin and a bile acid binding resin (colestimide), which is known to activate SREBP-2 in the liver, caused a reduction in the hepatic cholesterol level and the same differential responses in vivo, leading to increases in hepatic ABCA1 mRNA and protein and plasma HDL levels. We conclude that the dual promoter system driven by SREBP-2 and LXR regulates hepatic ABCA1 expression and may mediate the unique response of hepatic ABCA1 gene expression to cellular cholesterol status.

Keywords: ABCA1, cholesterol, SREBP-2

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Saito, Y., Katori, N., Soyama, A., Nakajima, Y., Yoshitani, T., Kim, S.R., Fukushima-Uesaka, H., Kurose, K., Kaniwa, N., Ozawa, S., Kamatani, N.[*1], Komamura, K.[*2], Kamakura, S.[*2], Kitakaze, M.[*2], Tomoike, H.[*2], Sugai, K.[*3], Minami, N.[*3], Kimura, H.[*3], Goto, Y.[*3], Minami, H.[*4], Yoshida, T.[*4], Kunitoh, H.[*4], Ohe, Y.[*4], Yamamoto, N.[*4], Tamura, T.[*4], Saijo, N.[*4] and Sawada, J.: ***CYP2C8* haplotype structures and their influence on pharmacokinetics of paclitaxel in a Japanese population**

Pharmacogenet. Genomics, **17**, 461-471 (2007)

OBJECTIVE: CYP2C8 is known to metabolize various drugs including an anticancer drug paclitaxel. Although large interindividual differences in CYP2C8 enzymatic activity and several nonsynonymous variations were reported, neither haplotype structures nor their associations with pharmacokinetic parameters of paclitaxel were reported. METHODS: Haplotype structures of the *CYP2C8* gene were inferred by an expectation-maximization based program using 40 genetic variations detected in 437 Japanese patients, which included cancer patients. Associations of the haplotypes and paclitaxel pharmacokinetic parameters were analyzed for 199 paclitaxel-administered cancer patients. RESULTS: Relatively strong linkage disequilibriums were observed throughout the *CYP2C8* gene. We estimated 40 haplotypes without an amino-acid change and nine haplotypes with amino acid changes. The 40 haplotypes were classified into six groups based on network analysis. The patients with heterozygous **IG* group haplotypes harboring several intronic variations showed a 2.5-fold higher median area under concentration-time curve of C3'-*p*-hydroxy-paclitaxel and a 1.6-fold higher median value of C3'-*p*-hydroxy-paclitaxel/paclitaxel area under concentration-time curve ratio than patients bearing no **IG* group haplotypes ($P < 0.001$ for both comparisons by Mann-Whitney U-test). No statistically significant differences, however, were observed between patients with and without the **IG* group (haplotypes) in clearance and area under concentration-time curve of paclitaxel, area under concentration-time curve of 6alpha-hydroxy-paclitaxel and 6alpha-, C3'-*p*-dihydroxy-paclitaxel, and area under concentration-time curve ratio of 6alpha-hydroxy-paclitaxel/paclitaxel. CONCLUSION: *CYP2C8***IG* group haplotypes were associated with increased area under concentration-time curve of C3'-*p*-hydroxy-paclitaxel and area under concentration-time curve ratio of C3'-*p*-hydroxy-paclitaxel/paclitaxel. Thus, **IG* group haplotypes might be associated with reduced CYP2C8 activity, possibly through its reduced protein levels.

Keywords: geneic polymorphism, *CYP2C8*, paclitaxel

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Minami, H.*, Sai, K., Saeki, M., Saito, Y., Ozawa, S., Suzuki, K., Kaniwa, N., Sawada, J., Hamaguchi, T.*, Yamamoto, N.*, Shirao, K.*, Yamada, Y.*, Ohmatsu, H.*, Kubota, K.*, Yoshida, T.*, Ohtsu, A.* and Saijo, N.*: **Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: roles of *UGT1A1*6* and **28***

Pharmacogenet. Genomics, **17**, 497-504 (2007)

OBJECTIVES: SN-38, an active metabolite of irinotecan, is detoxified by glucuronidation with UGT1A isoforms, 1A1, 1A7, 1A9, and 1A10. The pharmacogenetic information on *UGT1A* haplotypes covering all these isoforms is important for the individualized therapy of irinotecan. Associations between *UGT1A* haplotypes and pharmacokinetics/pharmacodynamics of irinotecan were investigated to identify pharmacogenetic markers. METHODS: Associations between *UGT1A* haplotypes and the area under concentration curve ratio (SN-38 glucuronide/SN-38) or toxicities were analyzed in 177 Japanese cancer patients treated with irinotecan as a single agent or in combination chemotherapy. For association analysis, diplotypes of *UGT1A* gene segments [(*1A1*, *1A7*, *1A9*, *1A10*), and Block C (common exons 2-5)] and combinatorial haplotypes (*1A9-1A7-1A1*) were used. The relationship between diplotypes and toxicities was investigated in 55 patients treated with irinotecan as a single agent. RESULTS: Among diplotypes of *UGT1A* genes, patients with the haplotypes harboring *UGT1A1*6* or **28* had significantly reduced area under concentration curve ratios, with the effects of *UGT1A1*6* or **28* being of a similar scale. A gene dose effect on the area under concentration curve ratio was observed for the number of haplotypes containing **28* or **6* (5.55, 3.62, and 2.07 for 0, 1, and 2 haplotypes, respectively, $P < 0.0001$). In multivariate analysis, the homozygotes and double heterozygotes of **6* and **28* (**6/*6*, **28/*28* and **6/*28*) were significantly associated with severe neutropenia in 53 patients who received irinotecan monotherapy. CONCLUSIONS: The haplotypes significantly associated with reduced area under concentration curve ratios and neutropenia contained *UGT1A1*6* or **28*, and both of them should be genotyped before irinotecan is given to Japanese and probably other Asian patients.

Keywords: geneic polymorphism, *UGT1A1*, irinotecan

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Fukushima-Uesaka, H., Saito, Y., Maekawa, K., Kamatani, N.*1, Kajio, H.*2, Kuzuya, N.*2, Noda, M.*2, Yasuda, K.*2 and Sawada J.: **Genetic variations and haplotype structures of transcriptional factor Nrf2 and its cytosolic reservoir protein Keap 1 in Japanese**

Drug Metab. Pharmacokinet., **22**, 212-219 (2007)

Transcriptional factor Nrf2 and its cytosolic reservoir protein Keap1 play important roles in induction of the expression of genes for xenobiotic metabolism and disposition, many of which are involved in protection from oxidative stress. In this study, 5 *NFE2L2* (encoding Nrf2) and 6 *KEAP1* exons and their flanking introns were comprehensively screened for genetic variations in 84 Japanese subjects. As for *NFE2L2*, 14 genetic variations were found, including 9 novel ones: 7 were located in the 5'-flanking region, 1 in the 5'-untranslated region (5'-UTR), 3 (1 synonymous and 2 nonsynonymous) in the coding exons, 1 in the intron, and 2 in the 3'-UTR. Two novel nonsynonymous variations, 697C>T (Pro233Ser) and 1094G>T (Ser365Ile), were heterozygously found with allele frequencies of 0.012 and 0.006, respectively. Regarding *KEAP1*, 18 genetic variations were detected, including 13 novel ones: 2 were located in the 5'-flanking region, 4 in the coding exons (4 synonymous), 5 in the introns, 4 in the 3'-UTR, and 3 in the 3'-flanking region. Based on the linkage disequilibrium (LD) profiles, both genes were analyzed as single LD blocks, where 14 (*NFE2L2*) and 18 (*KEAP1*) haplotypes were inferred. Six (*NFE2L2*) and 5 (*KEAP1*) haplotypes were relatively prevalent (>or=0.03 frequencies) and accounted for >or=88% of the inferred haplotypes. Haplotype-tagging variations of each gene were identified to capture these prevalent haplotypes. These data would be fundamental and useful information for pharmacogenetic studies on Nrf2-regulated genes for xenobiotic metabolism and disposition.

Keywords: genetic polymorphism, *NFE2L2*, *KEAP1*

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Kim, S.R., Sai, K., Tanaka-Kagawa, T., Jinno, H., Ozawa, S., Kaniwa, N., Saito, Y., Akasawa, A.*1, Matsumoto, K.*1, Saito, H.*1, Kamatani, N.*2, Shirao,

K.[*3], Yamamoto, N.[*3], Yoshida, T.[*3], Minami, H.[*3], Ohtsu, A.[*3], Saijo, N.[*3] and Sawada, J.: **Haplotypes and a novel defective allele of *CES2* found in a Japanese population**

Drug Metab. Dispos., **35**, 1865-1872（2007）

Human carboxylesterase 2（hCE-2）is a member of the serine esterase superfamily and is responsible for hydrolysis of a wide variety of xenobiotic and endogenous esters. hCE-2 also activates an anticancer drug, irinotecan（7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin, CPT-11), into its active metabolite, 7-ethyl-10-hydroxycamptothecin（SN-38). In this study, a comprehensive haplotype analysis of the *CES2* gene, which encodes hCE-2, in a Japanese population was conducted. Using 21 single nucleotide polymorphisms（SNPs), including 4 nonsynonymous SNPs, 100C>T（Arg34Trp, **2*), 424G>A（Val142Met, **3*), 1 A>T（Met 1 Leu, **5*), and 617G>A（Arg206His, **6*), and a SNP at the splice acceptor site of intron 8（IVS8-2A>G, **4*), 20 haplotypes were identified in 262 Japanese subjects. In 176 Japanese cancer patients who received irinotecan, associations of *CES2* haplotypes and changes in a pharmacokinetic parameter,（SN-38 + SN-38G)/CPT-11 area under the plasma concentration curve（AUC）ratio, were analyzed. No significant association was found among the major haplotypes of the **1* group lacking nonsynonymous or defective SNPs. However, patients with nonsynonymous SNPs, 100C>T（Arg34Trp）or 1 A>T（Met 1 Leu), showed substantially reduced AUC ratios. In vitro functional characterization of the SNPs was conducted and showed that the 1 A>T SNP affected translational but not transcriptional efficiency. These findings are useful for further pharmacogenetic studies on CES2-activated prodrugs.

Keywords: genetic polymorphism, *CES2*, function

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Kim, S.R., Saito, Y., Sai, K., Kurose, K., Maekawa, K., Kaniwa, N., Ozawa, S., Kamatani, N.[*1], Shirao, K.[*2], Yamamoto, N.[*2], Hamaguchi, T.[*2], Kunitoh, H.[*2], Ohe, Y.[*2], Yamada, Y.[*2], Tamura, T.[*2], Yoshida, T.[*2], Minami, H.[*2], Ohtsu, A.[*2], Saijo, N.[*2] and Sawada, J.: **Genetic variations and frequencies of major haplotypes in *SLCO1B1* encoding the transporter OATP1B1 in Japanese subjects: *SLCO1B1*17* is more prevalent than **15***

Drug Metab. Pharmacokinet., **22**, 456-461（2007）

A liver-specific transporter organic anion transporting polypeptide 1B1（OATP1B1, also known as OATP-C）is encoded by *SLCO1B1* and mediates uptake of various endogenous and exogenous compounds from blood into hepatocytes. In this study, 15 *SLCO1B1* exons（including non-coding exon 1）and their flanking introns were comprehensively screened for genetic variations in 177 Japanese subjects. Sixty-two genetic variations, including 28 novel ones, were found: 7 in the 5'-flanking region, 1 in the 5'-untranslated region（UTR), 13 in the coding exons（9 nonsynonymous and 4 synonymous variations), 5 in the 3'-UTR, and 36 in the introns. Five novel nonsynonymous variations, 311T>A（Met104Lys), 509T>C（Met170Thr), 601A>G（Lys201Glu), 1553C>T（Ser518Leu), and 1738C>T（Arg580Stop), were found as heterozygotes. The allele frequencies were 0.008 for 1738C>T（Arg580Stop）and 0.003 for the four other variations. Arg580Stop having a stop codon at codon 580 results in loss of half of transmembrane domain（TMD）11, TMD12, and a cytoplasmic tail, which might affect transport activity. In addition, novel variations, IVS12- 1 G>T at the splice acceptor site and -3A>C in the Kozak motif, were detected at 0.003 and 0.014 frequencies, respectively. Haplotype analysis using -11187G>A, -3A>C, IVS12-1G>T and 9 nonsynonymous variations revealed that the haplotype frequencies for **1b*, **5*, **15*, and **17* were 0.469, 0.000（not detected), 0.037, and 0.133, respectively. These data would provide fundamental and useful information for pharmacogenetic studies on OATP1B1-transported drugs in Japanese.

Keywords: genetic polymorphism, *SLCO1B1*, Japanese

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Ukaji, M., Saito, Y., Fukushima-Uesaka, H., Maekawa, K., Katori, N., Kaniwa, N., Yoshida, T.[*], Nokihara, H.[*], Sekine, I.[*], Kunitoh, H.[*], Ohe, Y.[*], Yamamoto, N.[*], Tamura, T.[*], Saijo, N.[*] and Sawada, J.: **Genetic variations of *VDR/NR1I1* encoding vitamin D receptor in a Japanese population**

Drug Metab. Pharmacokinet., **22**, 462-467（2007）

The vitamin D receptor (VDR) is a transcriptional factor responsive to 1α,25-dihydroxyvitamin D_3 and lithocholic acid, and induces expression of drug metabolizing enzymes CYP3A4, CYP2B6 and CYP2C9. In this study, the promoter regions, 14 exons (including 6 exon 1's) and their flanking introns of *VDR* were comprehensively screened for genetic variations in 107 Japanese subjects. Sixty-one genetic variations including 25 novel ones were found: 9 in the 5'-flanking region, 2 in the 5'-untranslated region (UTR), 7 in the coding exons (5 synonymous and 2 nonsynonymous variations), 12 in the 3'-UTR, 19 in the introns between the exon 1's, and 12 in introns 2 to 8. Of these, one novel nonsynonymous variation, 154A>G (Met52Val), was detected with an allele frequency of 0.005. The single nucleotide polymorphisms (SNPs) that increase VDR expression or activity, -29649G>A, 2T>C and 1592 (*308) C>A tagging linked variations in the 3'-UTR, were detected at 0.430, 0.636, and 0.318 allele frequencies, respectively. Another SNP, -26930A>G, with reduced VDR transcription was found at a 0.028 frequency. These findings would be useful for association studies on *VDR* variations in Japanese.

Keywords: genetic polymorphism, *VDR*, Japanese

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Maekawa, K., Saeki, M., Saito, Y., Ozawa, S., Kurose, K., Kaniwa, N., Kawamoto, M.[*1], Kamatani, N.[*1], Kato, K.[*2], Hamaguchi, T.[*2], Yamada, Y.[*2], Shirao, K.[*2], Shimada, Y.[*2], Muto, M.[*2], Doi, T.[*2], Ohtsu, A.[*2], Yoshida, T.[*2], Matsumura, Y.[*2], Saijo, N.[*2] and Sawada, J.: **Genetic variations and haplotype structures of the *DPYD* gene encoding dihydropyrimidine dehydrogenase in Japanese and their ethnic differences**

J. Hum. Genet., **52**, 804-819 (2007)

Dihydropyrimidine dehydrogenase (DPD) is an inactivating and rate-limiting enzyme for 5-fluorouracil (5-FU), and its deficiency is associated with a risk for developing a severe or fatal toxicity to 5-FU. In this study, to search for genetic variations of *DPYD* encoding DPD in Japanese, the putative promoter region, all exons, and flanking introns of *DPYD* were sequenced from 341 subjects including cancer patients treated with 5-FU. Fifty-five genetic variations, including 38 novel ones, were found and consisted of 4 in the 5'-flanking region, 21 (5 synonymous and 16 nonsynonymous) in the coding exons, and 30 in the introns. Nine novel nonsynonymous SNPs, 29C>A (Ala10Glu), 325T>A (Tyr109Asn), 451A>G (Asn151Asp), 733A>T (Ile245Phe), 793G>A (Glu265Lys), 1543G>A (Val515Ile), 1572T>G (Phe524Leu), 1666A>C (Ser556Arg), and 2678A>G (Asn893Ser), were found at allele frequencies between 0.15 and 0.88%. Two known nonsynonymous variations reported only in Japanese, 1003G>T (**11*, Val335Leu) and 2303C>A (Thr768Lys), were found at allele frequencies of 0.15 and 2.8%, respectively. SNP and haplotype distributions in Japanese were quite different from those reported previously in Caucasians. This study provides fundamental information for pharmacogenetic studies for evaluating the efficacy and toxicity of 5-FU in Japanese and probably East Asians.

Keywords: genetic polymorphism, *DPYD*, Japanese

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Hanioka, N.[*], Tsuneto, Y.[*], Saito, Y., Maekawa, K., Sawada, J. and Narimatsu, S.[*]: **Influence of *CYP2C19*18* and *CYP2C19*19* alleles on omeprazole 5-hydroxylation: in vitro functional analysis of recombinant enzymes expressed in Saccharomyces cerevisiae**

Basic Clin. Pharmacol. Toxicol., **102**, 388-393 (2008)

Omeprazole is one of the most widely used proton pump inhibitors for the treatment of gastric acid-related disorders. The major metabolic pathway of omeprazole is 5-hydroxylation, which is catalysed by CYP2C19. In this study, the effect of *CYP2C19*18* and *CYP2C19*19* alleles on omeprazole 5-hydroxylation was studied using recombinant CYP2C19 enzymes of wild-type (CYP2C19.1B having Ile331Val) and variants (CYP2C19.18 having Arg329His/Ile331Val and CYP2C19.19 Ser51Gly/Ile331Val) expressed in yeast cells. The *K*m value for omeprazole 5-hydroxylation of CYP2C19.1B was 1.46 microM. The *K*m value of CYP2C19.19 was significantly higher (1.5-fold) than that of CYP2C19.1B. *V*max and *V*max/*K*m values for omeprazole 5-hydroxylation of CYP2C19.1B on the basis of cytochrome P450 protein level were 8.09 pmol/min./pmol CYP and 5.45 microl/min./pmol CYP,

respectively. The *V*max value of CYP2C19.19 was significantly higher (1.8-fold) than that of CYP2C19.1B, whereas the *V*max/*K*m value was comparable to that of CYP2C19.1B. In contrast, *K*m, *V*max and *V*max/*K*m values of CYP2C19.18 were similar to those of CYP2C19.1B. These results suggest that *CYP2C19*19* allele decreases the affinity between CYP2C19 enzyme and the substrate in omeprazole metabolism.

Keywords: genetic polymorphism, CYP2C19, omeprazole

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Sai, K., Saito, Y., Sakamoto, H.*, Shirao, K.*, Kurose, K., Saeki, M., Ozawa, S., Kaniwa, N., Hirohashi, S.*, Saijo, N.*, Sawada, J. and Yoshida, T.*: **Importance of *UDP-glucuronosyltransferase 1A1 *6* for irinotecan toxicities in Japanese cancer patients**

Cancer Lett., **261**, 165-171 (2008)

Recent pharmacogenetic studies on irinotecan have revealed the impact of *UDP-glucuronosyltransferase* (*UGT*) *1A1*28* on severe irinotecan toxicities. Although the clinical role of *UGT1A1*6*, which is specifically detected in East Asian patients, in irinotecan toxicities is suggested, clear evidence remains limited. To examine the impact of **6*, the association of *UGT1A1* genotypes with severe irinotecan toxicities was retrospectively investigated in Japanese cancer patients. A significant **6*-dependent increase in the incidence of grade 3 or 4 neutropenia was observed in 49 patients on irinotecan monotherapy (p=0.012). This study further clarifies the clinical importance of **6* in irinotecan therapy in East Asians.

Keywords: irinotecan, *UGT1A1*, pharmacogenetics

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Sai, K., Saito, Y., Itoda, M., Fukushima-Uesaka, H., Ozawa, S., Kurose, K., Kaniwa, N., Kawamoto, M.*1, Kamatani, N.*1, Shirao, K.*2, Yamamoto, N.*2, Hamaguchi, T.*2, Kunitoh, H.*2, Ohe, Y.*2, Yamada, Y.*2, Tamura, T.*2, Yoshida, T.*2, Minami, H.*2, Matsumura, Y.*2, Ohtsu, A.*2, Saijo, N.*2 and Sawada, J. : **Genetic variations and haplotypes of *ABCC2* encoding MRP2 in a Japanese population**

Drug. Metab. Pharmacokinet., **23**, 139-147 (2008)

In this study, all 32 exons and the 5'-flanking region of *ABCC2* in 236 Japanese were resequenced, and 61 genetic variations including 5 novel nonsynonymous ones were detected. A total of 64 haplotypes were determined/inferred and classified into five **1* haplotype groups (**1A*, **1B*, **1C*, **1G*, and **1H*) without nonsynonymous substitutions and **2* to **9* groups with nonsynonymous variations. This study revealed that haplotype **1A*, which has lowered activity, is quite common in Japanese, and that the frequency of **1C*, another functional haplotype, was comparable to frequencies in Asians and Caucasians. In contrast, haplotype **1G*, which are reportedly common in Caucasians were minor in Japanese. These findings imply possible differences in MRP2-mediated drug responses between Asians and Caucasians.

Keywords: *ABCC2*, SNP, haplotypes

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Upham, B.L.*1, Bláha, L.*2, Babica, P.*1, Park, J.S.*1, Sovadinova, I.*1, Pudrith, C.*1, Rummel, A.M.*1, Weis, L.M.*1, Sai, K., Tithof, P.K.*3, Guzvić, M.*4, Vondrácek, J.*5, 6, Machala, M.*5 and Trosko, J.E.*1: **Tumor promoting properties of a cigarette smoke prevalent polycyclic aromatic hydrocarbon as indicated by the inhibition of gap junctional intercellular communication via phosphatidylcholine-specific phospholipase C**

Cancer Sci., **99**, 696-705 (2008)

Inhibition of GJIC and the activation of intracellular mitogenic pathways are characteristic of epithelial derived cancer cells. Our results indicate that PC-PLC is an important signaling enzyme needed for the inhibition of GJIC in response to a cigarette smoke relevant polycyclic aromatic hydrocarbon. This report clearly indicates that specific phospholipid signaling is involved in the regulation of GJIC, and that, in addition to reported Mek-dependent regulation of GJIC, the regulation of GJIC can be Mek-independent even though this MAPK pathway is activated. Thus, not all tumor promoters inhibit GJIC through the same signaling pathway, and this implicates that chemoprevention strategies relative to up-regulating GJIC activity probably can not be universally effective.

Keywords: PAH, GJIC, PC-PLC

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*6 Institute of Biophysics (Czech Republic)

Suzuki R*1, Furuno T*2, Okamoto*1, Teshima R., Nakanishi M*2: **ATP plays a role in neurite stimulation with activated mast cells.**
J. Neuroimmunol, **192**, 49-56 (2007)

Previously, we showed that nerve-mast cell cross-talk can occur bidirectionally and that substance P is a mediator to activate mast cells. Here, we have studied the mediators to activate nerves cocultured with mast cells. Addition of antigen to the cocultures of superior cervical ganglia (SCG) and rat basophilic leukemia cells (RBLs) elicited Ca(2+) response in RBLs and after a lag period induced Ca(2+) signal in SCG neurites. Pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (purinergic receptor antagonist) or apyrase (ATP-hydrolyzing enzyme) reduced the Ca(2+) signals in neurites, indicating that ATP released from activated mast cells was one of important mediators to activate nerves.
Keywords: ATP, neurite stimulation, mast cells

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Nakajima, O., Teshima, R., Takagi, K., Okunuki, H. and Sawada, J.: **ELISA method for monitoring human serum IgE specific for Cry1Ab introducing into genetically modified corn**
Regul Toxicol Pharmacol., **47**, 90-95 (2007)

ELISA-linked immunosorbent assay (ELISA) is the most convenient method of monitoring the occurrence of IgE antibodies specific for novel proteins in genetically modified (GM) foods. The levels of IgE specific for a recombinant protein, Cry1Ab, were determined using an ELISA method. A soluble form of the Cry1Ab protein purified from pCold1 vector-transformed *Escherichia coli* pTf16 / BL21 was used as the ELISA coating antigen, and 1 M NaCl was used as the washing buffer to remove IgE non-specifically bound to the coated antigen. Sera from 44 patients allergic to major food allergens were obtained, diluted 20-fold, tested, and found no identifiable IgE above background levels. We also tested sera from patients with corn allergy against whole extracts of non-GM and GM-corn (MON810) using immunoblotting. The staining patterns were similar for the two types of corn. These results indicate that significant levels of IgE antibodies specific to Cry1Ab were not found in the sera of Japanese patients with food allergies.

Nakamura, R., Teshima, R., Hachisuka, A., Sato, Y., Takagi, K., Nakamura, R., Woo, G.H., Shibutani, M.*1, Sawada, J.: **Effects of developmental hypothyroidism induced by maternal administration of methimazole or propylthiouracil on the immune system of rats**
Int. Immunopharmacol., 7, 1630-1638 (2007)

Methimazole (MMI) and propylthiouracil (PTU) are popularly used antithyroid drugs (ATDs) for the treatment of Graves' hyperthyroidism. The aim of the present study was to determine the effects of ATDs on the developing immune system of the rats. Maternal Sprague-Dawley rats were given drinking water containing 200 ppm of MMI, 12 ppm of PTU (high-dose PTU), or 3 ppm of PTU (low-dose PTU) between gestational day (GD) 10 and postnatal week (PNW) 3. Exposure to the ATDs was ceased upon weaning at PNW3, and the male offspring were sampled at PNWs 3 or 11. The serum thyroid-related hormone levels and the hematological components in the offspring were then determined. The expressions of surface markers in the spleen, thymus and peripheral blood were determined using flowcytometry. The weights of the body, spleen and thymus and the splenic and thymic cell numbers were decreased in the MMI-treated and the high-dose PTU-treated animals at PNWs 3 and 11. The serum levels of thyroid-related hormones were depressed in the MMI and high-dose PTU groups. FACS analysis revealed that the ATDs caused proportional changes in the lymphoid cell subpopulations. The proportion of B cells among the total lymphocytes was significantly decreased at PNW3, whereas that of T cells, especially of inactive T cells, was dramatically increased. Moreover, the proportion of $CD4^+CD25^+$ regulatory T cells was significantly increased in the spleen and peripheral blood at PNW3. Most of the above-described changes had recovered to normal levels at PNW11. These results suggest that

ATDs might have temporal immunomodulatory effects on the developing immune system.

Keywords; antithyroid, immune system, perinatal exposure, regulatory T cell

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Yamakawa, H.*1, Akiyama, H., Endo, Y.*1, Miyatake, K.*1, Sakata, K., Sakai, S., Toyoda, M.*2 and Urisu, A.*3: **A Specific Detection of Wheat Residues in Processed Foods Using Polymerase Chain Reaction**

Biosci. Biotech. Biochem., **71**, 2561-2564（2007）

A sensitive qualitative detection method for wheat in foods using polymerase chain reaction（PCR）was developed. Trace amounts of wheat in commercial food products could be qualitatively detected by this method. The sensitivity of the proposed PCR method appears to be similar to that of ELISA. The present method should be very useful for detecting wheat residues in processed foods.

Keywords : common wheat; Triticum aestivum L. polymerase chain reaction（PCR）

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Yano, T.*1, Sakai, Y.*1, Uchida, K.*1, Nakao, Y.*1, Ishihata, K.*2, Nakano, S.*2, Yamada, T.*2, Sakai, S., Urisu, A.*3, Akiyama, H. and Maitani, T.: **Detection of Walnut Residues in Processed Foods by Polymerase Chain Reaction**

Biosci. Biotech. Biochem., **71**, 1793-1796（2007）

A sensitive qualitative detection method for walnut（Juglans regia）using polymerase chain reaction（PCR）was developed. For detection of walnuts with high specificity, the primer pair WAL-F/WAL-R was designed based on walnut matK genes. Trace amounts of walnuts in commercial food products can be qualitatively detected using this method.

Keywords : walnut, pecan nut, PCR

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Akiyama, H., Sasaki N.*1, Sakata K., Ohmori K.*2, Toyota A.*3, Kikuchi, Y., Watanabe, T., Furui, S.*4, Kitta, K.*4 and Maitani, T.: **Identification and detection of GM Shanyou 63 line and unknown Bt rice line contaminating rice vermicelli products**

J. Agric. Food Chem., **55**, 5942-5947（2007）

We analyzed the DNA fragments extracted from four rice vermicelli products. The Bacillus thuringiensis（Bt）rice line, which has a construct similar to the GM Shanyou 63 line, was detected in some vermicelli products by identification of the junction region sequence between rice Act1 promoter and the Cry1Ac gene, and that between Cry1Ac and nos. In addition, we also detected a different Bt rice line by means of the junction region sequence between the maize ubiquitin promoter and cry1Ab gene and that between the cauliflower mosaic virus 35S promoter and the hygromycin phosphotransferase in some vermicelli products. Accordingly, we for the first time have detected the two transgenic Bt rice lines contaminating rice vermicelli samples. Furthermore, we developed a duplex real-time polymerase chain reaction（PCR）method for the simultaneous detection of both Bt rice lines.

Keywords: genetically modified rice, real-time PCR, Rice Bacillus thuringiensis

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Akiyama, H., Sakata K., Kondo K., Tanaka A.*1, Liu S. M.*2, Oguchi, T.*3, Furui, S.*3, Kitta, K.*3, Hino, A.*3 and Teshima, R.: **Individual Detection of Genetically Modified Maize Varieties in Non-Identity-Preserved Maize Samples**

J. Agric. Food Chem., **56**, 1977-1983（2008）

In many countries, the labeling of grains and feed- and foodstuffs is mandatory if the genetically modified organism（GMO）content exceeds a certain level of approved GM varieties. The GMO content in a maize sample containing the combined-trait（stacked）GM maize as determined by the currently available meth-

odology is likely to be overestimated. However, there has been little information in the literature on the mixing level and varieties of stacked GM maize in real sample grains. For the first time, the GMO content of non-identity-preserved (non-IP) maize samples imported from the United States has been successfully determined by using a previously developed individual kernel detection system coupled to a multiplex qualitative PCR method followed by multichannel capillary gel electrophoresis system analysis. To clarify the GMO content in the maize samples imported from the United States, determine how many stacked GM traits are contained therein, and which GM trait varieties frequently appeared in 2005, the GMO content (percent) on a kernel basis and the varieties of the GM kernels in the non-IP maize samples imported from the United States were investigated using the individual kernel analysis system. The average (+standard deviation) of the GMO contents on a kernel basis in five non-IP sample lots was determined to be 51.0 ± 21.6%, the percentage of a single GM trait grains was 39%, and the percentage of the stacked GM trait grains was 12%. The MON810 grains and NK603 grains were the most frequent varieties in the single GM traits. The most frequent stacked GM traits were the MON810 × NK603 grains. In addition, the present study would provide the answer and impact for the quantification of GM maize content in the GM maize kernels on labeling regulation.
Keywords: Combined-trait genetically modified maize, multiplex real-time PCR, capillary gel electrophoresi

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Oguchi, T.[*1], Onishi, M.[*2], Chikagawa, Y.[*2], Minegishi, Y.[*3], Kodama, T.[*4], Akiyama, H., Ohno, Y., Futo, S.[*2], Hino, A.[*1], Furui, S.[*1] and Kitta, K.[*1]: **Development of Event-Specific Quantitation Method for GA21 Maize, Which Is a GM Event without CaMV35S Promoter**
J. Food Hyg. Soc. Japan., **49**, 16-22 (2008)

A real-time PCR detection method was developed for event-specific quantitation of Roundup Ready maize, GA21. The developed PCR method was designed to amplify an artificial junction site between the native maize genome DNA and the recombinant DNA of GA21 maize, which provides only one target sequence per haploid of GA21 genome. Thus, the amplification efficiency of the event-specific target for GA21 became closely similar to the amplification of SSIIb, and the conversion factor (Cf) for the quantitation method was similar to the theoretical value. The developed method demonstrated better performance than the existing construct-specific method that has been used as a Japanese official method. The developed method can easily be combined with the real-time PCR targeting of the CaMV35S promoter, and the multiplexed method should be an effective screening method for GM maize.
Keywords: genetically modified (GM), GA21 maize, realtime PCR

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Seiki, K.[*1], Oda, H.[*1], Yoshioka, H.[*1], Sakai, S., Urisu, A.[*2], Akiyama, H. and Ohno, Y.: **A Reliable and Sensitive Immunoassay for the Determination of Crustacean Protein in Processed Foods**
J. Agric. Food Chem., **55**, 9345-9350 (2007)

Among food allergens, crustacea such as shrimps, crabs, and lobsters are a frequent cause of adverse food reactions in allergic patients. The major allergen has been identified as a muscular protein, tropomyosin. A novel sandwich enzyme-linked immunosorbent assay (ELISA) for the detection and quantification of crustacean protein in processed foods was developed using the sample dilution buffer that is added to porcine tropomyosin. The sandwich ELISA method was highly specific for the Decapoda group, apart from minor cross-reactivities to other crustacea and mollusks. The recovery ranged from 85 to 141%, while the intra- and interassay coefficients of variation were less than 2.8 and 8.4%, respectively.
Keywords: Crustacea, food allergy, ELISA

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Tanabe, S.[*1], Hase, M.[*2], Yano, T.[*3], Sato, M.[*4], Fujimura, T.[*5] and Akiyama, H.: **Real-Time Quantitative PCR Detection Method for Pork, Chicken, Beef,**

Mutton, and Horseflesh in Foods

Biosci. Biotech. Biochem., **71**, 3131-3135（2007）

A rapid real-time quantitative PCR method to detect the trace amount of pork, beef, chicken, mutton, and horseflesh in foods was developed. The primers and TaqMan MGB probes were designed upon the gene encoding cytochrome b for the specific detection of each species. The limit of quantification of this method was found to be 100 fg/μl of each mitochondrial DNA in 10 ng/μl of wheat mitochondrial DNA matrix. The calculated R2 values of the standard curves for five species were ranged between 0.994 and 0.999. This method would be useful particularly for the detection of 'hidden' meat mince in processed foods, which would verify food labeling and gain consumers' trust.

Keywords: real-time PCR, cytochrome b, meat

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森山達哉[*1]，光山英由[*1]，矢野えりか[*1]，大羽美香[*2]，橘田和美[*2]，川本伸一[*2]，穐山浩，宇理須厚雄[*3]，高橋浩司[*4]，羽鹿牧太[*5]，小川　正[*6]，河村幸雄[*1]：**食物アレルゲンタンパク質の近赤外蛍光標識プローブによる検出**

日本食品科学工学会誌, **54**, 468-476（2007）

We aimed to detect the allergen proteins in food materials using recently developed near-infrared fluorescent probes. Sensitivities of this method were comparable to chemiluminescence detection methods, which are known to be sensitive. In addition, the sensitivities of this near-infrared fluorescent method were at least 10-50-times higher than those of the conventional visible fluorescent methods using Cy3 and Cy5 dyes. This method was effectively applicable to immunoblotting, dot-blotting and plate-assay（direct FLISA：fluorescence-linked immunosorbent assay）with ELISA plate. Allergen levels of the food sample were quantified by standard curves using standard allergen protein using the dot-blotting technique. This highly sensitive detection system also provided multiple detections of different allergens for different antibodies and dyes with distinct properties of wavelength. This enables high-throughput screening of characteristic allergen contents of target food materials, or cultivars. Generally, allergen proteins are recognized by patient's serum IgE. Therefore, we tried to detect patient's IgE-binding proteins, the putative allergens in foodstuffs. In this detection system, it was possible to detect IgE-binding proteins with sensitivity almost equivalent to a chemiluminescent detection system. Taken together, it was shown that this novel detection system was an effective technique for the sensitive detection and screening of food allergens.

Keywords: food allergens, soybean allergen, rice allergens

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Amano, H.,[*1] Akiyama, H. and Bienenstock, J.[*2]: **Differential corticosterone responses to stress in the lung in two strains of Flinders rats**

Clin. Exp Allergy, **38**, 659-666（2007）

BACKGROUND: Acute stress affects a variety of organs and cellular systems. These include the hypothalamic-pituitary- adrenal（HPA）axis, corticotropin-releasing factor（CRF）, mast cells and nerves. Flinders-sensitive（FSL）rat strains have hypercholinergic responses and are more sensitive than Flinders-resistant rats（FRL）to anaphylaxis. OBJECTIVE: To investigate the effects of acute water avoidance stress（1 h）on FSL and FRL tracheal epithelial tissue. METHODS: We measured short circuit current（I(sc)）as a measure of tracheal response, and the effect of substance P（SP）on tracheal epithelium in Ussing chambers. Electron microscopy was performed to assess mast cell activation. RESULTS: Both strains showed increased I（sc）responses to stress, inhibited by prior injection of the CRF receptor 1 and 2 antagonist, alpha-helical CRF-(9-41). No increases in conductance were seen. Stress responses were accompanied by electron microscopic morphologic evidence for mast cell degranulation, which was not completely inhibited by alpha-helical CRF-(9-41) pre-treatment. Stress primed

the epithelium for an enhanced response to SP in FSL, but this again was not inhibited by alpha-helical CRF-(9-41). FRL had 2.5 times the corticosterone response of FSL. CONCLUSION: Acute stress affects the tracheal epithelium, not accompanied by changes in ion permeability, but associated with mast cell degranulation. Because blunted HPA axis responses are associated with vulnerability to inflammation, this may partially explain the findings. These stress effects on the lung have a genetic basis associated with relative corticosterone responses, are complex and only in part mediated by CRF.

Keywords: corticosterone, hypothalamic-pituitary-adrenal (HPA) axis, stress

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Amano, H.[*], Negishi, I.[*], Akiyama, H. and Ishikawa, O.[*]: **Psychological stress can trigger atopic dermatitis in NC/Nga mice: an inhibitory effect of corticotropin-releasing factor**

Neuropsychopharmacology. **33**, 566-73 (2008)

Atopic dermatitis (AD) is one of the most common inflammatory diseases of the skin and is usually associated with a family history of atopic diathesis. It has been well established that many environmental or psychological factors aggravate AD. However, it is not clear whether psychological stress by itself can trigger AD. We examined the effect of psychological stress on the onset of AD, using an animal model, the NC/Nga mouse. The animals were exposed to the water avoidance stress (WAS) test to induce psychological stress. Additionally, we examined how corticotropin-releasing factor (CRF) affected the development of AD induced by psychological stress. Under specific pathogen-free (SPF) conditions, NC/Nga mice did not develop AD-like skin lesions. In contrast, NC/Nga mice exposed to psychological stress developed AD-like skin lesions along with elevated levels of serum immunoglobulin E even when kept under SPF conditions. The AD-like skin lesions induced by WAS were completely blocked by pretreating the animals with CRF. Our data indicate that a psychological factor is capable of eliciting AD-like skin lesions in NC/Nga mice. It is possible that the inhibitory effect of CRF may be mediated by the functional modification of various cells that have CRF receptors.

Keywords: psychological stress; atopic dermatitis; corticotropin-releasing factor

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Kondo K, Watanabe A, Akiyama H, Maitani T.: **The metabolisms of agaritine, a mushroom hydrazine in mice**

Food and Chem. Toxicol.. **46**, 854 (2008)

The mushroom hydrazine agaritine was measured in mouse plasma and urine using LC/MS/MS, which is highly specific. Agaritineconcentration peaked 20 min after oral administration to mice (4.0 and 40 mg/kg). The concentration gradually decreased and returned to the basal level in 100 min. The maximum concentration, the time to the maximum concentration, and the half life were 0.37 lg/mlplasma, 0.33 h, and 0.71 h, respectively after administration of agaritine at 40 mg/kg body weight. One agaritine metabolite was found in the plasma and the urine from agaritine-administered mice. The structure of metabolites of agaritine by c-GT was next investigatedusing LC/MS. HMPH proved to be generated from agaritine. The oxidative stress marker 8-OHdG was detected in agaritine-administered mouse urine. After administration, the 8-OHdG level immediately tripled, and then decreased to the control level over 48 h. Its levelthen elevated again and remained high for 11 days. These results suggest that agaritine quickly metabolizes and disappears in the plasma,whereas DNA damage lasts for a long time after a single administration of agaritine to mice.

Keywords: agaritine, mushroom, metabolism, mice

Sakai, S., Hirano, K.[*1], Toyoda, H.[*1], Linhardt, R. J.[*2] and Toida, T.[*1]: **Matrix assisted laser desorption ionization-time of flight mass spectrometry analysis of hyaluronan oligosaccharides**.

Anal. Chim. Acta., **593**, 207-213 (2007)

A new method is presented for the identification of oligosaccharides obtained by enzymatic digestion of hyaluronan (HA) with bacterial hyaluronidase (E.C. 4.2.2.1, from Streptomyces hyalurolyticus) using matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOFMS). Mixtures containing HA oligosaccharides of tetrasaccharide (4-mer)-34-mer were analyzed using this method. The carboxyl groups

of the glucuronate residues in the prepared HA oligomers, were modified as the acidic form (-COOH), sodium salts (-COONa), organic ammonium salts, or methylesters before MALDI-TOFMS measurement. Among these samples, the methylester form of glucuronate residues in HA oligosaccharides, prepared by methylation using trimethylsilyl diazomethane, afforded high sensitivity for spectra. This simple modification method for carboxyl group methylation of acidic polysaccharides [Hirano et al., Carbohydr. Res., 340, (2005) 2297-2304] provides samples suitable for MALDI-TOF mass spectrometric analysis throughout a significantly enhanced range of masses.

Keywords: hyaluronan oligosaccharide, matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOFMS), methylester

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Kusano, S.*1, Ootani, A.*1, Sakai, S., Igarashi, N.*2, Takeguchi, A.*1, Toyoda, H.*2 and Toida, T.*2: **HPLC determination of chondrosine in mouse blood plasma after intravenous or oral dose**.
Biol. Pharm. Bull., **30**, 1365-1368 (2007)

The bioavailability of chondrosine was evaluated by its direct measurement as found in the blood plasma following removal of plasma proteins by perchloric acid. The postcolumn HPLC determination of chondrosine was performed on an SCX column (6 mm i.d. x 150 mm), 0.35 mol/l boric acid (pH 5.2 adjusted by 0.1 mol/l NaOH) as an eluent (0.9 ml/min), 0.5% 2-cyanoacetamide and 1.0 M NaOH as fluorogenic reagents (0.25 ml/min each) with a fluorescence detector (ex. 331nm, em. 383nm). Two separate animal studies were conducted. In study 1, adult male ddY mice (n=6) received i.v. chondrosine (1.0 mg/kg body weight) and the plasma samples were collected. In the second study, 6 adult male ddY mice received p.o. chondrosine (400 mg/kg body weight) and the plasma samples were collected. Blood plasma samples were deproteinized by perchloric acid, analyzed and the bioavailability of chondrosine was determined. Twenty five to fifty microliters of blood plasma were required for the assay. Chondrosine was absorbed after oral administration with two phases having two maximum values, 7.8+/−5.4 and 4.0+/−1.9 at 15 microg/ml and 120 min, respectively; it disappeared from the blood flow very quickly after intravenous administration. This study provides the first report of the bioavailability of orally administered chondrosine in mice.

Keywords: chondrosine, HPLC determination, oral dose, bioavailability

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Ohtsuki, T.*1, Yokosawa, E.*1, Koyano, T.*2, Preeprame, S.*3, Kowithayakorn, T.*4, Sakai, S., Toida, T.*1 and Ishibashi, M.*1: **Quinic acid esters from Pluchea indica with collagenase, MMP-2 and MMP-9 inhibitory activities**.
Phytother. Res., **22**, 264-266 (2007)

Investigation of collagenase inhibitory natural components afforded two quinic acid esters (1 and 2) and quercetin (3) from the leaves of Pluchea indica (Compositae). Of these, compounds 1 and 2 exhibited collagenase inhibitory activity (IC_{50}) at a concentration of less than 10 microm, and 1 showed matrix metalloproteinase (MMP)-2 and -9 inhibitory activity (IC_{50}) at 2.5 and 6.4 microm, respectively.

Keywords: quinic acid ester, collagenase, matrix metalloproteinase, Pluchea indica

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Sakai, S., Matsuda, R., Adachi, R., Akiyama, H., Maitani, T., Ohno, Y., Oka, M.*1, Abe, A.*2, Seiki, K.*3, Oda, H.*3, Shiomi, K.*4 and Urisu, A.*5: **Interlaboratory evaluation of two enzyme-linked immunosorbent assay (ELISA) kits for the determination of crustacean protein in processed foods**.
J. AOAC Int., **91**, 123-129 (2008)

The labeling of foods containing material derived from crustaceans such as shrimp and crab is to become mandatory in Japan because of increases in the number of allergy patients. To ensure proper labeling,

2 novel sandwich enzyme-linked immunosorbent assay (ELISA) kits for the determination of crustacean protein in processed foods, the N kit (Nissui Pharmaceutical Co., Ltd, Ibaraki, Japan) and the M kit (Maruha Nichiro Holdings, Inc., Ibaraki, Japan), have been developed. Five types of model processed foods containing 10 and/or 11.9 μg/g crustacean soluble protein were prepared for interlaboratory evaluation of the performance of these kits. The N kit displayed a relatively high level of reproducibility relative standard deviation (interlaboratory precision; 4.0-8.4% RSDR) and sufficient recovery (65-86%) for all the model processed foods. The M kit displayed sufficient reproducibility (17.6-20.5% RSD_R) and a reasonably high level of recovery (82-103%). The repeatability relative standard deviation (RSD_r) values regarding the detection of crustacean proteins in the 5 model foods were mostly < 5.1% RSD_r for the N kit and 9.9% RSDr for the M kit. In conclusion, the results of this interlaboratory evaluation suggest that both these ELISA kits would be very useful for detecting crustacean protein in processed foods.

Keywords: Interlaboratory evaluation, Enzyme-linked immunosorbent assay (ELISA), crustacean protein

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酒井信夫，安達玲子，柴原裕亮[*1]，岡道　弘[*1]，阿部晃久[*2]，清木興介[*3]，織田浩司[*3]，吉岡久史[*3]，塩見一雄[*4]，宇理須厚雄[*5]，穐山浩，手島玲子：**食品原材料中に含まれる「えび」，「かに」等の甲殻類タンパク質の実態調査**.

日本食品化学学会誌, **15**, 1-6 (2008)

我が国における「えび」及び「かに」等の甲殻類アレルギー患者の増加に伴い，厚生労働省は2008年４月よりそれらの表示義務化を予定している．本調査研究では，305検体の食品原材料に含まれる甲殻類タンパク質量を２種類のELISAを用いて定量した．その結果，137検体の食品原材料から甲殻類タンパク質が検出された(海苔，27検体；いわし稚魚，48検体；すり身，59検体；二枚貝３検体)．これらの結果より，それらの食品原材料が意図せざるコンタミネーションとして甲殻類種を含むこと，特にいわし稚魚及びすり身は，甲殻類タンパク質の検出頻度及び濃度が高いことが明らかになった．本研究は，消費者の食物アレルギー危害防止のために，甲殻類種のコンタミネーションの可能性について，ラベルに注意喚起を要することを示唆した．これらのデータは食品原材料段階での表示制度の参考資料となりうるであろう．

Keywords: crustacean soluble protein, shrimp, crab, allergen, ingredients

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Uneyama C, Toda M, Yamamoto M, Morikawa K.: **Arsenic in various foods: cumulative data**.

Food Addit Contam., **24**, 447-534 (2007)

Data for the arsenic content in various foods were collated. The number of collected values was about 2500 columns, which enables an estimation of the range of arsenic contents in each food group. Data were categorized into six groups (crops, milk/meat/egg, fish, algae, seafood, others) and expressed as a percentile graph. In addition, the inorganic arsenic ratio of each food group was estimated. This approach enabled the authors to understand the arsenic contents of some food groups at a glance. The intake of inorganic arsenic seems to be mostly from seafood. The contribution from other categories of food is small.

Keywords: arsenic food

Sato, S.[*1], Shirakawa, H.[*1], Tomita, S.[*2], Ohsaki, Y.[*1], Haketa, K.[*1], Tooi, O.[*3], Santo, N.[*4], Tohkin, M., Furukawa, Y.[*1], Gonzalez, F.J.[*5], Komai, M.[*1]: **Low-dose dioxins alter gene expression related to cholesterol biosynthesis, lipogenesis, and glucose metabolism through the aryl hydrocarbon receptor-mediated pathway in mouse liver**.

Toxicol. Appl. Pharmacol., **229**, 10-19 (2008)

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a common environmental contaminant. TCDD binds and activates the transcription factor aryl hydrocarbon receptor (AHR), leading to adverse biological responses via the alteration of the expression of various AHR target genes. Although small amounts of TCDD are consumed via contaminated daily foodstuffs and envi-

ronmental exposures, the effects of low-dose TCDD on gene expression in animal tissues have not been clarified, while a number of genes affected by high-dose TCDD were reported. In this study, we comprehensively analyzed gene expression profiles in livers of C57BL/6N mice that were orally administered relatively low doses of TCDD (5, 50, or 500 ng/kg body weight (bw) day-1) for 18 days. The hepatic TCDD concentrations, measured by gas chromatography-mass spectrometry, were 1.2, 17, and 1063 pg toxicity equivalent quantity (TEQ)/g, respectively. The mRNA level of the cytochrome P450 CYP1A1 was significantly increased by treatment with only TCDD 500 ng/kg bw day-1. DNA microarray and quantitative RT-PCR analyses revealed changes in the expression of genes involved in the circadian rhythm, cholesterol biosynthesis, fatty acid synthesis, and glucose metabolism in the liver with at all doses of TCDD employed. However, repression of expression of genes involved in energy metabolism was not observed in the livers of Ahr-null mice that were administered the same dose of TCDD. These results indicate that changes in gene expression by TCDD are mediated by AHR and that exposure to low-dose TCDD could affect energy metabolism via alterations of gene expression.

Keywords: Dioxin, Mouse liver, DNA microarray

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Kaniwa, N., Sugiyama, E., Kim, S., Saito, Y., Sawada, Y., Furuse, J.[*1], Ishii, H.[*1], Yoshida, T.[*2], Ueno, H.[*3], Okusaka, T.[*3] and Saijo, N.[*1]: **In reply to Mercier et al. "Genotype-based methods for anticipating gemcitabine-related severe toxicities may lead to false-negative results"**
J. Clin. Oncol., **25**, 4855-4856 (2007)

著者らが，ゲムシタビンの代謝酵素であるシチジンデアミネースをコードする遺伝子CDAのアミノ酸変異を伴う多型*CDA*208G>Aが，日本人においては抗がん剤ゲムシタビンのクリアランスを低下させ，グレード３以上の骨髄抑制の危険性を増加させると報告したが，これに対して，Mercierらが，遺伝子多型に基づく予測は重篤な副作用を見逃す恐れがあり，CDA活性に基づく予測の方が優れていると反論した．この反論に対し，著者らのデータは，CDA活性や薬物動態パラメータなどのフェノタイプとCDAの遺伝子タイプのいずれもが，重篤な好中球抑制の予測に有用なマーカーであることを示唆していることを示し，特に，白人では検出されない*CDA*208G>Aも，日本人においては有用なマーカーであることを示した．

Keywords: gemcitabine, cytidine deaminase, genetic polymorphism

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Okiyama, Y.[*1], Watanabe, H.[*1], Fukuzawa, K.[*2], Nakano, T., Mochizuki, Y.[*3], Ishikawa, T.[*3], Tanaka, S.[*1], and Ebina, K.[*1]: **Application of the fragment molecular orbital method for determination of atomic charges on polypeptides**
Chem. Phys. Lett., **449**, 329-335 (2007)

The electrostatic potential fitting methods for the determination of atomic charges are applied to polypeptides on the basis of the fragment molecular orbital (FMO) method. We show that the charges determined in the pair-approximation stage agree with those determined from the conventional molecular orbital method within an error of 1%. Analyzing the dependency of charges on the structural variation of glycine trimer and the reproducibility of electrostatic potential on the surface of the ligand-binding pocket of estrogen receptor, we also show the applicability of the FMO method for atomic charge determination using the electrostatic potential fitting.

Keywords: FMO, ESP, atomic charge

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Ito, M.[*1], Fukuzawa, K.[*2], Mochizuki, Y.[*3], Nakano, T., and Tanaka, S.[*1]: **Ab Initio Fragment Molecular Orbital Study of Molecular Interactions between Liganded Retinoid X Receptor and Its Coactivator: Roles of Helix 12 in the Coactivator Binding Mech-**

anism

J. Phys. Chem. B, **111**, 3525-3533 (2007)

On the basis of the fragment molecular orbital method we addressed molecular interactions of liganded retinoid X receptor (RXR) with steroid receptor coactivating factor-1 (SRC1) coactivator to examine the contribution of helix 12 (H12), which contains the core of the transcriptional activation function 2 activating domain, to the coactivator binding of RXR. The interaction between H12 and SRC1 was proved to be the main cause for the stabilization of the coactivator binding. In particular, highly conserved charged (Glu453) and hydrophobic (Phe450) residues in H12 were found to have stronger electrostatic and dispersion interactions with SRC1 than the other charged and hydrophobic residues in H12, respectively. In addition, the charge transfer (CT) from RXR to SRC 1 was found to occur mainly by the changes in charges of H12 residues. Large positive and negative charge changes were observed especially for Glu453 and for Lys631 and Ile632 in SRC1, respectively, indicating that Glu453 is an electron donor for Lys631 and Ile632 in this CT. Taken together, our findings quantitatively demonstrated that H12 and its highly conserved residues significantly contribute to the coactivator binding not only by the Coulomb and dispersion interactions but also by the CT described with the quantum-mechanical framework.

Keywords: FMO, RXR, coactivator

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Kurisaki, I.[*1], Fukuzawa, K.[*2], Komeiji, Y.[*3], Mochizuki, Y.[*4], Nakano, T., Imada, J.[*5], Chmielewski, A.[*5], Rothstein, S. M.[*5], Watanabe, H.[*1], and Tanaka, S.[*1]: **Visualization analysis of Inter-fragment interaction energies of CRP-cAMP-DNA complex based on the fragment molecular orbital method**

Biophys. Chem., **130**, 1-9 (2007)

Avisualization method for inter-fragment interaction energies (IFIEs) of biopolymers is presented on the basis of the fragment molecular orbital (FMO) method. The IFIEs appropriately illustrate the information about the interaction energies between the fragments consisting of amino acids, nucleotides and other molecules. The IFIEs are usually analyzed in a matrix form called an IFIE matrix. Analyzing the IFIE matrix, we detect important fragments for the function of biomolecular systems and quantify the strength of interaction energies based on quantum chemistry, including the effects of charge transfer, electronic polarization and dispersion force. In this study, by analyzing a protein-DNA complex, we report a visual representation of the IFIE matrix, a so-called IFIE map. We comprehensively examine what information the IFIE map contains concerning structures and stabilities of the protein-DNA complex.

Keywords: FMO, IFIE map, CRP-cAMP-DNA

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Ishikawa, T.[*1], Mochizuki, Y.[*1], Amari, S.[*2], Nakano, T., Tokiwa, H.[*1], Tanaka, S.[*3], and Tanaka, K.[*2]: **Fragment interaction analysis based on local MP2**

Theor. Chem. Acc., **118**, 937-945 (2007)

We have developed a fragment interaction analysis based on local MP2 (FILM) in the context of the fragment molecular orbital (FMO) scheme. The primary purpose of this work is to provide a tool for analyzing interfragment interaction associated with dispersion interactions in a large molecule such as protein and DNA. Our implementation of local MP2 (LMP2) is based on the algorithm developed by Pulay and Werner. A potential of FILM was demonstrated using the human immunodeficiency virus type 1 protease (HIV-1 PR) complexed with lopinavir (LPV). The total energy, binding affinity, and inter-fragment interaction energy (IFIE) by the FMO method using LMP2 were compared with those obtained by canonical MP2 and the site-specific information in dispersion interaction was obtained. It turned out that the FILM is a useful tool for analyzing the dispersion interaction between an amino acid residue and a specific site of a ligand.

Keywords: FMO, LMP2, FILM

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Watanabe, T.[*1], Inadomi, Y.[*2], Fukuzawa, K.[*3], Nakano, T., Tanaka, S.[*4], Nilsson, L.[*5], Nagashima, U.[*1]: **DNA and Estrogen Receptor Interaction Revealed by Fragment Molecular Orbital Calculations**

J. Phys. Chem. B, **111**, 9621-9627（2007）

Molecular orbital calculations of the complex between DNA-ERE（estrogen response element）and ER（estrogen receptor）-DBD（DNA-binding domain）were performed using the fragment molecular orbital（FMO）method, which enables large-scale MO（molecular orbital）calculations by reducing the computational cost and by significantly increasing efficiency for parallel computation. Such a large system, which contains 3354 atoms, is impractical via conventional MO methods due to the immense computational cost. Details of the interaction between DNA-ERE and ER-DBD were revealed in this study as follows by using the FMO calculations to analyze the interfragment interaction energies（IFIEs）and the electrostatic potentials（ESPs）. An area with a high positive ESP is identified on the DNA-binding side of ER-DBD and is the main driving force behind access to the DNA. The position of the ER-DBD monomer can be fixed on a phosphate group of DNA-ERE by the strong electrostatic interactions, whereas the rotation cannot be fixed. In contrast, both the position and rotation of the ER-DBD dimer can be fixed and can therefore form the stable（ER-DBD）2âââDNA-ERE complex. Dimerization of the ER-DBD monomers, each of which have a charge of +5, is mainly due to large attractive interaction energies of the second Zn fragments. The base pairs in the consensus sequence of DNA-ERE interact only with the recognition helix located in the major groove due to the large shielding effect of the phosphate groups of DNA. The recognition helix has weaker interactions with the base pairs than the electrostatic interactions with the phosphate groups. Thus, the DNA-binding machinery of the ER-DBD dimer, which can secure the recognition helix in the major groove of DNA, is crucial for interactions between the recognition helix and base pairs.

Keywords: FMO, ER-DBD, DNA-ERE

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Yoshida, K[*]., Hirabayashi, Y., Wada, S[*]., Watanabe, F[*]., Watanabe, K[*]., Aizawa, S[*]., and Inoue, T.: **p53（TRP53）deficiency-mediated antiapoptosis escape after 5 Gy X irradiation still induces stem cell leukemia in C3H/He mice: comparison between whole-body assay and bone marrow transplantation（BMT）assay**.

Radiat Res **167**, 703-710（2007）

Mice exposed to a lethal dose of radiation were repopulated with heterozygous p53（+/−）（TRP53（+/−））bone marrow cells and then exposed to doses of 1, 3 and 5 Gy 1 month later. This resulted in the transplanted bone marrow-specific diseases other than competitively induced nonhematopoietic neoplasms. Interestingly, the present study showed a high frequency of stem cell leukemia, i.e., leukemias characterized by a lack of differentiation due also to p53 deficiency, even after 5 Gy irradiation. The frequencies of stem cell leukemias（and those of total hematopoietic malignancies）were 16%（24%）at 1 Gy and 45%（75%）at 3 Gy. Furthermore, markedly high incidences of stem cell leukemias were observed at 5 Gy in p53（+/−）mice, i.e., 87%（100%）in the transplantation assay and 60%（83.3%）in the whole-body assay, whereas a conventional whole-body assay induced only 14% in wild-type mice. The high incidence of stem cell leukemias observed in this study using heterozygous p53-deficient mice agrees with results of a previous study of homozygous p53-deficient mice and is consistent with the high frequency of loss of heterozygosity in the p53 wild-type allele observed in leukemias. This suggests that the target cells for radiation-induced stem cell leukemias may be p53-deficient hematopoietic stem cells.

Keywords: p53, hematopoietic stem/progenitor cell, 5Gy-X-ray-irradiation

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Suzuki, H[*]., Inoue, T[*]., Matsushita, T[*]., Kobayashi, K[*]., Horii, I[*]., Hirabayashi, Y., and Inoue, T.: **In vitro gene expression analysis of hepatotoxic drugs in rat primary hepatocytes**.

J Appl Toxicol **28**, 227-236（2008）

The study examined the feasibility of screening for hepatotoxicity by an in vitro gene expression analysis

using rat primary hepatocytes and Affymetrix Rat Toxicology U34 arrays. Hepatocytes were exposed for 6 or 24 h to eight drugs, with different mechanisms of hepatotoxicity, at one third of the cytotoxic concentration TC50, i.e. acetaminophen, cyclophosphamide, clofibrate, chlorpromazine, lithocholic acid, cisplatin, diclofenac and disulfiram. The types of transcriptional changes observed in this study were generally consistent with previously reported in vivo data, although there were some differences. In hierarchical cluster analysis, drugs formed clusters depending on their mode of toxicity against cells. The number of transcripts affected by the cholestatic hepatotoxicants (lithocholic acid and chlorpromazine) or the drugs that rarely cause of hepatotoxicity (cisplatin, diclofenac and disulfiram) were limited compared with the other drugs (acetaminophen, clobifibrate and cyclophosphamide), where they did not induce transcriptional changes apparently related to toxicity. It is concluded that in vitro gene expression analysis of hepatocytes using microarray is a useful tool for evaluating the toxicological profile of drugs and in screening for the direct toxicity of drugs against hepatocytes.

Keywords: Gene expression, U34 rat array, hepatotoxicity

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Suzuki, H*., Inoue, T*., Matsushita, T*., Kobayashi, K*., Horii, I*., Hirabayashi, Y., and Inoue, T.: **In vitro gene expression analysis of nephrotoxic drugs in rat primary renal cortical tubular cells**.

J Appl Toxicol **28**, 237-248 (2008)

Rat primary renal cortical tubular cells were exposed to seven test substances, some with, and some without, known direct renal tubular cell toxicity. Cells were exposed to the substances at either one-third or one-tenth of the TC50 for cytotoxicity for 6 h or 24 h, so as not to induce cytotoxicity but to cause some transcriptional changes. Transcriptional profiles were investigated by using the Affymetrix Rat Toxicology U34 arrays, containing probes for more than 850 genes and ESTs. Four direct toxicants, cisplatin (CDDP), its less nephrotoxic analogue carboplatin (CBDCA), cephaloridine and gentamicin, were grouped together in a hierarchical clustering. In addition, the four direct toxicants affected more than 32 transcripts at their subcytotoxic concentrations at either 6 h or 24 h exposure. On the other hand, diclofenac, cyclosporine A and zinc, which are not considered to be directly toxic to tubules, affected less than 12 transcripts. Decreased Map3k12 and increased Hmox1 were commonly observed among the four direct toxicants, which appeared to be responses to cellular damage. Two platinum complexes, CDDP and CBDCA, induced similar changes, regardless of exposure duration or concentration. The types of transcriptional changes observed in this study were consistent with previously reported in vivo data, although there were some differences. These observations suggest that an in vitro gene expression analysis approach using GeneChip is feasible for screening for direct tubular toxicity of drugs and may help to clarify the underlying mechanisms of tubular toxicity.

Keywords: Gene expression, U34 rat array, nephrotoxicity

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Baniasadi, S.*1, 2, Chairoungdua, A.*2, Iribe, Y.*2, Kanai, Y.*2, Endou, H.*2, Aisaki, K., Igarashi, K., Kanno, J.: **Gene expression profiles in T24 human bladder carcinoma cells by inhibiting an L-type amino acid transporter, LAT1**.

Arch. Pharm. Res., **30**(4), 444-452 (2007)

Inhibition of LAT1 (L-type amino acid transporter 1) activity in tumor cells could be effective in the inhibition of tumor cell growth by depriving tumor cells of essential amino acids. Because of the high level of expression of LAT1 in tumor cells, LAT1 inhibitors would be useful for anticancer therapy in suppressing tumor growth without affecting normal tissues. In recent years, cDNA microarray technique is useful technology for anticancer drug development. It allows identifying and characterizing new targets for developments in cancer drug therapy through the understanding genes involved in drug action. The present study was designed to investigate gene expression profile induced by LAT1 inhibitor using gene chip technology. Human bladder carcinoma cells (T24 cells) were treated with classical system L inhibitor 2-aminobicyclo-(2, 2, 1)-heptane-2-carboxylic acid (BCH). Gene chip experiment was applied for treated and untreated cells after 3 and 12 h. Two independent experiments with a high degree of concordance identified the

altered expression of 151 and 200 genes after 3 and 12 h BCH treatment. Among these genes, 132 and 13 were up-regulated and 19 and 187 were down-regulated by 3 and 12 h BCH treatment respectively. We found that BCH affected the expression of a large number of genes that are related to the control of cell survival and physiologic behaviors. These data are useful for understanding of intracellular signaling of cell growth inhibition induced by LAT1 inhibitors as candidate for anticancer drug therapy.

Keywords: BCH, Gene expression, Microarray, Bladder carcinoma cells, LAT1

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Wetherill, Y.B.*1, 2, Akingbemi, B.T.*3, Kanno, J., McLachlan, J.A.*4, Nadal, A.*5, Sonnenschein, C.*6, Watson, C.S.*7, Zoeller, R.T.*8, Belcher, S.M.*9: **In vitro molecular mechanisms of bisphenol A action**.

Reprod. Toxicol., **24**(2), 178-198 (2007)

Bisphenol A (BPA, 2,2-bis (4-hydroxyphenyl) propane; CAS# 80-05-7) is a chemical used primarily in the manufacture of polycarbonate plastic, epoxy resins and as a non-polymer additive to other plastics. Recent evidence has demonstrated that human and wildlife populations are exposed to levels of BPA which cause adverse reproductive and developmental effects in a number of different wildlife species and laboratory animal models. However, there are major uncertainties surrounding the spectrum of BPA's mechanisms of action, the tissue-specific impacts of exposures, and the critical windows of susceptibility during which target tissues are sensitive to BPA exposures. As a foundation to address some of those uncertainties, this review was prepared by the "In vitro" expert sub-panel assembled during the "Bisphenol A: An Examination of the Relevance of Ecological, In vitro and Laboratory Animal Studies for Assessing Risks to Human Health" workshop held in Chapel Hill, NC, Nov 28-29, 2006. The specific charge of this expert panel was to review and assess the strength of the published literature pertaining to the mechanisms of BPA action. The resulting document is a detailed review of published studies that have focused on the mechanistic basis of BPA action in diverse experimental models and an assessment of the strength of the evidence regarding the published BPA research.

Keywords: Bisphenol A, Endocrine disruption, In vitro mechanisms

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Kato, Y.*1, Ikushiro, S.*2, Takiguchi, R.*3, Haraguchi, K.*4, Koga, N.*5, Uchida, S.*3, Sakaki, T.*2, Yamada, S.*3, Kanno, J., Degawa, M.*3: **A novel mechanism for polychlorinated biphenyl-induced decrease in serum thyroxine level in rats**.

Drug Meta. Dispos., **35**(10), 1949-1955 (2007)

We have previously suggested that the decrease in the levels of serum total thyroxine (T(4)) and free T(4) by a single administration to rats of Kanechlor-500 (KC500) at a dose of 100 mg/kg is not necessarily dependent on the increase in hepatic T(4)-UDP-glucuronosyltransferase (UDP-GT). In the present study, we determined whether or not a consecutive treatment with KC500 at a relatively low dose (10 mg/kg i.p., once daily for 10 days) results in a decrease in the level of serum total T(4) and further investigated an exact mechanism for the KC500-induced decrease in the T(4). At 4 days after final treatment with KC500, the serum total T(4) and free T(4) levels were markedly decreased in both Wistar and UGT1A-defi-

cient Wistar (Gunn) rats, whereas significant increases in hepatic T(4)-UDP-GT activity were observed in Wistar rats but not in Gunn rats. The level of serum thyroid-stimulating hormone was not significantly changed in either Wistar or Gunn rats. Clearance from serum of the [(125)I]T(4) administered to the KC500-pretreated Wistar and Gunn rats was faster than that to the corresponding control (KC500-untreated) rats. The accumulated level of [(125)I]T(4) was increased in several tissues, especially the liver, in the KC500-pretreated rats. The present findings demonstrated that a consecutive treatment with KC500 resulted in a significant decrease in the level of serum total T(4) in both Wistar and Gunn rats and further indicated that the KC500-induced decrease would occur through increase in accumulation of T(4) in several tissues, especially the liver, rather than increase in hepatic T(4)-UDP-GT activity.

Keywords: Polychlorinated biphenyl, Thyroxine, Rats

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Nakatsu, N.[*1], Nakamura, T.[*1,2], Yamazaki, K.[*1], Sadahiro, S.[*2], Makuuchi, H.[*2], Kanno, J., Yamori, T,[*1]: **Evaluation of action mechanisms of toxic chemicals using JFCR39, a panel of human cancer cell lines**.
Mol. Pharmacol., **72**(5), 1171-1180 (2007)

We previously established a panel of human cancer cell lines, JFCR39, coupled to an anticancer drug activity database; this panel is comparable with the NCI60 panel developed by the National Cancer Institute. The JFCR39 system can be used to predict the molecular targets or evaluate the action mechanisms of the test compounds by comparing their cell growth inhibition profiles (i.e., fingerprints) with those of the standard anticancer drugs using the COMPARE program. In this study, we used this drug activity database-coupled JFCR39 system to evaluate the action mechanisms of various chemical compounds, including toxic chemicals, agricultural chemicals, drugs, and synthetic intermediates. Fingerprints of 130 chemicals were determined and stored in the database. Sixty-nine of 130 chemicals (approximately 60%) satisfied our criteria for the further analysis and were classified by cluster analysis of the fingerprints of these chemicals and several standard anticancer drugs into the following three clusters: 1) anticancer drugs, 2) chemicals that shared similar action mechanisms (for example, ouabain and digoxin), and 3) chemicals whose action mechanisms were unknown. These results suggested that chemicals belonging to a cluster (i.e., a cluster of toxic chemicals, a cluster of anticancer drugs, etc.) shared similar action mechanism. In summary, the JFCR39 system can classify chemicals based on their fingerprints, even when their action mechanisms are unknown, and it is highly probable that the chemicals within a cluster share common action mechanisms.

Keywords: Cancer Cell Panel, 50% growth inhibition, Toxicity

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Nakamura, T.[*1,2], Imai, Y.[*1,3], Matsumoto, T.[*1,2], Sato, S.[*4], Takeuchi, K.[*1], Igarashi, K., Harada, Y.[*5], Azuma, Y.[*5], Krust, A.[*6], Yamamoto, Y.[*1], Nishina, H.[*4], Takeda, S.[*4], Takayanagi, H.[*4], Metzger, D.[*6], Kanno, J., Takaoka, K.[*3], Martin, T.J.[*7], Chambon, P.[*6], Kato, S.[*1,2]: **Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts**.
Cell. **130**(5), 811-23 (2007)

Estrogen prevents osteoporotic bone loss by attenuating bone resorption; however, the molecular basis for this is unknown. Here, we report a critical role for the osteoclastic estrogen receptor alpha (ERalpha) in mediating estrogen-dependent bone maintenance in female mice. We selectively ablated ERalpha in differentiated osteoclasts (ERalpha (DeltaOc/DeltaOc)) and found that ERalpha (DeltaOc/DeltaOc) females, but not males, exhibited trabecular bone loss, similar to the osteoporotic bone phenotype in postmenopausal

women. Further, we show that estrogen induced apoptosis and upregulation of Fas ligand (FasL) expression in osteoclasts of the trabecular bones of WT but not ERalpha (DeltaOc/DeltaOc) mice. The expression of ERalpha was also required for the induction of apoptosis by tamoxifen and estrogen in cultured osteoclasts. Our results support a model in which estrogen regulates the life span of mature osteoclasts via the induction of the Fas/FasL system, thereby providing an explanation for the osteoprotective function of estrogen as well as SERMs.

Keywords: Estrogen Receptor alpha, Osteoclast, Osteoporosis, Fas Ligand

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Takagi, A., Hirose, A.*1, Nishimura, T.*2, Fukumori, N.*3, Ogata, A.*3, Ohashi, N.*3, Kitajima, S., Kanno, J. : **Induction of mesothelioma in p53+/− mouse by intraperitoneal application of multi-wall carbon nanotube**.

J. Toxicol. Sci., **33**(1), 105-116 (2008)

Nanomaterials of carbon origin tend to form various shapes of particles in micrometer dimensions. Among them, multi-wall carbon nanotubes (MWCNT) form fibrous or rod-shaped particles of length around 10 to 20 micrometers with an aspect ratio of more than three. Fibrous particles of this dimension including asbestos and some man-made fibers are reported to be carcinogenic, typically inducing mesothelioma. Here we report that MWCNT induces mesothelioma along with a positive control, crocidolite (blue asbestos), when administered intraperitoneally to p53 heterozygous mice that have been reported to be sensitive to asbestos. Our results point out the possibility that carbon-made fibrous or rod-shaped micrometer particles may share the carcinogenic mechanisms postulated for asbestos. To maintain sound activity of industrialization of nanomaterials, it would be prudent to implement strategies to keep good control of exposure to fibrous or rod-shaped carbon materials both in the workplace and in the future market until the biological/ carcinogenic properties, especially of their long-term biodurability, are fully assessed.

Keywords: Multi-wall carbon nanotube (MWCNT), Mesothelioma, P53 heterozygous mouse

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Hirabayashi, Y., Yoon, B.I., Tsuboi, I., Huo, Y., Kodama, Y., Kanno, J., Ott, T.*1, Trosko, J.E.*2, Inoue, T. : **Protective role of connexin 32 in steady-state hematopoiesis, regeneration state, and leukemogenesis**.

Exp. Biol. Med., **232**, 700-712 (2007)

The role of gap junctions formed by connexins (Cxs) has been implicated in the homeostatic regulation of multicellular systems. Primitive hematopoietic progenitor cells form a multicellular system, but a previous report states that Cx32 is not expressed in the bone marrow. Thus, a question arises as to why Cx molecules are not detected in the hematopoietic tissue other than in stromal cells. Based on our preliminary study, which suggested a potential impairment of hematopoiesis in Cx32-knockout (KO) mice, the objectives of the present study were to determine whether Cx32 functions in the bone marrow during steady-state hematopoiesis and to examine its possible protective roles during regeneration after chemical abrasions and during leukemogenesis after the administration of a secondary genotoxic chemical, methyl nitrosourea (MNU). As a result, the Cx32 molecule, functioning in the hematopoietic stem cell (HSC) compartment during steady-state hematopoiesis, was observed for the first time; the expressions of Cx32 at the mRNA level, as determined by polymerase chain reaction analysis, and at the protein level, determined using an anti-Cx32 antibody, were observed only in the lin(−)c-kit(+) HSC fraction, using a combination of immunobead-density gradient and immunomagnetic bead separation. Hematopoiesis

was impaired in the absence of Cx32, and it was delayed during regeneration after chemical abrasion with 5-fluorouracil at 150 mg/kg body wt in Cx32-KO mice. Cx32-KO mice showed increased leukemogenicity compared with wild-type mice after MNU injection; furthermore, in a competitive assay for leukemogenicity in mice that had been lethally irradiated and repopulated with a mixed population of bone marrow cells from Cx32-KO mice and wild-type mice, the resulting leukemias originated predominantly from Cx32-KO bone marrow cells. In summary, the role of Cx32 in hematopoiesis was not previously recognized, and Cx32 was expressed only in HSCs and their progenitor cells. The results indicate that Cx32 in wild-type mice protects HSCs from chemical abrasion and leukemogenic impacts.

Keywords: connexin 32, hematopoietic stem/progenitor cell, MNU-induced leukemia

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Hirabayashi, Y., Yoon, B.I., Tsuboi, I., Huo, Y., Kodama, Y., Kanno, J., Ott, T.*1, Trosko, J.E.*2, Inoue, T.: **Membrane Channel Connexin 32 Maintains Lin(−)/c-kit(+) Hematopoietic Progenitor Cell Compartment: Analysis of the Cell Cycle**.
J. Membr. Biol., **217**, 105-113 (2007)

Membrane channel connexin (Cx) forms gap junctions that are implicated in the homeostatic regulation of multicellular systems; thus, hematopoietic cells were assumed not to express Cxs. However, hematopoietic progenitors organize a multicellular system during the primitive stage; thus, the aim of the present study was to determine whether Cx32, a member of the Cx family, may function during the primitive steady-state hematopoiesis in the bone marrow (BM). First, the numbers of mononuclear cells in the peripheral blood and various hematopoietic progenitor compartments in the BM decreased in Cx32-knockout (KO) mice. Second, on the contrary, the number of primitive hematopoietic progenitor cells, specifically the Lin(−)/c-kit(+)/Scal(+)fraction, the KSL progenitor cell compartment, also increased in Cx32-KO mice. Third, expression of Cx32 was detected in Lin(−)/c-kit(+)hematopoietic progenitor cells of wild-type mice (0.27% in the BM), whereas it was not detected in unfractionated wild-type BM cells. Furthermore, cell-cycle analysis of the fractionated KSL compartment from Cx32-KO BM showed a higher ratio in the G(2)/M fraction. Taken together, all these results imply that Cx32 is expressed solely in the primitive stem cell compartment, which maintains the stemness of the cells, i.e., being quiescent and noncycling; and once Cx32 is knocked out, these progenitor cells are expected to enter the cell cycle, followed by proliferation and differentiation for maintaining the number of peripheral blood cells.

Keywords: connexin 32, hematopoietic stem/progenitor cell, cell cycle

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Hirabayashi, Y., Yoon, B.I., Li, G.X., Kanno, J., Fujii-Kuriyama, Y.*, Inoue, T.: **Aryl hydrocarbon receptor suppresses spontaneous neoplasms and extends life span: possible mechanism implied by hematopoietic stem cell kinetics**.
Organohalogen Compounds **69**, 357-361 (2007)

The aryl hydrocarbon receptor (AhR) is an orphan receptor whose original physiological function remains unknown. Since AhR-knockout (KO) mice were found to show an earlier onset of spontaneous neoplasms than wild-type mice, AhR was assumed to play a suppressor gene function (Hirabayashi, 2006). However, because not all AhR-KO ($AhR^{-/-}$) mice or wild-type mice die of spontaneous neoplasms, the function of wild-type AhR may also be associated with a possible genomic stabilization, thereby consequently extending the life span of mice simultaneously. What are the underlying mechanisms that contribute to these suppressed cell cycle and extended longevity? The result of the evaluation of a reactive oxygen species (ROS) using a DCFH-DA dye showed a prominent increase in oxidative stress in unfractionated bone marrow cells as well as in hematopoietic progenitor cells in the $AhR^{-/-}$ mice. Hematopoietic progenitor cells are quiescent in an anoxic environment, and are regulated by a weak oxidative stimulation. Thus, the higher reactivity of the fraction to the DCFH-DA dye in the $AhR^{-/-}$ mice is in good agreement with the underlying mechanism of genomic stabilization under a low oxidative tension in combination with the suppressor gene function and the consequent longevity observed in wild-type, $AhR^{+/+}$

mice.

Keywords: Aryl hydrocarbon receptor, lifespan, hematopoietic stem/progenitor cell

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Shimazaki, M.[*1], Nakamura, K.[*2], Kii, I.[*1], Kashima, T.[*2], Amizuka, N.[*3], Li, M.[*3], Saito, M.[*4], Fukuda, K.[*5], Nishiyama, T.[*1], Kitajima, S., Saga, Y.[*6], Fukayama, M.[*2], Sata, M.[*2] and Kudo.[*1], A.: **Periostin is essential for cardiac healing after acute myocardial infarction**.

J Exp Med **205**, 295-303（2008）

Acute myocardial infarction（AMI）is a common and lethal heart disease, and the recruitment of fibroblastic cells to the infarct region is essential for the cardiac healing process. Although stiffness of the extracellular matrix in the infarct myocardium is associated with cardiac healing, the molecular mechanism of cardiac healing is not fully understood. We show that periostin, which is a matricellular protein, is important for the cardiac healing process after AMI. The expression of periostin protein was abundant in the infarct border of human and mouse hearts with AMI. We generated periostin（-/-）mice and found no morphologically abnormal cardiomyocyte phenotypes; however, after AMI, cardiac healing was impaired in these mice, resulting in cardiac rupture as a consequence of reduced myocardial stiffness caused by a reduced number of alpha smooth muscle actin-positive cells, impaired collagen fibril formation, and decreased phosphorylation of FAK. These phenotypes were rescued by gene transfer of a spliced form of periostin. Moreover, the inhibition of FAK or alphav-integrin, which blocked the periostin-promoted cell migration, revealed that alphav-integrin, FAK, and Akt are involved in periostin signaling. Our novel findings show the effects of periostin on recruitment of activated fibroblasts through FAK-integrin signaling and on their collagen fibril formation specific to healing after AMI.

Keywords: Periostin, cardiac healing, acute myocardial infarction

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David, R.[*1], Brenner, C.[*1], Stieber, J.[*2], Schwarz, F.[*1], Brunner, S.[*1], Vollmer, M.[*3], Mentele, E., Muller-Hoecker, J.[*5], Kitajima, S., Lickert, H.[*3], Rupp, R. and Franz, W.M.[*1]: **MesP1 drives vertebrate cardiovascular differentiation via Dkk-1 mediated blockage of wnt-signalling**.

Nat Cell Biol **10**: 338-345（2008）

ES-cell-based cardiovascular repair requires an in-depth understanding of the molecular mechanisms underlying the differentiation of cardiovascular ES cells. A candidate cardiovascular-fate inducer is the bHLH transcription factor MesP1. As one of the earliest markers, it is expressed specifically in almost all cardiovascular precursors and is required for cardiac morphogenesis. Here we show that MesP1 is a key factor sufficient to induce the formation of ectopic heart tissue in vertebrates and increase cardiovasculogenesis by ES cells. Electrophysiological analysis showed all subtypes of cardiac ES-cell differentiation. MesP1 overexpression and knockdown experiments revealed a prominent function of MesP1 in a gene regulatory cascade, causing Dkk-1-mediated blockade of canonical Wnt-signalling. Independent evidence from ChIP and in vitro DNA-binding studies, expression analysis in wild-type and MesP knockout mice, and reporter assays confirm that Dkk-1 is a direct target of MesP1. Further analysis of the regulatory networks involving MesP1 will be required to preprogramme ES cells towards a cardiovascular fate for cell therapy and cardiovascular tissue engineering. This may also provide a tool to elicit cardiac transdifferentiation in native human adult stem cells.

Keywords: MesP1, cardiovascular-fate inducer, formation of ectopic heart tissue

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Kiyosawa, N.[*1], Uehara, T.[*1], Gao, W.[*1], Omura, K.[*1], Hirode, M.[*1], Shimizu, T.[*1], Mizukawa, Y.[*2], Ono, A., Miyagishima, T.[*1], Nagao, T. and Urushidani, T.[*1, 2]: **Identification of glutathione depletion-responsive genes using phorone-treated rat liver**.

J Toxicol Sci, **32**, 469-486 (2007)

To identify candidate biomarker gene sets to evaluate the potential risk of chemical-induced glutathione depletion in livers, we conducted microarray analysis on rat livers administered with phorone (40, 120 and 400 mg/kg), a prototypical glutathione depletor. Hepatic glutathione content was measured and glutathione depletion-responsive gene probe sets (GSH probe sets) were identified using Affymetrix Rat Genome 230 2.0 GeneChip by the following procedure. First, probe sets, whose signal values were inversely correlated with hepatic glutathione content throughout the experimental period, were statistically identified. Next, probe sets, whose average signal values were greater than 1.5-fold compared to those of controls 3 hr after phorone treatment, were selected. Finally, probe sets without unique Entrez Gene ID were removed, ending up with 161 probe sets in total. The usefulness of the identified GSH probe sets was verified by a toxicogenomics database. It was shown that signal profiles of the GSH probe sets in rats treated with bromobenzene were strongly altered compared with other chemicals. Focusing on bromobenzene, time-course profiles of hepatic glutathione content and gene expression revealed that the change in gene expression profile was marked after the bromobenzene treatment, whereas hepatic glutathione content had recovered after initial acute depletion, suggesting that the gene expression profile did not reflect the hepatic glutathione content itself, but rather reflects a perturbation of glutathione homeostasis. The identified GSH probe sets would be useful for detecting glutathione-depleting risk of chemicals from microarray data.

Keywords: Gene Expression Profiling; Glutathione depletion; Toxicogenonics

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Takahashi, Y., Takagi, A., Hiraoka, S.[*1], Koseki, H.[*1], Kanno, J., Rawls, A.[*2] and Saga, Y.[*3]: **Transcription factors Mesp2 and Paraxis have critical roles in axial musculoskeletal formation**

Developmental Dynamics, **236**, 1484-1494 (2007)

Mesp2 and *Paraxis* are basic HLH-type transcription factors co-expressed in the presomitic mesoderm and are required for normal somite formation. Here we show that *Mesp2/Paraxis* double-null mice exhibit a distinct phenotype unexpected from either *Mesp2* or *Paraxis* single-null mice. In the posterior region of the body, most of the skeletal components of both the vertebral body and neural arches are severely reduced and only a rudimental lamina and ribs remain, indicating a strong genetic interaction in the sclerotomal cell lineage. However, yeast two-hybrid analyses revealed no direct interaction between Mesp2 and Paraxis. The *Mesp2/Paraxis* double-null embryo has caudalized somites, revealed by expanded *Uncx4.1* expression pattern observed in the *Mesp2*-null embryo, but the expression level of *Uncx4.1* was significantly decreased in mature somites, indicative of hypoplasia of lateral sclerotome derivatives. By focusing on vertebral column formation we found that expressions of *Pax1*, *Nkx3.1*, and *Bapx1* are regulated by Paraxis and that Pax9 expression was severely affected in the *Mesp2/Paraxis* double-null embryo. Furthermore, the expression of *Pax3*, a crucial factor for hypaxial muscle differentiation, is regulated by both Mesp2 and Paraxis in the anteriormost PSM and nascent somite region. The present data strongly suggest that patterning events by bHLH-type transcription factors have deep impacts on regional chondrogenic and myogenic differentiation of somitic cells, mainly via control of *Pax* genes.

Keywords: Mesp2, Paraxis, vertebra

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Sato, K., Akaishi, T.[*1], Matsuki, N.[*2], Ohno, Y., Nakazawa, K.: **β-Estradiol induces synaptogenesis in the hippocampus by enhancing brain-derived neurotrophic factor release from dentate gyrus granule cells**

Brain Res., **1150**, 108-120 (2007)

培養海馬切片を用いて海馬のシナプス形成に対するβ-エストラジオール（E2）の作用を検討した．E2は後シナプス部マーカーであるPSD95発現をCA3野 stratum

lucidum（CA3SL）で上昇させた．E2は CA3SLの樹状突起起始部のスパイン密度も上昇させ PSD95 はスパイン頭部に密集していた．E2の作用は培養一日目に歯状回（DG）を切除すると消失した．アンモン核神経細胞，DG神経細胞，およびこれらの混合細胞からなる海馬小領域培養系を用いたFM1-43解析では，E2はDG神経細胞を含む小領域培養において前シナプス部の数を増加させた．脳由来神経栄養因子（BDNF）受容体の強力な阻害薬であるK252aおよびBDNF機能中和抗体は海馬切片培養と小領域培養で見受けられた E2の作用を完全に阻害したが，核内エストロゲン受容体（nER）の強力な阻害薬である ICI182,780（ICI）は阻害しなかった．DG神経細胞でのBDNF発現量はアンモン核神経細胞より顕著に高く，E2は発現レベルに影響を与えなかった．E2は DG神経細胞からのBDNF放出を有意に促進した．PKAの選択的阻害薬である KT5720とcAMPの非水解性ジアステレオマーであり強力なPKA阻害薬であるRp-cAMPはE2によるBDNF放出促進を完全に抑制したが，ICIとMEK阻害剤であるU0126は阻害しなかった．これらの結果はE2はDG神経細胞からのBDNF放出をnER非依存的かつPKA依存的に促進することにより苔状線維－CA3神経細胞間のシナプス形成を誘導していることを示唆している．

Keywords: estrogen, hippocampus, synaptogenesis

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Ohkubo, S., Nagata, K.[*], Nakahata, N.[*]: **Adenosine uptake-dependent C6 cell growth inhibition**.
Eur J Pharmacol., **577**, 35-43（2007）

In C6 glioma cells, adenine nucleotides, especially AMP, and adenosine inhibited cell proliferation in time- and concentration-dependent manners. alpha,beta-methylene-ADP, an ecto-5'-nucleotidase inhibitor, suppressed the hydrolysis of AMP and reversed the inhibition of cell growth induced by AMP but not by adenosine. Adenosine deaminase eliminated both AMP- and adenosine-mediated growth inhibitions. 5'-N-ethylc arboxamidoadenosine, an adenosine receptor agonist, had little effect on the cell growth. Equilibrative nucleoside transporters, ENT-1 and ENT-2, were expressed in C6 cells by determining their mRNAs. ENT inhibitors, nitrobenzylthioinosine and dipyridamole, suppressed the uptake of [(3)H]adenosine into C6 cells, and attenuated AMP- or adenosine-mediated growth inhibition. Furthermore, an adenosine kinase inhibitor 5-iodotubercidin reversed the growth inhibition induced by AMP and adenosine. When uridine was added in the extracellular space, AMP- or adenosine-induced cell growth inhibition was completely reversed, suggesting that intracellular pyrimidine starvation would be involved in their cytostatic effects. These results indicate that extracellular adenine nucleotides inhibit C6 cell growth via adenosine, which is produced by ecto-nucleotidases including CD73 at the extracellular space and then incorporated into cells by ENT2. Intracellular AMP accumulation by adenosine kinase after adenosine uptake would induce C6 cell growth inhibition through pyrimidine starvation.

Keywords: AMP, Adenosine, Nucleoside transporter

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Matsuoka, R.[*1], Ohkubo, S., Yoshida, M.[*2], Nakahata, N.[*1]: **Alteration of Adenylyl Cyclase Type 6 Expression in Human Astrocytoma Cells After Exposure to Simulated Microgravity**
Journal of Health Science, **53**, 534-542（2007）

Although many physiological changes after space flight have been reported, it is not clear how microgravity influences our bodies. The focus of the present study was to clarify the changes in G-protein-coupled receptor-mediated intracellular signaling, especially Gs-adenylyl cyclase（AC）-adenosine 3', 5'-cyclic monophosphate（cyclic AMP）pathway, under simulated microgravity. Human astrocytoma 1321N1 cells were cultivated under vector-averaged microgravity conditions generated by clinostat rotation（20 rpm）for 24 hr. Isoproterenol, a β-adrenergic agonist and forskolin, a direct AC stimulant, increased intracellular cyclic AMP level in concentration dependent manners, however, both of which response were decreased in cells cultivated in clinostat rotation. While the level of Gαs or intracellular ATP, a substrate for AC, was not changed, the AC activity was significantly low in the membranes of clinostat-rotated cells. Reverse transcriptase-polymerase chain reaction（RT-PCR）analysis revealed that AC type 3（AC3）, AC6, and AC9 and to a lesser extent AC7 and AC8 were expressed in 1321N1 cells. Among them, the expression of AC6 mRNA was significantly decreased by clinostat rotation. These results indicate that intracellular cyclic AMP production by agonists may be decreased via a reduction in AC6

expression under simulated microgravity conditions.
Keywords: simulated microgravity, adenylyl cyclase, adenosine 3', 5'-cyclic monophosphate

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Kobayashi, D.*, Ohkubo, S., Nakahata, N.*: **Cooperation of calcineurin and ERK for UTP-induced IL-6 production in HaCaT keratinocytes**
Eur J Pharmacol., **573**, 249-52 (2007)

UTP causes IL-6 production in HaCaT keratinocytes, which is partially inhibited by PD98059, a mitogen-activated protein kinase kinase (MEK) inhibitor, suggesting that a pathway other than the extracellular signal-regulated kinase (ERK) pathway is involved in the production. In the present study, we examined the involvement of calcineurin in the UTP-induced interleukin (IL)-6 production in HaCaT keratinocytes. FK506 and cyclosporine A, calcineurin inhibitors, partially inhibited UTP-induced IL-6 mRNA expression and protein production. In addition, combined application of FK506 and PD98059 synergistically inhibited the UTP-induced IL-6 production. These results suggest that ERK and calcineurin are cooperatively involved in UTP-induced IL-6 production.
Keywords: Keratinocyte, Interleukin-6 (IL-6), Ca^{2+}

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Iwashita, M.*, Oka, N.*, Ohkubo, S., Saito, M.*, Nakahata, N.*: **Piperlongumine, a constituent of Piper longum L., inhibits rabbit platelet aggregation as a thromboxane A2 receptor antagonist**
Eur J Pharmacol., **570**, 38-42 (2007)

Piper longum L. has been used as a crude drug for the treatment of the disorder of peripherally poor blood circulation in Asia. In the present study, we examined the effect of piperlongumine, a constituent of P. longum L., on rabbit platelet aggregation. Piperlongumine concentration-dependently inhibited platelet aggregation induced by thromboxane A(2) receptor agonist U46619, but it only slightly inhibited thrombin-induced one. Piperlongumine also inhibited U46619-induced phosphatidylinositol hydrolysis and the binding of [(3)H]SQ29548 to thromboxane A(2) receptor with a similar concentration-dependency to the aggregation. It is assumed that piperlongumine inhibits platelet aggregation as a thromboxane A(2) receptor antagonist.
Keywords: Platelet aggregation, Piperlongumine, Thromboxane A_2 receptor

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Sasaki, M.*, Sukegawa, J.*, Miyosawa, K.*, Yanagisawa, T.*, Ohkubo, S., Nakahata, N.*: **Low expression of cell-surface thromboxane A2 receptor beta-isoform through the negative regulation of its membrane traffic by proteasomes**
Prostaglandins Other Lipid Mediat. **83**, 237-49 (2007)

Human thromboxane A_2 receptor (TP) consists of two alternatively spliced isoforms, TP alpha and TP beta, which differ in their cytoplasmic tails. To examine the functional difference between TP alpha and TP beta, we searched proteins bound to C termini of TP isoforms by a yeast two-hybrid system, and found that proteasome subunit alpha 7 and proteasome activator PA28 gamma interacted potently with the C terminus of TP beta. The binding of TP beta with alpha 7 and PA28 gamma was confirmed by co-immunoprecipitation and pull-down assays. MG-132 and lactacystin, proteasome inhibitors, increased cell-surface expression of TP beta, but not TP alpha. Scatchard analysis of [(3)H]SQ29548 binding revealed that the B(max) was higher in transiently TP alpha-expressing cells than TP alpha-expressing cells. In addition, TP-mediated phosphoinositide hydrolysis was clearly observed in TP alpha-, but not TP beta-expressing cells. These results suggest that TP beta binds to alpha 7 and PA28 gamma, and the cell-surface expression of TP beta is lower than that of TP alpha through the negative regulation of its membrane traffic by proteasomes.
Keywords: Thromboxane A_2 receptor, Proteasome, PA28γ

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Usami, M., Mitsunaga, K.* and Nakazawa, K.: **Comparative proteome analysis of the embryo proper and yolk sac membrane of day 11.5 cultured rat embryos**
Birth Defects Res. B Dev. Reprod. Toxicol., **80**, 383-395 (2007)

Protein expression in cultured postimplantation rat embryos were analyzed by two-dimensional electro-

phoresis (2-DE) and mass-spectrometric protein identification. Rat embryos were cultured from day 9.5 for 48 h or from day 10.5 for 24 h. About 800 and 1,000 protein spots were matched through the replicate 2-DE gels each from one embryo in the embryo proper and yolk sac membrane, respectively, and virtually the same protein spots were observed irrespective to the length of culture period. From protein spots specific to the embryo proper (126 spots) and yolk sac membrane (304 spots), proteins involved in tissue-characteristic functions, such as morphogenesis and nutritional transfer, were identified. Proteomic analysis of cultured postimplantation rat embryos will be a new approach in developmental biology and toxicology at the protein level.

Keywords: rat embryo, proteome, two-dimensional electrophoresis

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Nakajima, M.*, Takahashi, H.*, Nakazawa, K. and Usami, M.: **Fetal cartilage malformation by intravenous administration of indium trichloride to pregnant rats**

Reprod. Toxicol., **24**, 409-413 (2007)

The effects of indium on bone and cartilage development in rat fetuses were examined. Pregnant Sprague Dawley (SD) rats were treated with indium trichloride (0.1, 0.2, or 0.3mg/kg) by single intravenous administration on Day 10 of gestation, and their fetuses were examined on Day 21. Half of each litter was prepared for skeletal examinations using a skeletal double-staining technique to allow evaluation of cartilage as well as bone. Dose-related increased incidences of external and skeletal fetal malformations occurred at doses of 0.2mg/kg or more. The incidences of cartilage malformations in the vertebrae, ribs, and forepaw phalanges were significantly increased at 0.3mg/kg. Malformations of the axial bone were accompanied by cartilage malformations. It was concluded from these results that indium produced cartilage malformations, that were considered to be the underlying cause for the majority of fetal skeletal malformations observed in rats in this study.

Keywords: indium, developmental toxicity, rat

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Mimoto, A.*1, Fujii, M.*1, Usami, M., Shimamura, M.*1, 2, Hirabayashi, N.*1, 2, Kaneko, T.*2, Sasagawa, N.*1 and Ishiura, S.*1: **Identification of an estrogenic hormone receptor in Caenorhabditis elegans**

Biochem. Biophys. Res. Commun., **364**, 883-888 (2007)

Of the 284 known nuclear hormone receptors (NHRs) in C. elegans, nhr-14, nhr-69, and nhr-121 were analyzed potential estrogenic hormone receptors, because they share sequence similarity with the human estrogen receptor. First, the genes were cloned and expressed in Escherichia coli, and then the affinity of each protein for estrogen was determined using a surface plasmon resonance (SPR) biosensor. All three NHRs bound estrogen in a dose-dependent fashion. Semi-quantitative RT-PCR showed that vitellogenin expression was significantly reduced in an nhr-14 mutant. This suggests that NHR-14 is a C. elegans estrogenic hormone receptor and that it controls gene expression in response to estrogen.

Keywords: C. elegans, estrogenic hormone receptor

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Usami, M., Mitsunaga, K.*1, Nakazawa, K. and Doi, O.*2: **Proteomic analysis of selenium embryotoxicity in cultured postimplantation rat embryos**.

Birth Defects Res. B Dev. Reprod. Toxicol., **83**, 80-96 (2008)

Embryotoxicity of selenium (Se) was investigated by proteomic analysis of cultured rat embryos. Rat embryos at day 9.5 or 10.5 of gestation were cultured for 48 or 24 h, respectively, in the presence of sodium selenate (100 or 150 microM) or sodium selenite (20 or 30 microM). Proteins from the embryo proper and yolk sac membrane were analyzed by two-dimensional electrophoresis for quantitative changes from those in control embryos. By the analysis of the embryo proper, actin-binding proteins were identified as proteins with quantitative changes by selenate. Many proteins showed similar changes between selenate and selenite. In the yolk sac membrane, antioxidant proteins were identified for protein spots with quantitative changes by selenite. The identified proteins with quantitative changes by selenate or selenite were considered to be candidate proteins involved in Se embryotoxicity. These proteins may also be used as biomarkers in de-

velopmental toxicity studies.
Keywords: selenium, embryotoxicity, proteomics

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Kojima, H.[*4], Ando, T., Inagaki, K.[*1], Ohhira, M.[*2], Kosaka, T.[*3], Nakamura, Y.[*5], Torishima, H.[*6], Morikawa, N.[*7], Kanno, J., Kuboki, M.[*2], Genno, M.[*6], Nokata, M.[*1], Harada, T.[*3], Morimoto, T.[*5], Yoshimura, I[*8]. and Ohno, Y.: **Validation of human skin models for skin corrosivity tests in Japan**
Altern. Animal Test. EXperiment, **13**, 36-44 (2008)

As shown in OECD test guidelines 430 and 431, the human skin epidermal assay and Transcutaneous Electrical Resistance Test (TER) were validated and peer reviewed as an alternative method to corrosivity testing; however, these methods have not been used widely in Japan. The problems related to techniques and evaluation are not clear.

Therefore, we performed a validation study of EPI-200 (EpiDerm™), a 3-dimensional cultured epidermal model and Vitrolife-Skin™, a 3-dimensional cultured skin model made in Japan as a catch-up validation trial of alternatives for skin corrosivity testing using 13 chemicals including a positive control: 10% potassium hydroxide solution in Japan.

From the obtained data, we identified the potential of utilizing these models to evaluate the corrosivity of a chemical.
Keywords: Skin corrosivity, cultured epidermal model, cultured skin model, validation

*1 Nihon Nohyaku Co., Ltd.
*2 Nippon Soda Co.,Ltd.
*3 The Inst. Environ. Toxicol.
*4 Nippon Menard Cosmetic Co., Ltd.
*5 Sumitomo Chemical Co., Ltd.
*6 Kurabo Industries Ltd.
*7 Gunze Ltd.
*8 Tokyo Univ. Science

Omori, T. [*1], Ikarashi, Y., Kanazawa, Y. [*2], Ikarashi, Y., Idehara, K.[*3], Kojima, H., Sozu, T.[*4], Arima, K.[*5], Goto, H.[*6], Hanada, T.[*7], Inoda, T.[*8], Kosaka, T.[*9], Maki, E.[*10], Morimoto, T.[*11], Shinoda, S.[*12], Shinoda, N.[*13], Takeyoshi, M.[*14], Tanaka, M.[*15], Uratani, M.[*16], Usami, M.[*17], Yamanaka, A.[*18], Yoneda, T.[*19], Yoshimura I[*20], and Yuasa, A.[*21]: **Validation studies on an alternative endpoint for the local lymph node assay (LLNA-DA) : Importance of study management**, WC6 proceedings, **429** (2008)

We conducted validation studies for a modified version of the local lymph node assay (LLNA), which was designated as the LLNA-DA. A total of 17 laboratories tested the validity of the assay by using 14 chemicals. Here, in addition to the experimental protocol, we prepared the study protocols describing the study purpose, the role of the participants, etc. Technology transfer was conducted by the developer of the assay. Prior to the studies, preliminary tests using only a positive control chemical were conducted to determine whether the experimental protocol prescribed for the assay was appropriate. A formatted data file was developed for data management. Fortunately, the results of these studies revealed small interlaboratory variations, and we believe that one of the factors that contributed to the successful results was the development of strategies and tools for study management at the planning stage itself. However, issues related to the management of validation studies have rarely been discussed. Strategies or tools developed for study management should be easily accessible and should be shared with researchers intending to conduct validation studies in the future.
Keywords: Interlaboratory validation study, Study management, Protocol, Technical transfer, Data quality

*1 Kyoto Univ. SPH
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*3 Daicel Chemical Industries, Ltd.
*4 Osaka Univ.
*5 Taisho Pharmaceutical CO., LTD.
*6 Otsuka Pharmaceutical Co., Ltd.
*7 Nippon Shinyaku Co., Ltd.
*8 Nakano Seiyaku Co., Ltd.
*9 Institute of Environmental Toxicology
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*11 Sumitomo chemical Co., Ltd.
*12 Drug Safety Testing Center Co., LTD.
*13 Santen Pharmaceutical Co., Ltd.
*14 Chemicals Evaluation and Research Institute
*15 MEIJI SEIKA KAISHA, LTD.
*16 Ishihara Sangyo Kaisha, Ltd.
*17 Hoyu Co., Ltd.
*18 Pias Corporation

*19 TOAEIYO LTD.
*20 Tokyo University of Science
*21 Fuji Film Co., Ltd.

Nishikawa, A., Imazawa, T.[*1], Umemura, T., Yoshimura, Y.[*2], and Hirose, M.[*3]: **Rapid screening for chemopreventive agents in herbal extracts in a PhIP rat model with DNA adduct and cell proliferation as end-points**

J. Toxicol. Pathol., **20**, 49-54 (2007)

This study was designed to rapidly screen for chemopreventive effects of three natural products, cyanidine-3-glycoside (CG), acetoside and rosemaric acid (RA), against PhIP-induced colonic, pancreatic and prostatic carcinogenesis in rats, using DNA adduct and cell proliferation as end-points. Ten-week-old F344 male rats were maintained for 2 weeks on a powdered basal diet containing 0.03% PhIP alone, PhIP together with 1% or 5% CG, acetoside or RA, 1% or 5% CG, acetoside or RA alone or basal diet. Immunohistochemically, PhIP DNA adduct-positive cells as well as BrdU-positive cells induced by PhIP treatment were significantly (P<0.05) reduced by the combined treatment with 5% CG in the proximal colon and pancreatic acinar cells as compared to the PhIP alone group values. In addition, combined treatment with 5% CG significantly (P<0.05) decreased numbers of BrdU-positive cells in the ventral prostate. Combined treatment with 5% RA also significantly (P<0.05) reduced PhIP DNA adduct-positive cells in the proximal and distal colon, and ventral prostate as well as BrdU-positive cells in the lateral prostate and exocrine pancreas at 5%, and in the distal colon with 1% or 5%. However, co-treatment with acetoside did not significantly affect these parameters under the present experimental conditions. These results suggest that CG and rosemaric acid may have the potential to prevent PhIP-induced carcinogenesis in the colon, prostate and/or pancreas.

Keywords: Cancer chemoprevention, PhIP, DNA adduct

*1 National Institute of Biomedical Innovation
*2 Hoshi University
*3 Food Safety Commission

Wang, M.[*1], Lao, Y.[*1], Cheng, G.[*1], Shi, Y.[*1], Villalta, PW.[*1], Nishikawa, A., and Hecht, SS.[*1]: **Analysis of adducts in hepatic DNA of rats treated with *N*-nitrosopyrrolidine**

Chem. Res. Toxicol., **20**, 634-640, (2007)

N-Nitrosopyrrolidine (NPYR) is a hepatocarcinogen in rats. It is metabolically activated by cytochrome P450 enzymes in the liver leading to the formation of 4-oxobutanediazohydroxide (4) and related intermediates that react with DNA to form adducts. We analyzed hepatic DNA of NPYR-treated rats for several adducts: N^2-THF-dGuo (13), N^6-THF-dAdo (14), N^4-THF-dCyd (17), and dThd adducts 15 and 16. The rats were treated with NPYR in the drinking water, 600 ppm for 1 week, or 200 ppm for 4 or 13 weeks. Hepatic DNA was isolated, and analyzed by capillary LC-ESI-MS-SIM, which indicated the presence of adducts 13, 14, and 17. Because these adducts can be unstable at the deoxyribonucleoside level, further analyses were carried out using DNA treated with $NaBH_3CN$, which converts adducts 13-17 to N^2-(4-hydroxybut-1-yl)dGuo [N^2-(4-HOB)dGuo, 18], N^6-(4-HOB)dAdo (19), O^2-(4-HOB)dThd (20), O^4-(4-HOB)dThd (21), and N^4-(4-HOB)dCyd (22). [^{15}N]-Labeled analogues of adducts 18-20 and 22 were synthesized and used in this analysis, which was performed by capillary LC-ESI-MS/MS-SRM. Convincing evidence for the presence of adducts 18-22 was obtained. Levels of 18, 19, 20, and 21 were (μmol/mol dGuo): 3.41-5.39, 0.02-0.04, 2.56-3.87, and 2.28-5.05, respectively. Compound 22 was not quantified due to interfering peaks. The finding of dAdo and dThd adducts is of particular interest since previous studies have shown that NPYR causes mutations at AT base pairs in DNA of rat liver.

Keywords: *N*-Nitrosopyrrolidine, hepatocarcinogen, DNA adduct

*1 University of Minnesota

Furukawa, F.[*1], Nishikawa, A., Abe, H.[*2], and Hirose, M.[*3]: **Inhibitory effects of octreotide acetate, a somatostatin analog, on spontaneous chronic pancreatitis in WBN/Kob Rats**

J. Toxicol. Pathol., **20**, 71-75, (2007)

Effects of octreotide acetate, a somatostatin analog, on the development of spontaneous pancreatitis were investigated in WBN/Kob rats. Delivery of the agent continuously for 28 days via osmotic pumps implanted subcutaneously at 6 μg/day (group 1), 3 μg/day

(group 2) or 0 μg/day (saline) (group 3) resulted in comparable body weight gain in all three groups. Relative weights of the liver, kidney, testis, spleen and pancreas also did not significantly differ between the treatments. Blood glucose levels were lowered by the high, but not the low dose treatment, while plasma somatostatin levels were remarkably increased in both the octreotide treatment groups. Remarkable hemorrhage, inflammatory cell infiltration, fibrosis, vacuolation of acinar cells and ductular proliferation were observed in the pancreas of control rats in group 3. However, these findings were consistently less intense in the octreotide treatment groups, in line with morphometric data showing fibrotic areas to be significantly (P<0.01) reduced. Immunohistochemically, collagen fibers in the intralobular space were mainly of type-III and mixed with α-smooth muscle actin, reflecting fibrosis in all groups. The present experiment demonstrated that octreotide inhibits spontaneous pancreatitis in WBN/Kob rats.

Keywords: WBN/Kob Rat, pancreatitis, octreotide acetate

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Yokota, T.[*1], Matsuzaki, Y.[*1], Koyama, M.[*1], Hitomi, T.[*1], Kawanaka, M.[*1], Enoki-Konishi, M.[*1], Okuyama, Y.[*1], Takayasu, J.[*1], Nishino, H.[*1], Nishikawa, A., Osawa, T.[*2], and Sakai, T.[*1]: **Sesamin, a lignan of sesame, down-regulates cyclin D1 protein expression in human tumor cells**.

Cancer Sci., **98**, 1447-1453, (2007)

Sesamin, a major lignan constituent of sesame, has previously reported to induce growth inhibition in human cancer cells. The authors here report that sesamin induces growth arrest at the G1 phase in cell cycle progression in the human breast cancer cell line MCF-7, and dephosphorylates tumor-suppressor retinoblastoma protein. It is also shown that inhibition of MCF-7 cell proliferation by sesamin is correlated with down-regulated cyclin D1 protein expression, a proto-oncogene. In addition, sesamin-induced down-regulation of cyclin D1 was inhibited by proteosome inhibitors, suggesting that sesamin suppresses cyclin D1 protein expression by promoting proteasome degradation of cyclin D1 protein. Sesamin down-regulates cyclin D1 protein expression in various kinds of human tumor cells. Furthermore, depletion of cyclin D1 protein using small interfering RNA rendered MCF-7 cells insensitive to the growth inhibitory effects of sesamin, implicating that cyclin D1 is at least partially related to the antiproliferative effects of sesamin. Taken together, these results suggest that the ability of sesamin to down-regulate cyclin D1 protein expression through the activation of proteosome degradation could be one of the mechanisms of the antiproliferative activity of this agent.

Keywords: Sesamine, cyclin D1

[*1] Kyoto Prefectural University
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Yoshida, T.[*], Shiraishi, T.[*], Horinaka, M.[*], Nakata, S.[*], Yasuda, T.[*], Goda, AE.[*], Wakada, M.[*], Mizutani, Y.[*], Miki, T.[*], Nishikawa, A., and Sakai, T.[*]: **Lipoxygenase inhibitors induce death receptor 5/TRAIL-R2 expression and sensitize malignant tumor cells to TRAIL-induced apoptosis**.

Cancer Sci., **98**, 1417-1423, (2007)

Lipoxygenase inhibitors have been considered as promising anti-tumor agents. Combined treatment with nordihydroguaiaretic acid (NDGA), a lipoxygenase inhibitor, and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), one of the most promising candidates for new cancer therapeutics, markedly induced apoptosis in Jurkat T-cell leukemia cells at suboptimal concentrations for each agent. The combined treatment efficiently activated caspase-3, -8 and -10, and Bid. Although NDGA did not change the expression levels of anti-apoptotic factors, the expression of death receptor-related genes was showed that NDGA specifically up-regulated the expression of DR5 at mRNA and protein levels. Down-regulation of DR5 by small interfering RNA prevented the sensitizing effect of NDGA on TRAIL-induced apoptosis. NDGA sensitized prostate cancer and colorectal cancer cells to TRAIL-induced apoptosis, while NDGA neither enhanced TRAIL-induced apoptosis nor up-regulated DR5 expression in normal peripheral blood mononuclear cells. Another lipoxygenase inhibitor, AA861, also up-regulated DR5 and sensitized Jurkat and DU145 cells to TRAIL. These results indicate that lipoxygenase inhibitors augment the apoptotic efficiency of TRAIL through

DR5 up-regulation in malignant tumor cells, and raise the possibility that the combination of lipoxygenase inhibitor and TRAIL is a promising strategy for malignant tumor treatment.

Keywords: Lipoxygenases, 5/TRAIL-R2

* Kyoto Prefectural University

Umemura, T., Kuroiwa, Y., Tasaki, M., Okamura, T., Ishii, Y.[*1], Kodama, Y., Nohmi, T., Mitsumori, K.[*2], Nishikawa, A., and Hirose, M.[*3]: **Detection of oxidative DNA damage, cell proliferation and in vivo mutagenicity induced by dicyclanil, a non-genotoxic carcinogen, using gpt delta mice**.

Mutat. Res., **633**, 46-54, (2007)

Male and female *gpt* delta mice were given dicyclanil (DC), at a carcinogenic dose for 13 weeks. Whereas there were no changes in TBARS levels among the groups, Significant increases in 8-OHdG levels and centrilobular hepatocyte hypertrophy were observed in the treated mice of both genders. In contrast, BrdU-LIs and liver weights for the treated females, but not the males were significantly higher than those for the controls. Likewise, the gpt mutant frequencies (MFs) in the treated females were significantly elevated, GC:TA transversion mutations being predominant. No significant alterations were found in the gpt MFs of the males and the Spi- MFs of both sexes. The results for the transgenic mutation assays were consistent with DC carcinogenicity in terms of the sex specificity for females. Considering that 8-OHdG induces GC:TA transversion mutations by mispairing with A bases, it is likely that cells with high proliferation rates and a large amounts of 8-OHdG come to harbor mutations at high incidence. This is the first report demonstrating DC-induced genotoxicity, the results implying that examination of carcinogenic parameters concomitantly with reporter gene mutation assays is able to provide crucial information to comprehend the underlying mechanisms of so-called non-genotoxic carcinogenicity.

Keywords: Dicyclanil, *in vivo* mutagenicity, oxidative DNA damage

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

Imazawa, T.[*1], Nishikawa, A., Miyauchi, M., Okazaki, K., Takahashi, S.[*2], Umemura, T., and Hirose, M.[*3]: **DNA Adduct Formation, Nucleolar Segregation and Cell Proliferation in Rats Treated with 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine**

J. Toxicol. Pathol., **20**, 39-48, (2007)

To validate early biomarkers for chemical carcinogenesis, alterations of DNA damage and subsequent cell replication induced by PhIP were sequentially investigated in the rat colon and liver. Male 6-week-old SD rats were singly administered by gavage 300 mg/kg bw PhIP and control rats received vehicle alone. Immunohistochemically, in the colon, PhIP-DNA adduct already appeared at 4 hr and the positive ratios peaked at 24 hr after the PhIP exposure. Nucleolar alteration, demonstrable by electron microscopy as segregation of nucleolar components into granular and fibrillar compartments, was evident in cells of the target organ colon. Sequential observation clarified that such alteration was highest in frequency after 48 hr in colon cells, suggesting that nucleolar segregation occurs subsequent to generation of DNA adduction. Following these events, Ki-67-labeling in the colon was significantly increased at 72 hr. No significant PhIP-DNA adduct formation, nucleolar alteration or cell proliferation were noted in colons of the control rats nor in livers regardless of the PhIP treatment. Our results thus indicate an identity between the target cells for PhIP-DNA adduct formation, nucleolar segregation and enhanced cell replication, which correlated with DNA damage. These biomarkers could be useful for predicting the target organs of chemical carcinogenesis.

Keywords: PhIP, DNA adduct, nucleolar segregation

*1 National Institute of Biomedical Innovation
*2 Nagoya City University
*3 Food Safety Commission

Kitamura, Y., Umemura, T., Kanki, K., Kodama, Y., Kitamoto, S.[*1], Saito, K.[*1], Itoh, K.[*2], Yamamoto, M.[*3], Masegi, T.[*4], Nishikawa, A., and Hirose, M.[*5]: **Increased susceptibility to hepatocarcinogenicity of *Nrf2*-deficient mice exposed to 2-amino-3-methylimidazo[4,5-*f*] quinoline**.

Cancer Sci, **98**, 19-24, (2007)

To elucidate the roles of Nrf2 in hepatocarcinogenesis

induced by IQ, Nrf2-deficient (wild: +/+, homozygous: −/−) mice were treated with 300 ppm IQ in their diet for 1, 4 or 52 weeks. In the long-term experiment, the multiplicity and incidence of liver tumors in male and female IQ-treated −/− mice were significantly higher than those in their counterpart +/+ mice exposed to IQ. In the short-term experiment, although IQ exposure to +/+ mice of both sexes did not modify UGT values, GST values were significantly increased due to IQ treatment, in contrast to no alteration in male and female −/− mice. IQ-specific DNA adduct levels were elevated only in female −/− mice, although the increase was not significant. IQ treatment caused an increase in PCNA-LI only in male −/− mice. The present data clearly show that −/− mice of both sexes are susceptible to IQ hepatocarcinogenicity, which might result from IQ accumulation due to failure of metabolizing enzyme induction. In addition, inconsistent results concerning IQ-specific adducts and PCNA-LI in male and female −/− mice suggest the existence of different contributions of Nrf2 to IQ hepatocarcinogenesis between mice of the two sexes.

Keywords: nrf2, IQ, hepatocarcinogenesis

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[*4] Gifu University
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Kuroiwa, Y., Umemura, T., Nishikawa, A., Kanki, K., Ishii, Y., Kodama, Y., Masumura, K., Nohmi, T., and Hirose, M.[*]: **Lack of *in vivo* mutagenicity and oxidative DNA damage by flumequine in the livers of *gpt* delta mice**.

Arch Toxicol, **81**, 63-69, (2007)

Flumequine (FLU), an anti-bacterial quinolone agent, has been recognized as a non-genotoxic carcinogen for the mouse liver, but recent reports have suggested that some genotoxic mechanism involving oxidative DNA damage may be responsible for its hepatocarcinogenesis. In the present study, we investigated this possibility in the mouse liver using male and female B6C3F1 *gpt* delta mice fed diet containing 0.4% FLU, a carcinogenic dose, for 13 weeks. Measurements of 8-hydroxydeoxyguanosine levels in liver DNA, and *gpt* point and deletion mutations revealed no significant increases in any of these parameters in either sex. Histopathologically, centrilobular swelling of hepatocytes with vacuolation was apparent, however, together with significant increase in bromodeoxyuridine-labeling indices in the treated males and females. These results suggest that genotoxicity, including oxidative DNA damage, is not involved in mouse hepatocarcinogenesis by FLU, which might rather solely exert tumor-promoting effects in the liver.

Keywords: Flumequine, *in vivo* mutagenicity, oxidative DNA damage

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[*] Food Safety Commission

Kuroiwa, Y., Ishii, Y.[*1], Umemura, T., Kanki, K., Mitsumori, K.[*2], Nishikawa, A., Nakazawa, H.[*1], and Hirose, M.[*2]: **Combined treatment with green tea catechins and sodium nitrite selectively promotes rat forestomach carcinogenesis after initiation with *N*-methyl-*N'*- nitro-*N*-nitrosoguanidine**.

Cancer Sci, **98**, 949-957, (2007)

Combined treatment with several phenolic antioxidants and $NaNO_2$ has already shown to enhance rat forestomach carcinogenesis. In the present study, effects of green tea catechins (GTC) alone or in combination with $NaNO_2$ on gastric carcinogenesis were investigated in a rat two-stage carcinogenesis model. After 10-week MNNG initiation on the stomach, F344 male rats were received either drinking water containing 0.2% $NaNO_2$ and a diet supplemented with 1% GTC in combination, each individual chemical alone or a basal diet until the end of week 42. In the forestomach, incidences and multiplicities of neoplastic lesions were clearly increased by the combined treatment. In a short-term study, a significant increase of 8-OHdG levels in forestomach DNA occurred 24 h after the combined treatment. *In vitro* ESR analysis demonstrated hydroxyl radical formation after incubation of epigallocatechin gallate or epicatechin gallate with the NOC-7. Thus, GTC alone showed a weak chemopreventive effect on forestomach carcinogenesis, but in the presence of $NaNO_2$ it exerted a promotive effect which might involve hydroxyl-radical-associated oxidative DNA damage. However, no influence was exerted in the glandular stomach.

Keywords: Green tea catechins, sodium nitrite, carcinogenesis

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

Ishii, Y.*1, Ogara, A.*1, Katsumata, T.*1, Umemura, T., Nishikawa, A., Iwasaki, Y.*1, Ito, R*1, Saito, K.*1, Hirose, M.*2, and Nakazawa, H.*1: **Quantification of nitrated tryptophan in proteins and tissues by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry**. *J. Pharm. Biomed. Anal.*, **44**, 150-159, (2007)

Aromatic amino acids are targets of reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^-$) and nitrogen dioxide. It is known that tryptophan (Trp) as well as tyrosine is nitrated, generated isomers. In this study, we have developed a method for the quantification of Trp and NO_2Trp isomers, 2-, 4- and 6-NO_2Trp, which uses LC-ESI-MS/MS. We measured protein-bound NO_2Trp levels in ONOO- treated BSA and in liver of B6C3F1 mice at 2, 4, and 8h after administration of 300 mg/kg acetaminophen (APAP). The limits of quantification were 50, 3.0, 10 and 4.0 nM for Trp, 2-, 4- and 6-NO_2Trp, respectively. In *in vitro* experiments demonstrated that all isomers of NO_2Trp were detectable from BSA treated with $ONOO^-$ and the amount generated decreased in the order of 6-, 4- and 2-NO_2Trp. In in vivo experiments, 4- and 6-NO_2Trp were detected in the liver of mice administered APAP. The concentration range of 4- and 6-NO_2Trp per mol of Trp in the sample was 2.24-3.92 and 26.96-32.71 nmol/mol of Trp, and its existence in vivo was confirmed for the first time with our method. The LC-ESI-MS/MS method was able to determine protein-bound NO_2Trp in a small amount of tissue sample, and is therefore applicable not only as a biomarker of RNS, but also as a mean to clarify novel mechanisms underlying RNS-related tissue damage.

Keywords: Reactive nitrogen species, nitrotryptophan

*1 Hoshi University
*2 Food Safety Commission

Ishii, Y.*1, Ogara, A.*1, Okamura, T., Umemura, T., Nishikawa, A., Iwasaki, Y.*1, Ito, R.*1, Saito, K.*1, Hirose, M.*1, and Nakazawa, H.*1: **Development of quantitative analysis of 8-nitroguanine concomitant with 8-hydroxydeoxyguanosine formation by liquid chromatography with mass spectrometry and glyoxal derivatization**.

J. Pharm. Biomed. Anal., **43**, 1737-1743, (2007)

Under inflammatory conditions, both 8-nitroguanine (NO_2Gua) and 8-hydroxydeoxyguanosine (8-OHdG) are found in tissues. Measurements of the two types of damaged bases on nucleotides are expected to provide information pointing to the possible correlation between inflammation and carcinogenesis. In this study, a sensitive and precise method for the determination of NO_2Gua, which uses LC-MS and 6-methoxy-2-naphthyl glyoxal (MTNG) derivatization, was developed. The procedure for DNA digestion in this method is identical to that widely used for 8-OHdG measurement, which enables us to detect the two damaged bases in the same DNA sample. A mass spectrometer operated in the ESI- was set up with SIM at *m/z* 391 and 394 for NO_2Gua-MTNG and [^{13}C, $^{15}N_2$]-NO_2Gua-MTNG as surrogate standard, respectively. The limit of quantification was 3.0 nM for NO_2Gua. We measured NO_2Gua and 8-OHdG levels in calf thymus DNA treated with $ONOO^-$. As a result, both NO_2Gua and 8-OHdG levels were clearly increased with $ONOO^-$ dose dependency, the amount of NO_2Gua at the high dose $ONOO^-$ being almost the same as those of 8-OHdG. LC-MS was able to determine NO_2Gua in a small amount of DNA sample, and is therefore expected to be a very powerful tool for the evaluation of DNA damage induced by reactive nitrogen species.

Keywords: Reactive nitrogen species, nitroguanine, 8-OHdG

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*2 Food Safety Commission

Nishimura, J.*, Dewa, Y.*, Muguruma, M.*, Kuroiwa, Y., Yasuno, H.*, Shima, T.*, Jin, M.*, Takahashi, M., Umemura, T., and Mitsumori, K.*: **Effect of fenofibrate on oxidative DNA damage and on gene expression related to cell proliferation and apoptosis in rats**.

Toxicol. Sci., **97**, 44-54, (2007)

To investigate the relationship between fenofibrate (FF) and oxidative stress, enzymatic, histopathological, and molecular biological analyses were performed in the liver of male F344 rats fed 2 doses of FF (Exp. 1; 0

and 6000 ppm）for 3 weeks and 3 doses（Exp. 2; 0, 3000, and 6000 ppm）for 9 weeks. FF treatment increased the activity of enzymes, and catalase in the liver. However, it decreased those of SOD in the liver in both experiments. Increased 8-OHdG levels in liver DNA and lipofuscin accumulation were observed in the treated rats of Exp. 2. *In vitro* measurement of ROS in rat liver microsomes revealed a dose-dependent increase due to FF treatment. Microarray（only Exp. 1）or real-time RT-PCR analyses revealed that the expression levels of metabolism and DNA repair-related genes were increased in FF-treated rats, indicating a direct or indirect relationship between oxidative stress and FF treatment. Increased expression levels of cell cycle-related and cell proliferation-related genes and fluctuations in the expression levels of apoptosis -related genes suggest that cell proliferation induction, apoptosis suppression, and oxidative DNA damage due to are probably involved in the mechanism of hepatocarcinogenesis due to FF in rats.

Keywords: Fenofibrate, hepatocarcinogenesis, oxidative stress

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* Tokyo University of Agriculture and Technology

Muguruma, M.[*1], Unami, A.[*2], Kanki, M.[*2], Kuroiwa, Y., Nishimura, J.[*1, 3], Dewa, Y.[*1, 3], Umemura, T., Oishi, Y.[*2], and Mitsumori, K.[*1]: **Possible involvement of oxidative stress in piperonyl butoxide induced hepatocarcinogenesis in rats**.
Toxicology, **236**, 61-75,（2007）

To clarify the possible mechanism of non-genotoxic hepatocarcinogenesis induced by PBO, male F344 rats were administered an i.p. injection of DEN to initiate hepatocarcinogenesis. Two weeks later, the rats were administered a PBO-containing（0, 1, or 2%）diet for 6 weeks and subjected to a two-third partial hepatectomy 1 week later. After sacrificing them on week 8, their livers were histopathologically examined and analyzed for gene expression using a microarray and real-time RT-PCR. ROS products were also measured using liver microsomes. Hepatocytes exhibited centrilobular hypertrophy and increased GST-P positive foci formation. ROS products increased significantly in liver microsomes. In the microarray analysis, the expressions of genes related to metabolism, oxidative stress, multidrug resistance associated protein 3, and solute carrier family 7 member 5 were up-regulated in the PBO group in comparison to the 0% PBO. A significant up-regulation of stress response related genes was observed in PBO-treated groups in real-time RT-PCR. HPLC analysis revealed that the level of 8-OHdG in the 2% PBO was significantly higher than that in the 0% PBO. This suggests that PBO has the potential to generate ROS via metabolic pathways and induce oxidative DNA damage resulting in the induction of hepatocellular tumors in rats.

Keywords: Piperonyl butoxide, microarray, oxidative stress

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*2 Astellas Pharma Inc.
*3 Sciences, Gifu University

Shibutani, M.[*1], Lee, K-Y., Igarashi, K.[*1], Woo, G-H., Inoue, K., Nishimura, T., Hirose, M.[*2]: **Hypothalamus region-specific global gene expression profiling in early stages of central endocrine disruption in rat neonates injected with estradiol benzoate or flutamide**.
Dev Neurobiol., **67**, 253-269（2007）

To identify genes linked to early stages of disruption of brain sexual differentiation, hypothalamic region-specific microarray analyses were performed using a microdissection technique with neonatal rats exposed to endocrine-acting drugs. The expression fidelity of microarrays was first examined with two-round amplified antisense RNAs（aRNAs）from methacarn-fixed paraffin-embedded tissue（PET）in comparison with expression in unfixed frozen tissue（UFT）. The expression patterns for the 2x-amplified aRNAs were mostly identical between methacarn-fixed PET and UFT, suggesting no obvious influence of methacarn fixation and subsequent paraffin embedding on expression levels. Next, neonatal rats at birth were treated subcutaneously either with estradiol benzoate（EB; 10 microg/pup）or flutamide（FA; 250 microg/pup）, and medial preoptic area（MPOA）-specific microarray analysis was performed 24 h later using 2x-amplified aRNAs from methacarn-fixed PET. Numbers of genes showing constitutively high expression in the MPOA predominated in males, implying a link with male-type growth supported by perinatal testosterone. Around 60% of genes showing sex differences in expression were altered by EB treatment in females, suggesting

an involvement of genes necessary for brain sexual differentiation. When compared with EB, FA affected a rather small number of genes, but fluctuation was mostly observed in females, as with EB. Many selected genes common to EB and FA showed down-regulation in females with both drugs, suggesting a common mechanism for endocrine center disruption in females at early stages of post-natal development.

Keywords: Brain sexual differentiation, microarray, microdissection.

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

Woo, G-H., Shibutani, M.*1, Ichiki, T.*1, Hamamura, M.*1, Lee, K-Y., Inoue, K., Hirose, M.*2: **A repeated 28-day oral dose toxicity study of nonylphenol in rats, based on the 'Enhanced OECD Test Guideline 407' for screening of endocrine-disrupting chemicals**.
Arch Toxicol., **81**, 77-88 (2007)

A 28-day repeated oral dose toxicity study of nonylphenol (NP) was performed for an international validation of the 'Enhanced OECD Test Guideline 407' paying particular attention to the sensitivity of individual endocrine-related parameters. Male and female Sprague-Dawley rats were administered NP once daily by gavage at doses of 0 (control), 10, 50, or 250 mg/kg body weight. At 250 mg/kg, three females died or became moribund during the experiment. At this dose, anemia, increases of relative liver and kidney weights, centrilobular liver cell hypertrophy and a variety of renal tubular lesions and alteration of serum biochemical parameters were observed in both sexes, some of them being evident from 50 mg/kg in females. In addition, increase of thyroid weight in males was detected from 50 mg/kg. At 250 mg/kg, males exhibited reduction of relative weights of the ventral prostate and seminal vesicles, and females developed irregular estrous cyclicity and vaginal mucosal hyperplasia, while no magnitude in serum hormone levels were detected in both sexes. In summary, repeated oral doses of NP to rats for 28 days resulted in hepato-renal toxicity and effects on the endocrine system from 50 mg/kg and anemia at 250 mg/kg. The no-observed-adverse-effect level of NP was estimated to be 10 mg/kg per day.

Keywords: Nonylphenol, Enhanced OECD Test Guideline 407, rat

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

Woo, G-H., Shibutani, M.*1, Inoue, K., Fujimoto, H., Takahashi, M., Lee, K-Y., Hirose, M.*2: **Promoting potential of a Jamaica quassia extract in a rat medium-term hepatocarcinogenesis bioassay**.
Food Chem Toxicol., **45**, 1160-1164 (2007)

Jamaica quassia extract (JQE), a natural bittering agent, was investigated for hepatocarcinogenesis-promoting potential using a medium-term liver bioassay system. F344 male rats were given a single intraperitoneal injection of diethylnitrosamine (200mg/kg body weight) and then starting 2 weeks later, received JQE in the diet at concentrations of 500, 5000 or 30,000 ppm for 6 weeks. Animals for tumor promotion (+) and (−) controls were fed 500 ppm sodium phenobarbital (PB) and basal diet, respectively during the promotion phase in this model. All animals were subjected to two-thirds partial hepatectomy at week 3 and killed at week 8. As with the PB-promoted case, both numbers and areas of glutathione S-transferase placental form-positive liver cell foci were significantly increased by JQE at 30,000 ppm, with non-significant increases evident at 5000 ppm. The results thus indicate that JQE at high dose has promoting potential for rat hepatocarcinogenesis.

Keywords: jamaica quassia Extract, medium-term liver bioassay, tumor promotion

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Woo, G-H., Shibutani, M*1., Kuroiwa, K., Lee, K-Y., Takahashi, M., Inoue, K., Fujimoto, H., Hirose, M.*2: **Lack of preventive effects of dietary fibers or chlorophyllin against acrylamide toxicity in rats**.
Food Chem Toxicol., **45**, 1507-1515 (2007)

Dietary fibers and chlorophyllin have shown to exert anti-carcinogenic effects against co-administered carcinogens. To test the possibility of chemoprevention by such dietary supplements on subacutely induced acrylamide (ACR) toxicity, Sprague-Dawley male rats were administered 2.5% sodium alginate, 5% glucomannan, 5% digestion resistant maltodextrin, 2.5% chitin or 1% chlorophyllin in the diet, and starting one

week later, co-administered 0.02% ACR in the drinking water for 4 weeks. For comparison, untreated control animals given basal diet and tap water were also included. Neurotoxicity was examined with reference to gait abnormalities and by quantitative assessment of histopathological changes in the sciatic and trigeminal nerves, as well as aberrant dot-like immunoreactivity for synaptophysin in the cerebellar molecular layer. Testicular toxicity was assessed by quantitation of seminiferous tubules with exfoliation of germ cells into the lumen and cell debris in the ducts of the epididymides. Development of testicular toxicity as well as neurotoxicity was evident with ACR-treatment, but was not suppressed by dietary addition of fibers or chlorophyllin, suggesting no apparent beneficial influence of these dietary supplements on experimentally induced subacute ACR toxicity.

Keywords: Acrylamide, dietary fibers, chlorophyllin

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Onishi, M.[*1], Shimizu, K.[*1], Sugata, E.[*1], Fujii M.[*1], Yoshida, M., Honoki, K.[*2], and Tsujiuchi, T.[*1]: **Absence of Epidermal Growth Factor Receptor Gene Mutations in Lung and Liver Tumors in Rats**

J. Toxicol. Pathol., **20**, 65-69 (2007)

Epidermal growth factor receptor (EGFR), a receptor protein tyrosine kinase, is a transmembrane protein. Recent studies indicate that mutations in the gene encoding EGFR are present in several human cancers. To assess the involvement of these mutations in the development of rat tumors, we looked for the presence of mutations in exons 18-21, a region which encodes the tyrosine kinase domain of Egfr, in lung and liver tumors in rats. Lung adenocarcinomas were induced in rats by exposure to N-nitrosobis (2-hydroxypropyl) amine (BHP). We also induced hepatocellular carcinomas (HCCs) in rats with multiple hepatocarcinogens and a choline-deficient L-amino acid-defined (CDAA) diet. Genomic DNA was extracted from 12 lung adenocarcinomas, 8 HCCs induced by multiple hepatocarcinogens and 8 HCCs induced by the CDAA diet. To identify mutations in Egfr, polymerase chain reaction (PCR)- single-strand conformation polymorphism (SSCP) analysis was performed. No mutations were detected throughout exons 18-21, in either lung or liver tumors in rats. These results suggest that alterations to Egfr might not be involved in the development of lung and liver tumors in rats.

Keywords: Epidermal growth factor receptor, mutation, lung adenocarcinoma, hepatocellular carcinoma, rat

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Igarashi, M.[*1], Yoshida, M., Watanabe, M.[*2], Yamada, T.[*3], Sakurai, T.[*4], Endo, Y.[*2] Miyajima, N.[*2], Maekawa, A.[*5], Oikawa, T.[*6], Sugano, S.[*2] and Nakae, D.[*7]: **Involvement of mutation-based inhibition of β-catenin phosphorylation at Ser33 in the malignant pregression of lung (pre) neoplastic lesions induced by N-nitrobis (2-hydorxypropyl) amine in male Fischer 344 rats.**

Lung, **185**: 271-278 (2007)

This study was investigated the Ser33 phosphorylation status of β-catenin in lung (pre) neoplastic lesions induced by N-nitrobis (2-hydorxypropyl) amine (BHP) in male F344 rats. Six-week-old rats received 2000 ppm of BHP in the drinking water for 8 weeks and sacrificed 12 weeks thereafter. 69 of 75 rats demonstrated multiple lung (pre) neoplastic lesions, and nucleotide mutation analysis of the β-catenin gene detected a total of 33 mutations in 12 assessed the lung lesions. The mutations tended to accumulate in positions near the phosphorylation region of the gene, between codons 33 and 45. Immunohistochemical expression of β-catenin increased and its localization changed from the cell membrane to the nuclei with advancing malignancy. Phosphorylation β-catenin protein at Ser 33 was weakened in the lung lesions. These results suggest that BHP-induced mutation of the β-catenin gene results in amino acid conversions in its product protein, which in turn lead to inhibition of phosphorylation of the protein and escape from protein degradation. These findings might contribute to the malignant progression of the lung (pre) neoplastic lesions, which start the relative early stage in lung carcinogenesis.

Keywords: β-Catenin, mutation, Phopho-β-catenin (Ser 33), N-nitrobis (2-hydroxypropyl) amine

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Matsuoka, Y.[*1], Hamaguchi, T.[*1], Fukamachi, K.[*2], Yoshida, M., Watanabe, G.[*3], Taya, K.[*3], Tsuda, H.[*2], Tsubura A.[*1]: **Molecular analysis of rat mammary carcinogenesis: an approach from carcinogenesis research to cancer prevention**.

Med. Mol. Morphol., **40**, 185-90（2007）

A rat strain carrying the human c-Ha-ras proto-oncogene is highly susceptible to chemically induced mammary carcinogenesis. All the transgenic rats develop preneoplastic mammary lesions within 20 days of an injection of N-methyl-N-nitrosourea, and mammary carcinomas appear within 8 weeks of treatment with a variety of chemical carcinogens. In this review, we summarize molecular aspects of mammary carcinogenesis in transgenic rats and the potential application of this model for studies of breast cancer prevention.

Keywords: Mammary carcinogenesis, c-Ha-ras proto-oncogene

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Imai, T., Hasumura, M., Cho, Y.M., Onose, J., Hirose, M.[*]: **Depression of T cell-mediated immunity reduces sulfadimethoxine-induced capsular inflammation and inhibits associated development of invasive thyroid follicular cell carcinomas in rats**

Cancer Sci., **98**, 294-298（2007）

We previously demonstrated that thyroid capsular inflammation induced by continuous treatment with the antithyroidal agent sulfadimethoxine is associated with development of invasive follicular cell carcinomas in rats initiated with *N*-bis（2-hydroxypropyl）nitrosamine（DHPN）. The inflammatory changes are characterized by large numbers of macrophages and lymphocytes as well as fibroblasts and we hypothesized that it might be enhanced by interplay between macrophages and T cells. To clarify this hypothesis, a comparative study was conducted between athymic nude（*rnu/rnu*）rats and euthymic（*rnu/+*）littermates initiated with DHPN（2800 mg/kg, s.c.）followed by sulfadimethoxine treatment in drinking water（0.1%）for 10 weeks. In *rnu/+*rats, marked capsular thickening with inflammation was induced along with invasive follicular cell carcinomas（2.8 +/− 1.3/rat）. In *rnu/rnu* rats, limited fibrous capsular thickening was noted with or without minimal inflammatory change, and the multiplicity of invasive carcinomas was significantly lower（1.1 +/− 1.0/rat, $P < 0.01$）. Inducible nitric oxide synthase expression in the inflamed lesions was detected in three of 10 *rnu/+*rats but in none of the *rnu/rnu* animals. The results thus suggest that development of invasive carcinomas is enhanced by capsular inflammation mediated by T cells, and inducible nitric oxide synthase induction may play a role in tumor progression.

Keywords: Thyroid, inflammation, carcinogenesis, F344 rats

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*1 Food Safety Commission

Hasumura, M., Ueda, M., Onose, J., Imai, T., Hirose, M.[*1]: **Lack of a significant effect of arctiin on development of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in ovariectomized Sprague-Dawley rats**

Nutr. Cancer, **57**, 201-208（2007）

Arctiin, a plant lignan, is metabolized to hormone-like compounds with weak estrogenic and antioxidative activity in experimental animals and man. To clarify its influence on mammary carcinogenesis, female rats were administrated 7,12-dimethylbenz(*a*)anthracene（DMBA）once, and when the incidence of palpable mammary tumors reached 50%, subjected to ovariectomy（OVX）and divided into tumor-bearing［DMBA-Tumor（+）］and no-tumor-bearing［DMBA-Tumor（-）］groups, subgroups of each then being fed soybean-free diet containing 0, 40, 200, and 1000 ppm of arctiin for 31 wk. The incidence and multiplicity of palpable tumors in the 200 ppm DMBA-Tumor（+）subgroup from week 12 of arctiin treatment tended to be decreased as compared to the 0 ppm subgroup and at terminal sacrifice, the volume of histopathologically defined mammary tumors was decreased in the 40 ppm DMBA-Tumor（-）subgroup, but again without statistical significance. In conclusion, weak inhibitory effects of arctiin on DMBA-induced mammary tumor development were suggested

in OVX rats, but any further assessment is needed to obtain conclusive results.

Keywords: Plant lignan, mammary gland; carcinogenesis; F344 rats

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Imai, T., Fukuta, K.*, Hasumura, M., Cho, Y.M., Ota, Y., Takami, S., Nakagama, H.*, Hirose, M.: **Significance of inflammation-associated regenerative mucosa characterized by Paneth cell metaplasia and beta-catenin accumulation for the onset of colorectal carcinogenesis in rats initiated with 1,2-dimethylhydrazine**.

Carcinogenesis, **28**, 2199-2206 (2007)

Short-term dextran sodium sulfate (DSS) treatment has been shown to notably accelerate colorectal tumor development in rats initiated with 1,2-dimethylhydrazine (DMH). In the present study, to clarify mechanisms underlying the DSS influence, time-course studies of histopathological and immunohistochemical characteristics and beta-catenin gene mutations in colorectal mucosa in early stages of this model were conducted. F344 males were given three subcutaneous injections of DMH (40 mg/kg body wt) within a week, followed by free access to drinking water containing 1% DSS for a week. At weeks 1, 4, 6 and 8 after the DSS treatment, rats were euthanized and colorectal samples were collected. At week 1, the colorectal mucosa demonstrated extensive erosion along with significant inflammatory cell infiltration and neighboring reactive hyperplasia. By week 4, the mucosal damage was repaired and regenerative mucosa, partly characterized by Paneth cell metaplasia and altered subcellular localization of beta-catenin, was apparent. Areas with Paneth cells/beta-catenin accumulation were significantly more likely to be accompanied by interstitial inflammation and 17 of 24 dysplastic foci were found in regenerative mucosa with Paneth cells. Furthermore, adenomas/carcinomas frequently featured various degrees of Paneth cell differentiation. Point mutations mainly in codons 34 and 41 of beta-catenin gene were detected in 6 of 27 samples of regenerative mucosa with Paneth cells and four of nine dysplastic foci/adenomas/carcinomas. These findings indicate that inflammation-associated regenerative mucosa with Paneth cell metaplasia and alteration in the APC/beta-catenin/Tcf signal transduction pathway are possibly involved in the acceleration of colorectal carcinogenesis in this DMH-DSS rat model.

Keywords: Colon, inflammation, carcinogenesis, F344 rats

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Cho, Y.M., Takahashi, S.*, Asamoto, M.*, Suzuki, S.*, Tang, X.*, Shirai, T.*: **Suppressive effects of antiandrogens, finasteride and flutamide on development of prostatic lesions in a transgenic rat model**

Prostate Cancer Prostatic Dis., **10**, 378-83 (2007)

Transgenic (TG) rats bearing a probasin promoter/simian virus 40 T antigen (SV40 Tag) construct were treated with antiandrogens to examine their ability to suppress prostate carcinogenesis. Finasteride and flutamide were administered to 10-week-old TG rats five times a week for 2, 5 and 7 weeks. Antiandrogen-treated prostates exhibited atrophic glandular structures with almost no expression of SV40 Tag and only weak signals for androgen receptors. Furthermore, quantitative data for ventral prostate adenocarcinomas showed significant decrease with antiandrogen treatment. Both finasteride and flutamide had the ability to suppress SV40 Tag-driven carcinogenesis through their different antiandrogenic mechanisms, suggesting that this TG model is suitable for exploring the potential of agents to inhibit prostate cancer

Keywords: Prostate cancer, Transgenic rats, Antiandrogen

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Newwirth, E.*, Honma, M., and Grosovsky, A.*: **Interchromosomal crossover in human cell is associated with long gene conversion tracts**.

Mol. Cell. Biol., **27**, 5261-5274 (2007)

Crossovers have rarely been observed in specific association with interchromosomal gene conversion in mammalian cells. In this investigation two isogenic human B-lymphoblastoid cell lines, TI-112 and TSCER2, were used to select for I-*Sce*I-induced gene conversions that restored function at the selectable thymidine kinase locus. Additionally, a haplotype linkage analysis methodology enabled the rigorous detection of all crossover-associated convertants, whether or not they

exhibited loss of heterozygosity. This methodology also permitted characterization of conversion tract length and structure. In TI-112, gene conversion tracts were required to be complex in tract structure and at least 7.0 kb in order to be selectable. The results demonstrated that 85%（39/46）of TI-112 convertants extended more than 11.2 kb and 48% also exhibited a crossover, suggesting a mechanistic link between long tracts and crossover. In contrast, continuous tracts as short as 98 bp are selectable in TSCER2, although selectable gene conversion tracts could include a wide range of lengths. Indeed, only 16%（14/95）of TSCER2 convertants were crossover associated, further suggesting a link between long tracts and crossover. Overall, these results demonstrate that gene conversion tracts can be long in human cells and that crossovers are observable when long tracts are recoverable.

Keywords; Loss of heterozygosity, Haplotype linkage analysis, I-*Sce*I

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Yatagai, F.[*1], Umebayashi, Y.[*1], Suzuki, M.[*2], Abe, T.[*1], Suzuki, H.[*3], Shimazu, T.[*3], Ishioka, N.[*4], Iwaki, M.[*1], and Honma, M.: **Influence of low-dose and low-dose-rate ionizing radiation on mutation induction in human cells**.

Advan. Space Res., **40**, 470-473（2007）

This is a review paper to introduce our recent studies on the genetic effects of low-dose and low-dose-rate ionizing radiation（IR）. Human lymphoblastoid TK6 cells were exposed to γ-rays at a dose-rate of 1.2 mGy/h（total 30 mGy）. The frequency of early mutations（EMs）in the thymidine kinase（*TK*）gene locus was determined to be 1.7×10^{-6}, or 1.9-fold higher than the level seen in unirradated controls［Umebayashi, Y., Honma, M., Suzuki, M., Suzuki, H., Shimazu, T., Ishioka, N., Iwaki, M., Yatagai, F., Mutation induction in cultured human cells after low-dose and low-dose-rate γ-ray irradiation: detection by LOH analysis. J. Radiat. Res., 48, 7-11, 2007］. These mutants were then analyzed for loss of heterozygosity（LOH）events. Small interstitial-deletion events were restricted to the *TK* gene locus and were not observed in EMs in unirradated controls, but they comprised about half of the EMs（8/15）after IR exposure. Because of the low level of exposure to IR, this specific type of event cannot be considered to be the direct result of an IR-induced DNA double strand break（DSB）. To better understand the effects of low-level IR exposure, the repair efficiency of site-specific chromosomal DSBs was also examined. The pre γ-irradiation under the same condition did not largely influence the efficiency of DSB repair via end-joining, but enhanced such efficiency via homologous recombination to an about 40% higher level（unpublished data）. All these results suggest that DNA repair and mutagenesis can be indirectly influenced by low-dose/dose-rate IR.

Keywords; TK6 cell, Low-dose/low-dose rate γ-rays, LOH analysis

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Nakano, T.[*1], Morishita, S.[*1], Katafuchi, A., Matsubara, M.[*1], Horikawa, Y.[*1], Terato, H.[*1], Salem, A.M.H.[*1], Izumi, S.[*1], Pack, S. P.[*2], Makino, K.[*2], and Ide, H.[*1]: **Nucleotide excision repair and homologous recombination systems commit differentially to the repair of DNA-protein crosslinks**.

Mol. Cell, **28**, 147-158,（2007）

DNA-protein crosslinks（DPCs）-where proteins are covalently trapped on the DNA strand-block the progression of replication and transcription machineries and hence hamper the faithful transfer of genetic information. However, the repair mechanism of DPCs remains largely elusive. Here we have analyzed the roles of nucleotide excision repair（NER）and homologous recombination（HR）in the repair of DPCs both in vitro and in vivo using a bacterial system. Several lines of biochemical and genetic evidence show that both NER and HR commit to the repair or tolerance of DPCs, but differentially. NER repairs DPCs with crosslinked proteins of sizes less than 12-14 kDa, whereas oversized DPCs are processed exclusively by RecBCD dependent HR. These results highlight how NER and HR are coordinated when cells need to deal with unusually bulky DNA lesions such as DPCs.

Keywords: DNA-protein crosslinks, base excision repair, homologous recombination

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Sugo, N.[*1], Niimi, N., Aratani, Y.[*1], Masutani, M.[*2], Suzuki, H.[*3], and Koyama, H.[*1]: **Decreased PARP-1 levels accelerate embryonic lethality but attenuate neuronal apoptosis in DNA polymerase β-deficient mice**.

BBRC, **354**, 656-616 (2007)

In mammalian cells, DNA polymerase β (Polβ) and poly (ADP-ribose) polymerase-1 (PARP-1) have been implicated in base excision repair (BER) and single-strand break repair. Polβ knockout mice exhibit extensive neuronal apoptosis during neurogenesis and die immediately after birth, while PARP-1 knockout mice are viable and display hypersensitivity to genotoxic agents and genomic instability. To study this, we generate Polβ$^{-/-}$PARP-1$^{-/-}$ double mutant mice. Here, we show that the double mutant mice exhibit a profound developmental delay and embryonic lethality at mid-gestation. Importantly, the degree of the neuronal apoptosis was dramatically reduced in PARP-1 heterozygous mice in a Polβ null background. The reduction was well correlated with decreased levels of p53 phosphorylation at serine-18. These results indicate that functional interactions between Polβ and PARP-1 play important roles in embryonic development and neurogenesis.

Keywords: DNA polymerase β; Knockout mouse; Neuronal apoptosis

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Takeiri, A.[*], Mishima, M.[*], Tanaka, K.[*], Shioda, A.[*], Harada, A.[*], Masumura, K., and Nohmi, T.: **Mutation spectra in cisplatin- and transplatin-treated GDL1 cells clarified the different mode of action of these compounds in mammalian cells**.

Genes and Environ., **29**, 89-99 (2007)

We characterized the gene mutations induced by both cisplatin and transplatin isomers using cell line GDL 1 established from *gpt* delta transgenic mice. Our findings suggest that intrastrand crosslinks play key roles in the cytotoxicity and mutagenicity induced by threes two platinum compounds and that the more efficient formation of intrastrand cross links of cisplatin compared to transplatin may account for the potent cytotoxicity and clinical activity. The spectral analysis of mutations using GDL1 cells would provide valuable information on the mechanisms underlying the mutagenesis induced by the platinum stereoisomers.

Keywords: cisplatin, *gpt* delta mouse, GDL1 cells

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Aoki, Y.[*1], Hashimoto, A.H.[*1], Amanuma, K.[*1], Matsumoto, M.[*1], Hiyoshi, K.[*1], Takano, H.[*1], Masumura, K., Itoh, K.[*2], Nohmi, T., and Yamamoto, M.[*1]: **Enhanced spontaneous and benzo(*a*)pyrene-induced mutations in the lung of Nrf2-deficient *gpt* delta mice**.

Cancer Res., **67**, 5643-5648 (2007)

Transcription factor Nrf2 mediates inducible and constitutive expression of cytoprotective enzymes against xenobiotics and mutagens. To address whether Nrf2 is also involved in DNA protection, we generated *nrf2*$^{+/-}$::*gpt* and *nrf2*$^{-/-}$::*gpt* mice. The spontaneous mutation frequency of the gpt gene in the lung was approximately three times higher in *nrf2*-null (*nrf2*$^{-/-}$) mice than nrf2 heterozygous (*nrf2*$^{+/-}$) and wild-type (*nrf2*$^{+/+}$) mice. A single intratracheal instillation of benzo(*a*)pyrene (BaP) increased the lung mutation frequency 3.1- and 6.1-fold in *nrf2*$^{+/-}$ and *nrf2*$^{-/-}$ mice, respectively, compared with BaP-untreated *nrf2*$^{+/-}$ mice. Surprisingly, mutation profiles of the *gpt* gene in BaP-treated *nrf2*$^{+/-}$ mice was substantially different from that in BaP-untreated *nrf2*$^{-/-}$ mice. These results show that Nrf2 aids in the prevention of mutations in vivo and suggest that Nrf2 protects genomic DNA against certain types of mutations.

Keywords: Nrf2, mutant frequency, *gpt* delta mouse

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Hashimoto, A.H.[*1], Amanuma, K.[*1], Hiyoshi, K.[*2], Sugawara, Y.[*1, 3], Goto, S.[*3], Yanagisawa, R.[*1], Takano, H.[*1], Masumura, K., Nohmi, T., Aoki, Y.[*1]: **Mutations in the lungs of *gpt* delta transgenic mice following inhalation of diesel exhaust**.

Environ. Mol. Mutagen., **48**, 682-693 (2007)

Diesel exhaust (DE) is a major airborne pollutant of urban areas. It contains various polycyclic aromatic hydrocarbons (PAH) and nitrated PAHs. In this study,

gpt delta mice were treated with inhalation of DE, or a single intratracheal instillation of diesel exhaust particles (DEP) or DEP extract. In the lungs of mice treated with inhalation of 3 mg/m^3 DE for 12 weeks, the mutant frequency (MF) was 3.2-fold higher than that of the control group. An instillation of DEP and DEP extract resulted in a significant dose-dependent linear increase in MF. The mutagenic potency (MF/mg) of DEP extract (5.6 x 10^{-5}) was double that of DEP (2.7 x 10^{-5}), suggesting that the mutagenicity of the latter is derived primarily from compounds in the extract, which itself is responsible for ca. 50% of the weight of DEP.

Keywords: Diesel exhaust, mutant frequency, *gpt* delta mouse

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Yatagai, F.*1, Umebayashi, Y.*1, Honma, M., Sugasawa, K.*1, Takayama, Y.*1, and Hanaoka, F.*2: **Mutagenic radioadaptation in a human lymphoblastoid cell line**.

Mutat. Res., **638**, 48-55 (2008)

We investigated the mutagenic radioadaptive response of human lymphoblastoid TK6 cells by pretreating them with a low dose (5 cGy) of X-rays followed by a high (2 Gy) dose 6h later. Pretreatment reduced the 2-Gy-induced mutation frequency (MF) of the thymidine kinase (TK) gene (18.3 x 10^{-6}) to 62% of the original level (11.4 x 10^{-6}). A loss of heterozygosity (LOH) detection analysis applied to the isolated TK$^-$ mutants revealed the mutational events as non-LOH (resulting mostly from a point mutation in the TK gene), hemizygous LOH (resulting from a chromosomal deletion), or homozygous LOH (resulting from homologous recombination (HR) between chromosomes). For non-LOH events, pretreatment decreased the frequency to 27% of the original level (from 7.1 x 10^{-6} to 1.9 x 10^{-6}). cDNAs prepared from the non-LOH mutants revealed that the decrease was due mainly to the repression of base substitutions. The frequency of hemizygous LOH events, however, was not significantly altered by pretreatment. Mapping analysis of chromosome 17 demonstrated that the distribution and the extent of hemizygous LOH events were also not significantly influenced by pretreatment. For homozygous LOH events, pretreatment reduced the frequency to 61% of the original level (from 5.1 x 10^{-6} to 3.1 x 10^{-6}), reflecting an enhancement in HR repair of DNA double-strand breaks. Our findings suggest that the radioadaptive response in TK6 cells follows mainly from mutations at the base-sequence level, not the chromosome level.

Keywords; Adaptive response, TK6 cells, LOH detection system

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Yasui, M., Suenaga, E., Koyama, N.*1, Masutani, C.*2, Hanaoka, F.*2, Grúz, P., Shibutani, S.*3, Nohmi, T., Hayashi, M., and Honma M.: **Miscoding properties of 2'-deoxyinosine, a nitric oxide-derived DNA Adduct, during translesion synthesis catalyzed by human DNA polymerases**.

J. Mol. Biol., **377**, 1015-1023 (2008)

Chronic inflammation involving constant generation of nitric oxide (*NO) by macrophages has been recognized as a factor related to carcinogenesis. At the site of inflammation, nitrosatively deaminated DNA adducts such as 2'-deoxyinosine (dI) and 2'-deoxyxanthosine are primarily formed by *NO and may be associated with the development of cancer. In this study, we explored the miscoding properties of the dI lesion generated by Y-family DNA polymerases (pols) using a new fluorescent method for analyzing translesion synthesis. An oligodeoxynucleotide containing a single dI lesion was used as a template in primer extension reaction catalyzed by human DNA pols to explore the miscoding potential of the dI adduct. Primer extension reaction catalyzed by pol alpha was slightly retarded prior to the dI adduct site; most of the primers were extended past the lesion. Pol eta and pol kappaDeltaC (a truncated form of pol kappa) readily bypassed the dI lesion. The fully extended products were analyzed by using two-phased PAGE to quantify the miscoding frequency and specificity occurring at the lesion site. All pols, that is, pol alpha, pol eta, and pol kappaDeltaC, promoted preferential incorporation of 2'-deoxycytidine monophosphate (dCMP), the wrong base, opposite the dI lesion. Surprisingly, no incorporation of 2'-deoxythymidine monophosphate, the correct base, was observed opposite the lesion. Steady-state kinetic studies with pol alpha,

pol eta, and pol kappaDeltaC indicated that dCMP was preferentially incorporated opposite the dI lesion. These pols bypassed the lesion by incorporating dCMP opposite the lesion and extended past the lesion. These relative bypass frequencies past the dC:dI pair were at least 3 orders of magnitude higher than those for the dT:dI pair. Thus, the dI adduct is a highly miscoding lesion capable of generating A-->G transition. This *NO-induced adduct may play an important role in initiating inflammation-driven carcinogenesis.

Keywords; DNA adduct, inflammation, nitric oxide

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Yamauchi, K.[*1], Kakinuma, S.[*1], Sudo, S.[*1], Kito, S.[*1], Ohta, Y.[*1], Nohmi, T., Masumura, K., Nishimura, M.[*1], and Shimada, Y.[*1]: **Differential effects of low- and high-dose X-rays on *N*-ethyl-*N*-nitrosourea-induced mutagenesis in thymocytes of B6C3F1 *gpt*-delta mice**

Mutat. Res., **640**, 27-37 (2008)

We examined the occurrence of mutations in thymic DNA following exposure of B6C3F1 *gpt*-delta mice to both ionizing radiation and *N*-ethyl-*N*-nitrosourea (ENU). Mice were exposed weekly to whole body X-irradiation (0.2 or 1.0 Gy), ENU (200 ppm) in the drinking water, or X-irradiation followed by ENU treatment. ENU exposure alone increased *gpt* mutant frequency by 10-fold compared to untreated controls. X-irradiation alone, at either low or high dose, reduced mutant frequency. Combined exposure to 0.2 Gy X-rays with ENU dramatically decreased mutant frequency compared to ENU treatment alone. In contrast, 1.0 Gy X-rays enhanced mutant frequency by about 30-fold and appeared to accelerate clonal expansion of mutated cells. These results indicate that the mode of the combined mutagenic effect is dose dependent.

Keywords: X-ray, ENU, combined effect

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Hidaka, K.[*1], Yamada, M., Kamiya, H.[*2], Masutani, C.[*3], Harashima, H.[*2], Hanaoka, F.[*3], and Nohmi, T.: **Specificity of mutations induced by incorporation of oxidized dNTPs into DNA by human DNA polymerase eta**

DNA Repair, **7**, 497-506 (2008)

Here, we report that human DNA polymerase η (h Pol η) incorporates oxidized dNTPs, i.e., 2-OH-dATP and 8-OH-dGTP, into DNA in an erroneous and efficient manner, thereby inducing various types of mutations during in vitro gap-filling DNA synthesis. When 2-OH-dATP was present at a concentration equal to those of the four normal dNTPs in the reaction mixture, DNA synthesis by h Polη enhanced the frequency of G-to-T transversions eight-fold higher than that of the transversions in control where only the normal dNTPs were present. When 8-OH-dGTP was present at an equimolar concentration to the normal dNTPs, it enhanced the frequency of A-to-C transversions 17-fold higher than the control. It also increased the frequency of C-to-A transversions about two-fold. h Polη enhanced the frequency of single-base frameshifts and deletions with the size of more than 100 base pairs when 8-OH-dGTP was present in the reaction mixture. We suggest that h Polη may be involved in induction of various types of mutations through the erroneous and efficient incorporation of oxidized dNTPs into DNA in human cells.

Keywords: Oxidative mutagenesis, Nucleotide pool, human DNA polymerase η

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Ema, M., Fujii, S.[*1], Matsumoto, M., Hirata-Koizumi, M., Hirose, A. and Kamata, E.: **Two-generation reproductive toxicity study of the rubber accelerator N,N-dicyclohexyl-2-benzothiazolesulfenamide in rats**

Reprod. Toxicol., **25**, 21-38 (2008)

Male and female Crl:CD (SD) rats were fed a diet containing rubber accelerator *N,N*-dicyclohexyl-2-benzothiazolesulfenamide (DCBS) at 0, 80, 600 or 4500 ppm throughout the study beginning at the onset of a 10-week pre-mating period and continuing through the mating, gestation, and lactation periods for two generations. At 4500 ppm, decreases in the body weight, body weight gain, and food consumption were found in F0 males and females. No changes in the estrous cyclicity, copulation index, fertility index, gesta-

tion index, delivery index, number of implantations, precoital interval, or gestation length were observed in any generation at any dose of DCBS. Delayed preputial separation at 4500 ppm as well as delayed vaginal opening and higher body weight at the age of vaginal opening at 600 and 4500 ppm were found in the F1 generation. A transient change in performance in a water-filled multiple T-maze was found at 600 and 4500 ppm in F1 females. There were no compound-related changes in number of pups delivered, sex ratio of pups, viability of pups, anogenital distance, surface righting reflex, negative geotaxis reflex, mid-air righting reflex, pinna unfolding, incisor eruption, or eye opening in the F1 and F1 generations. The body weight of F1 and F2 male and female pups was lowered at 4500 ppm. Reduced uterine weight of the weanlings was noted in the F1 generation at 4500 ppm and in the F2 generation at 600 and 4500 ppm. The data indicate that the NOAEL of DCBS for two-generation reproductive toxicity is 80 ppm（5.2 mg/kg bw per day）in rats.

Keywords: *N,N*-Dicyclohexyl-2-benzothiazolesulfenamide, Rubber accelerator, Two-generation reproductive toxicity

*1 Safety Research Institute for Chemical Compounds Co. Ltd.

Ema, M., Fujii, S.*1, Yabe, K.*1, Matsumoto, M. and Hirata-Koizumi, M.: **Evaluation of reproductive and developmental toxicity of the rubber accelerator N,N-dicyclohexyl-2-benzothiazole sulfenamide in rats**

Cong. Anom., **49**, 149-155（2007）

Male and female Crl:CD（SD）rats were fed a diet containing the rubber accelerator *N,N*-dicyclohexyl-2-benzothiazolesulfenamide（DCBS）at 0, 1500, 3000, 6000 or 10000 ppm（0, 83, 172, 343 or 551 mg/kg bw/day in males and 0, 126, 264, 476 or 707 mg/kg bw/day in females）for a total of 57 days beginning 16 days before mating in males, and a total of 61-65 days from 16 days before mating to day 21 of lactation in females. Body weight gains and food consumption were reduced in males at 6000 ppm and higher and females at 3000 ppm and higher. The weights of the spleen at 6000 and 10000 ppm and of the thymus at 10000 ppm were decreased in females. No changes in estrous cyclicity, copulation index, fertility index, gestation index, delivery index, pre-coital interval, or gestation length were observed at any dose of DCBS. Numbers of implantations at 6000 and 10000 ppm and pups delivered at 10000 ppm were reduced. There were no changes in the sex ratio or viability of pups. The body weights of male and female pups were lowered at 6000 ppm and higher. Decreased weight of the spleen in weanlings was also observed in males at 1500 ppm and higher and in females at 3000 ppm and higher. The data indicate that DCBS possesses adverse effects on reproduction and development in rats.

Keywords: *N,N*-dicyclohexyl-2-benzothiazolesulfenamide, rubber accelerator, reproductive toxicity

*1 Safety Research Institute for Chemical Compounds Co., Ltd.

Ema, M., Hara, H.*1, Matsumoto, M., Hirata-Koizumi, M., Hirose, A. and Kamata, E.: **Evaluation of developmental neurotoxicity of polysorbate 80 in rats**

Reprod. Toxicol., **25**, 89-99（2008）

The developmental neurotoxicity of polysorbate 80（PS80）was evaluated in rats. Crl:CD（SD）rats were given drinking water containing PS80 at 0, 0.018, 0.13, 1.0, or 7.5%（0, 0.035, 0.245, 1.864, or 16.783 ml/kg bw/day）on day 0 of pregnancy through day 21 after delivery. Pregnant rats were allowed to deliver spontaneously. Potential adverse effects of pre- and post-natal exposure on the development and function of the nervous system in offspring of rats given PS80 were examined. Maternal body weight was lowered at 7.5%. Number of pups born was lowered at 7.5%. There were no compound-related effects on locomotor activity of offspring on postnatal days（PNDs）14-15, 17-18, 20-21 and 33-37. No compound-related changes were found in developmental landmarks, sexual maturation, or reflex responses. Although decreased rate of avoidance responses was noted on PNDs 23-27 in male and female offspring at 7.5%, no compound-related changes were found in performance in the conditioned avoidance response on PNDs 60-67. Histopathological examinations of the brain revealed no toxicological changes. Lowered body weight was observed in male and female offspring at 7.5%. The NOAEL in this study was considered to be 1.0%（1.864 ml/mg/kg bw/day）.

Keywords: Polysorbate 80; Tween 80; Developmental neurotoxicity

[*1] Ina Research Inc.

Ema, M., Ito, Y.[*1], Matsumoto, M., Hirose, A. and Kamata, E.: **Screening study for repeated dose and reproductive/developmental toxicity of rubber accelerator, N, N-dicyclohexyl-2-benzothiazolesulfenamide, in rats**

Drug Chem. Toxicol., **30**, 167-180 (2007)

A screening study for a vulcanization accelerator *N,N*-dicyclohexyl-2-benzothiazolesulfenamide (DCBS) was performed in rats. Rats were given DCBS by gavage daily at 0, 6, 25, 100, or 400 mg/kg. Males were dosed for a total of 44 days beginning 14 days before mating. Females were dosed for a total of 40-51 days beginning 14 days before mating to day 3 of lactation. Toxicologic changes were significantly noted only at 400 mg/kg. Three females died. An increased incidence of females showing decreased locomotor activity, soil of the lower abdominal fur, and reddish tears was observed. A lowered body weight was found in males and females. Increased urinary ketones and serum inorganic phosphorus and decreased serum glutamate pyruvate transaminase in males were found. Increased absolute and relative weights of the kidneys in males and decreased absolute weight of the thymus in both sexes were noted. Significant fatty degeneration of the renal tubular epithelia, vacuolation of the adrenocortical cells, and atrophy of the spleen were observed in females. Significant decreases in the gestation index, numbers of corpura lutea, implantations, pups born and pups born alive, live birth index, and viability index were detected. It is concluded that the No Observed Adverse Effect Levels (NOAELs) for repeat dose and reproductive/developmental toxicity are 100 mg kg^{-1} day^{-1} in this screening study.

Keywords: *N,N*-Dicyclohexyl-2-benzothiazolesulfenamide, Reproductive and developmental toxicity, Vulcanization accelerator

[*1] Research Institute for Animal Science in Biochemistry and Toxicology

Hirata-Koizumi, M., Matsuyama, T.[*1], Imai, T., Hirose, A., Kamata, E. and Ema, M.: **Gonadal influence on the toxicity of 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl) benzotriazole in rats**

Drug Chem. Toxicol., **31**, 115-126 (2008)

Previously, we showed that susceptibility of male rats to the toxicity of an ultraviolet absorber, 2-(2'-hydroxy-3',5'-di-tert-butylphenyl) benzotriazole (HDBB), was nearly 25 times higher than that of females. In the current study, we investigated the role of sex steroids in the mediation of the gender-related difference using castrated rats. Male and female castrated CD (SD) rats were given HDBB by gavage at 0, 0.5, 2.5, or 12.5 mg/kg/day for 28 days. No deaths, clinical signs of toxicity, or changes in body weight or food consumption were found at any doses. Blood biochemical changes suggestive of hepatic damage, such as increased levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and lactate dehydrogenase, were detected at 12.5 mg/kg/day in males. Absolute and relative liver weight increased at 0.5 mg/kg/day and above in males and at 12.5 mg/kg/day in females. In the liver, histopathological changes, such as nucleolar enlargement, increased mitosis, hypertrophy in hepatocytes, and/or focal necrosis were observed at 0.5 mg/kg/day and above in males, and at 2.5 mg/kg/day and above in females. These findings indicate that castration markedly reduced the gender-related differences in toxicity of HDBB in rats.

Keywords: Benzotriazole UV absorber, Castration, Gender-related difference.

[*1] Shin Nippon Biomedical Laboratories, Ltd.

Hirata-Koizumi, M., Matsuyama, T.[*1], Imai, T., Hirose, A., Kamata, E. and Ema, M.: **Lack of gender-related difference in the toxicity of 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl) benzotriazole in preweaning rats**

Drug Chem. Toxicol., **31**, 275-287 (2008)

In our previous toxicity studies using young rats, we showed that an ultraviolet absorber, 2-(2'-hydroxy-3',5'-di-tert-butylphenyl) benzotriazole (HDBB), principally affected the liver, and male rats had nearly 25 times higher susceptibility to the toxic effects than females. In the present study, the toxicity of HDBB was investigated in preweaning rats. HDBB was administered by gavage to male and female CD (SD) rats from postnatal days 4 to 21 at a dose of 0, 0.1, 0.5, 2.5, or 12.5 mg/kg/day. No substance-related deaths, clinical signs of toxicity, or body-weight changes were observed.

Increased levels of albumin, AST and ALP in both sexes, BUN in males, and LDH in females were found at 12.5 mg/kg. Liver weights increased at 2.5 mg/kg and above in both sexes. Histopathologically, hepatocellular findings, such as nucleolar enlargement, anisokaryosis, increased mitosis, and/or hypertrophy, were observed at 2.5 mg/kg and above in both sexes. These results indicate no gender-related differences in the susceptibility to the toxic effects of HDBB in preweaning rats.

Keywords: Benzotriazole UV absorber, Preweaning rat, Gender-related difference.

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*1 Shin Nippon Biomedical Laboratories, Ltd.

Hirata-Koizumi, M., Noda, A.*1, Hirose, A., Kamata, E. and Ema, M.: **Reproductive and developmental toxicity screening test of tetrahydrofurfuryl alcohol in rats**

Reprod. Toxicol., **25**, 231-238 (2008)

Twelve male and female rats per group were given tetrahydrofurfuryl alcohol (THFA) by gavage at 0, 15, 50, 150 or 500 mg/kg/day. Males were dosed for 47 days, beginning 14 days before mating, and females were dosed for 42-52 days beginning 14 days before mating to day 4 of lactation throughout the mating and gestation period. Changes in locomotor activity, inhibition of body weight gain, and/or histopathological changes in the thymus, spleen, testes and/or epididymides were observed in males and females at 150 mg/kg and above. No effects of THFA were found on the copulation index, fertility index, or the number of corpora lutea and implantations in pregnant females. At 500 mg/kg, no pregnant females delivered any pups. At 150 mg/kg, gestation length was prolonged, and the total number of pups born and the number of live pups on postnatal days 0 and 4 was markedly decreased. No effects of THFA were found on the sex ratio and body weight of live pups, or the incidence of pups with malformations or variations. Based on these findings, the NOAELs for parental and reproductive/developmental toxicity of THFA were concluded to be 50mg/kg/day in rats.

Keywords: Tetrahydrofurfuryl alcohol; Reproductive and developmental toxicity; Rat

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*1 Research Institute for Animal Science in Biochemistry & Toxicology,

Hirata-Koizumi, M., Ogata, H.*1, Imai, T., Hirose, A., Kamata, E. and Ema, M.: **A 52-week repeated dose toxicity study of ultraviolet absorber 2-(2'-hydroxy-3',5'-di-*tert*-butylphenyl) benzotriazole in rats**

Drug Chem. Toxicol., **31**, 81-96 (2008)

A 52-week repeated dose toxicity study of an ultraviolet absorber, 2-(2'-hydroxy-3',5'-di-tert-butylphenyl) benzotriazole (HDBB), was conducted according to OECD TG 452 under GLP. CD (SD) IGS rats were given HDBB by gavage at 0, 0.1, 0.5, or 2.5 mg/kg/day in males and 0, 0.5, 2.5, or 12.5 mg/kg/day in females. No substance-related deaths or clinical signs of toxicity were observed in any group; however, a lowered body weight was found from day 36 to the end of the 52-week administration period at 2.5 mg/kg in males. At the completion of the dosing period, a decrease in red blood cells at 0.5 mg/kg and higher, and in hematocrit at 2.5 mg/kg, was detected in males. Blood biochemical changes, including increases in the levels of alkaline phosphatase and glucose and the A/G ratio, were also found at 0.5 mg/kg and higher in males and at 12.5 mg/kg in females. At necropsy, absolute and relative liver weight was increased at 0.5 mg/kg and higher in males and at 12.5 mg/kg in females. Histopathological changes were observed in the liver; centrilobular hypertrophy of hepatocytes at 0.5 mg/kg and higher in males, and at 12.5 mg/kg in females, and altered hepatocellular foci at 0.5 mg/kg and higher, and cystic degeneration and lipofuscin deposition in hepatocytes at 2.5 mg/kg in males. Based on these findings, the no observed adverse effect level was concluded to be 0.1 mg/kg/day in male rats and 2.5 mg/kg/day in female rats.

Keywords: Benzotriazole UV absorber, Chronic toxicity, Gender-related difference.

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*1 Panapharm Laboratories Co., Ltd.

Hirata-Koizumi, M., Watari, N.*1, Mukai, D.*1, Imai, T., Hirose, A., Kamata, E. and Ema, M.: **A 28-day repeated dose toxicity study of ultraviolet absorber 2-(2'-hydroxy-3',5'-di-tert-butylphenyl) benzotriazole in rats**

Drug Chem. Toxicol., **30**, 327-341 (2007)

To examine the possible repeated-dose toxicity of an ultraviolet absorber, 2-(2'-hydroxy-3',5'-di-tert-butylphenyl) benzotriazole (HDBB), CD (SD) IGS rats were

administered HDBB by gavage at a dose of 0 (vehicle: corn oil), 0.5, 2.5, 12.5, or 62.5 mg kg(−1) day(−1) for 28 days. At the completion of the administration period, a decrease in red blood cells, hemoglobin, and hematocrit was noted only in males at 2.5 mg/kg and more. Blood biochemical changes were noted at 0.5 mg/kg and more in males and at 62.5 mg/kg in females. Histopathologic changes were observed principally in the liver (vacuolar degeneration and hypertrophy of hepatocytes, bile duct proliferation, etc.) and in the heart (degeneration and hypertrophy of myocardium and cell infiltration). These changes were noted at 0.5 mg/kg and more in males and at 12.5 mg/kg and more in females. At higher doses, hypertrophy of tubular epithelium in the kidneys and diffuse follicular cell hyperplasia in the thyroids in both sexes and increased severity of basophilic tubules in the kidneys and extramedullary hematopoiesis in the spleen in males were also detected. After the 14-day recovery period, these changes mostly recovered in females but not in males. Based on these findings, no observed adverse effect level (NOAEL) was concluded to be less than 0.5 mg kg(−1) day(−1) in male rats and 2.5 mg kg(−1) day(−1) in female rats.

Keywords: Benzotriazole UV absorber, Gender-related difference, Repeated dose toxicity,

[*1] Biosafety Research Center, Foods, Drugs and Pesticides (An-pyo Center)

Matsumoto, M., Furuhashi, T.[*1], Poncipe, C.[*2] and Ema, M.: **Combined repeated dose and reproductive/developmental toxicity screening test of the nitrophenolic herbicide dinoseb, 2-*sec*-butyl-4,6-dinitrophenol, in rats**

Environ. Toxicol., **23**, 169-183 (2008)

In a combined repeated dose toxicity study with reproduction/developmental toxicity screening test, Crj:CD (SD) IGS rats were dosed with dinoseb, 2-*sec*-butyl-4,6-dinitrophenol, by gavage at 0 (vehicle), 0.78, 2.33 or 7.0 mg/kg bw/day. Six males per group were dosed for a total of 42 days beginning 14 days before mating. Twelve females per group were dosed for a total of 44-48 days beginning 14 days before mating to day 6 of lactation throughout the mating and gestation period. Recovery groups of six males per group and non-pregnant six females per group were dosed for 42 days followed by a 14-day recovery period. No deaths were observed in males of any dose group or in females of the recovery groups. At 7.0 mg/kg bw/day, eight females died and two animals were moribund during late pregnancy, and a significant decrease in body weight gain was found in both sexes. Hematocrit was significantly higher at 0.78 mg/kg bw/day and above in the main group males at the end of administration period. Reduction in extramedullary hematopoiesis in the spleen was significant at 2.33 mg/kg bw/day in the main group females. Sperm analysis revealed a decrease in sperm motility and an increase in the rates of abnormal sperm, abnormal tail and abnormal head at 7.0 mg/kg bw/day. A number of dams delivered their pups and of dams with live pups at delivery was significantly lowered in the 7.0 mg/kg bw/day group. Based on these findings, the LOAEL for males and NOAEL for females were 0.78 mg/kg bw/day, and the NOAEL for reproductive/developmental toxicity was considered to be 2.33 mg/kg bw/day.

Keywords: Dinoseb, nitrophenolic herbicide, reproductive and developmental toxicity

[*1] Nihon Bioresearch Inc.
[*2] SafePharm Laboratories Ltd.

鎌田栄一：**化学物質の安全性試験結果公表システムとしての既存化学物質毒性データベース**

日本化学会情報化学部会誌, **25**, 96-98 (2007)

化学物質の審査及び製造等の規制に関する法律（化審法）は，新規化学物質のヒトへの安全性を評価する目的で，スクリーニング試験としてテストガイドライン(TG)が定められ，それに基づいて遺伝子突然変異を指標とする「細菌を用いる復帰突然変異試験」(AMES試験）及びDNAの損傷を指標とする「ほ乳類培養細胞を用いる染色体異常試験」(染色体試験)，更に，ヒトへの一般毒性を推測するためにラットを用いた「ほ乳類を用いる28日間反復投与毒性試験」(28日間試験）が実施されます．既存化学物質の安全性審査においても新規化学物質と同様の試験結果やOECDのTGに基づいた試験結果を参考に審査されます．既存化学物質毒性データベース（JECDB）は既存化学物質の安全性審査に用いられた試験結果を集積したデータベースで，格納されている安全性試験報告は全て統一されたテストガイドラインの元に実施されており，GLPに基づいた試験施設で試験は行われ，更に，使用するラットについても，動物の系統や生

産業者もある程度統一されていることから，試験の質が均一化されており，安全性評価の基礎的資料として非常に有用な資料となっています．

新規化学物質を化審法に基づいて申請する場合，まず，分解度試験を実施し，環境中で分解するか否かを検討します．もし，分解物がJECDBの中の物質の場合，このDBのデータが化学物質調査会に提出されて，安全性評価の資料と成っています．更に，OECDのHPV点検事業において，日本が分担しているHPV物質の報告書には，JECDBの試験報告が引用されて，更に，OECD加盟国についても同様に引用をしています．また，本年度から始まった（独）新エネルギー・産業技術総合開発機構（NEDO）のプロジェクトの内「構造活性相関手法による有害性評価手法開発」では，その第一期としてこのJECDBの内容を分析し，臓器毎に化学構造と毒性徴候を精査して，構造活性相関作製の為の毒性知識情報データベースを作製しています．以上の様にJECDBは日本のみならず外国でも利用されており，その存在意義は重要であることから，今後も品質の高い試験報告の数を増やしていきたいと考えています．

Keywords: Existing Chemicals, GINC, New Chemical Control Act in Japan