Sugiyama, K., Muroi, M., Tanamoto, K., Nishijima, M., Sugita-Konishi, Y.: **Deoxynivalenol and nivalenol inhibit lipopolysaccharide-induced nitric oxide production by mouse macrophage cells**

Toxicol. Lett., **192**, 150-154 (2010)

Deoxynivalenol (DON) and nivalenol (NIV), trichothecene mycotoxins, are secondary metabolites produced by Fusarium fungi. Trichothecene mycotoxins cause immune dysfunction, thus leading to diverse responses to infection. The present study evaluated the effect of DON and NIV on nitric oxide (NO) production by RAW 264 cells stimulated with lipopolysaccharide (LPS). LPS-induced NO production was reduced in the presence of these toxins. The transcriptional activation and expression of inducible NO synthase (iNOS) by LPS were also repressed by these toxins. DON or NIV inhibited LPS-induced expression of interferon-beta (IFN-beta), which plays an indispensable role in LPS-induced iNOS expression. These results indicate that DON and NIV inhibit the LPS-induced NO and IFN-beta production, which both play an important role for host protection against invading pathogens, and suggests that the inhibition of these factors may be involved in the immunotoxic effects of these mycotoxins.

Keywords: Lipopolysaccharide, Inducible nitric oxide synthase, Macrophage, Deoxynivalenol, Nivalenol

Shinkai-Ouchi, F.*, Yamakawa, Y.*, Hara, H., Tobiume, M.*, Nishijima, M., Hanada, K.*, Hagiwara, K.*: **Identification and structural analysis of C-terminally truncated collapsin response mediator protein-2 in a murine model of prion diseases**

Proteome Sci., **8**, 53 (2010)

Prion diseases are fatal neurodegenerative disorders that accompany an accumulation of the disease-associated form (s) of prion protein (PrPSc) in the central nervous system. The neuropathological changes in the brain begin with focal deposits of PrPSc, followed by pathomorphological abnormalities of axon terminal degeneration, synaptic loss, atrophy of dendritic trees, and eventual neuronal cell death in the lesions. However, the underlying molecular basis for these neuropathogenic abnormalities is not fully understood. In a proteomic analysis of soluble proteins in the brains of mice challenged intracerebrally with scrapie prion (Obihiro I strain), we found that the amount of the full-length form of collapsin response mediator protein-2 (CRMP-2; 61 kDa) decreased in the late stages of the disease, while the amount of its truncated form (56 kDa) increased to comparable levels observed for the full-length form. Detailed analysis by liquid chromatography-electrospray ionization-tandem mass spectrometry showed that the 56-kDa form (named CRMP-2-⊿C) lacked the sequence from serine518 to the C-terminus, including the C-terminal phosphorylation sites important for the regulation of axonal growth and axon-dendrite specification in developing neurons. The invariable size of the mRNA transcript in Northern blot analysis suggested that the truncation was due to post-translational proteolysis. By overexpression of CRMP-2-⊿C in primary cultured neurons, we observed the augmentation of the development of neurite branch tips to the same levels as for CRMP-2T514A/T555A, a non-phosphorylated mimic of the full-length protein. This suggests that the increased level of CRMP-2-⊿C in the brain modulates the integrity of neurons, and may be involved in the pathogenesis of the neuronal abnormalities observed in the late stages of the disease. We identified the presence of CRMP-2-⊿C in the brain of a murine model of prion disease. Of note, C-terminal truncations of CRMP-2 have been recently observed in models for neurodegenerative disorders such as ischemia, traumatic brain injury, and Wallerian degeneration. While the structural identity of CRMP-2-⊿C in those models remains unknown, the present study should provide clues to the molecular pathology of degenerating neurons in prion diseases in connection with other neurodegenerative disorders

Keywords: Prion

* 国立感染症研究所

Yamaoka, Y.*[1], Yu, Y.*[2], Mizoi, J.*[1], Fujiki, Y.*[1], Nishijima, M., Lee, Y.*[2], Nishida, I.*[1]: **Phosphatidylserine synthase 1 is required for microspore development in Arabidopsis thaliana**

Plant J., Epub ahead of print (2011)

Phosphatidylserine (PS) has many important biological roles, but little is known about its role in plants, partly because of its low abundance. We show here that PS is enriched in Arabidopsis floral tissues and that genetic disruption of PS biosynthesis decreased heterozygote fertility due to inhibition of pollen maturation. At1g

15110, designated PSS1, encodes a base-exchange-type PS synthase. Escherichia coli cells expressing PSS1 accumulated PS in the presence of l-serine at 23℃. Promoter-GUS assays showed PSS1 expression in developing anther pollen and tapetum. A few seeds with pss1-1 and pss1-2 knockout alleles escaped embryonic lethality but developed into sterile dwarf mutant plants. These plants contained no PS, verifying that PSS1 is essential for PS biosynthesis. Reciprocal crossing revealed reduced pss1 transmission via male gametophytes, predicting a rate of 61.6% pss1-1 pollen defects in PSS1/pss1-1 plants. Alexander's staining of inseparable qrt1-1 PSS1/pss1-1 quartets revealed a rate of 42% having three or four dead pollen grains, suggesting sporophytic pss1-1 cell death effects. Analysis with the nuclear stain 4',6-diamidino-2-phenylindole (DAPI) showed that all tetrads from PSS1/pss1-1 anthers retain their nuclei, whereas unicellular microspores were sometimes anucleate. Transgenic Arabidopsis expressing a GFP-LactC2 construct that binds PS revealed vesicular staining in tetrads and bicellular microspores and nuclear membrane staining in unicellular microspores. Hence, distribution and/or transport of PS across membranes were dynamically regulated in pollen microspores. However, among unicellular microspores from PSS1/pss1-2 GFP-LactC2 plants, all anucleate microspores showed little GFP-LactC2 fluorescence, suggesting that pss1-2 microspores are more sensitive to sporophytic defects or show partial gametophytic defects.

Keywords : Phosphatidylserine synthase 1

---

*1 Laboratory of Plant Molecular Physiology, Graduate School of Science and Engineering, Saitama University
*2 Division of Molecular Life Sciences, Pohang University of Science and Technology, Korea

Kurebayashi, H., Okudaira, K.*, Ohno, Y. : **Species difference of metabolic clearance of bisphenol A using cryopreserved hepatocytes from rats, monkeys and humans**

Toxicology Letters, **198**, 210-215 (2010)

In vitro metabolism of bisphenol A (BPA), an weak estrogen, was studied with cryopreserved hepatocytes from rat, monkey and human, and was compared with in vivo metabolism reported. The metabolites identified include a major metabolite, BPA glucuronide (BPAG) and BPA sulfate (BPAS). The metabolic rates of bisphenol A at 20μM by the hepatocytes (BPAG plus BPAS, nmol/10^6 cells/h) followed the order of rats (48+12)> monkeys (18+4)>humans (8.6+0.8), respectively. The rate of BPAG formation was much higher than that of BPAS formation in all these species. For the BPAG formation, we have determined the apparent K (m) (μM) of rats (3), monkeys (7), and humans (5). V (max) (nmol/10^6 cells/h) in hepatocytes followed the order of rats (55)>monkeys (22)>humans (11). The total CL (H) for the hepatic formation of BPAG plus BPAS (L/h/kg BW) estimated by well-stirred model with low f (B) value followed the order of rats (3.0)> monkeys (0.68)>humans (0.27), correlating well with in vivo studies of BPA subcutaneously injected rats and monkeys. This study showed that the cryopreserved hepatocytes could be a useful tool for assessing BPA metabolism and predicting systemic exposure of BPA.

Keywords : Bisphenol A, Species difference, Monkey, Human

---

* 東邦大学薬学部

Kosaka, N.*1, Inaba, H.*2, Okamoto, K.*3, Mizuno, M.*4, Sono, S.*5, Kato, Y.*6, Kishi, M.*6, Ashikaga, T.*5, Okamoto, Y.*4, Kuwahara, H.*3, Nakamura, T.*2, Sakaguchi, H.*1 and Ohno, Y. : **The Japanese Ring Study of a Human Cell Line Activation Test (h-CLAT) for Predicting Skin Sensitization Potential (5th Report) : A Study for Evaluating Preservative Skin Sensitization Potential Using h-CLAT**

AATEX, **15** (2), 71-80 (2010)

We have developed the human Cell Line Activation Test (h-CLAT) as an in vitro skin sensitization test. In this study, in order to examine whether h-CLAT can predict the skin sensitizing potential of a variety of preservatives, the ring study was performed in two independent laboratories. We selected a total of 16 preservatives, which have been used in cosmetic products at one time, and two different solvents, physiological saline (saline) and dimethylsulfoxide (DMSO). According to h-CLAT protocol, expression of CD86 and CD54 on THP-1 cells was measured by flow cytometry after 24 h treatment with each chemical at 8 doses. The skin sensitizing potential for 13 of 16 preservatives was correctly predicted in one laboratory and 12 of 16 preservatives in the other. These data indicate relatively high concordance with the animal tests. Furthermore,

five preservatives with sensitizing potential were tested using both solvents to compare the effect of saline and DMSO on the predicting capacity of h-CLAT. The sensitizing potential of these preservatives was correctly identified in both saline and DMSO. Our results from this small-scale ring study demonstrate the utility of h-CLAT for detecting the preservatives with skin sensitizing potential.

Keywords: h-CLAT, skin sensitization, alternatives, THP-1, preservative

*1 Kao Corporation
*2 Lion Corporation
*3 Kanebo Cosmetics Inc.
*4 KOSÉ Corporation
*5 Shiseido Co., Ltd.
*6 Nippon Menard Cosmetic Co., Ltd.

Okamoto, K.*1, Kato, Y.*2, Kosaka, N.*3, Mizuno, M.*4, Inaba, H.*5, Sono, S.*6, Ashikaga, T.*6, Nakamura, T.*5, Okamoto, Y.*4, Sakaguchi, H.*3, Kishi, M.*2, Kuwahara, H.*1 and Ohno, Y.: **The Japanese Ring Study of a Human Cell Line Activation Test (h-CLAT) for Predicting Skin Sensitization Potential (6th Report): A Study for Evaluating Oxidative Hair Dye Sensitization Potential Using h-CLAT**

AATEX, **15**(2), 81-88(2010)

We are conducting a Japanese ring study to develop the human Cell Line Activation Test (h-CLAT). The aim of this study is to confirm whether the h-CLAT can predict skin sensitization for oxidative hair dyes. In addition, we studied the effect of test chemical fluorescence on prediction performance. h-CLATs were independently performed in two laboratories for eight chemicals, yielding good reproducibility between the laboratories. Good concordance was obtained between LLNA results and study chemicals. *p*-Phenylenediamine, which exhibits fluorescence at around 530 nm, which is in the h-CLAT measurement range, was correctly evaluated as positive by h-CLAT. It was possible to compensate for the influence of fluorescence in CD86/CD54 expression measurements, suggesting that substances exhibiting auto-fluorescence, such as oxidative hair dyes, can also be evaluated correctly. While a notably high, concentration-dependent CD54 expression was observed with Bandrowski's base, this expression was not observed with *p*-phenylenediamine, a result which suggests that the sensitization responses of these two dyes may differ. In conclusion, it is suggested that h-CLAT is useful for evaluating the skin sensitization potential of oxidative hair dyes.

Keywords: h-CLAT, skin sensitization, alternatives, Bandrowski's base, hair dye

*1 Kanebo Cosmetics Inc.
*2 Nippon Menard Cosmetic Co., Ltd.
*3 Kao Corporation
*4 KOSÉ Corporation
*5 Lion Corporation
*6 Shiseido Co., Ltd.

Sono, S.*1, Mizuno, M.*2, Kosaka, N.*3, Okamoto, K.*4, Kato, Y.*5, Inaba, H.*6, Nakamura, T.*6, Kishi, M.*5, Kuwahara, H.*4, Sakaguchi, H.*3, Okamoto, Y.*2, Ashikaga, T.*1 and Ohno, Y.: **The Japanese Ring Study of a Human Cell Line Activation Test (h-CLAT) for Predicting Skin Sensitization Potential (7th Report): Evaluation of Volatile, Poorly Soluble Fragrance Materials**

AATEX, **15**(2), 89-96(2010)

A ring study was conducted to examine whether the human Cell Line Activation Test (h-CLAT) can predict the skin sensitization potential of fragrance materials with low solubility and high volatility. Seven fragrance materials which had previously been evaluated for skin sensitization potential in vivo (local lymph node assay; LLNA) were selected. In addition, to investigate the influence of volatility or solubility of test materials, we performed the assay with or without sealing of the plate or supersonic ultrasonic wave treatment of the sample solution. All experiments were performed independently in two laboratories. The reproducibility between the two laboratories was 100%. The accuracy with respect to LLNA was 86%: diethyl phthalate gave a false positive in the tests of both laboratories. Sealing the plate had little effect on the CV75 (75% cell viability) or CD86/CD54 RFI (relative fluorescence intensity of CD86/54 expression) values. Ultrasonic wave treatment of the sample solution altered the turbidity and CV75 values in some cases, but did not affect the CD86/CD54 RFI values. Our results indicate that h-CLAT is useful for evaluation of the skin sensitization potential of fragrance materials.

Keywords: skin sensitization, alternative test, fragrance,

h-CLAT

*1 Shiseido Co., Ltd.
*2 KOSÉ Corporation
*3 Kao Corporation
*4 Kanebo Cosmetics Inc.
*5 Nippon Menard Cosmetic Co., Ltd.
*6 LION Corporation

Izutsu, K., Fujii, K.*, Katori, C.*, Yomota, C., Kawanishi, T., Yoshihashi, Y.*, Yonemochi, E.*, Terada, K.*: **Effects of solute miscibility on the micro- and macroscopic structural integrity of freeze-dried solids**

J. Pharm. Sci., **99**, 4710-4719 (2010)

The purpose of this study was to elucidate the effect of solute miscibility in frozen solutions on their micro- and macroscopic structural integrity during freeze-drying. Thermal analysis of frozen solutions containing poly (vinylpyrrolidone) (PVP) and dextran showed single or multiple thermal transitions (T_g' : glass transition temperature of maximally freeze-concentrated solutes) depending on their composition, which indicated varied miscibility of the concentrated noncrystalline polymers. Freeze-drying of the miscible solute systems (e. g., PVP 10,000 and dextran 1060, single T_g' induced physical collapse during primary drying above the transition temperatures T_g'). Phase-separating PVP 29,000 and dextran 35,000 mixtures (two T_g's) maintained their cylindrical structure following freeze-drying below both of the T_g's (<−24℃). Primary drying of the dextran-rich systems at temperatures between the two T_g's (−20 to −14℃) resulted in microscopically disordered "microcollapsed" cake-structure solids. Freeze-drying microscopy (FDM) analysis of the microcollapsing polymer system showed locally disordered solid region at temperatures between the collapse onset (T_{c1}) and severe structural change (T_{c2}). The rigid dextran-rich matrix phase should allow microscopic structural change of the higher fluidity PVP-rich phase without loss of the macroscopic cake structure at the temperature range. The results indicated the relevance of physical characterization and process control for appropriate freeze-drying of multi-component formulations

Keywords : freeze-drying, collapse, formulation

* Faculty of Pharmaceutical Sciences, Toho University

Izutsu, K., Yomota, C. and Kawanishi, T. : **Stabilization of liposomes in frozen solutions through control of osmotic flow and internal solution freezing by trehalose**

J. Pharm. Sci., **100**, 2935-2944 (2011)

The purpose of this study was to elucidate the effect of trehalose distribution across the membrane on the freeze-related physical changes of liposome suspensions and their functional stability upon freeze-thawing. Cooling thermal analysis of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine liposome suspensions showed exotherm peaks of bulk (−15℃ to −25℃) and intraliposomal (approx. −45℃) solution freezing initiated by heterogeneous and homogeneous ice nucleation, respectively. The extent of the intraliposomal solution freezing exotherm depended on liposome size, lipid composition, cosolutes, and thermal history, suggesting that osmotic dehydration occurred due to the increasing difference in solute concentrations across the membrane. A freeze-thawing study of carboxyfluorescein-encapsulated liposomes suggested that controlling the osmotic properties to avoid the freeze-induced intraliposomal solution loss either by rapid cooling of suspensions containing trehalose in both sides of the membrane (retention of the intraliposomal supercooled solution) or by cooling of suspensions containing trehalose in the extraliposomal media prior to freezing (e. g., osmotic shrinkage) led to higher retention of the water-soluble marker. Evaluation and control of the osmotically mediated freezing behavior by optimizing the formulation and process factors should be relevant to the cryopreservation and freeze-drying of liposomes.

Keywords : liposome, formulation, stabilization

Yoshida, H., Nishikawa, M.*1, Kiyota, T.*1, Uno, S.*1, Toyota, H.*1, Takahashi, R.*2, Narita, M.*3, Takakura, Y.*1 : **5'-phosphate oligodeoxynucleotides enhance the phosphodiester-CpG DNA-induced inflammatory response in macrophages**

Eur. J. Immunol., **41**, 425-436 (2011)

We investigated whether nucleotides and nucleosides affect immune responses to phosphodiester-CpG DNA. Addition of non-CpG DNA to RAW264.7, murine macrophage-like cells, induced no significant TNF-α production irrespective of treatment with DNase I; however, DNase I-treated, but not untreated, non-CpG DNA increased the phosphodiester-CpG DNA-mediated

TNF-α production. Deoxynucleotides with a 5'-phosphate showed similar effects to those of DNase I-treated non-CpG DNA, but DNase II-treated DNA or deoxynucleosides did not. Subcutaneous injection of phosphodiester-CpG DNA into the mouse footpad induced little swelling of the paw ; however, significant swelling was observed when DNase I-treated DNA was co-injected with phosphodiester-CpG DNA. These results imply that phosphodiester-CpG DNA-dependent inflammatory responses are increased by DNA molecules with a 5'-phosphate.

Keywords : Cytokines, macrophages, CpG motif, DNase I, 5'-phosphate

---

*1 京都大学大学院薬学研究科
*2 同志社女子大学薬学部
*3 新潟大学大学院保健学研究科

Miyazaki, T., Aso, Y., Yoshioka, S.*, and Kawanishi, T. : **Differences in crystallization rate of nitrendipine enantiomers in amorphous solid dispersions with HPMC and HPMCP**

Int. J. Pharm., **407**, 111-118 (2011)

To clarify the contribution of drug-polymer interaction to the physical stability of amorphous solid dispersions, we studied the crystallization rates of nitrendipine (NTR) enantiomers with identical physicochemical properties in the presence of hydroxypropylmethylcellulose (HPMC), hydroxypropylmethyl-cellulose phthalate (HPMCP) and polyvinylpyrrolidone (PVP). The overall crystallization rate at 60℃ and the nucleation rate at 50-70℃ of (+)-NTR were lower than those of (−)-NTR in the presence of 10-20% HPMC or HPMCP. In contrast, similar crystallization profiles were observed for the NTR enantiomers in solid dispersions containing PVP. The similar glass transition temperatures for solid dispersions of (−)-NTR and (+)-NTR suggested that the molecular mobility of the amorphous matrix did not differ between the enantiomers. These results indicate that the interaction between the NTR enantiomers and HPMC or HPMCP is stereoselective, and that differences in the stereoselective interaction create differences in physical stability between (−)-NTR and (+)-NTR at 50-70℃. However, no difference in physical stability between the enantiomers was obvious at 40℃. Loss of the difference in physical stability between the NTR enantiomers suggests that the stereoselective interaction between NTR and the polymers may not contribute significantly to the physical stabilization of amorphous NTR at 40℃.

Keywords : enantiomer, chiral polymer, crystallization

---

* School of Pharmacy, University of Connecticut

Sato, Y.*, Yamamoto, N.*, Kunitoh, H.*, Ohe, Y.*, Minami, H.*, Laird, N. M.*, Katori, N., Saito, Y., Ohnami, S.*, Sakamoto, H.*, Sawada, J., Saijo, N.*, Yoshida, T.*, Tamura, T.* : **Genome-Wide Association Study on Overall Survival of Advanced Non-small Cell Lung Cancer Patients Treated with Carboplatin and Paclitaxel**

J. Thoracic Oncology, **6**, 132-138 (2011)

Purpose : Our goal was to identify candidate polymorphisms that could influence overall survival (OS) in advanced non-small cell lung cancer (NSCLC) patients treated with carboplatin (CBDCA) and paclitaxel (PTX). Methods : Chemotherapy-na-ve stage IIIB or IV NSCLC patients treated with CBDCA (area under the curve = 6 mg/mL/min) and PTX (200 mg/m2, 3-hour period) were eligible for this study. The DNA samples were extracted from peripheral blood mononuclear cells before treatment, and genotypes at approximately 110,000 gene-centric single-nucleotide polymorphisms (SNPs) were obtained by Illumina's Sentrix Human-1 Genotyping BeadChip. Statistical analyses were performed by the log-rank test and Cox proportional hazards model. Results : From July 2002 to May 2004, 105 patients received a total of 308 cycles of treatment. The median survival time (MST) of 105 patients was 17.1 months. In the genome-wide association study, three SNPs were associated significantly with shortened OS after multiple comparison adjustment : rs1656402 in the EIF4E2 gene (MST was 18.0 and 7.7 months for AG [n = 50] + AA [n = 40] and GG [n = 15], respectively ; p = 8.4 ! 10"8), rs1209950 in the ETS2 gene (MST = 17.7 and 7.4 months for CC [n = 94] and CT [n = 11] + TT [n = 0] ; p = 2.8 ! 10"7), and rs9981861 in the DSCAM gene (MST = 17.1 and 3.8 months for AA [n = 75] + AG [n = 26] and GG [n = 4] ; p = 3.5 ! 10"6). Conclusion : Three SNPs were identified as new prognostic biomarker candidates for advanced NSCLC treated with CBDCA and PTX. The agnostic genome-wide association study may unveil unexplored molecular pathways associated with the drug response, but our findings should

be replicated by other investigators.

Keywords: Advanced non-small lung cancer, Carboplatin, Paclitaxel, Genome-wide association study, Single-nucleotide polymorphisms

* National Cancer Center

坂本知昭，中山幸治[*1]，藤巻康人[*2]，笹倉大督[*3]，川西　徹，檜山行雄：**近赤外分光法を用いた医薬品の規格・基準の設定に関する研究　ー結晶レジボア型経皮吸収テープの品質評価法への応用と含量試験に用いる検量モデルの構築法に関する一例ー**

医薬品医療機器レギュラトリーサイエンス，**41**，971-982（2010）

結晶レジボアシステム（結晶形成型）をもつTDDSテープ中の主薬結晶の特異的検出をNIR測定により行った．TDDSテープは薬剤を含有する基剤をライナーで保護し，また基剤を不織布等の支持板で固定するため，調製後の外側からの品質確認が困難な剤形である．そこで，透過反射NIR光を用いてライナーを剥がすことなく非破壊での基剤中主薬結晶の検出を試みた．6430 cm^{-1}付近の出現する主薬成分の結晶化に由来する吸収を検出し，結晶化の経過に伴いその吸収強度が増大することが確認できた．また，NIR光をフォーカスモードにし，約3 mm径の照射点を用いてテープ剤全体のマッピング測定を行った．その結果，テープ剤中の主薬結晶の大まかな分布を調べることができた．以上の結果により，結晶レジボアシステムをもつTDDSテープにおける結晶化工程の非破壊評価ツールを提案することができた．

NIR分光法を用いた含量試験に使用する検量モデル構築法に関する検討を行った．テオフィリンを主薬成分とする錠剤モデルを作成し，主薬定量のための検量モデル構築に必要な実験計画並びに現実的に必要とされるサンプル数を評価した．定量に用いた吸収はテオフィリンのN–Hに由来する様々なスペクトル処理におけるPLS検量モデルの作成を行い，相関係数及びNIR値の対照値（HPLC値）からの誤差を基に検量モデルの評価を行った．検量モデルの作成のための主薬と各添加剤の混合比は主薬と線形相関が生じない混合比を用いた．検量モデルは5水準×6サンプル及び30水準×1サンプルの2種類を比較した．その結果，両検量モデルともに高精度の検量モデルを作成することができた．以上の結果から，検量モデルの作成に供するサンプルは必ずしも多くの水準を用いる必要はなかった．

Keywords: NIR, Transdermal tapes, Macroscopic mapping

[*1] 東和薬品（株）研究開発本部
[*2] （地独）東京都立産業技術研究センター
[*3] ブルカーオプティクス（株）

Sakai-Kato, K., Hasegawa, T.[*1], Takaoka, A.[*1], Kato, M.[*2], Toyo'oka, T.[*3], Utsunomiya-Tate, N.[*4], Kawanishi, T.: **Controlled structure and properties of silicate nanoparticle networks for incorporation of biosystem components**

Nanotechnology, **22**, 205702（2011）

Inorganic nanoparticles are of technological interest in many fields. We created silicate nanoparticle hydrogels that effectively incorporated biomolecules that are unstable and involved in complicated reactions. The size of the silicate nanoparticles strongly affected both the physical characteristics of the resulting hydrogel and the activity of biomolecules incorporated within the hydrogel. We used high-resolution transmission electron microscopy（TEM）to analyze in detail the hydrogel network patterns formed by the silicate nanoparticles. We obtained clear nanostructured images of biomolecule-nanoparticle composite hydrogels. The TEM images also showed that larger silicate nanoparticles（22 nm）formed more loosely associated silicate networks than did smaller silicate nanoparticles（7 nm）. The loosely associated networks formed from larger silicate nanoparticles might facilitate substrate diffusion through the network, thus promoting the observed increased activity of the entrapped biomolecules. This doubled the activity of the incorporated biosystems compared with that of biosystems prepared by our own previously reported method. We propose a reaction scheme to explain the formation of the silicate nanoparticle networks. The successful incorporation of biomolecules into the nanoparticle hydrogels, along with the high level of activity exhibited by the biomolecules required for complicated reaction within the gels, demonstrates the nanocomposites' potential for use in medical applications.

Keywords: nanotechnology, nanoparticle, nanocomposite

[*1] 大阪大学超高圧電子顕微鏡センター
[*2] 東京大学大学院薬学系研究科
[*3] 静岡県立大学薬学部
[*4] 武蔵野大学薬学部

橋井則貴，川崎ナナ，正田卓司，福原　潔，品川麻衣[*1]，榛葉信久[*1]，河合健蔵[*2]，嶋村英雄[*2]，余田光[*2]，奥田晴宏，山口照英：**ヘパリン純度試験に関する研究（第５報）^{1}H-NMR によるヘパリンナトリウム確認試験及び純度試験に関する研究**

医薬品医療機器レギュラトリーサイエンス，**41**，469-476（2010）

欧米国で発生した過硫酸化コンドロイチン硫酸（OSCS）混入ヘパリンナトリウムによる有害事象への対応として，日本薬局方医薬品各条ヘパリンナトリウムの改訂が検討されている．本研究では，^{1}H-NMR がヘパリンナトリウムの確認試験，並びに^{1}H-NMR OSCS 限度試験として適用可能であることを実証した．

Keywords：^{1}H-NMR，ヘパリンナトリウム，過硫酸化コンドロイチン硫酸

[*1] 味の素（株）ライフサイエンス研究所
[*2] 扶桑薬品工業（株）研究開発センター

Hashii, N., Kawasaki, N., Itoh, S., Qin, Y., Fujita, N.[*1], Hattori, T.[*1], Miyata, K.[*2], Bando, A.[*2], Sekimoto, Y.[*2], Hama, T.[*3], Kashimura, M.[*3], Tatsumi, M.[*4], Mabuchi, K.[*5], Namekawa, H.[*5], Sakai, T.[*6], Hirose, M.[*7], Dobashi, S.[*7], Shimahashi, H.[*8], Koyama, S.[*9], Herr, S. O.[*10], Kawai, K.[*11], Yoden, H.[*11], Yamaguchi, T.: **Heparin identification test and purity test for OSCS in heparin sodium and heparin calcium by weak anion-exchange high-performance liquid chromatography**

Biologicals, **38**, 539-543（2010）

Heparin sodium and heparin calcium, which are widely used as anti-coagulants, are known to potentially contain the natural impurity dermatan sulfate（DS）. Recently serious adverse events occurred in patients receiving heparin sodium in the US, and a contaminant oversulfated chondroitin sulfate（OSCS）was found to be a cause of the events. To ensure the quality and safety of pharmaceutical heparins, there is need of a physicochemical identification test that can discriminate heparin from the heparin-related substances as well as a sensitive purity test for OSCS. Recently, HPLC with a strong-anion exchange column was proposed as the methods for identifying heparin and determination of OSCS in heparin sodium. Although this method is convenient and easy to perform, the only column suitable for this purpose is the Dionex IonPac AS11-HC column. In this study, we developed alternative identification test and test for OSCS in both heparin sodium and heparin calcium using a weak anion-exchange column. The identification test allowed for separation of heparin from the impurity DS and contaminant OSCS in a shorter time. The purity test provided enough sensitivity, specificity, linearity, recovery and repeatability for OSCS. We believe that our methods will be useful for quality control of pharmaceutical heparins.

Keywords：heparin, oversulfated chondroitin sulfate, WAX-HPLC

[*1] 味の素製薬（株）研究開発本部創薬研究センター
[*2]（株）大塚製薬工場
[*3] Sanofi-aventis K.K.
[*4] 沢井製薬（株）
[*5] 沢井製薬（株）関東工場
[*6] 大洋薬品（株）関東工場
[*7] テルモ（株）
[*8] 日本臓器製薬（株）製剤研究所
[*9] 持田製薬工場（株）
[*10] Pharmaceutical Product Support, LEO Pharma
[*11] 扶桑薬品工業（株）研究開発センター

Ogawa, Y.[*1], Miura, Y.[*2], Harazono, A., Kanai-Azuma, M.[*3], Akimoto, Y.[*3], Kawakami, H.[*3], Yamaguchi, T., Toda, T.[*2], Endo, T.[*2], Tsubuki, M.[*4] and Yanoshita, R.[*5]: **Proteomic analysis of two types of exosomes in human whole saliva**

Biol. Pharm. Bull., **34**, 13-23（2011）

Saliva contains a large number of proteins that participate in the protection of oral tissue. Exosomes are small vesicles（30-100 nm in diameter）with an endosome-derived limiting membrane that are secreted by a diverse range of cell types. We have recently demonstrated that exosomes are present in human whole saliva. In this study, we found that whole saliva contained at least two types of exosomes（exosome I and exosome II）that are different in size and protein composition. Proteomic analysis revealed that both types of exosomes contained Alix, Tsg101 and Hsp70, all exosomal markers, immunoglobulin A and polymeric immunoglobulin receptor, whereas they had different protein compositions. Most of dipeptidyl peptidase IV known as CD26 in whole saliva, was present on the exosome II and metabolically active in cleaving chemokines（CXCL11 and CXCL12）. Human whole saliva exosomes might participate in the catabolism of bioactive peptides and play a regulatory role in local

immune defense in the oral cavity.

Keywords : exosome, human saliva, proteomic analysis

*1 帝京平成大学薬学部
*2 東京都立老人総合研究所
*3 杏林大学医学部
*4 星薬科大学医薬品化学研究所
*5 星薬科大学

Maeda, Y.*, Yusa, K., Nakano, Y.*, Harada, S.* : **Involvement of inhibitory factors in the inefficient entry of HIV-1 into the human CD4 positive HUT78 cell line**

Virus Res., **155**, 368-371 (2011)

Little is known about whether human CD4 positive T cells, the principal natural target of HIV-1, have intrinsic factors, other than the receptor/coreceptor molecules, which modulate the entry efficiency of HIV-1. In the present study, we found that human T cell lines, HUT78 and PM1, were less permissive to VSV-G-mediated HIV-1 infection compared with the Jurkat cell line. Furthermore, HUT78 cells were also less sensitive to HIV-1 Env-mediated infection, while PM1 cells became susceptible to HIV-1. Real-time PCR analyses showed that less susceptibility of the cells to HIV-1 was due to block at, or prior to, reverse transcription of viral RNA. To clarify the entry efficiency of HIV-1 into these cell lines, we analyzed the internalization of p24 Ag into the cytosolic and vesicular fractions of post-nuclear extracts at 4h post-infection. When the cells were infected with HIV-1 pseudotyped with VSV-G, the amount of p24 Ag in the cytosolic fractions in both HUT78 and PM1 cells was lower than that observed in Jurkat cells. In the case of HIV-1 Env-mediated infection, however, PM1 cells exhibited comparable amounts of p24 Ag in the cytosolic fraction compared with Jurkat cells, while the amount of p24 Ag in HUT78 cells remained low. Heterokaryon experiments between susceptible and less susceptible cell lines suggested that some inhibitory factors counteracted VSV-G-mediated viral entry in PM1 and HUT78 cells, and HIV-1 Env-mediated viral entry in HUT78 cells.

Keywords : inhibitory factor, $CD4^+$ T cells, HIV-1 p24

* Kumamoto University

Kiuchi, F.*1, Goda, Y., Isizaki, M.*2, Ito, H.*3, Kawasaki, T.*4, Kawahara, N.*5, Kanmoto, T.*6, Kikuchi, Y.*6, Kondo, S.*7, Sugimoto, C.*8, Narukawa, Y.*1, Higano, T.*9, Yamamoto, Y.*10 : **Crude drug identification tests with TLC in the Japanese Pharmacopoeia (1) On the TLC tests with 1-butanol/water/acetic acid solvent system**

Jpn. J. Pharmacog., **65**, 25-32 (2011)

The crude drug identification tests, which use 1-butanol/water/acetic acid solvent system for TLC in the Japanese Pharmacopoeia, were examined. Comparison of TLC chromatograms from 7 laboratories, using commercially available TLC plates purchased from Merck & Co. and Wako Pure Chemical Industries, revealed that the Rf values of the indicator spots were markedly influenced by the make of TLC plates, and in the tests of Lycium Bark and Plantago Herb, the Rf values observed were different from those indicated in the Japanese Pharmacopoeia. TLC chromatograms developed in two different lengths (7 cm and 10 cm) showed almost identical patterns for all the 12 identification tests examined. This indicates that it is possible to change the development length from 10 cm to 7 cm, which will save the time required for the development by about 45%, without affecting the results of the identification tests.

Keywords : TLC, the Japanese Pharmacopoeia, crude drug tests

*1 慶應義塾大学薬学部
*2 三和生薬（株）
*3 和光純薬工業（株）
*4 （株）ウチダ和漢薬
*5 （独）医薬基盤研究所薬用植物資源研究センター
*6 （株）ツムラ
*7 小太郎漢方製薬（株）
*8 救心製薬（株）
*9 大正製薬（株）
*10 （株）栃本天海堂

Amakura, Y.*1, Yoshimura, M.*1, Kawahara, N.*2, Goda, Y., Yoshida, T.*1 : **TLC-based identification test for the crude drug "Salviae miltriorrhizae Radix" and "Codonopsitis Radix"**

Jpn. J. Pharmacog., **65**, 18-24 (2011)

Rapid and simple methods for TLC-based identification of the crude drug "Salviae miltiorrhizae Radix"

（danshen；root of *Salvia miltiorrhiza*）as well as “Codonopsitis Radix”（tangshen；root of *Codonopsis pilosula and C. tangshen*）were developed. TLC and HPLC were preliminarily applied to characterization of possible chemical markers for evaluating their qualities, and two UV-sensitive compounds, lithospermic acid B and tanshinone IIA, were identified as TLC markers of danshen methanolic extracts prepared under a setting condition. On the other hand, although any UV-sensitive TLC marker of tangshen extract was not detected, a characteristic spot of D-fructose was definitely detected by spraying with diluted sulfuric acid test solution. The present data could be useful for development of convenient TLC test for identifying the danshen and tangshen.

Keywords：Salviae miltiorrhizae Radix, Codonopsitis Radix, thin-layer chromatography

*1 松山大学薬学部
*2（独）医薬基盤研究所薬用植物資源研究センター

Hirasawa, Y.*1, Hara, M.*1, Nugroho, A. E.*1, Sugai, M.*1, Zaima, K.*1, Kawahara, N., Goda, Y., Awang, K.*2, Hadi, A. H. A.*2, Litaudon, M.*3, Morita, H.*1：**Bisnicalaterines B and C, atropisomeric bisindole alkaloids from Hunteria zeylanica, showing vasorelaxant activity**

J. org. chem., **75**, 4218-4223（2010）

Two new bisindole alkaloids, bisnicalaterines B and C consisting of an eburnane and a corynanthe type of skeletons, were isolated from the bark of Hunteria zeylanica. -Their absolute structures were detected by combination of NMR, CD, and computational methods, and each of them was shown to be in an atropisomeric relationship. -Bisnicalaterines B and C showed potent vasorelaxant activity on isolated rat aorta.

Keywords：*Hunteria zeylanica*, atropisomeric bisindole alkaloid, bisnicalaterine

*1 Hoshi University
*2 University of Malaya
*3 Institut de Chimie de la Substances Naturelles, Centre Nationale de la Recherches Scientifique

Hosoe, J., Sugimoto, N., Goda, Y.：**Trial study to determine absolute purities of chemical reagents used as reference standards in Japanese Pharmacopoeia by using quantitative NMR（qNMR）**

Pharmaceutical and Medical Device Regulatory Science, **41**, 960-970（2010）

In the “Crude Drugs” section of the Japanese Pharmacopoeia（JP）, many chemical reagents that are commercially available and chemically specified in the section on “Reagents and Test Solutions” are used as reference standards for quantitative analyses. However, there is no information on the absolute purity of these standards because it is very difficult to obtain pure natural compounds and determine their purity with International System of Units（SI）traceability. Therefore, in a strict sense, quantitative regulation by such marker compounds on crude drugs and related products in JP is somewhat ambiguous. Recently, quantitative NMR（qNMR）using a certified reference material as a qNMR reference has been developed. This method qualifies as an absolute quantification method and is theoretically able to determine the purity of any compound with SI traceability. Therefore, we are proposing to introduce the qNMR method to JP for the specification of reagents used as marker compounds. In this report, in order to clarify practical issues that must be solved before the adoption of qNMR by JP, we applied qNMR to reagents（and a naturally purified compound）which are known to have wide-ranging purity. As a result, we found that the selection of the specific NMR signal（s）for calculating the purity of the target compound was very important. It is our view that a simple non-exchangeable signal such as a singlet or doublet should be selected and that the numbers of selected signals should be modified depending on the level of purity of the target compound. Coexisting signals from impurities in the integration section cause integration errors and increasing the number of the selected signals consequently enhances the likelihood of accurate integrations. Further studies and intensive discussion in the panel on crude drugs in JP are needed to reach a final consensus on this issue.

Keywords：quantitative NMR, the Japanese Pharmacopoeia, crude drug marker compounds

Kikuchi, H., Uchiyama, N., Ogata, J., Kikura-Hanajiri, R., Goda, Y.：**Chemical constituents and DNA sequence analysis of a psychotropic herbal product**

Forensic Toxicol., **28**, 77-83（2010）

In recent years, the distribution of a variety of psychotropic products, especially “spice” and “herbal

blends," which are advertised to have narcotic-like effects, has become more widespread in the Japanese illegal drug market. We recently found two synthetic annabinoids, cannabicyclohexanol and JWH-018, that serve as adulterants in herbal products purchased via the Internet. In this study, we focused on a herbal product being sold as incense, which showed unknown components by liquid chromatography-mass spectrometry (LC-MS). The product did not show any peak corresponding to the above synthetic cannabinoids, but seven other peaks were identified by high-performance liquid chromatography and LC-MS. We identified them as *N*-methyltyramine (1), (*R*)-normacromerine (2), (*R*)-macromerine (3), (*S*)-vasicine (4), mescaline (5), harmaline (6), and harmine (7) by polarimetry, LC-MS, gas chromatography-mass spectrometry, high-resolution mass spectrometry, and nuclear magnetic resonance spectroscopy. We also used DNA sequence analyses to identify the plant species of the product. As a result of the sequencing of *trnL-F*, internal transcribed spacer (ITS), and *rpl16* intron regions, three sequences derived from *Coryphantha macromeris* (Cactaceae), *Peganum harmala* (Zygophyllaceae), and *Turnera diffusa* (Turneraceae) were observed. Compounds 2 and 3, both phenethylamines, were reported to cause hallucinogenic effects and are frequently found in *Coryphantha genus* (Cactaceae). Therefore, the plant source of these compounds was considered to be *C.macromeris*. Compound 5 is known to be a psychoactive phenethylamine found in peyote (*Lophophora williamsii*) and San Pedro cactus (*Trichocereus pachanoi*). The β-carboline alkaloids 6 and 7 are known to be found in the seeds of *P.harmala*. Therefore, there seems to be no contradiction between the chemical constituents and the plant species estimated by DNA analyses, except for compound 5. This is the first report dealing with identification of the psychoactive cactus *C.macromeris* and its constituent compounds in a herbal product distributed in the illegal drug market.

Keywords: Herbal product, DNA analysis, LC-MS

丸山卓郎，近藤健児[*1]，四柳雄一[*2]，山本　豊[*3]，川崎武志[*4]，司馬真央[*1]，寺坂和祥[*5]，山根真由[*2]，Shu Zhu[*6]，坂田こずえ，藤田正雄[*4]，穐山　浩，西村直行[*2]，小松かつ子[*6]，水上　元[*5]，合田幸広：**PCR-RFLP法によるビャクジュツのソウジュツに対する純度試験に対する妥当性確認試験**

生薬学雑誌，**64**，96-101（2010）

An inter-laboratory validation study was performed for the purity test of Atractylodes Rhizome targeted for Atractylodes Lancea Rhizome based on a PCR-RFLP by 7 persons whose experience with PCR experiments ranges from 0 to 10 years. Twenty-five crude drugs derived from medicinal *Atractylodes* plants were distributed to each practitioner and the discrimination of them into Atractylodes Rhizome and Atractylodes Lancea Rhizome was carried out following the common experimental protocol. As a result, all practitioners achieved exact identification regardless of their experience with PCR or the kind of the instruments used in the test. This result indicates that the test is reliable for the discrimination of Atractylodes Rhizome and Atractylodes Lancea Rhizome.

Keywords: Atractylodes Rhizome, Atractylodes Lancea Rhizome, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

[*1] （株）ツムラ
[*2] （株）島津製作所
[*3] （株）栃本天海堂
[*4] （株）ウチダ和漢薬
[*5] 名古屋市立大学大学院薬学研究科
[*6] 富山大学和漢医薬学総合研究所

鎌倉浩之，丸山卓郎，杉村康司*，飯田　修*，合田幸広：**健康食品に使用されるパッションフラワーの基原種と成分について**

日本食品化学学会誌，**17**，198-206（2010）

In our continuing research on guarantee for the safety of dietary supplements derived from medicinal plants, commercial passion flower products were investigated for their botanical origin on the basis of nrDNA ITS1 and cpDNA *trn*L-F IGS sequences, as well as with analyses of flavone glycoside and β-carboline alkaloid composition using LC-PDA-MS. Both nuclear and chloroplast DNA sequences well distinguished *P.incarnata*, from other species of the same genus, such as *P.edulis*, *P.caerulea*, *P.quadranglaris* and others. Three ITS1 genotypes were found in passion flower products which were assigned to *P.incarnata*, *P.edulis* and *P.edulis f. flavicarpa* with reference to the sequences of referential *Passiflora* plants. Flavone glycoside composition showed the species- and forma-specific variation and the profile of each product supported the results of DNA sequence

analyses. Fourteen passion flower products were analyzed for their source plant species, and were shown to be made from *P.incarnata* (nine samples) and *P.edulis* sensu lato (five samples) on the basis of the DNA and LC-PDA-MS analyses. Furthermore, β-carboline alkaloids such as harmine and harmaline which were reported as the constituents of *P.incarnata* were not detected in the products. Plant materials legally restricted to medicinal use in Japan are specified by their scientific names and listed on the Pharmaceutical Affairs Bureau Notification. However, those for general herbal products are not specified. According to the results, plant material used for general herbal products are suggested to be specified by scientific names in order to ensure their safety.

Keywords: Passion flower, LC-PDA-MS analysis, DNA analysis

---

* (独) 医薬基盤研究所薬用植物資源研究センター

Sato, M.*, Anetai, M.*, Kamakura, H., Goda, Y.: **Migration of organophosphorus pesticides to decoctions of Kampo formula from crude drugs**

Pharmaceutical Medical Device Regulatory Science, **41**, 458-468 (2010)

During our studies on quality evaluation of crude drugs in Japan, we have detected organophosporus pesticides in several crude drugs. In Japan, about 90% of crude drugs are used as raw materials for Kampo products after industrial decoction followed by a spray-drying process. To assess human exposure, it is important to determine the fate of the pesticides during the extraction step. In this study, we prepared 3 Kampo decoctions from crude drugs contaminated with organo phosphorus pesticides and quantitatively determined residual pesticide concentrations in the decoctions and the crude drug residues. The maximum migration rate to the decoctions was 31% in the case of malathion in Citrus Unshiu Peel in Hochuekkito. The migration rates of the other pesticides, except malathion, parathion and parathion-methyl, were less then 20%. It is concluded that considerable amounts of these pesticides may remain in the crude drug residues or be lost by decomposition or vaporization during the decoction process. The amounts of organophosphorus pesticides, except tolclophos-methyl, in crude drug residues of Hochuekkito were related to log Kow, not to water solubility.

Keywords: Kampo formula, crude drugs, organophosphorus pesticide residue

---

* 北海道立衛生研究所

Sato, M.*, Hakamatsuka, T., Anetai, M.*, Kamakura, H., Goda, Y.: **Fate of organophosphorus pesticides in decoction based on a Kampo formula during drying**

Pharmaceutical Medical Device Regulatory Science, **41**, 816-822 (2010)

During quality evaluation of crude drugs, we have sometimes detected residual organophosphorus pesticides in them. In our previous study, we prepared 3 Kampo decoctions using crude drugs contaminated with organophosphorus pesticides and quantified the concentrations of the pesticides in the decoctions to evaluate the migration rates from crude drugs to decoctions. The migration rates were less than 28% for Hangekobokuto decoction. In Japan, about 90% of crude drugs are used as raw materials for Kampo dry extracts prepared from industrial decoctions. Therefore, in the present work, we investigated the fate of organophosphorus pesticides in a decoction based on a Kampo formula during the drying process. In the present work, we first prepared decoction of Hangekobokuto by utilizing Perilla Herb contaminated with parathion and parathion-methyl, and then freeze-dried or spray-dried the supernatant after centrifugation. The pesticide residues in the dried extract; and the removed herbal residue were measured by GC-FPD. The organophosphorus pesticide contents in the freeze-dried and spray-dried extracts were 43% (for both parathion and parathion-methyl) and less than 10% (parathion: 6.7%, parathion-methyl: 8.9%) of those in the decoction, respectively. On the other hand, 36% of parathion and 16% of parathion-methyl remained in the herbal residue obtained by centrifugation. These data suggest that substantial amounts of pesticides in the decoction were decomposed or vaporized during the drying process. It was calculated that more than 85% of residual organophosphorus pesticides contained in the orginal crude drug, Perilla Herb, is removed during the decoction and drying process. The final pesticide residue levels in the freeze-dried and spray-dried extracts were 10～14% and 2～3% of those in Perilla Herb, respectively.

Keywords: Kampo formula, drying process, organophosphorus pesticide residue

* 北海道立衛生研究所

Wakana, D., Kawahara, N., Goda, Y.: **Three new triterpenyl esters, codonopilates A-C, isolated from *Codonopsis pilosula***

J. Nat. Med., **65**, 18-23 (2011)

Three triterpenyl esters, codonopilates A-C, were isolated from *Codonopsis pilosula*, along with fourteen known compounds.Their structures were elucidated on the basis of chemical and spectroscopic investigations.

Keywords: *Codonopsis pilosula*, triterpenyl ester, Tojin

高橋市長*，長谷川貴志*，西條雅明*，永田知子*，若菜大悟，合田幸広：**いわゆる健康食品中から検出されたシルデナフィル構造類似体について**

千葉県衛研年報，**58**，55-60（2011）

国内で流通している「いわゆる健康食品」の試買検査を行った結果，分子量488 を示す化合物が検出された．本物質は国内では「いわゆる健康食品」から検出された例のないメチソシルデナフィルだった．

Keywords: methisosildenafil, dietary supplement, sildenafil analog

* 千葉県衛生研究所

Inagaki, S.*, Taniguchi, S.*, Hirashima, H.*, Higashi, T.*, Min, J. Z.*, Kikura-Hanajiri, R., Goda, Y., Toyo'oka, T.*: **HPLC enantioseparation of α,α-diphenyl-2-pyrrolidinemethanol and methylphenidate using a chiral fluorescent derivatization reagent and its application to the analysis of rat plasma**

J. Sep. Sci., **33**, 3137-3143 (2010)

Enantioseparation of α,α-diphenyl-2-pyrrolidinemethanol (D2PM) and methylphenidate (MPH; Ritalin®) using (*R*)-(−)-4-(*N*, *N*-dimethylaminosulfonyl)-7-(3-isothiocyanatopyrrolidin-1-yl)-2,1,3-benzoxadiazole as the chiral derivatization reagent has been achieved for the first time, and a simple, reliable detection method using HPLC with fluorescence detection has been developed. D2PM and MPH have been derivatized with (*R*)-(−)-4-(*N*,*N*-dimethylaminosulfonyl)-7-(3-isothiocyanatopyrrolidin-1-yl)-2,1,3-benzoxadiazole at 55℃ for 15 min. The derivatives of D2PM and MPH have been separated, completely and rapidly, using a reversed-phase system within 16 min (resolution factor (R_s)=1.60 and 2.53, respectively). The detection limits of (*R*)- and (*S*)-D2PM were found to be 6.8 and 13 ng/mL, respectively, and those of D- and L-*threo*-MPH were 61 and 66 ng/mL, respectively (S/N=3). The proposed method was successfully applied to the analysis of rat plasma, where the rats were separately dosed with D2PM and MPH (Ritalin).

Keywords: enantioseparation, fluorescence detection, HPLC

* School of Pharmaceutical Sciences, University of Shizuoka

Min, J. Z.*, Yamashita, K.*, Toyo'oka, T.*, Inagaki, S.*, Higashi, T.*, Kikura-Hanajiri, R., Goda, Y.: **Simultaneous and group determination methods for designated substances by HPLC with multi-channel electrochemical detection and their application to real samples**

Biomed. Chromatogr., **24**, 1287-1299 (2010)

Many psychotropic substances are illegally available on the streets and/or via the Internet. This wide distribution has become a serious social problem. To control this problem, many substances have been controlled as 'designated substances' (Shitei-Yakubutsu) in Japan since April 2007 by the Pharmaceutical Affairs Law, including tryptamines, phenethylamines and piperazines. In the present study, simultaneous determination methods using HPLC with multi-channel electrochemical detection (MECD) were developed for the designated substances. The proposed methods utilizing online electrochemical oxidation are the first report on the simultaneous determination of various designated substances. The methods involve direct determination and require no complicated pretreatments such as fluorescence labeling. The designated substances were separated by reversed-phase chromatography using a TSK-gel ODS-100V (4.6×250 mm, i.d., 3 μm) and gradient elution by a mixture of potassium phosphate buffer, methanol and acetonitrile. The total separation of 31 designated substances was successfully performed but required long chromatographic run times. Thus, the designated substances were divided into three groups: (1) tryptamines, (2) phenethylamines and (3) piperazines and others. They were then analyzed by HPLC-MECD as another separation method. The suitable applied voltages for each designated substance were determined based upon the hydrodynamic voltammogram. The limits of

detection (signal-to-noise ratio of 3) of the designated substances for the most suitable voltages were in the range of 17.1 pg (5-MeO-MIPT) to 117 ng (indan-2-amine). The calibration curves based on the peak heights were linearly related to the amounts of the designated substances ($R^2 > 0.999$). Good accuracy and precision by intra-day assay and inter-day assay were also obtained using the present procedures. The proposed methods were applied to the analyses of the designated substance in several real samples.

Keywords: designated substances, HPLC, multi-channel electrochemical detection

---

* School of Pharmaceutical Sciences, University of Shizuoka

Kikura-Hanajiri, R., Kawamura, M., Miyajima, A., Sunouchi, M., Goda, Y.: **Chiral analyses of dextromethorphan/levomethorphan and their metabolites in rat and human samples using LC-MS/MS**

Anal. Bioanal. Chem., **400**, 165-174 (2011)

In order to develop an analytical method for the discrimination of dextromethorphan (an antitussive medicine) from its enantiomer, levomethorphan (a narcotic) in biological samples, chiral analyses of these drugs and their *O*-demethyl and/or *N*-demethyl metabolites in rat plasma, urine, and hair were carried out using LC-MS/MS. After the i.p. administration of dextromethorphan or levomethorphan to pigmented hairy male DA rats (5 mg/kg/day, 10 days), the parent compounds and their three metabolites in plasma, urine and hair were determined using LC-MS/MS. Complete chiral separation was achieved in 12 min on a Chiral CD-Ph column in 0.1% formic acid-acetonitrile by a linear gradient program. Most of the metabolites were detected as being the corresponding *O*-demethyl and *N*, *O*-didemethyl metabolites in the rat plasma and urine after the hydrolysis of *O*-glucuronides, although obvious differences in the amounts of these metabolites were found between the dextro and levo forms. No racemation was observed through *O*- and/or *N*-demethylation. In the rat hair samples collected 4 weeks after the first administration, those differences were more clearly detected and the concentrations of the parent compounds, their *O*-demethyl, *N*-demethyl, and *N*, *O*-didemethyl metabolites were 63.4, 2.7, 25.1, and 0.7 ng/mg for the dextro forms and 24.5, 24.6, 2.6, and 0.5 ng/mg for the levo forms, respectively. In order to fully investigate the differences of their metabolic properties between dextromethorphan and levomethorphan, DA rat and human liver microsomes were studied. The results suggested that there might be an enantioselective metabolism of levomethorphan, especially with regard to the *O*-demethylation, not only in DA rat but human liver microsomes as well. The proposed chiral analyses might be applied to human samples and could be useful for discriminating dextromethorphan use from levomethorphan use in the field of forensic toxicology, although further studies should be carried out using authentic human samples.

Keywords: levomethorphan, dextromethorphan, enantioselective metabolism

Wada, M.*, Abe, K.*, Ikeda, R.*, Kikura-Hanajiri, R., Kuroda, N.*, Nakashima, K.*: **HPLC determination of methylphenidate and its metabolite, ritalinic acid, by high-performance liquid chromatography with peroxyoxalate chemiluminescence detection**

Anal. Bioanal. Chem., **400**, 387-393 (2011)

An HPLC-peroxyoxalate chemiluminescence (PO-CL) method for simultaneous determination of methylphenidate (MPH) and ritalinic acid (RA) was developed. The method was used to monitor MPH and RA after administration of MPH to rats. Deproteinized plasma spiked with 1-(3-trifluoromethylphenyl) piperazine (IS) was dried and labeled with 4-(*N*, *N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F). The labeled sample was cleaned with two kinds of solid-phase extraction cartridge, and the DBD-labels were separated on an ODS column with gradient elution using a mixture of CH_3CN and imidazole-HNO_3 buffer. Separation of MPH and RA can be achieved within 33-min. The LODs of MPH and RA at a signal-to-noise ratio of 3 were 2.2 and 0.4 ng mL^{-1}, respectively. Moreover, monitoring of MPH and RA after MPH administration (10 mg kg^{-1}) to rat could be performed. The concentration of RA 480 min after administration was eight times higher than that of MPH. The proposed HPLC-PO-CL method was useful for determination of MPH and RA in rat plasma and was successfully used to monitor these substances after MPH administration.

Keywords: methylphenidate, HPLC, peroxyoxalate chemiluminescence

* Graduate School of Biomedical Sciences, Nagasaki University

Sogawa, C.*1, Sogawa, N.*1, Ohyama, K.*1, Kikura-Hanajiri, R., Goda, Y., Sora, I.*2, Kitayama, S.*1: **Methylone and monoamine transporters: correlation with toxicity**

Current Neuropharmacology, **9**, 58-62 (2011)

Methylone 2-methylamino-1-[3,4-methylenedioxyphenyl] propane-1-one) is a synthetic hallucinogenic amphetamine analog, like MDMA (3,4-methylenedioxymethamphetamine), considered to act on monoaminergic systems. However, the psychopharmacological profile of its cytotoxicity as a consequence of monoaminergic deficits remains unclear. We examined here the effects of methylone on the transporters for dopamine (DAT), norepinephrine (NET), and serotonin (SERT), using a heterologous expression system in CHO cells, in association with its cytotoxicity. Methylone inhibited the activities of DAT, NET, and SERT, but not GABA transporter-1 (GAT1), in a concentration-dependent fashion with a rank order of NET > DAT > SERT. Methylone was less effective at inhibiting DAT and NET, but more effective against SERT, than was methamphetamine. Methylone alone was not toxic to cells except at high concentrations, but in combination with methamphetamine had a synergistic effect in CHO cells expressing the monoamine transporters but not in control CHO cells or cells expressing GAT1. The ability of methylone to inhibit monoamine transporter function, probably by acting as a transportable substrate, underlies the synergistic effect of methylone and methamphetamine.

Keywords: methylone, neurotransmitter transporter, monoaminergic systems

---

*1 Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences
*2 Tohoku University School of Medicine

Kitajima, M.*1, Iwai, M.*1, Kikura-Hanajiri, R., Goda, Y., Iida, M. *2, Yabushita, H.*2, Takayama, H.*1: **Discovery of indole alkaloids with cannabinoid CB1 receptor antagonistic activity**

Bioorg. Med. Chem. Lett., **21**, 1962-1964 (2011)

Three indole alkaloids, voacamine (1), 3,6-oxidovoacangine (2), and a new alkaloid, 5-hydroxy-3,6-oxidovoacangine (3), isolated from *Voacanga africana* were found to exhibit potent cannabinoid CB1 receptor antagonistic activity. This is the first example of CB1 antagonists derived from natural alkaloids.

Keywords: CB1 antagonist, indole alkaloid, voacanga

---

*1 Graduate School of Pharmaceutical Sciences, Chiba University
*2 Otsuka Pharmaceutical Co., Ltd

高橋美津子*, 桜井克巳*, 渡部健二朗*, 花尻（木倉）瑠理, 合田幸広：**無承認無許可医薬品及び違法ドラッグのスクリーニング分析を指向したLC/MSライブラリーの構築**

医薬品医療機器レギュラトリーサイエンス, **41**, 742-749 (2010)

インターネットを中心に販売されている法的に問題のある健康食品や違法ドラッグは含有形態が多成分系である場合が多く，その組み合わせも多様である．従って，検査すべき成分が容易に特定出来ない場合があり，特定出来た場合も同定用標準品の入手が困難である場合が多い．本研究では，このような問題を解決する一手法としてLC/MSライブラリー検索用ソフトを使用して，医薬品成分（類似成分も含む）及び指定薬物のMSデータとリテンションタイムに関するライブラリーを構築した．さらに同ライブラリーを利用してスクリーニング分析を行った結果，シブトラミン．フルオキセチン，ビサコジル，フロセミド，シルデナフィル及びグリベンクラミドを検出した．これより，検体の測定で得られたMSデータ及びリテンションタイムをライブラリーと照合することで．LC/MSによる未知成分の検索特定が可能であることが示された．また，標準品の入手が困難な指定薬物等の化合物においても，MSデータと照合することで物質の推定が可能となり，違法ドラッグに対し迅速な分析が可能になると考えられる．

Keywords: スクリーニング，液体クロマトグラフ質量分析計，無承認無許可医薬品

---

* 横浜市衛生研究所

高橋市長*, 長谷川貴志*, 西條雅明*, 永田知子*, 花尻（木倉）瑠理, 合田幸広：**千葉県における違法ドラッグ試験調査について（平成21年度）**

千葉県衛研年報, **58**, 51-54 (2011)

平成21年度に試買した84製品について試験検査したところ，2製品から指定薬物であるbk-MBDBが検出され，32製品から試買後に追加指定されたJWH-018，カ

ンナビシクロヘキサノール，4-メチルメトカチノン，JWH-073及びJWH-250を検出した．また，10製品から指定薬物の構造類似体である4-フルオロメトカチノン，4-メトキシメトカチノン及びJWH-200を検出した．
Keywords：違法ドラッグ，指定薬物，流通実態調査

＊千葉県衛生研究所

Uchiyama, N., Kawamura, M., Kikura-Hanajiri, R., Goda, Y.: **Identification and quantitatitation of two cannabimimetic phenylacetylindoles, JWH-251 and JWH-250, and four cannabimimetic naphthoylindoles JWH-081, JWH-015, JWH-200, and JWH-073 as designer drugs in illegal products**

Forensic Toxicol., **29**, 25-37（2011）

Six cannabimimetic indoles, including 4 new ones identified as designer drugs, have been found as adulterants in herbal or chemical products being sold illegally in Japan by gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), high-resolution MS and nuclear magnetic resonance (NMR) analyses. The first two were identified as phenylacetyl indoles: JWH-251 (2-(2-methylphenyl(1-pentyl-1*H*-indol-3-yl)ethanone, 1) and the demethylmethoxylated analog JWH-250 (2-(2-methoxyphenyl(1-pentyl-1*H*-indol-3-yl)ethanone, 2). Compound 2 was found in the UK and Germany in 2009. The third one was a naphthoylindole, JWH-081 (1-(4-methoxynaphthalenyl(1-pentyl-1*H*-indol-3-yl)methanone, 3). In addition, JWH-073 (1-naphthalenyl(1-butyl-1*H*-indol-3-yl)methanone, 4), which was reported in our previous study, was detected in several products. Two additional compounds were also found: a naphthoyl-2-methylindole, JWH-015 (1-naphthalenyl(2-methyl-1-propyl-1*H*-indol-3-yl)methanone, 5) and an *N*-morpholinylindole, JWH-200 (1-naphthalenyl(1-(2-(4-morpholinyl)ethyl)-1*H*-indol-3-yl)methanone, 6). Compounds 1 - 4 and 6 were reported to be synthetic cannabinoids with selective affinity for cannabinoid CB1 receptors. However, compound 5 was reported to be a selective CB2 receptor agonist causing immunosuppressive effects without psychotropic affects. This is the first report of the detection of synthetic cannabinoids possessing different types of activity in one illegal product. Quantitative analyses of the 6 cannabimimetic compounds in 20 products revealed that there was some variability among the concentrations of the detected compounds in each product. So far as herbal cutting products, the total amounts of these cannabinoids ranged from 26 mg to 100 mg.
Keywords: synthetic cannabinoid, designer drug

Yamaguchi, K.[*1], Okamoto, N.[*1], Tokuoka, K.[*1], Sugiyama, S.[*1], Uchiyama, N., Matsumura, H.[*1], Inaka, K.[*2], Urade, Y.[*3], Inoue, T.[*1]: **Structure of inhibitor complex of old yellow enzyme from *Trypanosoma cruzi***

J. Synchrotron Rad., **18**, 66-69（2011）

Old yellow enzyme (OYE) is an NADPH oxidoreductase which contains flavin mononucleotide as prosthetic group. The X-ray structures of OYE from *Trypanosoma cruzi* (TcOYE) which produces prostaglandin (PG) $F_{2\alpha}$ from PGH_2 have been determined in the presence or absence of menadione. The binding motif of menadione, known as one of the inhibitors for TcOYE, should accelerate the structure-based development of novel anti-chagasic drugs that inhibit $PGF_{2\alpha}$ production specifically.
Keywords: X-ray structure, Inhibitor complex, Prostaglandin synthase

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

Liu, Y.[*1,2], Nugroho, A. E.[*1], Hirasawa, Y.[*1], Nakata, A.[*1], Kaneda, T.[*1], Uchiyama, N., Goda, Y., Shirota, O.[*3], Morita, H.[*1], Aisa, H. A.[*2]: **Vernodalidimers A and B, novel orthoester elemanolide dimers from seeds of *Vernonia anthelmintica***

Tetrahedron Lett., **51**, 6584-6587（2010）

Two novel elemanolide dimers, vernodalidimers A and B, possessing a rare tricyclic ortho estermoiety, were isolated from the seeds of Vernonia anthelmintica. Their structures were elucidated by 1D and 2D NMR data and CD spectra. Vernodalidimers A and B exhibited potent cell growth inhibitory activity against HL-60 cells (IC_{50} 0.72 and 0.47 μM, respectively).
Keywords: orthoester elemanolide dimer, vernodalidimers A and B, *Vernonia anthelmintica*

[*1] 星薬科大学
[*2] Chinese Academy of Sciences
[*3] 徳島文理大学薬学部

Rasmussen, I.[*1], Pedersen, L.[*1], Byg, L.[*1], Suzuki, K., Sumimoto, H.[*2], Vildhardt, F.[*1]: **Effects of F/G-actin ratio and actin turn-over rate on NADPH oxidase activity in microglia**

BMC Immunol., **11**, 44 (2010)

Most in vivo studies that have addressed the role of actin dynamics in NADPH oxidase function in phagocytes have used toxins to modulate the polymerization state of actin and mostly effects on actin has been evaluated by end point measurements of filamentous actin, which says little about actin dynamics, and without consideration for the subcellular distribution of the perturbed actin cytoskeleton. Here, we in addition to toxins use conditional expression of the major actin regulatory protein LIM kinase-1 (LIMK1), and shRNA knock-down of cofilin to modulate the cellular F/G-actin ratio in the Ra2 microglia cell line, and we use Fluorescence Recovery after Photobleaching (FRAP) in β-actin-YFP-transduced cells to obtain a dynamic measure of actin recovery rates (actin turn-over rates) in different F/G-actin states of the actin cytoskeleton. Our data demonstrate that stimulated NADPH oxidase function was severely impaired only at extreme actin recovery rates and F/G-actin ratios, and surprisingly, that any moderate changes of these parameters of the actin cytoskeleton invariably resulted in an increased NADPH oxidase activity. Actin polymerization and depolymerization both increase the FMLP and PMA-stimulated NADPH oxidase activity of microglia, which is directly correlated with neither actin recovery rate nor F/G- actin ratio. Our results indicate that NADPH oxidase functions in an enhanced state of activity in stimulated phagocytes despite widely different states of the actin cytoskeleton.

Keywords: superoxide, actin cytoskeleton, cofilin

[*1] Copenhagen University, Denmark
[*2] 九州大学大学院医学研究院

Nishimura, K.[*1], Sano, M.[*1], Ohtaka, M.[*1], Furuta, B., Umemura, Y.[*1], Nakajima, Y.[*1], Ikehara, Y.[*1], Kobayashi, T.[*2], Segawa, H.[*1], Takayasu, S.[*1], Sato, H.[*2], Motomura, K.[*1], Uchida, E., Kanayasu-Toyoda, T., Asashima, M.[*1], Nakauchi, H.[*2], Yamaguchi, T., Nakanishi, M.[*1]: **Development of defective and persistent Sendai virus vector: a unique gene delivery/expression system ideal for cell reprogramming**

J. Biol. Chem., **286**, 4760-4771 (2011)

The ectopic expression of transcription factors can reprogram differentiated tissue cells into induced pluripotent stem cells. However, this is a slow and inefficient process, depending on the simultaneous delivery of multiple genes encoding essential reprogramming factors and on their sustained expression in target cells. Moreover, once cell reprogramming is accomplished, these exogenous reprogramming factors should be replaced with their endogenous counterparts for establishing autoregulated pluripotency. Complete and designed removal of the exogenous genes from the reprogrammed cells would be an ideal option for satisfying this latter requisite as well as for minimizing the risk of malignant cell transformation. However, no single gene delivery/expression system has ever been equipped with these contradictory characteristics. Here we report the development of a novel replication-defective and persistent Sendai virus (SeVdp) vector based on a noncytopathic variant virus, which fulfills all of these requirements for cell reprogramming. The SeVdp vector could accommodate up to four exogenous genes, deliver them efficiently into various mammalian cells (including primary tissue cells and human hematopoietic stem cells) and express them stably in the cytoplasm at a prefixed balance. Furthermore, interfering with viral transcription/replication using siRNA could erase the genomic RNA of SeVdp vector from the target cells quickly and thoroughly. A SeVdp vector installed with Oct4/Sox2/Klf4/c-Myc could reprogram mouse primary fibroblasts quite efficiently; 1% of the cells were reprogrammed to Nanog-positive induced pluripotent stem cells without chromosomal gene integration. Thus, this SeVdp vector has potential as a tool for advanced cell reprogramming and for stem cell research.

Keywords: Sendai virus vector, gene delivery, reprogramming

[*1] (独) 産業技術総合研究所
[*2] 東京大学医科学研究所

小木美恵子*，石丸幸大*，西脇基晃*，宮脇英明*，内田恵理子，得永嘉昭*：**遺伝子導入用インパルス応力波素子開発のための実験的検討**

信学技報 IEICE Technical Report，**US2011-1**，31-34 (2011)

細胞への遺伝子導入のための創発的インパルス応力波

（EISW）を発生させるための素子構造やその材料，実験システムについて検討した．素子構造は黒色天然ゴムと透明高分子膜を接着剤で結合させた Confined 型応力波素子を提案する．その上で，EISW を測定するための計測部を含む実験試料を使って測定を行い，Confined 型の優位性を黒色天然ゴムのみで構成される Direct 型と比較して明らかにした．

Keywords：gene transfer，stress wave

＊金沢工業大学

佐藤陽治：**再生医療・細胞治療の規制等に関する欧米の動向ー臨床応用に関する規制当局の支援の比較ー**

ヒューマンサイエンス，**22**，28-32（2011）

ヒトの臓器や組織の確保が難しいわが国の状況下において，重篤で生命を脅かす疾患や身体の機能を著しく損なう病態などのうち，治療法に乏しいものに対しての再生医療や細胞治療の実用化を望む声が高まっている．これらの医療に用いることを目的として加工（培養・活性化・足場との複合化等）を施された細胞や組織，あるいは加工された細胞・組織を含む製品は「細胞・組織加工製品」（細胞・組織加工医薬品ないし細胞・組織加工医療機器）と呼ばれ，その開発では世界的にも熾烈な競争が展開されている．ただし細胞・組織加工製品は，細胞という動的で複雑な成分を含むと同時に，製品の態様や特性，臨床上の適用法は多種多様であり，また，その臨床応用に関して限られた経験と知識しか存在しないため，科学的根拠に基づいた品質や安全性等の確保のあり方や開発の合理的な進め方が課題となっている．比較的進んでいると言われる欧米においても，当局は細胞・組織加工製品の実用化を促進するための試行を繰り返しながら規制の枠組みの整備を進めている．本総説では，再生医療・細胞治療の規制等に関する欧米の動向を，臨床応用に関する規制当局の支援を比較しながら解説した．

Keywords：細胞・組織加工製品，再生医療，規制動向

佐藤陽治：**再生医療・細胞治療の規制に関する国際動向**

PHARMSTAGE，**10**，1-2（2011）

治療法に乏しく，重篤・致死的ないし QOL を著しく損なう疾病・損傷に対する活路として，再生医療や細胞治療には非常に大きな期待が集まっている．これらの先進的な医療に用いることを目的として加工（培養・活性化・足場との複合化等）を施された細胞や組織，あるいは加工された細胞・組織を含む製品は「細胞・組織加工製品」（細胞・組織加工医薬品ないし細胞・組織加工医療機器）と呼ばれ，その開発は世界的にも熾烈な競争が展開している．ただし細胞・組織加工製品は，細胞という動的で複雑な成分を含むと同時に，その臨床応用に関して限られた経験と知識しか存在しないため，明確な科学的根拠に基づいた品質や安全性等の確保が課題となっている．本稿では，比較的進んでいると言われる欧米にける，実用化を促進するために試行を繰り返しながら進められている当局の規制の枠組みの整備について解説した．

Keywords：細胞・組織加工製品，再生医療，規制動向

Jin, M. H.[*1], Yokoyama, U.[*1], Sato, Y., Shioda, A.[*1], Jiao, Q.[*2], Ishikawa, Y.[*1] and Minamisawa, S.[*1,2]: **DNA microarray profiling identified a new role of growth hormone in vascular remodeling of rat ductus arteriosus**

J. Physiol. Sci., **61**, 167-179（2011）

The ductus arteriosus（DA）, a fetal arterial connection between the pulmonary artery and the aorta, has a character distinct from the adjacent arteries. We compared the transcriptional profiles of the DA and the aorta of Wistar rat fetuses on embryonic day 19（preterm）and day 21（near-term）using DNA microarray analyses. We found that 39 genes were expressed 2.5-fold greater in the DA than in the aorta. Growth hormone（GH）receptor（GHR）exhibited the most significant difference in expression. Then, we found that GH significantly promoted migration of DA smooth muscle cells（SMCs）, thus enhancing the intimal cushion formation of the DA explants. GH also regulated the expression of cytoskeletal genes in DA SMCs, which may retain a synthetic phenotype in the smooth muscle-specific cytoskeletal genes. Thus, the present study revealed that GH-GHR signal played a role in the vascular remodeling of the DA.

Keywords：ductus arteriosus, growth hormone, DNA microarray

[*1] 横浜市立大学大学院医学研究科
[*2] 早稲田大学理工学術院

佐藤陽治，鈴木和博，早川堯夫＊：**EU における細胞・組織加工製品の規制動向**

医薬品・医療機器レギュラトリーサイエンス，**42**，142-148（2011）

バイオテクノロジーや幹細胞学等の進展に伴い，再生医療・細胞治療などの先端医療で使用することを目的として，培養・活性化等の加工が施された生細胞を含む医

薬品・医療機器（細胞・組織加工製品）が国内外で数多く開発されつつあり，今まで治療が困難であった疾患や重度の損傷への高い効果が期待されている．これらの開発の勢いに呼応し，細胞・組織加工製品の品質および安全性を確保するための行政施策・規制をいち早く整備することは，細胞・組織加工製品の実用化を促進して患者のもとにいち早く届けるという意味の上からも，製品の国際競争力確保の意味の上からも大きな課題である．また，製品の効率的な国際流通を視野に入れた場合，世界各国・各地域における承認審査での有効性・安全性・品質評価に関する考え方についての理解および国際的協調が不可欠である．欧州連合（EU）では，細胞・組織加工製品は体細胞治療薬（somatic cellular therapy products）または組織工学製品（tissue engineered products）の範疇に分類されている．従来，体細胞治療薬は遺伝子治療薬とともに先端医療医薬品（ATMP，advanced therapy medicinal products）という医薬品の一類型に分類されていたが，2008年12月より組織工学製品もATMPとして規制を受けることになった．また，同時にATMPの審査に特化した先端医療委員会（CAT）が創設されるなど，積極的な開発支援策が取られている．本稿ではEUにおける，これらの新しい取り組みについて概説した．

Keywords：細胞・組織加工製品，ATMP，規制動向

＊近畿大学薬学総合研究所

西田基宏＊，斎木翔太＊，北島直幸＊，仲矢道雄＊，佐藤陽治，黒瀬　等＊：**TRPCチャネルのリン酸化による心血管機能制御**

YAKUGAKU ZASSHI, **130**, 1427-1433（2010）

Calcium ions（Ca^{2+}）play an essential role in homeostasis and the activity of cardiovascular cells. Ca^{2+} influx across the plasma membrane induced by neurohumoral factors or mechanical stress elicits physiologically relevant timing and spatial patterns of Ca^{2+} signaling, which leads to the activation of various cardiovascular functions, such as muscle contraction, gene expression, and hypertrophic growth of myocytes. A canonical transient receptor potential protein subfamily member, TRPC6, which is activated by diacylglycerol and mechanical stretch, works as an upstream regulator of the Ca^{2+} signaling pathway required for pathological hypertrophy. We have recently found that the inhibition of cGMP-selective phosphodiesterase 5（PDE5）suppresses agonist- and mechanical stretch-induced hypertrophy through inhibition of Ca^{2+} influx in rat cardiomyocytes. The inhibition of PDE5 suppressed the increase in frequency of Ca^{2+} spikes induced by receptor stimulation or mechanical stretch. Activation of protein kinase G by PDE5 inhibition phosphorylated TRPC6 proteins at Thr69 and prevented TRPC6-mediated Ca^{2+} influx. Substitution of Ala for Thr69 in TRPC6 abolished the antihypertrophic effects of PDE5 inhibition. These results suggest that phosphorylation and functional suppression of TRPC6 underlies the prevention of cardiac hypertrophy by PDE5 inhibition. As TRPC6 proteins are also expressed in vascular smooth muscle cells and reportedly participate in vascular remodeling, TRPC6 blockade may be an effective therapeutic strategy for preventing pathologic cardiovascular remodeling.

Keywords：TRPC, phosphodiesterase, cardiac hypertrophy

＊九州大学大学院薬学研究科

Nishida, M.[*1], Suda, R.[*1], Nagamatsu, Y.[*1], Tanabe, S., Onohara, N.[*1], Nakaya, M.[*1], Kanaho, Y.[*2], Shibata, T.[*3], Uchida, K.[*3], Sumimoto, H.[*4], Sato, Y. and Kurose, H.[*1]：**Pertussis toxin upregulates angiotensin type 1 receptors through Toll-like receptor 4-mediated Rac activation**

J. Biol. Chem., **285**, 15268-15277（2010）

Pertussis toxin（PTX）is recognized as a specific tool that uncouples receptors from Gi and Go through ADP-ribosylation. During the study analyzing the effects of PTX on Ang II type 1 receptor（AT1R）function in cardiac fibroblasts, we found that PTX increases the number of AT1Rs and enhances AT1R-mediated response. Microarray analysis revealed that PTX increases the induction of interleukin（IL）-1β among cytokines. Inhibition of IL-1β suppressed the enhancement of AT1R-mediated response by PTX. PTX increased the expression of IL-1β and AT1R through NF-κB, and a small GTP-binding protein, Rac, mediated PTX-induced NF-κB activation through NADPH oxidase-dependent production of reactive oxygen species. PTX induced biphasic increases in Rac activity, and the Rac activation in a late but not an early phase was suppressed by IL-1β siRNA, suggesting that IL-1β-induced Rac activation contributes to the amplification of Rac-dependent signaling induced by PTX. Furthermore, inhibition of TLR4（Toll-like receptor 4）abolished

PTX-induced Rac activation and enhancement of AT1R function. However, ADP-ribosylation of Gi/Go by PTX was not affected by inhibition of TLR4. Thus, PTX binds to two receptors; one is TLR4, which activates Rac, and another is the binding site that is required for ADP-ribosylation of Gi/Go.

Keywords: pertussis toxin, angiotensin receptor, Toll-like receptor

---

*1 九州大学大学院薬学研究科
*2 筑波大学大学院人間総合科学研究科
*3 名古屋大学大学院生命農学研究科
*4 九州大学大学院医学研究科

鈴木孝昌：**日本の体外診断用医薬品の規制をめぐる動向～DNA チップを用いた遺伝子型判定装置に関する評価指標の策定**

PHARMSTAGE, **7**, 1-2（2010）

個の医療の実現に向け，遺伝子診断の重要性が高まっている。一方で，DNA チップ（マイクロアレイ）等の革新的な分析ツールが開発され，診断ツールとしての応用が可能となりつつある．個の医療を推進する上でも，こうした革新的な診断ツールを積極的に活用していくことは重要であり，その有効性を担保するための評価指標の作成が早急に望まれていた．こうした流れを受け，平成18年度に次世代医療機器評価指標策定事業の一環として，厚生労働省を母体として「テーラーメイド医療用診断機器（DNA チップ）」の審査に関するワーキンググループが，立ち上がり，経済産業省を母体とする開発側の WG と相互に連携をとりながら開発と審査に関するガイドラインを同時に作成した．DNA チップは「ジェノタイピング」用のツールと，「発現プロファイリング」用のツールに大別でき，後者はより課題が多く当面指標策定が難しいと考えられることから，より判定結果が明瞭であり，市場導入の可能性も高いジェノタイピング用に的を絞ってガイドラインを作成することとなった．その内容に関して概説する．

Keywords: 体外診断薬，DNA チップ，評価指標

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

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

骨組織適合性の高い Ti-Zr-Nb 合金にアルカリ処理後カルシウム導入のための表面処理を施し，そのアパタイト形成能を評価し，加えて，カルシウム導入した Ti-Zr-Nb 合金の細胞毒性試験及び骨芽細胞適合性試験を行って生物学的安全性を評価した．その結果，アルカリ処理後 $Ca(OH)_2$ 処理は，Ti や Ti-Zr-Nb 合金等に高いアパタイト形成能を付与することができ，さらに，本研究で骨芽細胞の分化を促進させることが分った．Ti-Zr-Nb 合金は力学的性質にも特長があり，有効性及び安全性の高い金属材料として，埋植医療機への応用が期待できる．

Keywords: チタン合金，アパタイト形成能，骨芽細胞適合性

Sakagami, H.*1, Kawano, M.*2, Thet, M. M.*2, Hashimoto, K.*1, Satoh, K.*3, Kanamoto, T.*4, Terakubo, S.*1, Nakashima, H.*1, Haishima, Y., Maeda, Y.*5 and Sakurai, K.*5: **Anti-HIV and immunomodulation activities of cacao mass lignin-carbohydrate complex**

In vivo, **25**, 229-236（2011）

アルカリ条件下でオートクレーブ処理することにより，溶解性を高めたカカオマス由来滅菌リグニン配糖体（LCC）の生物活性について検討した結果，カカオマス LCC は LPS と異なり，高い抗 HIV 活性と NO radical-scavenging 活性を示すが，iNOS 及びサイトカイン産生を誘導しないことが明らかになった．これらの結果から，カカオマス LCC と LPS の作用点は異なることが推測された．

Keywords: Anti-HIV activity, Cacao lignin-carbohydrate complex, LPS

---

*1 Division of Pharmacology, Meikai University School of Dentistry
*2 Meikai Pharmaco-Medical Laboratory, Meikai University School of Dentistry
*3 Depertment of Anatomy, School of Medicine, Showa University
*4 Department of Microbiology, St. Marianna University School of Medicine
*5 Lotte Co., Ltd.

澤田留美，山田貴史，土屋利江*，松岡厚子：**ヒト間葉系幹細胞の網羅的遺伝子発現解析　ー無血清培地を用いた *in vitro* 培養期間中の遺伝子発現の変化についてー**

薬学雑誌，**130**（10），1387-1393（2010）

We examined the effects of serum-free medium on the gene expression changes in human mesenchymal stem cells (hMSCs) during the in vitro culture using a DNA microarray analysis. In this study, we cultured hMSCs with two kinds of medium; 1) MSCGM (contain 10% fetal bovine serum) or 2) STK2 (serum-free medium

developed for mesechymal stem cells multiplication), and compared hMSCs proliferation, cell morphology, and gene expression changes until 50 days culture. Expression analysis was performed with Affymetrix GeneChip Human Genome U133 Plus 2.0 Array. hMSC proliferation was significantly higher in STK2 medium than in MSCGM medium. The cell morphology of hMSC cultured with STK2 was not significantly changed in 50 days culture. The gene expression changes in hMSCs during the in vitro culture were significantly higher in STK2 than in MSCGM. After 50 days culture, 1991 genes were significantly changed the expression levels compared with 3 days in STK2 but not MSCGM. The expressions of genes related to cell cycle, cancer, proliferation, and cell growth were significantly changed by STK2 for 50 days culture. It was also changed by STK2 that the expressions of genes related to the signaling pathways contain various growth factors, such as IGF-1, FGF, TGF-β, EGF, proliferation, and cell cycle. These results suggest that STK2 may be useful to obtain an enough number of hMSC cells for tissue engineered medical devices in short-term, however, it should be recognized that STK2 would alter the expressions of genes related to a variety of signaling pathways in hMSC if the culture period would be extended to obtain a large number of cells.

Keywords: human mesenchymal stem cells, gene expression, serum-free medium, proliferation, *in vitro* culture

* 大阪大学医学部附属病院未来医療センター

Nakaoka, R., Yamakoshi, Y.*, Isama, K., and Tsuchiya, T.: **Effects of surface chemistry prepared by self-assembled monolayers on osteoblast behavior**

J. Biomed. Mater. Res., **94A**, 524-532 (2010)

A surface of biomaterials is known to affect the behavior of cells after their adhesion on the surface, indicating that surface characteristics of biomaterials play an important role in cell adhesion, proliferation and differentiation. To assess the effects of functional groups on biomaterial surface, normal human osteoblasts (NHOsts) were cultured on surfaces coated with self-assembled monolayers (SAMs) containing various functional groups, and the adhesion, proliferation, differentiation, and gap junctional intercellular communication (GJIC) of the NHOsts were investigated. In the case of SAM with terminal methyl groups (hydrophobic surface), NHOst adhesion and proliferation was less prevalent. In contrast, NHOsts were adhered well on SAMs with hydroxyl, carboxyl, amino, phosphate and sulfate group, which are relatively hydrophilic, their proliferation and differentiation level were dependent on the type of functional groups. Especially, when they were cultured on either SAMs with phosphate or sulfate group, both their alkaline phosphate activity and the calcium deposition by them were enhanced more than those cultured on a collagen-coated dish. More interestingly, GJIC of NHOsts, which has been reported to play a role in cell differentiation as well as homeostasis of cells, were not significantly different among the SAM surfaces tested. These suggest that a specific functional group on a material surface can regulate NHOst adhesion, proliferation and differentiation via cell-functional group interaction without influencing their homeostasis.

Keywords: surface chemistry, cell differentiation, gap junctional intercellular communication

* Department of Radiology and Department of Chemistry, University of Pennsylvania

追田秀行，石川　格，脇谷滋之*1，天正恵治*2，佐藤道夫，松岡厚子：**人工関節用超高分子量ポリエチレンのフラクトグラフィに関する基礎的研究**

臨床バイオメカニクス，**31**，187-191（2010）

人工関節用超高分子量ポリエチレンへのフラクトグラフィの応用により，生体中における材料の長期変化に関する知見が得られる可能性があるが，現在までに充分な知見が得られていない．そこで，２種の試料（未劣化と劣化）を３つの条件（液体窒素中，引張破断，引張疲労破断）で破断させ，電子顕微鏡で観察した．未劣化試料と劣化試料では，破断面が大きく異なり，両者の識別は容易であったが，引張破断と引張疲労破断という破断条件の違いでは，差が観察されなかった．

Keywords: fractography, UHMWPE, fracture

*1 大阪市立大学
*2 信州大学

Sakoda, H., Ishikawa, I., Jung, D. Y., Wakitani, S.*1, Tensho, K.*2, Sato, M. and Tsuchiya, T.: **Direct evaluation of fatigue property of ultra-high molecular weight polyethylene components of retrieved knee implants using small specimens**

Strength, Fracture and Complexity, **6**, 103-114（2010）

人工関節用超高分子量ポリエチレンには，摩耗特性向上のために放射線照射による架橋処理がしばしば行われるが，この処理により疲労特性が低下することが指摘され，不具合発生が懸念されている．しかし，不具合を生じた人工関節の疲労特性評価法がなかったため，疲労特性の低下と不具合発生との関係は不明であった．抜去品から試験試料が作製可能な小さな試験片を用いた疲労特性評価法を開発し，不具合抜去品の疲労特性を評価したところ，酸化の進行，疲労特性，不具合の発生の間に関係があることがわかった．

Keywords : Joint implant, oxidation, fatigue

---

*1 大阪市立大学
*2 信州大学

迫田秀行，石川　格，松岡厚子，西井　孝*，菅野伸彦*：**破損したバイポーラ型人工骨頭の不具合要因分析**

日本人工関節学会誌，**40**，550-551（2010）

バイポーラ型人工骨頭の破損例について，インプラントと臨床情報の分析により不具合要因分析を行った．超高分子量ポリエチレンの酸化劣化，骨頭保持のために設けられた切り欠き，摩耗，インピンジによる引張力などが破損の原因として考えられた．バイポーラ型人工骨頭では，デザインコンセプト上骨頭保持のための機構が必要であること，インピンジが回避できないことから，摩耗の抑制だけでなくこれらの部分の耐久性が重要である．

Keywords : 人工関節，破損，要因分析

---

* 大阪大学

石川　格，迫田秀行，菅野伸彦*1，松岡厚子，土屋利江*2：**光学式３Ｄデジタイザによる抜去人工股関節UHMWPEライナーの摩耗測定**

臨床バイオメカニクス，**31**，299-304（2010）

Wear measurement of retrieved acetabular liners is necessary to elucidate the mechanisms of arthroplasty failure. This study evaluated the wear volume of retrieved acetabular liners using an optical 3D digitizer. In order to calculate the wear volume from measured geometry, information on the unworn geometry of the liner is needed, but the lack of this information becomes a problem. In this study, we introduced a novel calculation method to identify the unworn geometry of retrieved liners. This method assumes that the geometry of the unworn sliding surface was a sphere and the center of the sphere was on the central axis of the liner. As a result of the verification test using test data, this method could identify the unworn geometry correctly. We applied this method to the geometry data of the retrieved liner measured by optical 3D digitizer, so that the unworn geometry of the sliding surface was estimated. It seemed that the unworn part of the sliding surface which is estimated by this method was consistent with visual examination of the liner. Using this estimated unworn geometry, the wear volume of the retrieved acetabular liner could be calculated. It is thought that the unworn surface estimation method proposed in this study can be applied to similar geometric data measured by other devices, such as a contact-type coordinate measurement machine or micro-CT.

Keywords : retrieved acetabular liner, wear measurement, optical 3D digitizer

---

*1 大阪大学大学院医学系研究科
*2 大阪大学医学部附属病院未来医療センター

Ohkawara, S.*, Tanaka-Kagawa, T., Furukawa, Y., Nishimura, T. and Jinno, H. : **Activation of the Human Transient Receptor Potential Vanilloid Subtype 1 by Essential Oils**

Biol. Pharm. Bull., **33**, 1434-1437（2010）

Transient receptor potential vanilloid subtype 1（TRPV1）is a non-selective cation channel activated by capsaicin. TRPV1 is expressed not only on human sensory neurons but also on human epidermal and hair follicle keratinocytes. Therefore, TRPV1 could have the potential to be a therapeutic target for skin disorders. To search for novel TRPV1 agonists, we screened 31 essential oils by using human TRPV1-expressing HEK 293 cells. TRPV1 was activated by 4 essential oils : rose, thyme geraniol, palmarosa, and tolu balsam. The dose-response curves for TRPV1 activation by the essential oils revealed a rank order potency［the half-maximal effective concentration（EC_{50}）］of rose>palmarosa>thyme geraniol>tolu balsam, and rank order efficiency（% activity in response to 1 μM capsaicin）of tolu balsam>rose>palmarosa>thyme geraniol. Moreover, the dose-response curves for TRPV1 activation by citronellol（main constituent of rose oil）and geraniol（main constituent of thyme geraniol and palmarosa oils）were consistent with the potency and efficiency of each

essential oil. In contrast, benzyl cinnamate and benzyl benzoate (main constituent of tolu balsam oil) and geranyl acetate (main constituent of thyme geraniol oil) did not show TRPV1 activity. In this first-of-its-kind study, we successfully investigated the role of some essential oils in promoting human TRPV1 activation, and also identified two monoterpenes, citronellol and geraniol, as new human TRPV1 agonists.

Keywords: transient receptor potential vanilloid subtype 1, monoterpene, essential oil

---

* 武蔵野大学薬学部

Ohkawara, S.*, Tanaka-Kagawa, T., Furukawa, Y., Nishimura, T. and Jinno, H.: **Development of a SYBR Green Real-time Polymerase Chain Reaction Assay for Quantitative Detection of Human N-methyl-D-aspartate Receptors Subtype 1 Splice Variants**

J. Health Sci., **56**, 527-533 (2010)

N-methyl-D-aspartate receptors (NMDAR) belong to the ionotropic glutamate receptor subclass and are widely distributed in the vertebrate brain. Molecular cloning has revealed the existence of seven NMDAR subunits: one NMDAR1 (NR1), four different NMDAR 2 (NR2A-D), and two different NMDAR3 (NR3A, B). Alternative splicing of the single NR1 gene generates eight isoforms with distinct functional properties. So far, the transcripts of the NR1 splice variants have been discriminated by Northern blot, in situ hybridization, or competitive polymerase chain reaction (PCR) methods all of which have their intrinsic limitations. In this study, we have developed a method to quantify the mRNAs of the NR1 splice variants by real-time PCR with the double-stranded DNA-binding dye SYBR Green I. The implementation of this assay will allow a better understanding of the regulatory mechanisms of the NR1 splice variants, and hence, their role in neuronal disease pathogenesis.

Keywords: N-methyl-D-aspartate, splice variants, real-time polymerase chain reaction

---

* 武蔵野大学薬学部

Hanioka, N.*[1], Tanabe, N.*[1], Jinno, H., Tanaka-Kagawa, T., Nagaoka, K.*[1], Naito, S.*[2], Koeda, A.*[3] and Narimatsu, S.*[1]: **Functional characterization of human and cynomolgus monkey UDP-glucuronosyltransferase 1A1 enzymes**

Life Sci., **87**, 261-268 (2010)

AIMS: UDP-glucuronosyltransferase 1A1 (UGT1A 1) plays important roles in the glucuronidation of various drugs and endogenous substances. Cynomolgus monkeys are regarded as experimental animals closer to humans in studies on safety evaluation and biotransformation for drug development. In this study, the similarities and differences in the enzymatic properties of UGT1A1 between humans and cynomolgus monkeys were precisely identified. MAIN METHODS: Human and cynomolgus monkey UGT1A1s (humUGT1A1 and monUGT1A1, respectively) were cloned, and the corresponding proteins were heterologously expressed in insect cells. The enzymatic properties of UGT1A1 proteins were characterized by kinetic analysis of 7-hydroxy-4-trifluoromethylcoumarin (7-HFC), estradiol at 3-hydroxy position (E-3OH) and 7-ethyl-10-hydroxy-camptothecin (SN-38) glucuronidation. KEY FINDINGS: There were no significant differences in the levels of kinetic parameters for 7-HFC, E-3OH and SN-38 glucuronidation between humans and cynomolgus monkeys in both enzyme sources of liver microsomes and recombinant UGT1A1s. 7-HFC and E-3OH glucuronidation by human liver microsomes exhibited biphasic and sigmoidal kinetics, respectively, whereas the kinetics by cynomolgus monkey liver microsomes fitted the typical Michaelis-Menten model. SN-38 glucuronidation by human and cynomolgus monkey liver microsomes exhibited autoactivation kinetics. In recombinant UGT1 A1 enzymes expressed in insect cells, the kinetics of 7-HFC, E-3OH and SN-38 glucuronidation fitted the substrate inhibition (7-HFC glucuronidation) or Hill equation (E-3OH and SN-38 glucuronidation), and each glucuronidation showed the same kinetic profile between humans and cynomolgus monkeys. SIGNIFICANCE: These findings suggest that the enzymatic properties of human and cynomolgus monkey UGT1A1 enzymes are very similar.

Keywords: UDP-glucuronosyltransferase 1A1, human and cynomolgus monkey, drug metabolism

---

*[1] 岡山大学大学院薬学研究科
*[2] 大塚製薬工場（株）
*[3] イナリサーチ（株）

Hanioka, N.*, Yamamoto, M.*, Tanaka-Kagawa, T., Jinno, H. and Narimatsu, S.*: **Functional characterization of human cytochrome P450 2E1 allelic variants: in vitro metabolism of benzene and toluene by recombinant enzymes expressed in yeast cells**

Arch. Toxicol., **84**, 363-371 (2010)

Benzene and toluene are common organic solvents currently in worldwide industrial usage, which are metabolized mainly by hepatic cytochrome P450 2E1 (CYP2E1) in humans. Genetic polymorphism of *CYP2E1* in 5′-flanking and coding regions has been found previously in Caucasian and Chinese populations. In this study, the effects of *CYP2E1* alleles causing amino acid substitutions (*CYP2E1**2, *CYP2E1**3 and *CYP2E1**4; wild-type, *CYP2E1.1A*) on benzene hydroxylation and toluene methylhydroxylation were studied using recombinant CYP2E1 enzymes of wild-type (CYP2E1.1) and variants (CYP2E1.2 having Arg76His, CYP2E1.3 having Val389 Ile and CYP2E1.4 having Val179Ile) expressed in yeast cells. The K_m, V_{max} and CL_{int} values of CYP2E1.1 were 10.1 mM, 9.38 pmol/min/pmol CYP and 0.99 nL/min/pmol CYP for benzene hydroxylation, and 3.97 mM, 19.9 pmol /min/pmol CYP and 5.26 nL/min/pmol CYP for toluene methylhydroxylation, respectively. The K_m, V_{max} and CL_{int} values for benzene and toluene metabolism of CYP2E 1.2, CYP2E1.3 and CYP2E1.4 were comparable to those of wild-type CYP2E1. These findings may mean that the polymorphic alleles of *CYP2E1* causing amino acid substitutions are not directly associated with the metabolic activation of benzene and toluene. The information gained in this study should help to identify the variations in the toxicity of environmental pollutants.

Keywords: benzene, toluene, cytochrome P450 2E1 (CYP2E1)

* 岡山大学大学院薬学研究科

Todo, H.*, Kimura, E.*, Yasuno, H.*, Tokudome, Y.*, Hashimoto, F.*, Ikarashi, Y. and Sugibayashi, K.*: **Permeation pathway of macromolecules and nanospheres through skin**

Biol. Pharm. Bull., **33**, 1394-1399 (2010)

The permeation pathway of macromolecules and nanospheres through skin was evaluated using fluorescent isothiocyanate (FITC)-dextran (average MW, 4 kDa) (FD-4) and nanospheres (500 nm in diameter) in hairless rat abdominal skin and porcine ear skin as well as a three-dimensional cultured human skin model (cultured skin model). A low molecular hydrophilic compound, sodium fluorescein (FL) (MW, 376 Da), was used for comparison. FL penetrated the stratum corneum and permeated the viable epidermis of hairless rat skin, whereas less permeation of FL was observed through the cultured skin model, suggesting that the primary permeation pathway for the hydrophilic material may be skin appendages through the rat skin. A macromolecular compound, FD-4, was distributed through the hair follicles of the rat skin. In addition, nanospheres were detected in the hair follicles of porcine skin, although no skin permeation was detected. These findings suggest that appendage routes such as hair follicles can be a penetration pathway of macromolecules and nanospheres through skin.

Keywords: silicate macromolecule, nanosphere, skin permeation pathway

* 城西大学薬学部

田原麻衣子，杉本直樹，久保田領志，西村哲治：**液体クロマトグラフィー/質量分析計による水道水中のハロ酢酸類の定量法の確立**

水道協会雑誌，**79**(4)，18-22（2010）

ハロ酢酸類であるモノクロロ酢酸，ジクロロ酢酸及びトリクロロ酢酸の水道水質基準に係る検査法は，長時間，かつ，発がん性物質による抽出や誘導体化を必要とする．この問題を解消するため，液体クロマトグラフ/質量分析計（LC/MS）を用い，前処理なしで直接定量可能な分析法を構築した．本法により3種ハロ酢酸の検出下限値は0.5mg/L，定量下限値は2 mg/Lとなった．水道水における2 mg/Lの添加試験は回収率103–111%で，3試行の相対標準偏差が5%以内と再現性よく定量できた．また，硝酸態窒素によるイオン化妨害や分解は観察されなかった．構築したLC/MS法は水道水質基準に係る検査法として期待できる．

Keywords: ハロ酢酸類，LC/MS，硝酸態窒素

Tahara, M., Kubota, R., Shimizu, K., Sugimoto, N. and Nishimura, T.: **Risk assessment of fenthion oxide derivatives in aqueous environment**

J. Water. Environ. Technol., **8**(3), 215-221 (2010)

Fenthion (MPP), an organophosphorus pesticide, is widely used as an agricultural and household insecticide. The oxons are known to be the actual toxic forms

of organophosphorus pesticides. Using an *in vitro* cytochrome P450 (CYP) metabolism system, MPP was metabolized to produce five metabolites: MPP sulfoxide, MPP sulfone, MPP oxon, MPP oxon sulfoxide and MPP oxon sulfone. MPP sulfoxide was the main product, while MPP oxon sulfone and the other metabolites were produced in small amounts. On the other hand, MPP was converted to MPP oxon sulfone by chlorination in a water purification system, raising the possibility of human exposure to MPP oxon sulfone through drinking water. MPP oxon sulfone showed the highest acute toxicity among MPP and its metabolites. In addition, MPP oxon sulfone was not metabolized by CYP3A4, the major CYP isomer in humans. It is important that MPP and its oxides are monitored and their health risk assessed to control drinking water safety because MPP was detected in river water.

Keywords: MPP, oxide derivative, risk assessment

Kubota, R., Tahara, M., Shimizu, K., Sugimoto, N. and Nishimura, T.: **Determination of EDTA in water samples by SPE-gaschromatography/mass spectrometry**

J. Water. Environ. Technol., **8**(4), 347-353(2010)

Japan's recommended method of EDTA determination is complex and time-consuming. In this study, a new method to prepare the solution to determine EDTA in water by solid-phase extraction-GC/MS was developed. Recovery yields were excellent with values ranging from 98.1 to 100.5%. Due to this method's ease and simplicity, it is suggested that this approach be adopted as Japan's recommended method for EDTA analysis. The method was applied to assess the concentrations of EDTA in river water from three regions of Japan. Median concentration of EDTA in river water samples was 115μg/L, and the concentrations ranged from 18.8 to 443μg/L. The highest concentration of EDTA (443μg/L) was observed in Tsurumi River. Sewage treatment plant (STP) effluent significantly contributed to high EDTA levels.

Keywords: EDTA, GC/MS, solid-phase extraction

杉本直樹，多田敦子，末松孝子[*1]，有福和紀[*1]，齋藤剛[*2]，井原俊英[*2]，吉田雄一[*3]，田原麻衣子，久保田領志，清水久美子，山崎　壮，河村葉子，西村哲治：**定量 NMR を用いたダッタンソバ乾麺中のクエルセチンの迅速定量**

日本食品化学学会誌，**17**，179-184（2010）

Quantitative NMR (qNMR) method was applied for the quantification of quercetin in tartary buckwheat (*Fagopyrum tataricum* L.) noodle. In the reagent market, quercetin is generally provided as quercetin + X hydrate of which the purity is not determined exactly. Hence, if using the reagent as the reference material for LC quantification, the reliability of analysis data will not be assured. qNMR is based on the fact that the signal intensities of a given NMR resonance are directly proportional to the molar amount of that nucleus in the sample, and is able to determine the contents with trace ability to International System of Units (SI units). The content of quercetin was calculated from the ratio of the signal intensities of a proton at H-2' on quercetin to eighteen protons of the methyl groups on hexamethyldisilane (HMD) used as the internal standard, after the concentration of HMD was corrected using potassium hydrogen phthalate (PHP), which is one of certified reference material (CRM). In the result, the content of quercetin in tartary buckwheat noodle was determined with SI-traceability to 1.58±0.14 mg/g as the anhydrate formula. The quantitative value was verified using general LC method after the purity of reagent was determined exactly. qNMR does not need its reference compound, the calibration curve and separation column like LC method. Our procedure in this study is rapid and simple with overall analysis time of only 10 min, and also the result is traceable to SI units.

Keywords: quantitative NMR, quercetin, tartary buckwheat noodle

[*1] 日本電子（株）
[*2] （独）産業技術総合研究所
[*3] 和光純薬工業（株）

鈴木俊也*，小杉有希*，保坂三継*，矢口久美子*，小縣昭夫*，西村哲治，中江　大*：**多摩川流域の下水処理場における医薬品の存在実態**

東京都健康安全研究センター研究年報，**61**，333-339（2010）

多摩川流域の下水処理場の水試料を対象に，医薬品の存在実態調査を実施した．調査対象の医薬品約100種類のうち，流入下水及び処理下水からそれぞれ38及び35医薬品が検出された．それらの検出濃度は，流入下水及び処理下水でそれぞれ数十 ng/L から十 μg/L 及び数十 ng/L から数 μg/L の範囲であった．解熱鎮痛消炎剤の下

水処理場への負荷量は冬季に著しい増加が認められたが，高脂血症薬や高血圧症治療薬などの負荷量の変動は小さく，服用の実態に応じた結果が得られた．下水処理場から多摩川水系への負荷量は調査期間中ほぼ一定であり，河川水中の濃度は降雨などによる河川水量の影響を強く受けることが示唆された．スリンダク，アマンタジン，エピナスチン，メトプロロール，プロプラノロール，ロサルタン，スルピリド，ハロペリドロール，フルボキサミン及びロラゼパムの除去率は20%未満と他の医薬品に比べ低く，微生物による分解性が低いためと推察された．

Keywords：pharmaceutical, sewage treatment plant, analysis

＊東京都健康安全研究センター

菅野文子*，冨澤朋絵*，西以和貴*，岸　智裕*，河上強志，高橋保雄*，小野寺祐夫*：**身体保護製品の昆虫忌非剤（DEET）および鎮痒剤（Crotamiton）による水環境汚染：千葉県北西地域の河川水と水道水におけるそれらの存在，季節変動および濃度の比較**

環境化学，**20**，121-125（2010）

Amberlite XAD-2 resin extracts of river and drinking water sampled each month during the period from January to December 2008 from Northwest Area of Chiba Prefecture were investigated in order to characterize and determine the organic pollutants. On the basis of GC/FID determinations of the extracts, over 90% of XAD extractable organic substances present in the river water concentrates was found to be reduced after processing in water treatment plant. Personal care products（DEET and Crotamiton）were also identified by GC/MS to be present at low concentrations, ranging from ND to 140 ng/L. The concentrations of these organic pollutants were dependent on the application periods. Although amounts of these organic pollutants in river water samples decreased remarkably after processing in the water treatment plant, it is necessary to conduct continuous bio-assays in order to evaluate the health risk effects of these personal care products and their chlorination byproducts in drinking water.

Keywords：river water, drinking water, personal care product

＊東京理科大学大学院薬学研究科

中島晴信*1，富山健一*2，河上強志，伊佐間和郎：**家庭用品に含有されるトリブチルスズ，トリフェニルスズの分析法ー公定分析法の改定に向けてー**

薬学雑誌，**130**，945-954（2010）

In preparing for the revision of the authorized analytical method for tributyltin（TBT）and triphenyltin（TPT），which are banned from using according to the “Act on the Control of Household Products Containing Harmful Substances”, an examination was conducted on the detection method of these substances using gas chromatography/mass spectrometry（GC/MS），after derivatizing them（ethyl-derivatizing method and hydrogen-derivatizing method）. Ethyl-derivatized compounds had stability, which enabled the detection of TPT with a higher sensitivity. In addition, a preparation suitable for the following analytical objects was established：（1）textile products,（2）water-based products（such as water-based paint），（3）oil-based products（such as wax），and（4）adhesives. Addition-recovery experiments were conducted using the prescribed pretreatment method, when each surrogate substances（TBT-d27, TPT-d15）were added and the data were corrected, good recovery rates（94.5-118.6% in TBT, and 86.6-110.1% in TPT）were obtained. When TBT and TPT in 31 commercially available products were an alyzed based on the developed analytical method, an adhesive showed 13.2 μg/g of TBT content, which exceeded the regulatory criterion（1 μg/g as tin）. Next, when the same products with different manufacturing date were analyzed, TBT（10.2-10.8 μg/g），which exceeded the regulatory criterion, was detected in 4 products among 8 products, and simultaneously, a high concentration（over 1000 μg/g）of dibutyltin（DBT）was detected. It was suggested that TBT as an impurity of DBT remained, and the manufacturer chose the voluntary recall of the product. The new method is considered sufficiently applicable as a revised method for the conventionally authorized method.

Keywords：GC/MS, organotin compounds, household products

*1 大阪府立公衆衛生研究所
*2（独）国立精神・神経医療研究センター

Kawakami, T., Isama, K., Nakashima, H.*, Tsuchiya, T. and Matsuoka, T.：**Analysis of primary aromatic amines originated from azo dyes in commercial**

textile products in Japan

J. Environ. Sci. Health Part A, **45**, 1281-1295 (2010)

The purpose of this study was to clarify the actual condition of 26 types of carcinogenic primary aromatic amines (PAAs) originated from azo dyes in commercial textile products in Japan. In the case of textiles made of various fibers of various colors, the fibers were separated by color and analyzed. A total of 86 textile products (117 samples) were analyzed. Twenty-one kinds of PAAs were detected in the samples and almost all the PAAs were detected at low concentrations. However, the concentrations of benzidine, 3,3'-dimethoxybenzidine, and 2,4-diaminotoluene (56-440 μg g^{-1}) in placemats made of cotton were found to exceed EU regulation limits of 30 μg g^{-1}. Although such placemats do not always come into contact with the user's skin, it is thought that they should be handled more carefully. Finally, 7 products (8 samples) contained PAAs at concentrations that exceeded the regulation limits. Two sample preparation methods (with and without solvent extraction) were performed on the same sample in order to compare the PAAs in samples in which it is difficult to separate the component materials, such as a cotton fabric that contained polyester fibers. In a comparison of the results obtained from the two methods, it was observed that the concentrations and/or kinds of PAAs detected in the samples were different. It was therefore thought that textile products that present this particular challenge should be analyzed by both methods.

Keywords: Aromatic amine, azo dye, textile products

* 大阪府立公衆衛生研究所

Hexig, B.[*1], Isama, K., Haishima, Y., Inoue, Y.[*1], Tsuchiya, T.[*2] and Akaike, T.[*1]: **Self-Organization of the Compositional Gradient Structure in Hyaluronic Acid and Poly(N-isopropylacrylamide)**

J. Biomater. Sci., Polym. Ed., **21**, 1957-1970 (2010)

A compositional gradient structure in hyaluronic acid (HA) and poly (N-isopropylacrylamide) (PIPAAm) blend film was self-organized from a homogeneous aqueous solution in a plasma-treated polystyrene dish (PTPSD), and the formation mechanisms of the gradient structure were studied by casting the same solution on PTPSD and a non-treated polystyrene dish (NTPSD) under ambient and vacuum conditions. The formation of a compositional gradient structure in HA/PIPAAm blend film was confirmed by scanning electron microscopy, energy dispersive X-ray (EDX) mapping analysis and step-scan photoacoustic Fourier transformed infrared spectroscopy (PAS-FT-IR) measurements. The EDX mapping measurements for Na element revealed that the HA component gradually decreases from the dish-side to the air-side of the film cast on PTPSD, while for the film cast on NTPSD no such obvious change was observed on the cross-section. Further studies on the films prepared on PTPSD and NPTPSD under ambient and vacuum conditions demonstrated that the hydrophilic interaction and the solvent evaporation rate were the most significant factors leading to the formation of a compositional gradient structure in the HA/PIPAAm blend system.

Keywords: self-organization, hyaluronic acid, poly (N-isopropylacrylamide)

[*1] 東京工業大学
[*2] 大阪大学医学部附属病院

Kanno, A.*, Nishi, I.*, Kishi, T.*, Kawakami, T., Takahashi, Y.* and Onodera, S.*: **Cholinesterase-inhibiting Potentials of Amberlite XAD-2 Resin Extracts Collected from River and Drinking Waters in Northwest District of Chiba Prefecture, Japan**

J. Health Sci., **56**, 664-674 (2010)

Amberlite XAD-2 resin extracts of river and drinking water sampled in each month during the period from January to December 2008 from the Northwest district of Chiba Prefecture were investigated to characterize and determine their cholinesterase (ChE)-inhibiting potentials and pesticide levels. The XAD-2 extracts from river water collected during the mid-spring to mid-summer periods exhibited strong inhibition effect to horse serum ChE, reflecting the application of organophosphorus and carbamate pesticides to paddy fields. Gas chromatographicmass spectrometric (GC/MS) determinations of the XAD-2 extracts of the river water collected during spring to summer periods also showed to be comparatively high levels of agricultural chemicals, such as herbicides, insecticides and fungicides, as compared with those detected in the drinking water. Although a considerable reduction in the ChE-inhibiting potentials and in the GC/MS detectable compound

levels was observed for the river water samples, it is particularly interest that ChE-inhibiting potentials still remained in the drinking water.

Keywords: river water, drinking water, ChE-inhibiting activity

* 東京理科大学大学院薬学研究科

Kanno, A.*, Nishi, I.*, Kishi, T.*, Kawakami, T., Takahashi, Y.* and Onodera, S.*: **Mutagenic potentials of Amberlite XAD-2-resin extracts obtained from river and drinking waters in the Northwest district of Chiba, Japan**

J. Toxicol. Sci., **35**, 817-826 (2010)

Amberlite XAD-2 resin extracts of river and drinking water sampled from the Northwest district of Chiba Prefecture in each month during the period from January to December 2008 were investigated to characterize and determine their mutagenic potentials and polycyclic aromatic hydrocarbon (PAH) levels. The extracts from the river water were shown to be mutagenic in Salmonella typhimurium TA98 (a flameshift mutagen) without S9 mix, with higher mutagenic responses in summer and early fall seasons. While the drinking water extracts exhibited weak mutagenicity in both the TA98 and TA100 strains (a base-pair substitution mutagen) without S9 mix, with high mutagenic responses in fall and early winter seasons. GC/MS determinations of the water concentrates showed some seasonal scatter in PAH levels in river water. In contrast, comparatively high concentrations of PAHs were observed for drinking water samples collected during warmer seasons. Statistical studies revealed that there is a lower correlation between the levels of flameshift mutagenicity and the concentrations of PAH in the river water concentrations, but a higher correlation between them in the drinking water samples.

Keywords: river water, drinking water, ChE-inhibiting activity

* 東京理科大学大学院薬学研究科

松田りえ子，渡邉敬浩，根本　了，前田　守*，下山晃*，青島陽子*：**食品中の残留農薬分析結果の不確かさの推定　ー試験室内妥当性評価結果を用いてー**

食品衛生研究，**60**(6)，25-31（2010）

農薬分析法の試験室内妥当性評価結果から，その機関における分析値の不確かさ推定を試みた．厚生労働省の通知試験法の中で有機リン系農薬のグループ試験法であるEPN等試験法の妥当性評価した結果推定された室内精度から，不確かさを推定した．

Keywords: 不確かさ，試験室内妥当性評価，農薬分析

* （財）日本冷凍食品検査協会

Nakamura, M.*, Noda, S.*, Kosugi, M.*, Ishiduka, N.*, Mizukoshi, K.*, Taniguchi, M.*, Nemoto, S.: **Determination of Dithiocarbamates and Milneb Residues in Foods by Gas Chromatography-Mass Spectrometry**

Food Hyg. Saf. Sci., **51**(5), 213-219 (2010)

A highly sensitive gas chromatographic-mass spectrometric (GC-MS) method was developed for dithiocarbamates (DTCs) and milneb in foods. DTCs and milneb were extracted from foods with cysteine-EDTA solution as sodium salts, and methylated with methyl iodide. Methyl derivatives of DTCs and milneb were cleaned up on a neutral alumina mini column and determined by GC-MS. The mean recoveries of DTCs and milneb were in the range of 72-120%, except for methiram. The quantification limits were 0.01 mg/kg (as CS2) in foods except tea (0.1 mg/kg as CS2). The developed method was applied to 10 compounds (4 dimethyldithiocarbamates, 3 ethylene-bisdithiocarbamates, polycarbamates, propineb and milneb).

Keywords: dithiocarbamate, methylation, GC-MS

* Japan Food Research Laboratories

上野英二*，大野春香*，棚橋高志*，大島晴美*，三上栄一*，根本　了，松田りえ子：**LC-MSによる畜水産物およびはちみつ中アセフェート，メタミドホスおよびオメトエートの分析**

食品衛生学雑誌，**51**(3)，122-127（2010）

畜水産物およびはちみつ中のアセフェート，メタミドホスおよびオメトエートを定量するための同時分析法を検討した．牛筋肉，豚ギョーザ，はちみつなど12種類の試料（5～10g）から，無水硫酸ナトリウムで脱水しながら酢酸エチルで抽出し，GPCおよびPSAカラムクロマトグラフィーにより脱脂・精製したのち，カラムスイッチング付きESI-SIMモードLC-MSで測定した．回収率（2併行×5日）は，はちみつを除いて71.4～98.4%（併行精度≦12.5%，室内精度≦14.1%）と良好であった．なお，はちみつに高純度のサロゲート物質を用いる内標準法を適用したところ，回収率が97.6～98.6%と大

きく改善された．

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

---

* 愛知県衛生研究所

高橋邦彦*，松本隆二*，根本　了，松田りえ子：**LC-MS/MS による農産物中のヒドラメチルノンの分析**

食品衛生学雑誌，**52**(1)，47-50（2011）

高速液体クロマトグラフィータンデム型質量分析計（LC-MS/MS）を用いた農産物中のヒドラメチルノンの分析法を検討した．試料にリン酸を添加してホモジナイズ後，アセトンで油出した．この抽出液に飽和塩化ナトリウム溶液を加えてヘキサンで転溶した．茶ではヘキサン転溶操作の前に凝固液による処理を行った．精製はシリカゲルミニカラム（500mg）を用いた．測定条件として分析カラムに C18，移動相は10mM 酢酸アンモニウム含有−メタノール−水（８：２），イオン化モードは ESI のポジティブモードを用いた．検量線は0.002〜0.2μg/mL の範囲で直線性を示した．パイナップルなど10種の農産物からの回収率（n＝５）は約82〜110%であり，相対標準偏差は２〜12%であった．

Keywords：hydramethylnon，agricultural product，LC-MS/MS

---

* 埼玉県衛生研究所

川上宏之*1，天倉吉章，堤　智昭，佐々木久美子，池津鮎美*2，稲崎端恵*2，久保田恵美*2，豊田正武*2：**マグロ肉における脂質含有量とダイオキシン類，総水銀およびメチル水銀レベルの関係について**

食品衛生学会誌，**51**(5)，258-263（2010）

天然/畜養クロマグロおよび畜養ミナミマグロの赤身，中トロおよび大トロのダイオキシン類および総水銀を分析し，部位，畜養/天然および種差について検討した．検討の結果，ダイオキシン類濃度は，脂質含有量との間に正の相関が見られ，部位別濃度は赤身＜中トロ＜大トロであった．クロマグロは，畜養と天然産で差がなく，畜養ミナミマグロに対して約２〜10倍ほど高い値を示した．総水銀濃度は，脂質含有量との間に負の相関を示し，部位別濃度は赤身＞中トロ＞大トロであった．畜養クロマグロの総水銀濃度は，天然産と同レベルの蓄積であったが，畜養ミナミマグロの約２〜３倍高い値を示した．

Keywords：tuna, dioxins, mercury

---

*1（財）日本冷凍食品検査協会
*2 実践女子大学生活科学部

渡邉敬浩，堀口容正*1，後藤浩文*2，槇島慎一*3，高附　巧，松田りえ子：**即席めん類の酸価および過酸化物価測定法の改良と性能評価**

食品衛生研究，**60**(12)，25-33（2010）

即席めん類の成分規格により規定された酸価ならびに過酸化物価測定法について，有害試薬を使用しない方法への改良を検討した．検討に当たっては，これらの方法が Codex 委員会の定める定義分析法（Type I）であることを考慮し，現行の告示法からの変更を最小とすることを方針とし，国内外で運用されている方法との整合を図った．

検討した分析法の性能評価の結果，滴定に必要な量の油脂を安定して抽出することが可能であり，告示法との間に性能の違いは認められなかった．また，酸価ならびに過酸化物価ともに，検討法と告示法との間で得られる測定値とそのばらつきに明らかな違いは認められなかった．さらに，酸価あるいは過酸化物価が異なる種々の即席めん類および一部の菓子類，総菜類についても適用可能であることが示された．

Keywords：acid value，peroxide value，noodle

---

*1 サンヨー食品（株）
*2（財）日本食品分析センター
*3 明治製菓（株）

渡邉敬浩，松田りえ子：**収去検査に伴うサンプリングの現状調査**

食品衛生研究，**61**(2)，19-30（2011）

国内の各自治体等で実施されている検査として，食品衛生法に準じた収去検査が挙げられる．しかし，この検査の実施に係るサンプリング（サンプリング計画ならびに手順）および，判定とそれに伴い講じられる措置（分析値の運用）については，参照可能な文書等が示されておらず，各検査実施者に一任されているのが現状である．本研究では，収去検査等を前提としたサンプリング計画や手順およびそれを通じて得られる分析値の運用指標について検討するため，この検討に不可欠な現状把握を目的に，自治体担当者を対象としたアンケート調査を実施した．92の自治体から得られた回答を集計した結果，サンプリングに関する理解の違いや運用指標（判定の基準とそれに伴いされる措置の内容）の不明確さと共に，収去検査の性質上の制限が明らかとなった．

Keywords：food sanitation law，inspection，sampling，questionnaire Survey

高附　巧，渡邉敬浩，松田りえ子：**ワイン，魚介類，精米，乳，粉乳およびヨーグルト中の過塩素酸塩濃度の実態調査**

食品衛生学会誌，**52**，78-85（2011）

過塩素酸塩は，天然および人工物が存在し，甲状腺へのヨウ素の取り込み阻害および甲状腺機能を抑制する．我が国における食品中の過塩素酸塩濃度の実態を調査するため，ワイン28試料，魚介類20試料，精米10試料，乳（牛乳，成分調整牛乳，低脂肪牛乳，加工乳，乳飲料）30試料，粉乳10試料およびヨーグルト10試料中の過塩素酸塩濃度を測定した．ワイン，乳，粉乳およびヨーグルトは全ての試料から過塩素酸が検出され，濃度範囲はワイン0.2～103ng/g，乳2～11ng/g，粉乳3～35ng/gおよびヨーグルト2～11 ng/gであった．魚介類試料中8試料は定量限界（0.8ng/g）未満であり，12試料から0.8～72ng/gの過塩素酸塩が検出された．精米は全試料が定量限界（1.0ng/g）未満であった．

Keywords：perchlorate，IC-MS/MS，dairy products

Amakura, Y.[*1], Tsutsumi, T., Nakamura, M.[*2], Handa, H.[*2], Yoshimura, M.[*1], Matsuda, R., Yoshida, T.[*1]: **Aryl hydrocarbon receptor ligand activity of commercial health foods**

Food Chemistry, **126**, 1515-1520（2011）

The aryl hydrocarbon receptor（AhR）is a ligand-activated transcription factor that mediates toxicological effects by binding to agonists such as dioxins. We previously reported the presence of natural dioxin-like ligands in foods. To further characterise natural ligands with dioxin-like activity, we examined the influence of 50 kinds of commercial supplement and health food on the AhR, using a reporter gene assay. Some samples, prepared using soybean, sesame, or propolis as an ingredient, were revealed to show AhR-binding activity, similar to that of 2,3,7,8-tetrachlorodibenzo-p-dioxin（TCDD）, at high concentrations. To characterise the AhR-activating substances in eight active samples, the respective extracts were subjected to fractionation with n-hexane, ethyl acetate, and water, followed by estimating their AhR activities. The n-hexane fraction of the propolis extract sample, and the ethyl acetate fractions of the other samples, showed AhR activity similar to that of TCDD, at a high concentration range. HPLC analysis of the active fractions identified isoflavones, such as daidzein and glycitein, and flavones, such as tectochrysin and chrysin, in the samples. Among these compounds, tectochrysin exhibited marked AhR activation. Flavonoids, which are characterised as natural AhR ligands, are known to have representative beneficial effects on human health. The natural AhR ligands identified in this study are known to be useful for human health. Therefore, it is considered that AhR may play a beneficial regulatory role in humans.

Keywords：aryl hydrocarbon receptor, health food, dioxin

[*1] Matsuyama university
[*2] Hiyoshi Corporation

Nakagawa, R.*, Murata, S.*, Ashizuka, Y.*, Shintani, Y.*, Hori, T.*, Tsutsumi, T.: **Hexabromocyclododecane determination in seafood samples collected from Japanese coastal areas**

Chemosphere, **81**, 445-452（2010）

The levels of three hexabromocyclododecane（HBCD）isomers and ΣHBCDs in 54 wild and 11 farmed seafood samples collected from four regions of Japan were determined by LC/MS/MS. For the fish classified as Anguilliformes, Perciformes, Clupeiformes and farmed Salmoniformes, the medians（ranges）of ΣHBCDs are 2.09（0.05-36.9）, 0.75（ND-26.2）, 0.12（0.09-77.3）and 1.29（1.09-1.34）ng/g ww, respectively. However, HBCDs were not detected in samples classified as Crustacea, Mollusca, Pleuronectiformes and Scorpaeniformes, or if detected, the levels were very low. The rank correlation between ΣHBCDs（or α-HBCD）and fat content could not be found except for the Japanese sea bass of the Tohoku region. In HBCD isomer profiles, for fish samples above 20 ng/g ww, the trend was found that γ-HBCD was predominant, which suggests the influence of discharge from a nearby industrial plant. In the other wild fish and the farmed fish samples, on the other hand, α-HBCD was mostly predominant, which suggests biomagnification via the food chain. Additionally, to assess the risk to human health, based on the determined HBCD median concentrations for Anguilliformes, farmed Salmoniformes and Perciformes, the daily intake of HBCDs from fish by an average Japanese adult was tentatively calculated to be 3.7, 2.3 and 1.3 ng/kg body weight/day, respectively.

Keywords：hexabromocyclododecane, LC/MS/MS, dietary intake

* Fukuoka Institute of Health and Environmental

Sciences

秋山卓美，佐々木　亮[*1]，山崎　壮，棚元憲一[*2]，山形一雄[*1]，河村葉子：**SDS-PAGE による既存添加物酵素のタンパク質分離パターン**

日本食品化学学会誌，**17**，88-95（2010）

日本では，食品用酵素は既存添加物名簿に収載されているが，その名称は酵素機能を表しており，酵素タンパク質を特定できる名称ではない．そのため，一つの酵素品目に異なる基原に由来する製品が含まれている．酵素製品に含まれるタンパク質を化学的に分析して基原生物種や菌種の確認ができれば，酵素の簡便な基原確認試験法として利用が期待できる．そこで，α–アミラーゼ，β–アミラーゼ，カタラーゼ，β–ガラクトシダーゼ，グルコアミラーゼ，セルラーゼ，プロテアーゼ，ヘミセルラーゼについて，日本国内で現在流通している製品を可能な限り網羅的に収集した．103製品を試料として用い，SDS-PAGE により分析した．含有量の大きいタンパク質の分子量を求めたところ，多くの製品に関して基原によって特徴的なタンパク質分離パターンを示した．植物由来酵素では，種の確認が可能であった．細菌・真菌由来酵素では，菌種の確認が可能な例が多かったが，*Bacillus* 属のように属の確認しかできない例もあった．

Keywords：food manufacturing enzyme，SDS-PAGE，origin

[*1] 日本大学
[*2] 武蔵野大学

秋山卓美，林　歩美[*1]，山崎　壮，多田敦子，杉本直樹，尹　永淑[*1]，功刀　彰[*1]，棚元憲一[*2]，河村葉子：**TLC と GC/MS を用いたテルペノイド系ガムベースの識別法**

食品衛生学雑誌，**51**，264-271（2010）

既存添加物名簿に収載もしくはかつて収載されていたガムベースのうち，トリテルペノイドを多く含有するとされているマスチック，ダンマル樹脂，ニュウコウ，ベンゾインガムおよびエレミ樹脂と，ジテルペノイドを多く含有するとされているロシンおよびコーパル樹脂について，簡便なクロマトグラフィー手法による識別法を検討した．TLC では品目ごとに互いに異なる特徴的なパターンを示したことから，品目の識別が可能であった．次に，試料をメチルエステル化した誘導体を GC/MS で分析した．TIC クロマトグラムは品目ごとに互いに異なる特徴的なパターンを示し，明らかな差異が見られた．また，各品目の主要構成成分を検出することができ，品目ごとに特徴的な，判別の指標成分となり得る化合物が存在した．今回検討した TLC 分析法は簡便な確認試験法として，GC/MS 分析法は含有成分の確認試験法として利用できることから，テルペノイド系ガムベースの体系的分析法としても品目間の判別法としても有用な試験法である．

Keywords：gum base，terpenoid，chromatography

[*1] 東京薬科大学
[*2] 武蔵野大学

秋山卓美，山崎　壮，棚元憲一[*]：**改良ジエチルジチオアセタール化法による増粘多糖類構成糖の分析**

食品衛生学雑誌，**52**，40-46（2011）

ウロン酸含有増粘多糖類の構成糖組成比を GC 分析する方法を検討した．単糖にジエチルジチオアセタール化と TMS 化を施して GC/MS で分析する既報の方法の反応スケールを 5 倍にし，さらに溶媒抽出を用いた調製法に変更し，操作性と再現性を向上させた．多糖類 7 品目を加水分解し，改良操作法により誘導体化して GC/FID で分析した．構成糖の誘導体をクロマトグラム上でいずれも単一のピークとして検出し，互いに分離することができ，同定と定量が可能であった．構成糖の種類と含量比から 7 品目を区別することができた．さらに加水分解反応の最適化を試みたところ，グルクロン酸は中性糖よりも加水分解に時間がかかることが示された．品目間の識別は 6 時間の加水分解で十分であるが，構成糖組成比の精密な比較を行う場合には，構成糖毎に加水分解時間の最適化を行う必要があると考えられた．

Keywords：thickening polysaccharide，diethyldithioacetal，gas chromatography

[*] 武蔵野大学

多田敦子，高橋加奈，杉本直樹，末松孝子[*1]，有福和紀[*1]，齋藤　剛[*2]，井原俊英[*2]，吉田雄一[*3]，石附京子，西村哲治，山崎　壮，河村葉子：**定量 NMR に基づく既存添加物中のクエルセチンおよびクエルセチン配糖体の絶対定量**

食品衛生学雑誌，**51**，205-212（2010）

我々は，国際単位系（SI）にトレーサブルな有機化合物の絶対定量法として，定量 NMR（quantitative NMR：qNMR）の開発を行っている．本研究では qNMR を応用し，既存添加物ルチン（抽出物），ルチン酵素分解物およびクエルセチンの各添加物製品中のルチン，イソクエルシトリンおよびクエルセチンや，これら化合物の市販試薬の絶対定量を行った．今回新たに，計量学的に正確に値付けされた1,4-ビストリメチルシリルベンゼン-d_4

（1,4-BTMSB-d_4）をqNMR基準物質として用い，そのメチル基と測定化合物の各2′位プロトンとのシグナル積分値比から含量を算出し，より簡便な1段階のqNMR測定を行った．その結果，qNMRを用いることにより，分離操作を行うことなく，かつ，測定対象化合物と同一の標準品を必要とせずに，ルチン，イソクエルシトリンおよびクエルセチンの定量が可能であることを見出した．

Keywords：quercetin glycoside，qNMR，food additive

*1 日本電子（株）
*2 （独）産業技術総合研究所
*3 和光純薬工業（株）

Ito, Y., Onobori, K., Yamazaki, T., and Kawamura, Y.: **Tigloylshikonin, a new minor shikonin derivative, from the roots and the commercial root extract of *Lithospermum erythrorhizon***

Chem. Pharm. Bull., **59**, 117-119（2011）

Tigloylshikonin, a new shikonin derivative esterified with tiglic acid ((*E*)-2-methylbut-2-enoic acid), was isolated as a minor pigment from a food colorant "Shikon color", a commercial root extract from *Lithospermum erythrorhizon* SIEBOLD *et* ZUCCARINI. The structure of tigloylshikonin was elucidated using ^{1}H, ^{13}C, the difference NOE, and 2D NMR techniques. Its stereochemistry was determined by chiral-phase HPLC analysis. Tigloylshikonin was also found in the roots of *L. erythrorhizon*, which indicated that this new shikonin derivative is a typical component of naphthoquinone pigments in the roots of *L. erythrorhizon*.

Keywords：Shikon color, shikonin, tiglic acid

石附京子，多田敦子，杉本直樹，松本　清*1,2，受田浩之*3，松藤　寛*4，山崎　壮，河村葉子：**既存添加物ドクダミ抽出物の品質評価**

日本食品化学学会誌，**17**，192-197（2010）

ドクダミ抽出物は天然由来の酸化防止剤の一つで，既存添加物に関連した通知（1996年，既存添加物名簿収載品目リスト）には，「ドクダミ科ドクダミ（*Houttuynia cordata* THUNB.）の葉より，エタノールで抽出し，精製して得られたものである．主成分はイソクエルシトリンである．」と記載されている．既存添加物ドクダミ抽出物として提供された製品のLC/MSによる成分分析の結果，イソクエルシトリンよりむしろクエルシトリン，ヒペリンが主に検出された．しかし，その成分組成は，ドクダミを基原とする生薬ジュウヤクの葉部からの抽出物と類似し，基原の妥当性が確認された．また，既存添加物製品のDPPHラジカル消去活性を測定した結果，明らかな抗酸化活性が確認された．

Keywords：food additive，Dokudami extract，*Houttuynia cordata* THUNB

*1 九州大学大学院農学研究院
*2 崇城大学生物生命学部
*3 高知大学農学部
*4 日本大学生物資源科学部

六鹿元雄，山口未来，大野浩之*，河村葉子：**ナイロン製品からのモノマーおよび芳香族第一級アミン類の溶出**

食品衛生学雑誌，**51**，228-236（2010）

ナイロン製品21試料について，熱分解ガスクロマトグラフィー（Py-GC/MS）を用いてそのナイロンの種類を判別するとともに，モノマー2種類及び芳香族第一級アミン類（PAAs）21種類の溶出量をLC/MS/MSにより測定した．試料の材質はナイロン6が1検体，ナイロン66が15検体，ナイロン6/66共重合体が3検体，ナイロンとPE，PPのラミネートが2検体であった．ただし，ナイロン66製品はナイロン6のモノマーであるε-カプロラクタム（CPL）も含有していた．また，20%エタノール60℃30分間でのモノマー及びPAAsの溶出量は，ラップフィルム1検体を除くすべての検体からCPLが0.015～38μg/mL，すべてのナイロン66製品とナイロン6/66製品1検体から1,6-ヘキサメチレンジアミンが0.002～0.013μg/mL検出された．また，4,4′-ジアミノジフェニルメタンが3検体から0.006～4.3μg/mL，アニリンが4検体から0.032～0.23μg/mL，その他4-クロロアニリンが2検体から各0.001μg/mL，2-トルイジン及び1-ナフチルアミンがそれぞれ1検体ずつから0.002及び0.066μg/mL検出された．

Keywords：nylon, caprolactam, primary aromatic amine

* 名古屋市衛生研究所

六鹿元雄，四柳道代，河村葉子：**食品用金属製器具の材質中鉛試験法**

食品衛生学雑誌，**52**，10-17（2011）

金属製品の材質中鉛試験法を確立した．測定法としてフレーム－原子吸光分析（AAS）法，フレームレス－原子吸光分析（GFAA）法，誘導結合プラズマ発光分析（ICP）法及び蛍光X線分析（XRF）法の比較を行い，試験溶液の調製法についても検討を行った．試料100mgを精秤し，塩酸・硝酸（3：1）混酸（チタンは塩酸）

を2.5mL 加えて溶解した．これに水を加えて50mL としたものを試験原液とし，0.1mol/L 硝酸で適宜希釈して試験溶液とした．鉛を0.1%添加した時の回収率は90～118%と良好であった．鉛を0.0098～0.11%含有する標準物質を測定したところ，各定量値は認証値とほぼ近い値を示した．本法はアルミニウム，鉄，ステンレス鋼，銅，スズ，チタン製品に適用でき，AAS 法，GFAA 法，ICP 法ともに鉛試験法として適用可能であった．また，XRF 法はスクリーニング法または簡易定量法として有用であった．ICP 法を用いて市販金属製器具22検体の鉛含有量を測定したところ，6検体から0.011～0.040%の鉛が検出された．

Keywords：metal utensil，lead，test method

阿部　裕，山口未来，六鹿元雄，平原嘉親，河村麻衣子，花尻（木倉）瑠理，合田幸広，河村葉子：**DART-TOF/MS を用いたポリ塩化ビニル中の可塑剤の検索およびフタル酸エステルのスクリーニング法の検討**

食品衛生学雑誌，**51**，160-169（2010）

Direct analysis in real time（DART）イオン化装置に time of flight/mass spectrometry（TOF/MS）を組み合わせた DART-TOF/MS を用いて，ポリ塩化ビニル（PVC）中の可塑剤の検索とフタル酸エステルのスクリーニング法を検討した．可塑剤40種を DART-TOF/MS で測定したところ，ほとんどの可塑剤でプロトン付加体の擬分子イオン［M＋H］$^+$が得られ，分子量が容易に推定された．また，PVC 製シート及び玩具中の可塑剤を測定したところ，それぞれの可塑剤に相当するマススペクトルが得られ，容易に可塑剤の検索ができた．さらに，DART-TOF/MS におけるフタル酸エステルのイオン強度を検出限界または最適な目安値で選抜することにより，フタル酸エステル含有量が0.1%を超える試料を見逃すことなくスクリーニングできることを示した．DART-TOF/MS 測定は操作が簡便で，瞬時に結果が得られるため PVC 中の可塑剤の検索やフタル酸エステルのスクリーニングに有用である．

Keywords：DART-TOF/MS，polyvinyl chloride，phthalate

Ohno, H.* and Kawamura, Y.: **Analysis of acrylonitrile, 1,3-butadiene, and related compounds in acrylonitrile-butadiene-styrene copolymers for kitchen utensils and children's toys by headspace gas chromatography/mass spectrometry**

J. AOAC International, **93**, 1965-1971 (2010)

A headspace gas chromatography/mass spectrometry method was developed for the simultaneous determination of the residual levels of acrylonitrile (AN), 1,3-butadiene (1,3-BD), and their related compounds containing propionitrile (PN) and 4-vinyl-1-cyclohexene (4-VC) in acrylonitrile-butadiene-styrene (ABS) copolymers for kitchen utensils and children's toys. A sample was cut into small pieces, then *N*,*N*-dimethylacetamide and an internal standard were added in a sealed headspace vial. The vial was incubated for 1 h at 90℃ and the headspace gas was analyzed by gas chromatography/mass spectrometry. The recovery rates of the analytes were 93.3-101.8% and the coefficients of variation were 0.3-6.5%. In ABS copolymers, the levels were 0.3-50.4 μg/g for AN, ND-4.5 μg/g for PN, 0.06-1.58 μg/g for 1,3-BD, and 1.1-295 μg/g for 4-VC. The highest level was found for 4-VC, which is a dimer of 1,3-BD, and the next highest was for AN, which is one of the monomers of the ABS copolymer. Furthermore, the method was also applied to acrylonitrile-styrene (AS) copolymers and polystyrenes (PS) for kitchen utensils, and nitrile-butadiene rubber (NBR) gloves. In AS copolymers, AN and PN were detected at 16.8-54.5 and 0.8-6.9 μg/g, respectively. On the other hand, the levels in PS and NBR samples were all low.

Keywords: acrylonitrile, 1,3-butadiene, headspace gas chromatography/mass spectrometry

---

* Nagoya City Public Health Research Institute

大野浩之*，鈴木昌子*，河村葉子：**4種擬似溶媒による合成樹脂製食品用器具の蒸発残留物量の検討**

食品衛生学雑誌，**52**，66-70（2011）

4種擬似溶媒（水，4%酢酸，20%エタノール及びヘプタン）を用い，合成樹脂製食品用器具12樹脂71検体の蒸発残留物量を調査した．測定は規格試験法に準じて行った．定量限界は5 μg/mL であった．ポリエチレン，ポリプロピレン，ポリスチレン，アクリロニトリル・スチレン樹脂，アクリロニトリル・ブタジエン・スチレン樹脂，ポリ塩化ビニル，ポリ塩化ビニリデン，ポリメチルペンテン，ポリメタクリル酸メチル及びポリエチレンテレフタレート製品では，蒸発残留物量はヘプタンの場合が最も高く，その他の溶媒は非常に低かった．一方，メラミン樹脂及びナイロン製品では，4%酢酸または20%エタノールの場合が最も高く，ヘプタンが最も低かった．この結果，合成樹脂の種類別に最適な溶出溶媒の選択が可能となり，迅速かつ効率良く試験を行うことができるようになると考えられた．

Keywords：evaporation residue，migration test，food-

simulating solvent

---

* 名古屋市衛生研究所

尾崎麻子*，大嶋智子*，大垣寿美子*，河村葉子：**ポリ乳酸製器具・容器包装の含有物質の検討および溶出液の変異原性**

食品衛生学雑誌，**51**，220-227（2010）

ポリ乳酸製器具・容器包装7検体について食品衛生法における規格試験を実施した．さらに，その他の含有物質や溶出物質の検討をICP-AES及びGC/MSを用いて行い，溶出液について2種類の変異原性試験を実施した．その結果，全ての試料が食品衛生法における規格基準を満たしており，金属の溶出もほとんど見られなかった．溶出液のGC/MSによるピーク検索の結果，大きなピークは見られず，レックアッセイ及び*umu*−テストの両方の試験において全ての試料が陰性を示した．*umu*−テストにおいて汁椀の溶出液がβ-ガラクトシダーゼ活性を若干増加させたが，素地であるポリ乳酸からの溶出物によるものではなく，塗装面のポリウレタンによるものと推測された．

Keywords：polylactic acid，rec-assay，*umu*-test

---

* 大阪市立環境科学研究所

Suzuki, T.*1, Ota, Y., Kasuya, Y.*1, Mutsuga, M., Kawamura, Y., Tsumoto, H.*1, Nakagawa, H.*1, Finn, M. G.*2 and Miyata, N.*1: **An unexpected example of copper-mediated *in situ* click chemistry**

Angew Chem. Int. Ed., **49**, 6817-6820 (2010)

In the course of a search for histone deacetylase (HDAC) inhibitors using *in situ* click chemistry, we unexpectedly observed the first example of protein-Cu acceleration of the azide-alkyne cycloaddition reaction. Adventitious cuprous ion bound to HDAC8 accelerated triazole formation between one azide-alkyne pair among 30 possibilities, showing that the protein target guided the powerful Cu-accelerated reaction, presumably by selective binding in the active site. In addition to providing a new route to HDAC inhibitors, these results provide a basis for developing new types of protein-based catalysts for click chemistry.

Keywords：copper, cycloaddition, protein

---

*1 Graduate School of Pharmaceutical Sciences, Nagoya City University

*2 The Scripps Research Institute

山本茂貴：**食品安全におけるリスクアナリシス**

日本リスク研究学会誌，**20**(3)，185-187（2010）

Risk analysis framework introduced into food safety. Codex Alimentarius Commission recommended this framework into food safety to set the international standards. Risk analysis consists of three elements, risk management, risk assessment and risk communication. Since 2003, Japanese food safety policy introduced this framework. Ministry of Health, Labor and Welfare and Ministry of Agriculture, Forest, and Fisheries are risk management bodies. Food safety commission in the Cabinet Office is the risk assessment body. Risk analysis in food safety is necessary and important in the world food trade.

Keywords：Food safety, Risk analysis, Risk management

Pinto, A. F.*1, Todorovic, S.*1, Hildebrandt, P.*1, Yamazaki, M.*2, Amano, F.*3, Igimi, S., Rom-o, C. V.*1, Teixeira, M.*1: **Desulforubrerythrin from *Campylobacter jejuni*, a novel multidomain protein**

Journal of Biological Inorganic Chemistry, **16**(3), 501-510 (2011)

A novel multidomain metalloprotein from *Campylobacter jejuni* was overexpressed in *Escherichia coli*, purified, and extensively characterized. This protein is isolated as a homotetramer of 24-kDa monomers. According to the amino acid sequence, each monomer was predicted to contain three structural domains: an N-terminal desulforedoxin-like domain, followed by a four-helix bundle domain harboring a non-sulfur μ-oxo diiron center, and a rubredoxin-like domain at the C-terminus. The three predicted iron sites were shown to be present and were studied by a combination of UV-vis, EPR, and resonance Raman spectroscopies, which allowed the determination of the electronic and redox properties of each site. The protein contains two FeCys (4) centers with reduction potentials of +240 mV (desulforedoxin-like center) and +185 mV (rubredoxin-like center). These centers are in the high-spin configuration in the as-isolated ferric form. The protein further accommodates a μ-oxo-bridged diiron site with reduction potentials of +270 and +235 mV for the two sequential redox transitions. The protein is rapidly reoxidized by hydrogen peroxide and has a significant NADH-linked hydrogen peroxide reductase activity of 1.8 μmol H (2) O (2) min (-1) mg (-1). Owing to its building blocks and its

homology to the rubrerythrin family, the protein is named desulforubrerythrin. It represents a novel example of the large diversity of the organization of domains exhibited by this enzyme family.

Keywords: Desulforubrerythrin, *Campylobacter jejuni*, multidomain

---

*1 Universidade Nova de Lisboa
*2（財）微生物化学研究会
*3 大阪薬科大学

Saito, E.*, Yoshida, N.*, Kawano, J.*, Shimizu, A.*, Igimi, S.: **Isolation of *Staphylococcus aureus* from raw fish in relation with culture methods**

J. Vet. Med. Sci., **73**(3), 287-292(2011)

Five hundred and fifty fish samples from various stages in the course of distribution in Hyogo Prefecture (209 retailed in super markets, 173 obtained from fishery cooperatives at a harbor, 91 caught by trawling and 77 caught by rod fishing) were examined for contamination with *Staphylococcus aureus* (*S.aureus*). *S.aureus* was detected in 41 (19.6%) of the retail fish samples and 46 (26.6%) of the samples from the fishery cooperatives. No *S.aureus* was isolated from the live fish (91 trawled and 77 fished by rod). With regard to the retail fish, the contamination rate of processed fish (26.0%) was significantly higher than that of unprocessed fish (14.2%). For 88 samples, the efficacy of the selective medium was compared using Baird-Parker agar and mannitol salt agar supplemented with egg yolk (MSEY agar) by the direct plate and enrichment culture methods. Using the direct culture method, the *S.aureus* positive rate with the Baird-Parker agar (30.7%) was significantly higher ($P<0.01$) than that with the MSEY agar (6.8%). The enrichment culture method remarkably raised the *S.aureus* detection rate. Seventy-eight (85.7%) of 91 isolates belonged to the human ecovar. Sixty-two (68.1%) of the 91 isolates had some enterotoxin genes, including 44 (48.4%) with the sea gene. These data showed that the fish were contaminated with *S.aureus* after landing and that Baird-Parker agar had an advantage in detecting *S.aureus* with a direct plate culture.

Keywords: culture methods, *Staphylococcus aureus*, raw fish

---

* 神戸大学

Asakura, H., Churin, Y., Bauer, B., Boettcher, J. P., Bartfeld, S., Hashii, N., Kawasaki, N., Mollenkopf, H. J., Jungblut, P. R., Brinkmann, V., Meyer, T. F.*: ***Helicobacter pylori* HP0518 affects flagellin glycosylation to alter bacterial motility**

Mol. Microbiol., **78**, 1130-1144(2010)

ピロリ菌は，感染性胃潰瘍・胃癌のリスク因子として，ヒトの胃組織に定着するが，運動性はその定着過程に必須とされる．本研究では，HP0518遺伝子変異がG27株の運動性を亢進させることを見い出し，これが鞭毛のO型糖鎖修飾の亢進に因ることを実証した．更に，高い運動性を示すHP0518変異菌株はAGS細胞における付着性とCagAリン酸化，NF-κB活性化を亢進させた．以上の成績より，HP0518は鞭毛における脱糖鎖性を有し，病原体–宿主相互作用の中で重要な役割を担う鞭毛の調節因子として機能することを明らかにした．

Keywords: *Helicobacter pylori*, HP0518, flagellar glycosylation

---

* Max Planck Institute for Infection Biology, Germany

Kawasaki, M.* and Machii, K.: **Basic Research on Developing Scallop Tissue Reference Material for Quality Assurance of Diarrhetic Shellfish Poisoning (DSP) Mouse Bioassay (MBA): -Free Fatty Acid (FFA) in Homogenized Frozen Scallop Slurry and its Effect on MBA**

J. Environ. Chem., **21**, 75-78(2011)

Diarrheic shellfish poisoning (DSP) is one of the gastrointestinal illness caused by the consumption of shellfish contaminated with toxigenic dinoflagellates. The main toxins responsible for DSP are Okadaic acid (OA) and its derivatives. Remarkable increase of free fatty acid (FFA) in the hepatopancreas (HP) of scallops during storage in a freezer is occasionally observed and it results in pseudo-positive with the MBA for DSP. In the process of making reference material (RM) for MBA, which is consisted of a set of a vial containing a piece of filter infused with OA and DSP negative slurry of homogenized scallop whole meat (WH), we investigated the concentration of FFA. The determination of OA and FFA concentrations was performed using liquid chromatography with a fluorometric detector for anthryl diazomethane (ADAM) derivatives. In this study FFA composition and toxicity were surveyed in homogenized scallop tissue stored in a freezer at-70℃ for 4 months. Most of the samples were

nontoxic as determined by mouse bioassay and showed low FFA concentration; one sample showed both toxic and high FFA concentrations. These results suggest that the determination of FFA concentration in scallop tissue by HPLC coupled with the MBA for DSP is important for RM.

Keywords: Diarrhetic Shellfish Poisoning, free fatty acid, reference material

*（財）食品薬品安全センター

Iwahori1, J.[*1], Yamamoto, A.[*2], Suzuki, H., Yamamoto, T.[*3], Tsutsui, T.[*3], Motoyama, K.[*4], Sawada, M.[*4], Matsushita, T.[*5], Hasegawa, A.[*5], Osaka, K.[*6], Toyofuku, H.[*7] and Kasuga, F.: **Quantitative risk assessment of *Vibrio parahaemolyticus* in finfish: a model of raw horse mackerel consumption in Japan**

Risk Analysis, **30**, 1817-1832（2010）

アジの刺身による腸炎ビブリオ感染の確率論的リスクアセスメントモデルを作成し，各リスク因子の影響度を比較した．輸送中に高温に曝されることにより，発症確率が50%上昇することを明らかにした．

Keywords: Dose-response model, quantitative risk assessment, *Vibrio parahaemolyticus*

[*1] 高知大学医学部
[*2] 兵庫県立健康環境科学研究センター
[*3]（独）農業・食品産業技術総合研究機構・動物衛生研究所
[*4]（株）日立東日本ソリューションズ
[*5]（株）三菱総合研究所
[*6] 東北大学大学院歯学研究科
[*7] 国立保健医療科学院

Teunis, P. F. M.[*1], Kasuga, F., Fazil, A.[*2], Ogden, I. D.[*3], Rotariu, O.[*4] and Strachan, N. J. C.[*4]: **Dose response modeling of Salmonella using outbreak data**

Int. J. Food Microbiol., **144**, 243-249（2010）

サルモネラによる集団感染事例のデータを基に，摂取菌数と感染率および発症率との相関関係を別々に関数化した．ID50として，発症には感染の成立よりも5倍多くの菌数が必要であることが示された．

Keywords: *Salmonella*, dose response, risk assessment

[*1] National Institute of Public Health and the Environment, The Netherlands
[*2] Public Health Agency of Canada
[*3] School of Medicine, University of Aberdeen
[*4] School of Biological Sciences, University of Aberdeen

Iizuka, S.[*1], Oka, T.[*2], Tabara, K.[*1], Omura, T.[*1], Katayama, K.[*2], Takeda, N.[*3] and Noda, M.: **Detection of sapoviruses and noroviruses in an outbreak of gastroenteritis linked genetically to shellfish**

J. Med. Virol., **82**, 1247-1254（2010）

Norovirus（NoV）and sapovirus（SaV）are important pathogens of human gastroenteritis. Compared to NoV, the transmission route of SaV is unclear. An outbreak of gastroenteritis occurred at a restaurant in June 2008, and SaV and NoV were detected in fecal specimens from 17 people who ate at the restaurant and one asymptomatic food handler and also in stripped shellfish and liquids remaining in the shellfish packages by reverse transcription-polymerase chain reaction（RT-PCR）and/or real-time RT-PCR. Nucleotide sequencing analysis of the RT-PCR products corresponding to the partial capsid region revealed 99.3-100% identities for SaV and 98.6-99.3% identities for NoV among the digestive diverticulum of the frozen stripped shellfish（*Ruditapes philippinarum*）, "Asari," the package liquid, and feces from symptomatic or asymptomatic guests. These results suggested a link between the consumption of contaminated shellfish and clinical features in the patients. While the transmission of NoV by shellfish has been reported, this report shows that SaV can also be transmitted by shellfish.

Keywords: sapovirus, shellfish, gastroenteritis outbreak

[*1] 島根県保健環境研究所
[*2] 国立感染症研究所
[*3] Research Collaboration Center on Emerging and Re-emerging Infections

Ueki, Y.[*1], Shoji, M.[*1], Okimura, Y.[*1], Miyota, Y.[*1], Masago, Y.[*2], Oka, T.[*3], Katayama, K.[*3], Takeda, N.[*3], Noda, M., Miura, T.[*4], Sano, D.[*4], and Omura, T.[*2]: **Detection of Sapovirus in oysters**

Microbiol. Immunol., **54**, 483-486（2010）

SaV sequences which are either genetically identical or similar were detected from oysters, feces from gastroenteritis patients, and domestic wastewater samples in geographically close areas. This is the first report of the detection of SaV in oysters which meet the legal requirements for raw consumption in Japan.

Keywords: commercial oyster, human sapovirus, water contamination

*1 宮城県保健環境センター
*2 東北大学大学院工学研究科
*3 国立感染症研究所
*4 北海道大学工学部

Motomura, K.*1, Yokoyama, M.*1, Ode, H.*1, Nakamura, H.*1, Mori, H.*1, Kanda, T.*1, Oka, T.*1, Katayama, K.*1, Noda, M., Tanaka, T.*2, Takeda, N.*1, Sato, H.*1, Norovirus Surveillance Group of Japan.: **Divergent evolution of norovirus GII/4 by genome recombination from May 2006 to February 2009 in Japan**

J. Virol., **84**, 8085-8097 (2010)

Norovirus GII/4 is a leading cause of acute viral gastroenteritis in humans. We examined here how the GII/4 virus evolves to generate and sustain new epidemics in humans, using 199 near-full-length GII/4 genome sequences and 11 genome segment clones from human stool specimens collected at 19 sites in Japan between May 2006 and February 2009. Phylogenetic studies demonstrated outbreaks of 7 monophyletic GII/4 subtypes, among which a single subtype, termed 2006b, had continually predominated. Phylogenetic-tree, bootscanning-plot, and informative-site analyses revealed that 4 of the 7 GII/4 subtypes were mosaics of recently prevalent GII/4 subtypes and 1 was made up of the GII/4 and GII/12 genotypes. Notably, single putative recombination breakpoints with the highest statistical significance were constantly located around the border of open reading frame 1 (ORF1) and ORF2 ($P< 0.000001$), suggesting outgrowth of specific recombinant viruses in the outbreaks. The GII/4 subtypes had many unique amino acids at the time of their outbreaks, especially in the N-term, 3A-like, and capsid proteins. Unique amino acids in the capsids were preferentially positioned on the outer surface loops of the protruding P2 domain and more abundant in the dominant subtypes. These findings suggest that intersubtype genome recombination at the ORF1/2 boundary region is a common mechanism that realizes independent and concurrent changes on the virion surface and in viral replication proteins for the persistence of norovirus GII/4 in human populations.

Keywords: norovirus, genome recombination, GII/4

*1 国立感染症研究所
*2 堺市衛生研究所

末永朱美*，田中寛子*，蔵田和正*，花木陽子*，毛利好江*，野田　衛，石村勝之*，池田義文*，笠間良雄*，吉岡嘉暁*：**広島市で分離された腸管出血性大腸菌 O157：H7の分子疫学的解析**

広島県獣医学会雑誌，**25**，75-79（2010）

2008～2009年に広島市で分離された腸管出血性大腸菌O157：H7，15事例28株を，パルスフィールドゲル電気泳動法（PFGE），IS-printing 法，Multiple-Locus Variablenumbertandem repeat Analysis（MLVA）の3法で分子疫学的解析を行い，比較検討した．IS-printing 法では15事例28株を12のコード型に分けることができ，同一事例の株は全て同一コード型に分類された．PFGE 法，MLVA 法によるクラスタ解析において，類似度を適切に設定することで同一事例の株は同一型として分けられ，3法でほぼ一致した型別を行うことができた．これにより集団事例や家族間感染事例では一致した型となり疫学的関連の裏付けとなった．一方，散発事例で同一型となった株もあり，疫学的関連性を示すデータは認められないが，共通の感染源を持つ可能性が示唆された．

Keywords: enterohemorrhagic Escherichia coli, IS-printing system, multiple-locus variable-number tandem repeat analysis

* 広島市衛生研究所

横井　一*1，田中俊光*1，小林圭子*1，岩撫晴子*1，野口喜信*1，三井良雄*1，岡本　明*1，若岡未記*2，西郡恵理子*2，渡部展彰*2，清田智子*2，加曽利東子*2，大山照雄*2，西村正樹*2，本橋　忠*2，小川さやか*2，小山大雅*2，長嶋真美*2，大野喜昭*2，大塚正毅*2，中台啓二*2，池上　宏*2，石井孝司*3，野田　衛：**A 型肝炎ウイルスによる食中毒事例–千葉市**

病原微生物検出情報，**32**，78-79（2011）

2011年1月に千葉市内の飲食店（寿司屋）を原因施設とする A 型肝炎ウイルスによる食中毒事例が発生したので，その概要について報告した．

Keywords: hepatitis A virus, food poisoning, Sushi restaurant

*1 千葉市衛生研究所
*2 千葉市保健所
*3 国立感染症研究所

入谷展弘*1，久保英幸*1，改田　厚*1，関口純一朗*1，後藤　薫*1，長谷　篤*1，齊藤武志*2，石黒正博*2，鎌

倉和哉[*2]，吉田英樹[*2]，清原知子[*3]，石井孝司[*3]，野田衛：**大阪市で認められたA型肝炎3症例について**

病原微生物検出情報，**31**，296-297（2010）

2010年10週以降にA型肝炎が国内で急増した状況において，2010年4月～6月に大阪市で認められた3症例のA型肝炎について分子疫学的解析を実施した．その結果，本3症例に疫学的な関連性はなく，個別の感染によるものであったと考えられた．今回実施したA型肝炎ウイルス（HAV）の分子疫学的解析は，各症例間の関連性や感染地域の推定に有用であり，原因究明に重要な情報になると考えられた．

Keywords：hepatitis A virus，molecular epidemiology

[*1] 大阪市環境科学研究所
[*2] 大阪市保健所
[*3] 国立感染症研究所

石井孝司*，清原知子*，吉崎佐矢香*，佐藤知子*，脇田隆字*，中村奈緒美*，島田智恵*，中島一敏*，多田有希*，野田　衛：**2010年春季に日本で多発したA型肝炎の分子疫学的解析**

病原微生物検出情報，**31**，287-289（2010）

日本でのA型肝炎患者数は2007年以降非常に低いレベル（150人/年程度）で推移していたが，2010年は3月から全国各地でA型肝炎が多発し，最終的には年間342人の患者発生を見た．全国の地方衛生研究所と共同で，A型肝炎患者の糞便または血清からA型肝炎ウイルスゲノムの配列を決定し，流行状況を分子疫学的に解析した．その結果，今年の流行株はgenotype 1Aの2つのクラスターと3Aの1つのクラスターに大部分が分類されることが判明した．本年にA型肝炎が多発した理由は，従来日本に常在していた株に加え，東南アジア由来と考えられる株が新たに日本で流行し，また韓国で大流行した株も一部日本に侵淫してきたためであると考えられた．

Keywords：hepatitis A，molecular epidemiology，diffuse outbreak

* 国立感染症研究所

吉田徹也[*1]，宮坂たつ子[*1]，畔上由佳[*1]，内山友里恵[*1]，笠原ひとみ[*1]，上田ひろみ[*1]，長瀬　博[*1]，藤田暁[*1]，石井孝司[*2]，野田　衛：**長野県におけるA型肝炎事例の疫学的分析**

病原微生物検出情報，**31**，294-295（2010）

2010年春期に長野県内において，A型肝炎患者3例の届出があった．疫学調査の結果，3例中2例は海外渡航歴があり，患者糞便から検出されたA型肝炎ウイルス（HAV）はそれぞれ遺伝子型IAおよびIIIAに分類された．海外渡航歴のなかった1例は，発病の約1か月前にアサリの生塩漬けを喫食しており，検出されたHAVの遺伝子型はIIIAに分類された．遺伝子型IAに分類された1株は，2010年我が国における主流行株やフィリピン河川水由来株と同じクラスターに分類された．疫学情報とHAVの遺伝子型等の情報が迅速かつ広域に収集・解析されることにより，原因究明や予防対策に役立つと考えられた．

Keywords：hepatitis A virus，molecular epidemiology

[*1] 長野県環境保全研究所
[*2] 国立感染症研究所

増本久人[*1]，南　亮仁[*1]，野田日登美[*1]，江口正宏[*1]，原崎孝子[*1]，鶴田清典[*1]，北島正章[*2]，片山浩之[*2]，清原知子[*3]，石井孝司[*3]，野田　衛：**A型肝炎ウイルス検出事例の分子疫学的検討–佐賀県**

病原微生物検出情報，**31**，292-294（2010）

佐賀県で2010年3月から6月の間に計7事例のA型肝炎発生届出があり，そのうち5事例の患者便検体およびその1事例の患者自宅敷地内の井戸水1件についてA型肝炎ウイルス（HAV）のVP1/2A遺伝子領域の検出を試みた．その結果，4事例の患者便検体と井戸水1件からHAVを検出した．検出HAVは全てIA型に分類され，それらは2種類のクラスターに分類された．井戸水を感染源と特定するには至らなかった．

Keywords：hepatitis A virus，molecular epidemiology，well water

[*1] 佐賀県衛生薬業センター
[*2] 東京大学大学院工学系研究科
[*3] 国立感染症研究所

吉田徹也*，宮坂たつ子*，畔上由佳*，内山友里恵*，笠原ひとみ*，上田ひろみ*，長瀬　博*，藤田　暁*，野田　衛：**掃除機内ダストからのノロウイルスおよびサポウイルス汚染実態調査**

病原微生物検出情報，**31**，317-319（2010）

ダストから簡便で効率よくノロウイルス（NoV）を回収するための検出法を確立し，ダスト中のNoV等の汚染実態調査を実施した．一般家庭のダスト59検体中2検体（3.4%）がNoV陽性，1検体（1.7%）がサポウイルス（SaV）陽性であった．2008/09シーズンは35検体中NoVあるいはSaV陽性がそれぞれ1検体（2.9%），2009/10シーズンは24検体中1検体（4.2%）がNoV陽

性であった．汚染ダストの中にはウイルス量が10^6コピー/gを超えるものも存在していた．さらに，ダストのNoV，SaVの汚染は長期間にわたり継続したことから，ダストがNoV，SaVの感染源となる可能性が示唆された．

Keywords：norovirus，sapovirus，dust

---

* 長野県環境保全研究所

長岡宏美*，湊　千壽*，山田俊博*，川森文彦*，杉山寛治*，野田　衛：**2009～2010年に静岡県で発生したノロウイルス集団胃腸炎事例について**

病原微生物検出情報，**31**，320-321（2010）

2009年4月～2010年10月までに静岡県（政令市を除く）で発生した集団胃腸炎のうち，54例（2009年39事例うち食中毒7事例，2010年15事例うち食中毒2事例）から，ノロウイルス（NoV）が検出された．これら54事例について，遺伝子型を調べ，流行遺伝子型の傾向を解析した．また，食中毒事例の食品について，その処理方法に若干の改良を加え，より高感度な検出を試みた．

Keywords：norovirus，outbreak，PCR

---

* 静岡県環境衛生科学研究所

岡智一郎*1，片山和彦*1，小林慎一*2，飯高順子*3，野田　衛：**愛知県と川崎市の食中毒事例から検出されたサポウイルスGI/2の塩基配列の比較**

病原微生物検出情報，**31**，324-325（2010）

2010年1月に愛知県で発生した給食弁当を原因とした食中毒事例および2010年4月に神奈川県川崎市の中華料理店で発生した食中毒事例から検出されたサポウイルス（SaV）株は，カプシドの部分配列（333塩基）を用いた系統樹解析により，いずれもGI/2に分類された．そのため，両事例で検出された株の異同性を把握するために，両株の塩基配列を比較したところ，333塩基のうち，331塩基（99.4%）の配列が一致し，株間で異なった2塩基はアミノ酸変異を伴わない同義置換であった．また，川崎市の事例で検出されたSaV株の塩基配列は2009年に北海道と宮城県の急性胃腸炎患者糞便から検出された株と100%一致した．

Keywords：sapovirus，genotype，GI/2

---

*1 国立感染症研究所
*2 愛知県衛生研究所
*3 川崎市衛生研究所

Tanaka, H., Sugita-Konishi, Y., Takino, M.*1, Tanaka, T.*2, Toriba, A.*3, Hayakawa, K.*3：**A Survey of the Occurrence of *Fusarium* Mycotoxins in Biscuits in Japan by Using LC/MS**

J.Health Sci., **56**(2), 188-194(2010)

By adopting a rapid and sensitive method for simultaneous detection of nivalenol (NIV), deoxynivalenol (DON), fusarenon-X (FX), 3-acetyl deoxinivalenol (3ADON), HT-2 toxin (HT-2), T-2 toxin (T-2) and zearalenone (ZEN), the natural occurrence of these mycotoxins in biscuits made of wheat (201 samples) in Japan was surveyed. Samples were analyzed by LC/MS with atmospheric pressure photo ionization (APPI). Further confirmation was performed by liquid chromatography/time of flight mass spectrometry (LC/TOFMS). The average contamination of each *Fusarium* mycotoxin was 3.1, 23, 0.7, 0.1 and 4.2 ng/g for NIV, DON, HT-2, T-2 and ZEN, respectively. Multiple toxins were observed in 120 samples while FX and 3ADON were not detected. The incidence of these toxins was 41% for NIV, 98% for DON, 19% for HT-2, 11% for T-2 and 2% for ZEN. There were no significant differences in the concentration and incidence between conventional biscuits made of wheat and biscuits made of wheat for infants. This is the first report concerning the presence of NIV, DON, HT-2, T-2 and ZEN in biscuits in Japan.

Keywords：*Fusarium* mycotoxin, contamination survey, LC/MS/<LC/time of flight mass spectrometry, biscuit, Japan

---

*1 Agilent Technologies Japan, Limited
*2 Kobe Institute of Health
*3 Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa

Kimura, J.*1, Abe, H.*1, Kamitani, S.*1, Toshima, H*1, Fukui, A*1, Miyake, M.*2, Kamata, Y., Sugita-Konishi, Y., Yamamoto, S., Horiguchi, Y.*1：***Clostridium perfringens* Enterotoxin Interacts with Claudins via Electrostatic Attraction**

Journal of Biological Chemistry., **285**(1), 401-408(2010)

Clostridium perfringens enterotoxin (CPE), a causative agent of food poisoning, is a pore-forming toxin disrupting the selective permeability of the plasma membrane of target cells, resulting in cell death. We previously identified claudin as the cell surface receptor for CPE. Claudin, a component of tight junctions, is a tetratrans-

membrane protein and constitutes a large family of more than 20 members, not all of which serve as the receptor for CPE. The mechanism by which the toxin distinguishes the sensitive claudins is unknown. In this study, we localized the region of claudin responsible for interaction with CPE to the C-terminal part of the second extracellular loop and found that the isoelectric point of this region in sensitive claudins was higher than insensitive claudins. Amino acid substitutions to lower the pI resulted in reduced sensitivity to CPE among sensitive claudins, whereas substitutions to raise the pI endowed CPE-insensitive claudins with sensitivity. The steric structure of the claudin-binding domain of CPE reveals an acidic cleft surrounded by Tyr^{306}, Tyr^{310}, Tyr^{312}, and Leu^{315}, which were reported to be essential for interaction with the sensitive claudins. These results imply that an electrostatic attraction between the basic claudin region and the acidic CPE cleft is involved in their interaction.

Keywords: *Clostridium perfringens*, enterotoxin, receptor, binding, claudin

*1 Department of Molecular Bacteriology, Research Institute for Microbial Diseases, Osaka University
*2 Laboratory of Veterinary Public Health, Department of Veterinary Environmental Sciences, Osaka Prefecture University

Hosokawa, M.*1, Asakawa, H.*2, Kaido, T.*1, Sugaya, C.*3, Inoue, Y.*3, Tsunoda, M.*3, Itai, K.*4, Kodama, Y., Sugita-Konishi, Y., Aizawa, Y.*3: **Deterioration of Renal Function in ICR-derived Glomerulonephritis (ICGN) Mice by Subacute Administration of Fluoride in drinking water**

Fluoride., **43**(1), 1-44(2010)

Sodium fluoride was administered at 0, 25, 50, 100, and 150 ppm F in drinking water for 4 weeks to Institute of Cancer Research (ICR) derived glomerulonephritis (ICGN) mice. Fluoride was also administered to ICR mice at 0 and 150 ppm. Blood was sampled from the tail artery of each mouse twice a week for the determination of blood urea nitrogen (BUN) and creatinine (CRE). All ICGN mice in the 150 ppm F group and 4 of 9 in the 100 ppm F group died before the end of four weeks, but no ICR control mice died. The mean values of BUN and CRE in the serum of the 150 ppm ICGN mice were significantly higher than those in the ICGN control mice at the end of the exposure period. The mean relative liver weight of the 150 ppm ICGN mice was significantly lower than that of the ICGN control mice. We conclude that F significantly exacerbates renal dysfunction.

Keywords: Blood urea nitrogen, Fluoride and lomerulonephritis, ICGN mice, Kidney dysfunction, Renal insufficiency, Serum creatinine

*1 Kitasato University Graduate School of Medical Sciences
*2 Kitasato University School of Allied Health Sciences
*3 Kitasato University Department of Preventive Medicine and Public Health
*4 Department of Hygiene and Public Health, School of Medicine Iwate Medical University

Poapolathep, A.*1, Poapolathep, S.*1, Sugita-Konishi, Y., Wongpanit, K.*2, Machii, K., Itoh, Y., Kumagai, S.*3: **The Effect of Naringenin on the Fate and Disposition of Deoxynivalenol in Piglets**

J. Vet. Med. Sci., **72**(10), 1289-1294(2010)

This research was conducted to evaluate the effect of naringenin (NAG) on fate and dispositions of deoxynivalenol (DON) in piglets following intravenous (i.v.) administration. Three piglets (Group 1) were pretreated orally with NAG at a dosage of 25 mg/kg bw, once a day for 3 consecutive days, followed by a single i.v. injection of DON at a dosage of 1 mg/kg bw. The other three piglets (Group 2) were intravenously administered with DON at the same dosage. The level of DON in the plasma and various piglets tissues were measured using liquid chromatography/tandem mass spectrometry. The plasma levels of DON were higher in the NAG-untreated piglets than in the NAG-pretreated piglets at each time point. However, the plasma DON concentrations in the piglets pretreated with NAG was lower than those of NAG-untreated piglets. The elimination half-life was longer in the NAG-untreated piglets than in the piglets pretreated with NAG. The initial peak concentration, area under the curve and mean residence time were higher in the NAGuntreated piglets than in the piglets pretreated with NAG. Plasma biomarker enzyme activities were also monitored and the levels of gamma glutamyltranspeptidase, aspartate aminotransferase, alanine aminotransferase, creatine phosphokinase, blood urea nitrogen, and creatinine were considerably lower in the piglets pretreated with NAG than in the NAG-

untreated piglets. The toxicokinetic data and blood biochemical parameters indicate that NAG enhances the excretion of DON and reduces the opportunity for damage in piglets. Consequently, its toxicity is greater in NAG-untreated piglets than in piglets pretreated with NAG.

Keywords: blood chemistry, deoxynivalenol, dispositions, naringenin, piglets

[*1] Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University
[*2] Faculty of Natural Resources and Agro-Industry, Chalermphrakiat Sakonnakhon Province Campus, Kasetsart University
[*3] Research Center for Food Safety, The University of Tokyo

Hamada, M.[*1], Satsu, H.[*1], Ashida, H.[*2], Sugita-konishi, Y., Shimizu, M.[*1]: **Metabolites of Galangin by 2,3,7,8-Tetrachlorodibenzo-pdioxin-Inducible Cytochrome P450 1A1 in Human Intestinal Epithelial Caco-2 Cells and Their Antagonistic Activity toward Aryl Hydrocarbon Receptor**

J. Agric. Food Chem., **58**(13), 8111-8118(2010)

Galangin, a dietary flavonoid, inhibited cytochrome P450 1A1 (CYP1A1) expression induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). This inhibitory activity remained after permeating human intestinal epithelial Caco-2 cell monolayers, but was reduced when galangin permeated TCDD-pretreated Caco-2 cells. The present study tested whether TCDD affected the intestinal metabolism of flavonoids. LC-MS/MS analyses showed that galangin and two galangin glucuronoconjugates were reduced 0.7-fold, whereas kaempferol (a galangin oxidate) and kaempferol glucuronoconjugate were increased 1.5-fold by permeating TCDD-pretreated Caco-2 cells, as compared to untreated Caco-2 cells. An assay using recombinant human CYP1A1 and the CYP1 A1 inhibitor R-naphthoflavone revealed that CYP1A1 oxidized galangin to kaempferol. These results indicated that galangin was metabolized to kaempferol by TCDD-inducible CYP1A1 in Caco-2 cells. A previous study revealed that kaempferol had much weaker inhibitory activity than galangin toward TCDD-induced CYP1A1 expression. Therefore, the oxidative metabolism of galangin to kaempferol in TCDD-pretreated Caco-2 cells implicated reduction in the inhibitory activity of galangin.

Keywords: Caco-2, CYP1A1, galangin, keampferol, metabolism, TCDD

[*1] Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo
[*2] Department of Agrobioscience, Applied Chemistry in Bioscience Division, Graduate School of Agricultural Sciences, Kobe University

Tanaka, H., Takino, M.[*1], Sugita-Konishi, Y., Tanaka, T.[*2], Leeman, D.[*3], Toriba, A.[*4], Hayakawa, K.[*4]: **Determination of *Fusarium* mycotoxins by liquid chromatography/tandem mass spectrometry coupled with immunoaffinity extraction**

Rapid Communications in Mass Spectrometry., **24**(16), 2445-2452(2010)

A method for the simultaneous quantitative determination of deoxynivalenol (DON), T-2 toxin (T-2), HT-2 toxin (HT-2) and zearalenone (ZEN) in wheat and biscuit by liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) coupled with immunoaffinity extraction is described. A clean-up was carried out using a DZT MS-PREP® immunoaffinity column (IAC), and the effect of the sample dilution rate and sample loading was investigated. Furthermore, the effects of ion suppression of a multifunctional column (MFC) and the IAC in the clean-up were compared. The results with the DZT MS-PREP® IAC showed that it is possible to make the sample dilution rate low, and indicated a higher solvent-tolerance than usual with an IAC. Sample loading was optimized at 0.25μg. Ion suppression was lowered by urification of the toxins using the DZT MS-PREP- IAC. Recoveries of each mycotoxin from wheat and biscuit samples spiked at two levels ranged from 78 to 109%. The limits of detection in wheat and biscuit was in the range of 0.03-0.33-ng$\cdot$g^{-1}. From these studies, it is suggested that use of an IAC is effective in the clean-up of each mycotoxin, and, when combined with LC/ESI-MS/MS, it is good for the determination of mycotoxins in foodstuffs due to its rapidity and high sensitivity.

Keywords: LC/ESI-MS/MS, deoxynivalenol (DON), T-2 toxin (T-2), HT-2 toxin (HT-2) and zearalenone (ZEN)

[*1] Agilent Technologies Japan, Ltd

*2 Kobe Institute of Health
*3 R-Biopharm Rhone Ltd
*4 Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University

Matsukane, Y.*1, Sato, H.*1, Tanaka, S.*2, Kamata, Y., Sugita-Konishi, Y.: ***Kudoa iwatai* and two novel *Kudoa* spp., *K. trachuri* n. sp. and *K. thunni* n. sp. (Myxosporea: Multivalvulida), from daily consumed marine fish in western Japan**

Parasitol Res., **108**, 913-926 (2011)

Abstract Infection of marine fish by certain myxosporean species of the genus Kudoa results in unsightly cyst formation in the trunk muscle or post-mortem myoliquefaction, causing a great economic loss to aquaculture industries, capture fisheries, and fish dealers. In addition, consumers encountering unsightly Kudoa cysts in fish fillets believe them to be unknown foreign materials acquired during processing. To identify prevalent Kudoa spp. encountered in daily life by the Japanese population, fresh fish slices (sashimi) or fish fillets with whitish spots were collected during a 7-month period (May to December 2008) at local markets in the city of Yamaguchi, western Japan. Kudoa cysts were found in three Japanese seaperches (Lateolabrax japonicus), two black sea bream (Acanthopagrus schlegelii), two Japanese jack mackerel (Trachurus japonicus), and one albacore (Thunnus alalunga). Kudoa iwatai was identified in all the examined Japanese seaperch and black sea bream from Japan's Inland Sea, as assessed by morphology and genetic analysis of the 18S and 28S ribosomal RNA gene (rDNA). Kudoa trachuri n. sp. from two Japanese jack mackerel fished in the Japanese Sea off Nagasaki and Kudoa thunni n. sp. from one albacore fished in the Pacific Ocean had a spore, which was semiquadrate in shape in apical views and ovoid in lateral views, with four equal shell valves and drop-like polar capsules. Scanning electron microscopy revealed that these three Kudoa species had different types of small projections at the apex of each valve. The 18S and 28S rDNA sequences of K. trachuri n. sp. and K. thunni n. sp. were found to be closely related to those of Kudoa crumena; however, these sequences were distinct in each of the species, which additionally exhibited different morphological features.

Keywords: 粘液胞子虫，サバ，ビンチョウマグロ，黒ダイ，市販魚肉

---

*1 Laboratory of Veterinary Parasitology, Faculty of Agriculture Yamaguchi University
*2 Laboratory of Plant Pathology, Faculty of Agriculture, Yamaguchi University

Nakatani, Y.*1, Satoh, T.*2, Saito, S.*2, Watanabe, M.*3, Yoshiike, N.*4, Kumagai, S.*5, Sugita-Konishi, Y.: **Simulation of deoxynivalenol intake from wheat consumption in Japan using the Monte Carlo method**

Food Additives and Contaminants., **28** (4), 471-476 (2011)

The aim of this study was to evaluate the current advisory level in Japan for deoxynivalenol (DON) in foods. To this end, we estimated the intake of DON based on its presence in wheat using a probabilistic computer simulation method. Values for the concentration of DON in wheat were based on those reported in surveys of 638 wheat samples conducted from 2002 to 2004. Data regarding consumption of 108 wheat-based products according to age group were obtained from the 2002 Japan national survey on food consumption. Two data sets on the consumption of wheat-based products and contamination of DON in wheat were analysed using three DON regulatory scenarios: no regulation, 1100 mgkg_1 and 2000 mgkg_1. Because consumption distributions contained two peaks for each age category, it was assumed that two log-normal distributions for each age category were needed to achieve a better fit to the distribution models. The results of simulated DON intake using the Monte Carlo method showed that children aged 1-6 years have the highest DON intake. However, the 95th percentile of simulated intake of DON in each age group was below the provisional maximum tolerable daily intake (TDI) of 1 mgkg_1 body weight using any regulation scenario. The 99th percentile of simulated DON intake in the 1-6-year-old group was greater than TDI at approximately 2 mgkg_1 body weight. These results suggest that the current dietary intake of DON from wheat consumption does not exert a significant health effect, but we may need to reconsider the current regulation value for the 1-6-year-old age group. In addition, we may need a better method to fit the distribution to the log-normal distribution better.

Keywords: exposure assessment, risk assessment, mycotoxins, Fusarium, bakery products, bread

*1 Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University
*2 Kitasato Clinical Research Center, School of Medicine, Kitasato University
*3 Department of Preventive Medicine and Public Health, School of Medicine, Kitasato University
*4 Faculty of Health Sciences, Aomori University of Health and Welfare
*5 Research Center for Food Safety, The University of Tokyo

Lee, K.*1, French, N. P.*2, Hara-Kudo, Y., Iyoda, S.*3, ideki Kobayashi, H.*4, Sugita-Konishi, Y., Tsubone, H.*1, Kumagai, S.*1: **Multivariate Analyses Revealed Distinctive Features Differentiating Human and Cattle Isolates of Shiga Toxin-Producing *Escherichia coli* O157 in Japan**

Journal of Clinical Microbiology., **49**(4), 1495-1500(2011)

Genotypes of Shiga toxin-producing *Escherichia coli* (STEC) O157 isolated from humans and cattle were analyzed by uni- and multivariable logistic regression, and population structure methods, to gain insight into transmission and the nature of human infection. Eleven genotyping assays, including PCR typing of five virulence factors (*stx*$_1$, *stx*$_2$, *stx*$_{2c}$, *eae*, and *ehxA*) and a lineage-specific polymorphism assay using six markers (LSPA 6), were considered in the analyses. The prevalence of the *stx*$_1$, *stx*$_2$, and *stx*$_{2c}$ virulence factors was significantly different between human and cattle isolates. However, multivariable regression revealed that the presence of only the *stx*$_2$ gene was significantly associated with human isolates after controlling for confounding effects. LSPA6 typing demonstrated an apparent difference in the distribution of LSPA6 lineages between human and cattle isolates and a strong association between *stx* genotypes and LSPA6 genotypes. Population genetics tools identified three genetically distinct clusters of STEC O157. Each cluster was characterized by *stx* genotypes and LSPA6 genotypes. The human isolates typically comprised LSPA6 lineage I with *stx*$_1$ *stx*$_2$ strains and LSPA6 lineage I/II with *stx*$_2$ or *stx*$_2$ *stx*$_{2c}$ strains. In contrast, the cattle isolates comprised LSPA6 lineage II strains with *stx*$_{2c}$ or *stx*$_2$ *stx*$_{2c}$ strains in addition to the clusters identified for the human isolates. Our analyses provide new evidence that the *stx*$_2$ gene is the most distinctive feature in human isolates compared to cattle isolates in Japan, and only a subset of the genetically diverse population isolated from cattle is involved in human illnesses. Our results may contribute to international comparisons and risk assessments of STEC O157.

Keywords: Shiga toxin-producing *Escherichia coli* O157, *stx* genotype, LSPA6 genotype, molecular epidemiology, population genetics

*1 Graduate School of Agricultural and Life Sciences, the University of Tokyo
*2 Institute of Veterinary, Animal and Biomedical Sciences, Massey University
*3 Department of Bacteriology, National Institute of Infectious Diseases
*4 National Agriculture and Food Research Organization, National Institute of Animal Health

Sugiyama, K., Kawakami, H.*, Kamata, Y. and Sugita-Konishi, Y.: **Effect of a combination of deoxynivalenol and nivalenol on lipopolisaccharide-induced nitric oxide production by mouse macrophages**

Mycotoxin Res., **27**, 57-62(2011)

Deoxynivalenol (DON) and nivalenol (NIV) are trichothecene mycotoxins produced by Fusarium fungi as secondary metabolites. Both compounds have the immunotoxic effects that the productions of inflammatory mediators by activated macrophages is disturbed. Cocontamination with DON and NIV can occur; however, the effects of simultaneous contamination are not well known. The present study investigated the combined effects of DON and NIV on nitric oxide (NO) production by mouse macrophages stimulated with lipopolisaccharide (LPS). The inhibitory effect of DON and NIV on NO release from activated macrophages has already been reported as an appropriate indicator of immunotoxic effect of the both compounds. LPS-induced NO production in macrophages was inhibited by both of these toxins individually in a dose dependent manner, and toxin mixtures at the same concentration inhibited NO production in the same manner. These results suggest that the combined effects of DON and NIV can be predicted based on addition of each compound alone.

Keywords: deoxynivalenol, nivalenol, nitric oxide

* 共立女子大学

宮原美知子，荒川英二*：**市販二枚貝での腸炎ビブリオの季節変動**

防菌防黴誌，**38**，515-520（2010）

東京で市販しているアサリとハマグリについて腸炎ビブリオとその毒素産生性を4月から10月まで毎週3検体ずつ検討した．腸炎ビブリオの菌数についてはMPN法で検討した．腸炎ビブリオが確認されたのは77%の検体からで，その内の27%が *tdh* 産生遺伝子を，また29%が *trh* 産生遺伝子を保有していることがPCRで確認できた．最近日本での腸炎ビブリオ食中毒の発生は激減しているが，市販二枚貝のほとんどに腸炎ビブリオが検出され，海水温度の上昇とともに菌数も上昇し，その約30%に毒素産生性遺伝子が確認されたことから，二枚貝の調理や取り扱いには今後も気を付けなければならない．

Keywords：腸炎ビブリオ，二枚貝，季節変動

* 国立感染症研究所

Miyahara, M., Taguchi, M.*1, Kanki, M.*1, Kai, A.*2, Ishihara, T.*3, Kimata, H.*4, Gunji, A.*5 and Tsukamoto, T*6.: **A Collaborative Study on a Method to Detect *Salmonella* in Food**

Biocontrol Science, **15**, 69-73（2010）

Fourteen laboratories with expertise in *Salmonella* detection in food joined in a collaborative study. The laboratories performed qualitative analyses of ground pork samples using the proposed detection method. *Salmonella* Typhimurium（hydrogen sulfide-producing strain）and *Salmonella* Senftenberg（hydrogen sulfide-nonproducing strain）were used for inoculation. Three levels of *Salmonella* contamination were used for the study（0, 1-10, and 11-100 cfu/25 g）. We evaluated the presence of *Salmonella* in each sample and the serological O group. Unmarked samples delivered to the laboratories were accurately judged to be inoculated or not inoculated with *Salmonella* at a 99.8%（419 / 420）detection rate in this collaborative study. The proposed method is suitable as a standard method to detect *Salmonella* in food.

Keywords：collaborative study, *Salmonella* detection, standard method

*1 Osaka Prefectural Institute of Public Health
*2 Tokyo Metropolitan Institute of Public Health
*3 Kanagawa Prefectural Institute of Public Health
*4 Kobe Institute of Health
*5 Japan Food Research Laboratories（Osaka branch）
*6 TBL

Iibuchi, R.*, Hara-Kudo, Y., Hasegawa, A.* and Kumagai, S.*: **Survival of *Salmonella* on a polypropylene surface under dry conditions in relation to biofilm-formation capability**

J. Food Prot., **73**, 1506-1510（2010）

This study was conducted to gain insights into the survival of Salmonella on a polypropylene surface in relation to the ability of these bacteria to form a biofilm. We selected Salmonella strains known for the relative ease or difficulty with which they formed biofilms based on microtiter plate assays and studied the survival of these strains on polypropylene discs in a desiccation chamber by sequentially counting CFUs. The biofilm-forming strains survived longer on the plastic disc surface than did biofilmdeficient strains. The biofilm-forming strains remained at over 104 CFU per plate until day 175, whereas the biofilm-deficient strains decreased to below 102 CFU per plate on day 20 or below 104 CFU per plate on day 108. Extracellular materials on the polypropylene surface were observed by scanning electron microscopy and crystal violet staining for the biofilm-forming strains but not for the biofilm-deficient strains. The extracellular polymeric materials on the polypropylene surface may have protected the bacterial cells from dryness, although the possibility of some inherent resistance to environmental stresses linked to biofilm formation could not be excluded. These results indicate that Salmonella strains with high biofilm productivity may be a greater risk to human health via food contamination by surviving for longer periods compared with strains with low biofilm productivity.

Keywords：Salmonella, Survival, biofilm

* Tokyo University

田中廣行*1，土屋　禎*2，大島赴夫*2，鈴木達也*2，工藤由起子：**技能試験データに基づく細菌数の不確かさの推定**

日本食品微生物学会雑誌，**27**，158-162（2010）

食品衛生外部精度管理調査における各試験所の試験実施条件に関する情報の解析を行った．なお，解析の対象とした情報は，①試験実施者の実務年数，②試料調製時におけるフィルター処理（ろ過処理）の有無，③ホモジナイザーなどによる試料調製時間および④集落の計数方

法の4項目とし，トップダウン方式の1手法として，技能試験データに基づき細菌数の不確かさの推定を試みるとともに，技能試験における各試験所の試験実施条件に関する情報の解析を行った．
Keywords：不確かさ，細菌数

*1（財）日本食品分析センター
*2（財）食品薬品安全センター

森　哲也*1，田中廣行*2，和田真太郎*1，伊藤　武*1，宇田川藤江*2，工藤由起子：**市販の生食用カット野菜，カット果実およびスプラウトの微生物汚染調査**
日本食品微生物学会雑誌，**27**，163-170（2010）

本研究では，国内主要地域で流通・販売されている生食用カット野菜，カット果実およびスプラウトを対象として，一般生菌数を測定し，季節別，月別．地域別に比較した．また大腸菌（E. coli），サルモネラ，腸管出血性大腸菌（EHEC）および腸管毒素原性大腸菌（ETEC）の汚染状況を調べた．カット野菜，カット果実およびスプラウトの一般生菌数は，カット果実が4.3±1.1 Log CFU/g，カット野菜が5.7±1.1 Log CFU/g，スプラウトが7.7±0.5 Log CFU/gであった．カット野菜およびカット果実は夏季に菌数が高かった（$p<0.01$）が，スプラウトでは冬季，夏季で菌数の差は認められなかった．また，購入地域別での一般生菌数では一部地域で差が認められた．メロンの一般生菌数は，国産が4.1±1.3 Log CFU/g，輸入が5.0±1.2 Log CFU/gであり，輸入の菌数が国産と比較して高かった（$p<0.01$）．大腸菌（E. coli）は，カット野菜1,127検体中45検体（4.0%），カット果実504検体中3検体（0.6%），スプラウト470検体中20検体（4.3%）で陽性であった．サルモネラ，EHEC，ETECは，いずれの検体も陰性であった．以上の結果より，国内で市販されているカット野菜，カット果実およびスプラウトのサルモネラおよび病原大腸菌による汚染は低いことが推察された．しかし，一般生菌数および大腸菌（E. coli）の陽性率が夏季に高いことから，製造工程の衛生管理を徹底し一般生菌数を抑えることが，より衛生的な製品の供給に重要であり，今後病原微生物の検出が増加した場合には微生物基準の設定を考慮する必要がある．
Keywords：カット野菜，カット果実，微生物汚染

*1（財）東京顕微鏡院
*2（財）日本食品分析センター

Ohtsuka, K.*1, Tanaka, M.*2, Ohtsuka, M.*3, Takatori, K. and Hara-Kudo, Y.: **Comparison of detection methods for *Escherichia coli* O157 in beef livers and carcasses**
Foodborne Pathogen and Disease, **7**, 1563-1567 (2010)

Beef organ meat such as liver, and beef are major food sources contaminated with *Escherichia coli* O157. This study investigated the detection method of *E. coli* O157 in beef liver and carcass.In an experiment with beef liver inoculated with *E. coli* O157, the direct plating method, plating after the immunomagnetic separation (IMS) method, and Shiga toxin (Stx)-producing E. coli detection and *E. coli* O157 detection loop-mediated isothermal amplification (LAMP) assays were compared for the detection of Stx-producing *E. coli* O157.Fifty and 45 % of samples were positive by Stx-producing *E. coli* detection LAMP assay and *E. coli* O157 detection LAMP assay, respectively. Thirty-five and 10 % of samples were positive by the IMS method and direct plating method, respectively. In an examination of beef swab samples, contamination frequencies with *E. coli* O157 were analyzed by LAMP assays and the IMS method. *E. coli* O157 was detected in 12 of 230 samples (5.2 %). There was no sample in which is positive for *E. coli* O157 isolation but negative for LAMP assays for Stx gene and O157 antigen gene. Four samples (1.7 %) were positive by both LAMP assays but negative by the IMS method. Because there was no sample in which the O157 antigen gene was positive but not the Stx gene, this indicated that the IMS method failed to detect *E. coli* O157. Twenty-nine samples (12.6 %) were positive for the Stx gene but not the O157 antigen gene, indicating that screening Stx gene and O157 gene by LAMP assays are effective to save time and effort to isolate *E. coli* O157 by the IMS method because the LAMP assay is more sensitive. This suggested that samples positive for Stx gene and O157 gene should be examined by the IMS method to isolate *E. coli* O157.
Keywords: *E. coli* O157, beef, carcasses

*1 Saitama Institute of Public Health
*2 Saitama Prefectural Meat Inspection Center
*3 Department of Public Health and Medical Services, Food and Safety Division, Saitama Prefectural Government

Kadota, T.*1,2, Takezawa, Y.*1, Hirano, S.*1, Tajima, O.*1, Maragos, M. C.*3, Nakajima, T.*4, Tanaka, T.*5, Kamata, Y. and Sugita-Konishi, Y.: **Rapid detection**

of nivalenol and deoxynivalenol in wheat using surface plasmon resonance immunoassay

Analytica Chimica Acta, **673**, 173-178（2010）

小麦中のニバレノールとデオキシニバレノールの濃度を測るためにモノクローナル抗体を用いての表面プラズモン共鳴免疫測定法を開発した．

モノクローナル抗体を用いての拮抗阻害アッセイ法では，ニバレノールとデオキシニバレノールに交差反応が見られた．これらの結果は，共鳴プラズモンアッセイ法がニバレノールとデオキシニバレノールの共汚染を迅速にスクリーニングする有用な方法であった．

Keywords: Mycotoxin, Surface, Plasmon Resonance, Immunoassay

[*1] Central Laboratories for Frontier Technology, Center for Food Safety Science, Kirin Holdings Company, Limited
[*2] United Graduate School of Veterinary Sciences, Gifu University
[*3] Mycotoxin Research Unit, National Center for Agricultural Utilization Research, USDA-ARS
[*4] Research Team for Fusarium Heasd Blight Control, National Agricultural Research Center for Kyusyu-Okinawa Region
[*5] Kobe Institute of Health

Matsukane, Y.*, Sato, H.*, Tanaka, S., Kamata, Y. and Sugita-Konishi, Y.: **Kudoa septempunctata n. sp.（Myxosporea : Multivalvulida）from an aquacultured olive flounder（Paralichthys olivaceus）imported from Korea**

Parasitol Res, **107**, 865-872（2010）

韓国から輸入された養殖ヒラメ中の筋肉に存在した新しいミクソスポレアン種クドア・セプテンプンクタータについて記載する．この種は，炎症反応を起こすことなく筋肉中に偽シストを形成した．この種のスポアーは不規則な放線上で7つの極嚢を持っていた．スポアーは，幅11.8厚さ9.4長さ8.5マイクロメーターだった．この新しい種の，リボゾーマルRNA遺伝子はクドアサラソニーに97.6パーセント同一だった．この新しい種は，今まで知られているクドア属のすべてとスポアーの形とSSU rDNA配列が異なっていた．

Keywords: Kudoa septempunctata, Flounder, Aqua Culture

* 山口大学

小沼ルミ*1，渡辺麻衣子，工藤由起子，小西良子，高鳥浩介*2，一戸正勝*3，瓦田研介*1：**炭素源資化性分析を用いた環境汚染糸状菌の同定および同定精度の向上**

防菌防黴，**38**，363-369（2010）

MicroLog Systemを用いた炭素源資化性分析による環境汚染糸状菌4属30菌種での同定について検討した．その結果，⑴正しく同定されたのは供試真菌種のうち非好乾性の*Aspergillus*属で81.8%，*Penicillium*属で41.7%および*Cladosporium*属で100%であった；⑵正しく同定された菌種でもSIM規定値を下回る場合があり，同定精度に問題があった；⑶好乾性真菌用の前培養用平板培地の検討を行う必要があった；⑷供試真菌株でSIM値を補正した後のデータベースを用いて分析を行った結果，供試真菌株では比較的高い精度で菌種を同定できた；以上のことが明らかとなった．よって，炭素源資化性分析はSIM値の補正を行うことで形態学的および分子生物学的同定を補完する同定法として有効であることが示唆された．

Keywords: environmental filamentous fungi, identification, carbon source utilization

*1（地独）東京都立産業技術研究センター
*2 NPO法人カビ相談センター
*3 東京家政大学

Watanabe, M., Lee, K.*1, Goto, K.*2, Kumagai, S.*1, Sugita-Konishi, Y. and Hara-Kudo, Y.: **Rapid and effective DNA extraction method for a large amount of fungal DNA**

J. Food Prot., **73**, 1077-1084（2010）

To identify a rapid method for extracting a large amount of DNA from fungi related with food hygiene, extraction methods using fungal pellets formed rapidly in liquid media were compared. Combinations of physical, and chemical or enzymatic methods were evaluated with three species of yeast, 10 species of ascomycetous molds and four species of zygomycetous molds. Bead grinding was used as the physical method, followed by chemical methods using sodium dodecyl sulfate (SDS), cetyl trimethyl ammonium bromide (CTAB) and benzyl chloride, and two commercial kits. Quantity calculated by UV absorbance at 260 nm, quality by the ratio of UV absorbance at 260 and 280 nm, gene amplifications and electrophoresis profiles of whole genomes were analyzed. From the results, the combinations of bead grinding and SDS method, and bead grinding and CTAB method

were the most effective for DNA extraction for yeasts and ascomycetous molds, and zygomycetous molds, respectively. For both groups of molds, the combination of bead grinding and CTAB method was the best method. Because this combination is relatively effective for yeasts, it is effective to extract of a large amount of DNA from a wide range of fungi. The DNA extraction method is useful to develop gene indexes to identify fungi using a molecular method, such as DNA fingerprinting.

Keywords: fugal DNA extraction, liquid medium, bead grinding

---

*1 東京大学大学院
*2 三井農林（株）食品総合研究所

Watanabe, M., Masaki, H.*1, Mori, T.*2, Tsuchiya, T.*3, Konuma, H.*4, Hara-Kudo, Y. and Takatori, K.: **Inactivation effects on yeasts and molds in mineral water by UV irradiation and ozone treatment**

J. Food Prot., **73**, 1537-1542(2010)

In recent years, bottled mineral water goes through inactivation methods other than traditional heat treatment during the production process; however, there are fewer reports of the effects of these inactivation methods on yeasts and molds in mineral water than on bacteria and protozoan oocysts. In this study, we selected UV irradiation and ozone treatment as non-heat treatments and evaluated their effects on the yeast and the mold inoculated into mineral water compared with heat treatment at 85℃. The 5-log reduction occurred with 31, 433 μJ/cm^2 UV irradiation for *S.cerevisiae* or 588, 285 μJ/cm^2 for *P.pinophilum*. The treatment time for 5-log reduction estimated for UV irradiation was about 0.6 min for *S.cerevisiae* and about 10.7 min for *P.pinophilum*, whereas for the ozone concentration of 0.1 ppm, it was 1.75 min for *S.cerevisiae* and 2.70 min for *P.pinophilum*, and for the concentration of 0.6 ppm, it was 0.32 min for S. cerevisiae and 0.57 min for *P.pinophilum*. Comparison of the inactivation effects among the three methods showed that UV irradiation and ozone treatment had relatively less inactivation effect than heat treatment at 85℃. Therefore, when UV irradiation and ozone treatment are applied for inactivation of mineral water, it seems that they need the combination with heat treatment to achieve a definite effect. Furthermore, yeast cells are more sensitive to all three inactivation methods than mold spores, and there is a possibility that the sensitivity of yeast cells and mold spores to these inactivation methods varies among genera.

Keywords: UV irradiation, ozone treatment, fugal inactivation

---

*1 埼玉県衛生研究所
*2 （財）東京顕微鏡院
*3 （財）日本食品分析センター
*4 東海大学

Watanabe, M., Tsutsumi, F.*1, Lee, K.*2, Sugita-Konishi, Y., Kumagai, S.*2, Takatori, K.*3, Hara-Kudo, Y. and Konuma, H.*1: **Enumeration methods for fungal contaminants in fruits by the most probable number method**

J. Food Sci., **75**, 564-567(2010)

In this study, enumeration methods for fungi in foods were evaluated using fruits which are often contaminated by fungi in the field and rot because of fungal contaminants. As the test methods, we used the standard most probable number (MPN) method with liquid medium in test-tubes, which is traditionally used as the enumeration method for bacteria, and the plate-MPN method with agar plate media, in addition to the surface plating method as the traditional enumeration method for fungi. We tested 27 samples of nine commercial domestic fruits using their surface skin. The results indicated that the standard MPN method showed slow recovery of fungi in test-tubes and lower counts than the surface plating method and plate-MPN method in almost all samples.The fungal count on the fourth day of incubation was approximately the same as on the tenth day by the surface plating method or the plate-MPN method, indicating little difference between the fungal counts obtained by these two methods. Because fungal counts are estimated based on the number of plates with growing colonies in the plate-MPN method, the statistical procedure in this method can provide a more logical count than counting the number of colonies in the surface plating method. Moreover, the plate-MPN method is a little laborious. These advantages demonstrated that the plate-MPN method is a superior and rapid method for enumeration of fungi.

Keywords: most probable number method, fungal contamination, fruit

*1 東海大学
*2 東京大学大学院
*3 NPO 法人カビ相談センター

大西貴弘：**国産ミネラルウォーターのエンドトキシン濃度測定による水源およびその製造所における細菌汚染検出の試み**

日本食品微生物学雑誌，**27**，141-145（2010）

ミネラルウォーターの水源およびその製造所における細菌汚染の指標としてエンドトキシンを利用できるか検討を行った．国産ミネラルウォーター41銘柄中３銘柄において非殺菌のヨーロッパ産ミネラルウォーターの約５～15倍，水道水の約2.5～7.7倍という非常に高いエンドトキシン濃度を示した．グラム陰性菌とエンドトキシン濃度の関係を表す回帰曲線を作成したところ，この３銘柄のエンドトキシン濃度は約8.9×10^{3}～2.2×10^{4}cfu/mlのグラム陰性菌に相当した．さらにこの３銘柄中１銘柄から大腸菌群の遺伝子が検出された．以上の結果から，ミネラルウォーター中のエンドトキシン濃度を測定することはミネラルウォーターにおける殺菌・除菌前の細菌汚染をスクリーニングするのに有用であることが明らかになった．

Keywords：mineral water，endotoxin，limulus test

Moe, K.*1, Mimura, J.*1, Ohnishi, T., Wake, T.*1, Yamazaki, W.*2, Nakai, M.*1 and Misawa, N.*1.: **The mode of biofilm formation on Smooth surfaces by *Campylobacter jejuni***

J. Vet. Med. Sci., **74**, 411-416（2010）

Campylobacter jejuni has the ability to form biofilm. When bacterial suspensions in Brucella broth were incubated in microplate wells with a glass coverslip, microcolonies 0.5～2 mm in diameter were formed on the coverslip within 2 hr from the start of incubation. These microcolonies gradually grew and formed a biofilm of net-like connections within 6 hr. Transmission electron microscopy indicated that massive amounts of extracellular material masked the cell surface, and this material bound ruthenium red, suggesting the presence of a polysaccharide moiety. Scanning electron microscopy indicated that the flagella acted as bridges, forming net-like connections between the organisms. To determine the genes associated with biofilm formation, aflagellate (flaA−) and flagellate but non-motile (motA−) mutants were constructed from strain 81-176 by natural transformation-mediated allelic exchange. The flaA− and motA− mutants did not form the biofilm exhibited by the wild-type strain. These findings suggest that flagella-mediated motility as well as flagella is required for biofilm formation in vitro.

Keywords：*Campylobacter jejuni*, biofilm

---

*1 Miyazaki University
*2 Osaka Prefectural Institute of public Health

Horinouchi, T.*, Nakagawa, H.*, Suzuki, T.*, Fukuhara, K., Miyata, N.*: **Photoinduced nitric oxide release from a nitrobenzene derivative in mitochondria**

Chem. Eur. J., **17**, 4809-4813（2011）

We report a novel NO donor (RpNO), containing a 2,6-dimethylnitrobenzene moiety for photocontrollable NO release and a rhodamine moiety for targeting to mitochondria. Photorelease of NO from RpNO in aqueous solution was confirmed by means of ESR analysis. Cellular release of NO from RpNO was confirmed with the aid of DAF-FM-DA, an NO-specific fluorescence probe. RpNO was colocalized with MitoTracker Green-FM, a mitochondrial stain, in HCT116 colon cancer cells and exhibited photodependent cytotoxicity. Our results indicate that RpNO is an effective NO donor for time-controlled, mitochondria-specific NO treatment

Keywords：mitochondria, NO donor, photolysis

---

* 名古屋市立大学大学院薬学研究科

Horinouchi, T.*, Nakagawa, H.*, Suzuki, T.*, Fukuhara, K., Miyata, N.*: **A novel mitochondria-localizing nitrobenzene derivative as a donor for photo-uncaging of nitric oxide**

Bioorg. Med. Chem. Lett., **21**, 2000-2002（2011）

We report a novel green-fluorescent NO donor, NBDNO, bearing a 2,6-dimethylnitrobenzene moiety for photocontrollable NO release and a triphenylphosphonium moiety for targeting to mitochondria. Photorelease of NO from NBDNO was confirmed by means of ESR analysis in aqueous solution. Intracellular release of NO from NBDNO was confirmed by using DAR-4M AM, an NO-specific fluorescence probe. NBDNO was colocalized with MitoRed, a mitochondrial stain, in HCT116 colon cancer cells. Our results indicate that NBDNO is an effective NO donor for time-controlled, mitochondria-specific NO treatment.

Keywords：Nitric oxide, Photoinduced release, Mitochondria

* 名古屋市立大学大学院薬学研究科

Demizu, Y., Doi, M.*1, Kurihara, M., Okuda, H., Nagano, M.*2, Suemune, H.*2, Tanaka, M.*3: **Conformational studies on peptides containing α,α-disubstituted α-amino acids: chiral cyclic α,α-disubstituted α-amino acid as an α-helical inducer**

Org. Biomol. Chem., **9**, 3303-3312 (2011)

Four types of α,α-disubstituted amino acids {*i.e.*, α-aminoisobutyric acid (Aib), 1-aminocyclopentanecarboxylic acid (Ac_5c), (*3S*,*4S*)-1-amino-(3,4-dimethoxy) cyclopentanecarboxylic acid [(*S*,*S*)-Ac_5c^{dOM}] and its enantiomeric (*R*,*R*)-Ac_5c^{dOM}} were introduced into L-leucine-based hexapeptides and nonapeptides. The dominant conformations of eight peptides: Cbz-(L-Leu-L-Leu-dAA)$_2$-OMe [dAA = 1: Aib; 2: Ac_5c; 3: (*S*,*S*)-Ac_5c^{dOM}; 4: (*R*,*R*)-Ac_5c^{dOM}] and Boc-(L-Leu-L-Leu-dAA)$_3$-OMe [dAA = 5: Aib; 6: Ac_5c; 7: (*S*,*S*)-Ac_5c^{dOM}; 8: (*R*,*R*)-Ac_5c^{dOM}], were investigated by IR, CD spectra and X-ray crystallographic analysis. The CD spectra revealed that Aib hexapeptide 1 and Ac_5c hexapeptide 2 formed right-handed (*P*) 3_{10}-helices, while Ac_5c^{dOM} hexapeptides 3 and 4 formed a mixture of (*P*) 3_{10}- and α-helices. The Aib nonapeptide 5 formed a (*P*) 3_{10}-helix, the Ac_5c nonapeptide 6 formed a mixture of (*P*) 3_{10}- and α-helices, and the Ac_5c^{dOM} nonapeptides 7 and 8 formed (*P*) α-helices. X-ray crystallographic analysis revealed that the Aib hexapeptide 1 formed a (*P*) 3_{10}-helix, while (*S*,*S*)-Ac_5c^{dOM} hexapeptide 3 formed a (*P*) α-helix. In addition, the Ac_5c nonapeptide 6 and (*R*,*R*)-Ac_5c^{dOM} nonapeptide 8 formed (*P*) α-helices. The Aib and achiral Ac_5c residues have the propensity to form 3_{10}-helices in short peptides, whereas the chiral Ac_5c^{dOM} residues have a penchant for forming α-helices.

Keywords: peptide, helix, secondary structure

*1 大阪薬科大学
*2 九州大学薬学部
*3 長崎大学薬学部

Yamagata, N., Demizu, Y., Sato, Y., Doi, M.*1, Tanaka, M.*2, Nagasawa, K.*3, Okuda, H., Kurihara, M.: **Design of a stabilized short helical peptide and its application to catalytic enantioselective epoxidation of (*E*)-chalcone**

Tetrahedron Lett., **52**, 798-801 (2011)

Stabilized short helical heptapeptides containing a combination of an α-aminoisobutyric acid as a helical promoter and L/D-serine derivatives to produce cross-linked units were synthesized. The cyclic peptide $R_{3,7}R$-2, which had D-serine derivatives at its 3rd and 7th positions, formed a stable right-handed (*P*) α-helix in solution and the crystalline state. Furthermore, its N-terminal free helical peptide catalyzed the enantioselective epoxidation of (*E*)-chalcone to afford the epoxide in a high yield and moderate enantioselectivity.

Keywords: helix, X-ray crystallographic analysis, organocatalyst

*1 大阪薬科大学
*2 長崎大学薬学部
*3 東京農工大学大学院

Demizu, Y., Nakatsu, A., Honzawa, S.*1, Yamashita, A.*1, Sugiura, T.*1, Kittaka, A.*1, Kato, S.*2, Okuda, H., Kurihara, M.: **Facile synthesis of stereoisomers of the non-secosteroidal ligand LG190178 and their evaluation using the mutant vitamin D receptor**

Lett. Org. Chem., **8**, 43-47 (2011)

We developed a facile synthesis process for producing optically active non-secosteroidal ligands (YR301-304), which are stereoisomers of LG190178, and evaluated their performance in transcriptional assays using mutant vitamin D receptor (VDR). It was found that all of them had stronger activities than the natural ligand 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$]. In particular, YR301 showed potent activity for both wild-type and mutant Arg274Leu VDR.

Keywords: Mutant vitamin D receptor, Non-secosteroidal ligand, Transcriptional assay

*1 帝京大学
*2 東京大学大学院

Demizu, Y., Doi, M.*1, Sato, Y., Tanaka, M.*2, Okuda, H., Kurihara, M.: **Three-dimensional structure control of diastereomeric Leu-Leu-Aib-Leu-Leu-Aib sequences in the solid state**

J. Org. Chem., **75**, 5234-5239 (2010)

Three diastereomeric -Leu-Leu-Aib-Leu-Leu-Aib-peptides composed of the same numbers of L-Leu, D-Leu, and Aib residues were synthesized: Boc-L-Leu-L-Leu-Aib-D-Leu-D-Leu-Aib-OMe (1), Boc-L-Leu-D-Leu-

Aib-L-Leu-D-Leu-Aib-OMe(2), and Boc-L-Leu-D-Leu-Aib-D-Leu-L-Leu-Aib-OMe(3). The crystals of the three peptides were characterized by X-ray crystallographic analysis as follows: (1) orthorhombic, $P2_12_12_1$, a = 21.383 Å, b = 11.070 Å, c = 19.560 Å, Z = 4, R_1 = 0.0527, and R_w = 0.1562; (2) monoclinic, $P2_1$, a = 9.391 Å, b = 21.278 Å, c = 11.662 Å, β = 99.125, Z = 2, R_1 = 0.0507, and R_w = 0.1447; and (3) triclinic, P_1, a = 12.545 Å, b = 14.913 Å, c = 15.330 Å, α = 77.622, β = 66.601, γ = 78.839, Z = 2, R_1 = 0.0775, and R_w = 0.1971. The three diastereomeric peptides, 1, 2, and 3, showed unique conformations. That is to say, 1 was folded into a left-handed (*M*) 3_{10}-helical structure, 2 was folded into a distorted β-hairpin nucleated by a type II' β-turn-like structure, and 3 was folded into an S-shape turn structure based on two type II'/III β-turns.

Keywords: peptide, secondary structure, conformational analysis

*1 大阪薬科大学
*2 長崎大学薬学部

Demizu, Y., Tanaka, M.*1, Doi, M.*2, Kurihara, M., Okuda, H., Suemune, H.*3: **Conformations of peptides containing a chiral cyclic α,α-disubstituted α-amino acid within the sequence of Aib residues**

J. Pept. Sci., **16**, 621-626(2010)

A single chiral cyclic α,α-disubstituted amino acid, (*3S*,*4S*)-1-amino-(3,4-dimethoxy) cyclopentanecarboxylic acid [(*S*,*S*)-Ac_5c^{dOM}], was placed at the N-terminal or C-terminal positions of achiral α-aminoisobutyric acid (Aib) peptide segments. The IR and ^{1}H NMR spectra indicated that the dominant conformations of two peptides Cbz-[(*S*,*S*)-Ac_5c^{dOM}]-$(Aib)_4$-OEt (1) and Cbz-$(Aib)_4$-[(*S*,*S*)-Ac_5c^{dOM}]-OMe (2) in solution were helical structures. X-ray crystallographic analysis of 1 and 2 revealed that a left-handed (*M*) 3_{10}-helical structure was present in 1 and that a right-handed (*P*) 3_{10}-helical structure was present in 2 in their crystalline states.

Keywords: α-aminoisobutyric acid, chiral cyclic α,α-disubstituted amino acid, conformational analysis

*1 長崎大学薬学部
*2 大阪薬科大学
*3 九州大学薬学部

Nagano, M.*1, Doi, M.*2, Kurihara, M., Suemune, H.*1, Tanaka, M.*3: **Stabilized α-helix-catalyzed enantioselective epoxidation of α, β-unsaturated ketones**

Org. Lett., **12**, 3564-3566(2010)

Chiral cyclic α-amino acid containing oligopeptide catalyzed highly enantioselective epoxidation of α, β-unsaturated ketones and the α-helical secondary structure of the peptide catalyst were revealed by X-ray crystallographic analysis.

Keywords: peptide, X-ray crystallographic analysis, organocatalyst

*1 九州大学薬学部
*2 大阪薬科大学
*3 長崎大学薬学部

Itoh, Y.*, Ishikawa, M.*, Naito, M., and Hashimoto, Y.*: **Protein knockdown using methyl bestatin-ligand hybrid molecules: design and synthesis of inducers of ubiquitination-mediated degradation of cellular retinoic acid-binding proteins**

J. Am. Chem. Soc., **132**, 5820-5826(2010)

Induction of selective degradation of target proteins by small molecules (protein knockdown) would be useful for biological research and treatment of various diseases. To achieve protein knockdown, we utilized the ubiquitin ligase activity of cellular inhibitor of apoptosis protein 1 (cIAP1), which is activated by methyl bestatin (MeBS, 2). We speculated that formation of an artificial (nonphysiological) complex of cIAP1 and a target protein would be induced by a hybrid molecule consisting of MeBS (2) linked to a ligand of the target protein, and this would lead to cIAP1-mediated ubiquitination and subsequent proteasomal degradation of the target protein. To verify this hypothesis, we focused on cellular retinoic acid-binding proteins (CRABP-I and -II) and designed hybrid molecules (compounds 4) consisting of MeBS (2) coupled via spacers of various lengths to all-trans retinoic acid (ATRA, 3), a ligand of CRABPs. Compounds 4 induced selective loss of CRABP-I and -II proteins in cells. We confirmed that 4b induced formation of a complex of cIAP1 and CRABP-II in vitro and induced proteasomal degradation of CRABP-II in cells. When neuroblastoma IMR-32 cells were treated with 4b, the level of CRABP-II was reduced and cell migration was inhibited, suggesting potential value of CRABP-II-targeting therapy for controlling tumor metastasis. Our results indicate that 4b possesses sufficient activity, permeability, and stability in cells to be employed in

cellular assays. Hybrid molecules such as 4 should be useful not only as chemical tools for studying the biological/physiological functions of CRABPs but also as candidate therapeutic agents targeting CRABPs.

Keywords : ubiquitin, IAP, protein knockdown

---

* 東京大学分子細胞生物学研究所

Dohgu, S.*, Sumi, N.*, Nishioku, T.*, Takata, F.*, Watanabe, T.*, Naito, M., Shuto, H.*, Yamauchi, A.* and Kataoka, Y.* : **Cyclosporin A induces hyperpermeability of the blood-brain barrier by inhibiting autocrine adrenomedullin-mediated up-regulation of endothelial barrier function**

Eur. J. Pharmacol., **644**, 5-9 (2010)

Cyclosporin A, a potent immunosuppressant, can often produce neurotoxicity in patients, although its penetration into the brain is restricted by the blood-brain barrier (BBB). Brain pericytes and astrocytes, which are periendothelial accessory structures of the BBB, can be involved in cyclosporin A-induced BBB disruption. However, the mechanism by which cyclosporin A causes BBB dysfunction remains unknown. Here, we show that in rodent brain endothelial cells, cyclosporin A decreased transendothelial electrical resistance (TEER) by inhibiting intracellular signal transduction downstream of adrenomedullin, an autocrine regulator of BBB function. Cyclosporin A stimulated adrenomedullin release from brain endothelial cells, but did not affect binding of adrenomedullin to its receptors. This cyclosporin A-induced decrease in TEER was attenuated by exogenous addition of adrenomedullin. Cyclosporin A dose-dependently decreased the total cAMP concentration in brain endothelial cells. A combination of cyclosporin A (1microM) with an adenylyl cyclase inhibitor, 9-(tetrahydro-2-furanyl)-9H-purin-6-amine (SQ22536 ; 10microM), or a protein kinase A (PKA) inhibitor, N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride (H89 ; 1microM), markedly increased sodium fluorescein permeability in brain endothelial cells, whereas each drug alone had no effect. Thus, these data suggest that cyclosporin A inhibits the adenylyl cyclase/cyclic AMP/PKA signaling pathway activated by adrenomedullin, leading to impairment of brain endothelial barrier function.

Keywords : blood-brain barrier, cyclosporin A

---

* 福岡大学薬学部

Okuhira, K., Fitzgerald, M. L.*, Tamehiro, N.*, Ohoka, N., Suzuki, K., Sawada, J., Naito, M. and Nishimaki-Mogami, T. : **Binding of PDZ-RhoGEF to ATP-binding cassette transporter A1 (ABCA1) induces cholesterol efflux through RhoA activation and prevention of transporter degradation**

J. Biol. Chem., **285**, 16369-16377 (2010)

ATP-binding cassette transporter A1 (ABCA1)-mediated lipid efflux to apolipoprotein A1 (apoA-I) initiates the biogenesis of HDL. Here we show the Rho guanine nucleotide exchange factors PDZ-RhoGEF and LARG bind to C-terminus of ABCA1 by a PDZ-PDZ interaction, and prevent ABCA1 protein degradation by activating RhoA. ABCA1 is a protein with a short half-life, and apoA-I stabilizes ABCA1 protein, however depletion of PDZ-RhoGEF/LARG by RNAi suppressed the apoA-I stabilization of ABCA1 protein in human primary fibroblasts. Exogenous PDZ-RhoGEF expression activated RhoA and increased ABCA1 protein levels and cholesterol efflux activity. Likewise, forced expression of a constitutively-active RhoA mutant significantly increased, whereas a dominant-negative RhoA mutant decreased ABCA1 protein levels. The constitutively-active RhoA retarded ABCA1 degradation, thus accounting for its ability to increase ABCA1 protein. Moreover, stimulation with apoA-I transiently activated RhoA, and the pharmacological inhibition of RhoA or the dominant-negative RhoA blocked the ability of apoA-I to stabilize ABCA1. Finally, depletion of RhoA or RhoGEFs/RhoA reduces the cholesterol efflux when transcriptional regulation via PPARgamma is eliminated. Taken together, our results have identified a novel physical and functional interaction between ABCA1 and PDZ-RhoGEF/LARG, which activates RhoA resulting in ABCA1 stabilization and cholesterol efflux activity.

Keywords : atherosclerosis, HDL, ABCA1

---

* Massachusetts General Hospital, Harvard Medical School

Sakai, S.*[1], Ohoka, N., Onozaki, K.*[1], Kitagawa, M.*[2], Nakanishi, M.*[3], Hayashi, H.*[1] : **Dual mode of regulation of cell division cycle 25 A protein by TRB3**

Biol. Pharm. Bull., **33** (7), 1112-1116 (2010)

We have recently demonstrated that TRB3, a novel

stress-inducible protein, is an unstable protein regulated by the ubiquitin-proteasome system. The expression level of TRB3 protein is down-regulated by anaphase-promoting complex/cyclosome-cell division cycle division 20 homolog 1（APC/C(Cdh1)）through its D-box motif. Here we demonstrate that TRB3 regulates the stability of cell division cycle 25 A（Cdc25A), an essential activator of cyclin dependent kinases（CDKs). The expression level of Cdc25A protein is suppressed by over-expression of TRB3, while knockdown of TRB3 enhances the endogenous Cdc25A expression level. On the other hand, Cdc25A degradation induced by DNA damage is significantly rescued by TRB3. When serine residues in the DSG motif, which is the critical sequences for the degradation of Cdc25A induced by DNA damage, is mutated to alanine（Cdc25A(DSG2X)), both stimulatory and protective effects of TRB3 on the Cdc25A degradation is disappeared. TRB3 protein interacts with both wild Cdc25A and mutant Cdc25A (DSG2X). Expression level of the endogenous TRB3 protein is down-regulated in a genotoxic condition. These results suggest TRB3 is a regulator for adjusting the expression level of Cdc25A both in a normal and a genotoxic conditions.

Keywords : TRB3, Cdc25A, ubiquitin

---

*1 名古屋市立大学薬学研究科
*2 浜松医科大学
*3 名古屋市立大学医学研究科

Kim, S., Ohoka, N., Okuhira, K., Sai, K., Nishimaki-Mogami, T., Naito, M.: **Modulation of RIP1 ubiquitylation and distribution by MeBS to sensitize cancer cells to tumor necrosis factor α-induced apoptosis**

Cancer Sci., **101**(11), 2425-2429(2010)

Overexpression of anti-apoptosis protein cIAP1 due to its genetic amplification is found in certain cancers such as esophageal squamous cell carcinoma, hepatocellular carcinoma, cervical cancer and lung cancer, and plays a significant role in resistance to cancer therapy. We previously reported that a class of small molecules represented by (−)-N-[(2S, 3R)-3-amino-2-hydroxy-4-phenyl-butyryl]-L-leucine methyl ester（MeBS) activates auto-ubiquitylation of cIAP1 for proteasomal degradation, and enhances apoptosis of various cancer cells. However, the molecular mechanism of how MeBS sensitizes cancer cells to apoptosis via downregulation of cIAP1 is not well understood. Here, we show that ubiquitylation and distribution of RIP1, a protein ubiquitylated by cIAP1, is modulated by MeBS. Upon tumor necrosis factor (TNF) α stimulation, ubiquitylated RIP1 associates with the TNF-receptor (TNFR) complex, whereas non-ubiquitylated RIP1 associates with caspase 8. MeBS reduces the ubiquitylated RIP1 in the TNFR complex and increases non-ubiquitylated RIP1 bound to caspase8. Downregulation of RIP1 by siRNA reduces apoptosis induced by TNFα plus MeBS treatment. These results indicate an important role of RIP1 in apoptosis induced by combined treatment with TNFα and MeBS, suggesting that MeBS sensitizes cancer cells to apoptosis by modulating RIP1 ubiquitylation and distribution.

Keywords : MeBS, TNFα, RIP1

Iguchi, Y.*, Yamaguchi, M.*, Sato, H.*, Kihira, K.*, Nishimaki-Mogami, T. and Une, M.*: **Bile alcohols function as the ligands of membrane-type bile acid-activated G protein-coupled receptor**

J. Lipid Res., **51**, 1432-1441(2010)

TGR5 is a G protein-coupled receptor that is activated by bile acids, resulting in an increase in cAMP levels and the subsequent modulation of energy expenditure in brown adipose tissue and muscle. Therefore, the development of a TGR5-specific agonist could lead to the prevention and treatment of various metabolic disorders related to obesity. In the present study, we evaluated the ability of bile alcohols, which are structurally and physiologically similar to bile acids and are produced as the end products of cholesterol catabolism in evolutionarily primitive vertebrates, to act as TGR5 agonists. In a cell-based reporter assay and a cAMP production assay performed in vitro, most bile alcohols with a side chain containing hydroxyl group(s) were highly efficacious agonists for TGR5 comparable to its most potent ligand in the naturally occurring bile acid, lithocholic acid. However, the abilities of the bile alcohols to activate TGR5 varied with the position and number of the hydroxyl substituent in the side chain. Additionally, the conformation of the steroidal nucleus of bile alcohols is also important for its activity as a TGR5 agonist. Thus, we have provided new insights into the structure-activity relationships of bile alcohols as TGR5 agonists.

Keywords : TGR5, bile alcohol, bile acid

＊広島国際大学薬学部

Iguchi, Y.＊, Nishimaki-Mogami, T., Yamaguchi, M.＊, Teraoka, F.＊, Kaneko, T.＊ and Une, M.＊: **Effects of chemical modification of ursodeoxycholic acid on TGR5 activation**

Biol. Pharm. Bull., **34**, 1-7 (2011)

The aim of this study is to examine the ability of the bile acid analogues obtained by chemical modification of ursodeoxycholic acid (UDCA) for TGR5 activation. Eleven UDCA analogues including 3- or 7-methylated UDCAs and amino acid conjugates were investigated as to their ability to activate TGR5 by means of the luciferase assay. It was noteworthy that 7α-methylated UDCA, namely 3α, 7β-dihydroxy-7α-methyl-5β-cholanoic acid, had a significantly high affinity for and ability to activate TGR5 as compared to UDCA. Additionally, FXR activation ability of 7α-methylated UDCA was low relative to that of UDCA. However, other modification of UDCA, such as the introduction of methyl group at its C-3 position and oxidation or epimerization of hydroxyl group in the C-3 position, could not elicit such remarkable effect. The present findings would provide a useful strategy for the development of TGR5-selective agonist.

Keywords: TGR5, bile alcohol, bile acid

＊広島国際大学薬学部

Cui, H., Okuhira, K., Ohoka, N., Naito, M., Kagechika, H.＊, Hirose, A. and Nishimaki-Mogami, T.: **Tributyltin chloride induces ABCA1 expression and apolipoprotein A-I-mediated cellular cholesterol efflux by activating LXRalpha/RXR**

Biochem. Pharmacol., **81**, 819-824 (2011)

Organotins, including tri-butyltin chloride (TBTC), are widely used in agricultural and chemical industries and cause persistent and widespread pollution. TBTC has been shown to activate nuclear receptor retinoid X receptor (RXR)/PPARγ signaling by interacting with RXR to modulate adipogenesis. However, whether TBTC affects liver X receptor (LXR)/RXR activity and subsequently the expression of cholesterol mobilizing genes is not known. In this study, we evaluated the ability of TBTC to activate LXR/RXR and ABC transporter A1 (ABCA1) expression. ABCA1 plays a critical role in HDL generation, maintaining cholesterol homeostasis, and cholesterol accumulation-induced diseases, such as atherosclerosis and pancreatic islet dysfunction. In a reporter gene assay, TBTC activated LXRα/RXR but not LXRβ/RXR. In mouse macrophage RAW264 cells, TBTC activated the ABCA1 promoter in an LXR-responsive element dependent manner and increased ABCA1 mRNA expression. TBTC augmented ABCA1 protein levels and apolipoprotein A-I-dependent cellular cholesterol efflux (HDL generation). The LXR-target fatty acid synthase and Spα mRNA levels were also increased by TBTC exposure. We conclude that TBTC has the ability to activate permissive LXRα/RXR signaling and thereby modulate cellular cholesterol efflux.

Keywords: Tributyltin, RXR, ABCA1

＊東京医科歯科大学

Sai, K., Saito, Y., Tatewaki, N., Hosokawa, M.＊[1], Kaniwa, N., Nishimaki-Mogami, T., Naito, M., Sawada, J., Shirao, K.＊[2], Hamaguchi, T.＊[2], Yamamoto, N.＊[2], Kunitoh, H.＊[2], Tamura, T.＊[2], Yamada, Y.＊[2], Ohe, Y.＊[2], Yoshida, T.＊[2], Minami, H.＊[2], Ohtsu, A.＊[2], Matsumura, Y.＊[2], Saijo, N.＊[2], Okuda, H.: **Association of carboxylesterase 1A genotypes with irinotecan pharmacokinetics in Japanese cancer patients**

Br. J. Clin. Pharmacol., **70**, 222-233 (2010)

AIMS Human carboxylesterase 1 (CES1) hydrolyzes irinotecan to produce an active metabolite SN-38 in the liver. The human CES1 gene family consists of two functional genes, *CES1A1* (*1A1*) and *CES1A2* (*1A2*), which are located tail-to-tail on chromosome 16q13-q22.1 (*CES1A2-1A1*). The pseudogene *CES1A3* (*1A3*) and a chimeric *CES1A1* variant (*var1A1*) are also found as polymorphic isoforms of *1A2* and *1A1*, respectively. In this study, roles of *CES1* genotypes and major SNPs in irinotecan pharmacokinetics were investigated in Japanese cancer patients. ***METHODS*** *CES1A* diplotypes [combinations of haplotypes A (*1A3-1A1*), B (*1A2-1A1*), C (*1A3-var1A1*) and D (*1A2-var1A1*)] and the major SNPs (-75T>G and -30G>A in *1A1*, and -816A>C in *1A2* and *1A3*) were determined in 177 Japanese cancer patients. Associations of *CES1* genotypes, number of functional *CES1* genes (*1A1*, *1A2* and *var1A1*) and major SNPs, with the AUC ratio of (SN-38 + SN-38G)/irinotecan, a parameter of in vivo CES activity, were analyzed for 58 patients treated with irinotecan monotherapy. ***RESULTS*** The median

AUC ratio of patients having three or four functional *CES1* genes (diplotypes A/B, A/D or B/C, C/D, B/B and B/D; n= 35) was 1.24-fold of that in patients with two functional *CES1* genes (diplotypes A/A, A/C and C/C; n= 23) [median (25th-75th percentiles): 0.31 (0.25-0.38) vs. 0.25 (0.20-0.32), P= 0.0134]. No significant effects of *var1A1* and the major SNPs examined were observed. ***CONCLUSION*** This study suggests a gene-dose effect of functional *CES1A* genes on SN-38 formation in irinotecan-treated Japanese cancer patients.

Keywords: carboxylesterase, irinotecan, genetic polymorphism

*1 Faculty of Pharmaceutical Sciences, Chiba Institute of Science

*2 National Cancer Center

Nakamura, R., Satoh, R., Nakamura, R., Shimazaki, T.*1, Kasuga, M.*2, Yamaguchi-Shinozaki, K.*2, Kikuchi, A.*1, Watanabe, K. N.*2, Teshima, R.: **Immunoproteomic and 2D-DIGE Analysis of *Arabidopsis* DREB1A-Transgenic Potato**

Biol. Pharm. Bull., **33**(8), 1418-1425(2010)

To produce crops that are more tolerant to stresses such as heat, cold, and salt, transgenic plants have been produced those express stress-associated proteins. In this study, we used immunoproteomic and two-dimensional difference gel electrophoresis (2D-DIGE) methods to investigate the allergenicity of transgenic potatoes expressing Arabidopsis DREB1A (dehydration responsive element-binding protein 1A), driven by the rd29A promoter or the 35S promoter. Immunoproteomic analysis using sera from potato-allergic patients revealed several immunoglobulin E (IgE)-binding protein spots. The patterns of protein binding were almost the same between transgenic and non-transgenic potatoes. The IgE-binding proteins in potato were identified as patatin precursors, a segment of serine protease inhibitor 2, and proteinase inhibitor II by matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) MS/MS. 2D-DIGE analysis revealed several differences in protein expression between non-transgenic potato and transgenic potato; those showing increased expression in transgenic potatoes were identified as precursors of patatin, a major potato allergen, and those showing decreased expression in transgenic potatoes were identified as lipoxygenase and glycogen (starch) synthase. These results suggested that transgenic potatoes may express slightly higher levels of allergens, but their IgE-binding patterns were almost the same as those of control potatoes. Further research on changes in protein expressions in response to environmental factors is required to confirm whether the differences observed in this study are due to gene transfection, rather than environmental factors.

Keywords: transgenic crops, DREB1A, potato

*1 Graduate School of Life and Environmental Sciences, University of Tsukuba

*2 Biological Resources Division, Japan International Research Center for Agricultural Sciences (JIRCAS)

Nakamura, R., Nakamura, R., Watanabe, K.*, Oka, K.*, Ohta, S.*, Mishima, S.*, Teshima, R.: **Effects of propolis from different areas on mast cell degranulation and identification of the effective components in propolis**

Int. Immunopharmacol., **10**(9), 1107-12(2010)

Propolis is considered to down-regulate type I allergy, but the effective components of propolis remain unknown. In addition, propolis components vary depending on the area from which they are collected due to variations among wild plants in an area. Therefore, we compared the effects of water and ethanol extracts of propolis from Brazil and China on mast cell degranulation and cytokine production, thereby identifying effective components in propolis. The amount of released beta-hexosaminidase via high-affinity IgE receptor I (Fc epsilon RI) from rat basophilic leukemia (RBL-2H3) cells was used as an index of degranulation. All propolis extracts inhibited degranulation from antigen-stimulated RBL-2H3 cells, but the effective doses differed according to collection areas. The ethanol extract of Chinese propolis, which was the strongest inhibitor of mast cell degranulation, was divided into compounds using normal- and reversed-phase liquid chromatography. The isolated anti-allergic components were identified as chrysin, kaempferol and its derivative, and chrysin was revealed to inhibit IL-4 and MCP-1 production from antigen-stimulated RBL-2H3 cells. HPLC quantification also revealed the Brazilian propolis extract to contain only small amounts of these flavonoids, which suggested that variation in propolis components could affect anti-allergic properties.

Keywords : Propolis, Mast cells, Flavonoids

---

* Research Center, API Co., Ltd.

Nakamura, R., Uchida, Y., Higuchi, M., Nakamura, R., Tsuge, I.*, Urisu, A.* and Teshima, R. : **A convenient and sensitive allergy test : IgE crosslinking-induced luciferase expression in cultured mast cells**

Allergy, **65**, 1266-1273 (2010)

Background : For the detection of allergen-specific IgE in sera, solid-phase IgE-binding assays like the CAP test are commonly used. Although such immunochemical methods are very sensitive, they frequently produce false positives. Degranulation of the human IgE receptor (FcεRI)-transfected rat mast cell (RBL) lines seems to be a possible indicator for human IgE, but spontaneous mediator release from these cells in the presence of human sera is not negligible.

Methods : The nuclear factor of activated T-cells (NFAT)-responsive luciferase reporter gene was stably transfected into human FcεRI-expressing RBL-SX38 cells. One established clone (RS-ATL8) was sensitized with 1 : 100 dilution of sera from egg white allergy patients and then stimulated with purified or a crude extract of egg white allergen.

Results : Sensitization with 15pg/ml IgE was sufficient to detect IgE crosslinking-induced luciferase expression (EXiLE) by anti-IgE stimulation. Allergen-specific EXiLE was elicited by as little as 1fg/ml of egg white protein without cytotoxicity. There was a good correlation between results with EXiLE and oral food challenge tests on egg-allergy patients (P=0.001687, Fisher's exact test). The measured values of EXiLE and the CAP test also correlated well (R=0.9127, Spearman's test).

Conclusion : The EXiLE test using RS-ATL8 cells is a promising *in vitro* IgE test to evaluate the biological activity of the binding between IgE and allergens.

Keywords : IgE, allergy test, mast cell

---

* 藤田保健衛生大学医学部

Teshima, R., Nakamura, R., Satoh, R., Nakamura, R. : **2D-DIGE analysis of rice proteins from different cultivars**

Regul. Toxicol. Pharmacol., **58** (3 Suppl.), S30-S35 (2010)

The 2D-DIGE (2-Dimensional Fluorescence Difference Gel Electrophoresis) method was applied to proteomic phenotyping of natural variants in 10 varieties of rice (Nipponbare, Koshihikari, Sasanishiki, Akitakomachi, Hitomebore, Hinohikari, Kasalath, Rexark, Bleiyo, Choko) from the world rice collection (WRC) in the Gen Bank of the National Institute of Agrobiological Sciences (NIAS), Japan.

Salt-soluble protein extracts of Nipponbare brown rice were labeled with Cy2 fluorochrome and used as an internal standard. Protein extracts from nine other rice varieties were labeled with Cy3 or Cy5 fluorochrome and applied to 2D-PAGE (13-cm gel length) analysis. Approximately 700 spots of rice proteins were observed. Fluorescence intensities of each of these spots for the nine rice varieties were expressed as relative ratios to that of Nipponbare. Statistical analysis revealed the spot numbers of five Japanese rice varieties above threshold five (relative ratio of each variety to Nipponbare exceeded 5-fold or was less than 1/5) to be less than three, while those of four varieties from other countries were more than five (especially, Kasalath which was 29 and Bleiyo which was 23). The 2D-DIGE method seems to be useful for analyzing natural varieties of different cultivars and also for comparing the expression of allergen proteins.

Keywords : Rice seed, Allergen, Proteomics

Satoh, R., Koyano, S., Takagi, K., Nakamura, R., Teshima, R. : **Proteomic analysis of known and candidate rice allergens between non-transgenic and transgenic plants**

Regul. Toxicol. Pharmacol., **59** (3), 437-444 (2011)

Salt-soluble proteins extracted from non-transgenic and transgenic rice were evaluated for the presence of known and potential allergens by proteomic techniques. The salt-soluble proteins were extracted, separated by 1D and 2D electrophoresis, and analyzed by Western blotting. 1D immunoblot analysis with patients' sera revealed few qualitative differences between the IgE-binding proteins of the non-transgenic and transgenic rice. 1D immunoblot with antigen-specific-animal sera revealed no qualitative or quantitative differences in two known allergens, RAG2 and glyoxalase I, between non-transgenic and transgenic rice. Multiple spots containing known and novel IgE-binding proteins were detected among the salt-soluble proteins of non-transgenic rice by 2D immunoblotting. Two globulin-like

proteins, a 52 kDa protein and a 63 kDa protein, were identified as novel IgE-binding proteins that are candidates for rice allergens. These globulin-like proteins were homologous to Cupin superfamily allergens. Quantitative analysis of 19, 52, and 63 kDa globulins with protein-specific-animal sera showed no significant differences in the expression of these proteins between the transgenic rice and non-transgenic rice. These results indicate that none of the known or novel endogenous IgE-binding proteins detected in this study appear to be altered by genetic modification.

Keywords: Rice, Transgenic, IgE-binding protein, Allergen, Proteomics

Oguchi, T.[*1], Onishi, M.[*2], Mano, J.[*1], Akiyama, H., Teshima, R., Futo, S.[*2], Furui, S.[*1], Kitta, K.[*1]: **Development of multiplex PCR method for simultaneous detection of four events of genetically modified maize: DAS-59122-7, MIR604, MON863 and MON 88017**

Food Hyg. Saf. Sci., **51**, 92-100 (2010)

A novel multiplex PCR method was developed for the simultaneous event-specific detection of four events of GM maize, i.e. DAS-59122-7, MIR604, MON88017, and MON863. A single laboratory validation study suggested that the limits of detection (LOD) of the mutiplex PCR method were 0.16% for MON863, MIR604, and MON 88017, and 0.076% for DAS-59122-7. We have previously developed a nonaplex (9plex) PCR method for eight events of GM maize, i.e. Bt11, Bt176, GA21, MON810, MON863, NK603, T25, and TC1507. Together with the nonaplex PCR method, the newly developed method enabled to detect and identify eleven GM maize events, which are frequently used for a commercial purpose. In addition, this combinational analysis may be useful for the detection of combined event products of GM maize.

Keywords: genetically modified (GM), maize (Zea mays), multiplex PCR

[*1] National Food Research Institute
[*2] Fasmac Co., Ltd.

Sato, Y., Akiyama, H., Matsuoka, H., Sakata, K., Nakamura, R., Ishikawa, S.*, Inakuma, T.*, Totsuka, M., Sugita-Konishi, Y., Ebisawa, M., Teshima, R.: **Dietary Carotenoids Inhibit Oral Sensitization and the Development of Food Allergy**

J. Agric. Food Chem., **58**, 7180-7186 (2010)

Type I allergic disorders, and particularly food hypersensitivities, are becoming increasingly common worldwide. This study investigated whether dietary enrichment with carotenoids inhibited oral sensitization to an antigen and the development of food allergies. The effects of a diet high in carotenoids were investigated in B10A mice that were orally sensitized to ovalbumin (OVA). The serum titers of OVA-specific immunoglobulin E (IgE), IgG1, and IgG2a were inhibited in mice fed *ad libitum* on a diet high in α-carotene or β-carotene, compared with the control mice, when orally sensitized to OVA. High α-carotene and β-carotene diets inhibited the immediate reduction in body temperature and rise in serum histamine associated with active systemic anaphylaxis in OVA-sensitized B10A mice. After re-stimulation with OVA in vitro, the production of T-helper 2-type cytokines by splenocytes from mice fed a diet high in carotenoids was lower than in control mice. Furthermore, the proportion of $CD4^+$ $CD103^+$ T cells in the Peyer's patches of mice fed a carotenoid-rich diet was significantly lower than in control mice. These results suggest that an increased oral intake of carotenoids inhibits OVA-specific IgE and IgG1 production, and antigen-induced anaphylactic responses, by inhibiting specific T-cell activation in the mucosal immune system. A diet high in carotenoids might therefore prevent the development of food allergies.

Keywords: β-carotene, food allergy, IgE

* Research Institute, Kagome Company, Limited

Abbott, M.[*1], Hayward, S.[*1], Ross, W.[*1], Godefroy, S. B.[*1], Ulberth, F.[*2], Van Hengel, A. J.[*2], Roberts, J.[*3], Akiyama, H., Popping, B.[*4], Yeung, J. M.[*5], Wehling, P.[*6], Taylor, S. L.[*7], Poms, R. E.[*8], Delahaut, P.[*9]: **Validation Procedures for Quantitative Food Allergen ELISA Methods: Community Guidance and Best Practices**

J. AOAC Int., **93**, 442-450 (2010)

This document provides supplemental guidance on specifications for the development and implementation of studies to validate the performance characteristics of quantitative ELISA methods for the determination of food allergens. It is intended as a companion document to other existing publications on method validation. The guidance is divided into two sections: information

to be provided by the method developer on various characteristics of the method, and implementation of a multilaboratory validation study. Certain criteria included in the guidance are allergen-specific. Two food allergens, egg and milk, are used to demonstrate the criteria guidance. These recommendations will be the basis of the harmonized validation protocol for any food allergen ELISA method, whether proprietary or nonproprietary, that will be submitted to AOAC and/or regulatory authorities or other bodies for status recognition. Regulatory authorities may have their own particular requirements for data packages in addition to the guidance in this document. Future work planned for the implementation and validation of this guidance will include guidance specific to other priority allergens.

Keywords: allergen, detection, ELISA, validation

*1 Health Canada, Food Directorate, Health Products and Food Branch
*2 European Commission, Joint Research Centre, Institute for Reference Materials and Measurements
*3 National Measurement Institute, Department of Innovation, Industry, Science and Research
*4 Eurofins Scientific
*5 Nestlé Nutrition, Product Technology Center-Fremont
*6 General Mills, Inc.
*7 Food Allergy Research and Resource Program, University of Nebraska
*8 ICC-International Association for Cereal Science and Technology
*9 CER Groupe-Laboratoire d'Hormonologie

Akiyama, H., Sakata, K., Spiegelhalter, F.*1, Furui, S.*2, Nakashima, A.*3, Kitta, K.*2, Teshima, R.: **Interlaboratory Validation of an Event-Specific Real time Polymerase Chain Reaction Detection Method for Genetically Modified DAS59132 maize**

Food Hyg. Saf. Sci., **51**, 65-70 (2010)

A real-time polymerase chain reaction (PCR) method specific for genetically modified (GM) maize event DAS 59132 (E32) was adapted for qualitative detection of low level presence of E32. The method was validated by a collaborative trial with eight participating Japanese laboratories. Sensitivity was assessed with three different samples of corn flour fortified to 0%, 0.05% and 0.1% (w/w) E32 respectively. In addition, a 0.01% E32 DNA solution was used. The detection limit with DNA solution was estimated to be approximately 0.01%. In conclusion, the results of the study confirmed this real-time PCR method as a reliable tool for qualitative detection of E32 maize.

Keywords: genetically modified maize, recombinant DNA, real time PCR, DAS59132

*1 Eurofins GeneScan, Inc.
*2 National Food Research Institute
*3 Hiroshima Prefectural Institute of Public Health and Environment

Mano, J.*, Yanaka, Y.*, Akiyama, H., Teshima, R., Furui, S.*, Kitta, K.*: **Improvement of polymerase chain reaction-based Bt11 maize detection method by reduction of non-specific amplification**

Food Hyg. Saf. Sci., **51**, 32-36 (2010)

The Bt11 maize-specific qualitative detection method based on polymerase chain reaction (PCR) in the JAS analytical test handbook has been widely used for administrative monitoring of GM crops and quality control of commercially distributed grains. In the present investigation, some apparently false-positive detections were observed in assays using the Bt11 maize-specific method, and these erroneous results were proved to have been caused by non-specific DNA amplification. We improved the detection method to reduce non-specific amplification by decreasing the concentration of magnesium ions in the PCR mixture. The subsequent evaluation of analytical performance demonstrated no marked difference between the currently used and the improved methods, except for the reduced non-specific amplification. We conclude that the currently used standard method should be replaced with the improved method for the reliable detection of Bt11 maize.

Keywords: polymerase chain reaction (PCR), genetically modified organism (GMO), non-specific amplification

* National Food Research Institute

清木興介*1，織田浩司*1，柴原裕亮*2，蒲生玲子*3，有馬優美*3，酒井信夫，中村　厚，安達玲子，塩見一雄*4，穐山　浩，手島玲子：**加工食品中の甲殻類タンパク質定量検査法における標準品調製法の検討**

食品衛生学雑誌，**51**，133-138（2010）

えび・かに定量検査法における標準品の調製法に関して検討を行った結果，抽出液にプロテアーゼインヒビタ

ーを添加し，さらに100℃，10分間加熱することにより，試料中のプロテアーゼの影響を低減させ，安定した標準品原液を調製することが可能となった．また，本調製法に基づき，標準品原液を3ロット調製し電気泳動とタンパク質の定量を行った．電気泳動像においては160，41，37kDa付近にそれぞれ1本，20～16kDaの範囲に4本の明瞭なバンドが認められた．また，タンパク質の定量値から，標準的な濃度範囲は2.74～4.10mg/mLであった．

Keywords：えび・かに定量検査法，食物アレルギー，特定原材料

*1（株）マルハニチロホールディングス
*2日水製薬（株）
*3（株）ニッポンジーン
*4東京海洋大学

Sakai, Y.*1, Ishihata, K.*2, Nakano, S.*2, Yamada, T.*2, Yano, T.*3, Uchida, K.*1, Nakao, Y.*1, Urisu, A.*4, Adachi, R., Teshima, R., Akiyama, H.: **Specific detection of banana residue in processed foods using polymerase chain reaction**

J. Agric. Food Chem., **58**, 8145-8151（2010）

Specific polymerase chain reaction（PCR）methods were developed for the detection of banana residue in processed foods. For high banana specificity, the primer set BAN-F/BAN-R was designed based on the large subunit of ribulose-1,5-bisphosphate carboxylase（*rbcL*）genes of chloroplasts and used to obtain amplified products specific to banana by both conventional and real-time PCR. To confirm the specificity of these methods, genomic DNA samples from 31 other species were examined; no amplification products were detected. Subsequently, eight kinds of processed foods containing banana were investigated using these methods to confirm the presence of banana DNA. Conventional PCR had a detection limit of 1 ppm（w/w）banana DNA spiked in 50 ng of salmon testis DNA, while SYBR Green I real-time semi-quantitative PCR had a detection limit as low as 10 ppm banana DNA. Thus, both methods show high sensitivity and may be applicable as specific tools for the detection of trace amounts of banana in commercial food products.

Keywords: allergen, real-time polymerase chain reaction, ribulose-1,5-bisphosphate carboxylase gene

*1 Nagahama Branch, Oriental Yeast Co., Ltd.
*2 Food Safety Research Institute, Nissin Foods Holdings Co., Ltd.
*3 Graduate School of Regional Innovation Studies, Mie University
*4 Fujita Health University, The Second Teaching Hospital

峰松和彦*1，中村公亮，穐山　浩，張替直輝*2，中島治，橘田和美*3，手島玲子，飯塚太由*1：**コンニャク製粉含有コメ粉からのコメDNA抽出精製法の検討**

食品衛生学雑誌，**51**，247-252（2010）

安全性未承認の遺伝子組換え（GM）コメの試験法として，polymerase chain reaction（PCR）を用いたGMコメDNAの検知法が厚生労働省から通知されている．コメ加工品として中国から輸入されるコンニャク製粉含有コメ粉のDNA検査は，コンニャク多糖成分の妨害によってDNA抽出液の調製が困難であったため，これまで「検知不能」の判定結果となる場合が多かった．本研究では，DNA抽出操作前にコンニャク多糖を分離し，その後，コメ粉のDNAを精製する方法を確立した．本法で得られたコメDNA試料は，定性PCR及びリアルタイムPCR法による検査が可能であることが確認された．

Keywords：遺伝子組換えコメ，コンニャク製粉，コメ加工食品

*1（財）食品環境検査協会
*2武庫川女子大学薬学部
*3（独）農研機構食品総合研究所

Harikai, N.*1, Akiyama, H., Kondo, K., Kitta, K.*2, Teshima, R., Yoshida, Y.*1: **A novel chromogenic method for determining the genetically modified soybean content in soybean powder with primer extension**

Jpn. J. Food Chem. Safety, **17**, 110-115（2010）

A conventional method for determining the Roundup Ready soybean（RRS）content in soybean powder was developed. The RRS DNA-specific oligodeoxyribonucleotide immobilized on a plastic plate was used as the primer of the primer extension reaction（PEXT）with the RRS specific PCR product obtained from the DNA extracts of soybean powder as the templates. The PEXT product was labeled with biotin and visualized by chromogenic reaction using avidin and biotin-conjugated alkaline phosphatase. This method could detect 10^9 copies of the RRS specific DNA sequence in assay

solution, and good correlation (r=0.99) was observed between the logarithm of copy number and the color intensity up to 10^{12} copies of RRS specific DNA sequence. By optimizing PCR conditions for amplifying the RRS specific sequence, this method could detect the RRS content in the soybean powder between 0.1 and 5.0%.

Keywords: arrayed primer extension, DNA microarray, genetically modified organism

---

*1 Mukogawa Women's University
*2 National Food Research Institute

Takabatake, R.*1, Onishi, M.*2, Koiwa, T.*3, Futo, S.*2, Minegishi, Y.*4, Akiyama, H., Teshima, R., Furui, S.*1, Kitta, K.*1: **Establishment and Evaluation of Event-Specific Quantitative PCR Method for Genetically Modified Soybean MON89788**

Food Hygiene and Safety Science, **51**, 242-246 (2010)

A novel real-time PCR-based analytical method was developed for the event-specific quantification of a GM soybean event; MON89788. The conversion factor (Cf) which is required to calculate GMO amount was experimentally determined. The method was evaluated by in-house analyses and the blind tests in a multilaboratory trial using real-time PCR instruments. The limit of quantitation for the method was estimated to be 0.1%. The trueness and precision were evaluated as bias and reproducibility of relative standard deviation (RSD_R), and the determined bias and RSD_R values for the method were both less than 20%. These results suggest that the developed method would be suitable for practical analyses for the quantification of MON 89788.

Keywords: MON89788, event-specific, genetically modified (GM), real-time PCR, Soybean

---

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

Takabatake, R.*1, Kodama, T.*2, Matsuoka, T.*2, Futo, S.*3, Minegishi, Y.*4, Watai, M.*5, Sawada, C.*6, Nakamura, K., Akiyama, H., Teshima, R., Furui, S.*1, Akihiro, H.*1, Kitta, K.*1: **Evaluation of quantitative PCR methods for genetically modified maize (MON 863, NK603, TC1507 and T25)**

Food Sci. Technol. Res., **16**, 421-430 (2010)

Novel real-time PCR-based quantitative methods were developed for three GM maize event: MON863, NK603, and TC1507. The quantitative methods were designed to amplify an event-specific segment for MON863, NK 603, and a construct-specific segment for TC1507. We also redeveloped an event-specific quantitative method for T25 because the target sequence for T25 used in our previous method was also introduced into TC1507. The conversion factor (Cf) which is required for calculating GMO amount, for each GM event was determined with three types of real-time PCR equipments, ABI PRISM 7700, 7900HT, and 7500. The quantitative methods for four GM events were evaluated by a blind test in an interlaboratory study using two instruments, ABI PRISM 7700 and 7900HT. The blind test was also conducted in a multilaboratory trial using ABI PRISM 7500. The trueness, the precision, and the limit of quantitation were determined. Although the bias expressing the trueness, for MON863, TC1507, and T25 were slightly high, all the data suggested that these developed methods are suitable for the practical identification and quantification of these GM maize events.

Keywords: genetically modified (GM), interlaboratory study, maize (*Zea maize*), real-time PCR system

---

*1 National Food Research Institute
*2 Food and Agricultural Materials Inspection Center
*3 Fasmac Co., Ltd.
*4 Nippon Gene Co., Ltd.
*5 Japan Food Research Laboratories
*6 Japan Frozen Foods Inspection Corp

Kodama, T.*1, Kasahara, M.*1, Minegishi, Y.*2, Futo, S.*3, Sawada, C.*4, Watai, M.*5, Akiyama, H., Teshima, R., Kurosawa, Y.*6, Furui, S.*6, Hino, A.*6, Kitta, K.*6: **Interlaboratory Study of Qualitative PCR Method for Roundup Ready Soybean**

J. AOAC Int., **94**, 224-231 (2010)

Quantitative and qualitative methods based on polymerase chain reaction (PCR) have been developed for genetically modified organism (GMO), and we previously conducted interlaboratory studies for GMO quantitative methods. In this study, we conducted an interlaboratory study for a qualitative method for GM soybean, Roundup Ready soy (RRS) with primer pairs previously designed for the quantitative method of RRS.

Fourteen laboratories in Japan participated in this study. Each participant extracted DNA from 1.0 g each of soy samples containing 0, 0.05, and 0.10 % of RRS, and performed PCR with primer pairs for an internal control gene (*Le1*) and RRS, followed by agarose gel electrophoresis. The PCR product amplified in this PCR system for Le1 was detected from all samples. The sensitivity, specificity, false negative rate, and false positive rate of the method were obtained from the results of RRS detection. False negative rates at the level of 0.05 and 0.10 % of RRS samples were 6.0 and 2.3 %, respectively, revealing that the limit of detection of the method was 0.10 %. The current study demonstrated that the qualitative method would be practical for monitoring the labeling system of GM soy.

Keywords: Roundup Ready Soybean, PCR, detection

*1 Food and Agricultural Materials Inspection Center
*2 Nippon Gene Co., Ltd.
*3 Fasmac Co., Ltd.
*4 Japan Frozen Foods Inspection Corp
*5 Japan Food Research Laboratories
*6 National Food Research Institute

Akiyama, H., Makiyama, D, Nakamura, K., Sasaki, N.*1, Minegishi, Y.*2, Mano, J.*3, Kitta, K.*3, Ozeki, Y.*1, Teshima, R.: **A Novel Detection System for the Genetically Modified Canola (Brassica rapa) Line RT73**

Anal. Chem., **82**, 9909-9916 (2010)

The herbicide-tolerant genetically modified Roundup Ready canola (Brassica napus) line RT73 has been approved worldwide for use in animal feed and human food. However, RT73 Brassica rapa lines derived from interspecific crosses with RT73 B. napus have not been approved in Japan. Here, we report on a novel system using individual kernel analyses for the qualitative detection of RT73 B. rapa in canola grain samples. We developed a duplex real-time polymerase chain reaction (PCR) method to discriminate B. napus and B. rapa DNA using scatter plots of the end-point analyses; this method was able to discriminate a group comprising B. rapa and Brassica juncea from a group comprising B. napus, Brassica carinata, and Brassica oleracea. We also developed a duplex real-time PCR method for the simultaneous detection of an RT73-specific sequence and an endogenous FatA gene. Additionally, a DNA-extraction method using 96-well silica-membrane plates was developed and optimized for use with individual canola kernels. Our detection system could identify RT73 B. rapa kernels in canola grain samples enabling the accurate and reliable monitoring of RT73 B. rapa contamination in canola, thus playing a role in its governmental regulation in Japan.

Keywords: Roundup Ready canola, Brassica rapa, real-time polymerase chain reaction

*1 Tokyo University of Agriculture and Technology
*2 Nippon Gene Co., Ltd.
*3 National Food Research Institute

Nakamura, K., Fujioka, S.*1, Fukumoto, S.*1, Inoue, N.*2, Sakamoto, K.*1, Hirata, H.*3, Kido, Y., Yabu, Y.*4, Suzuki, T.*4, Watanabe, Y.*1, Saimoto, H.*4, Akiyama, H., Kita, K*1: **Trypanosome alternative oxidase, a potential therapeutic target for sleeping sickness, is conserved among Trypanosoma brucei subspecies**

Parasitol. Int., **59**, 560-564 (2010)

Trypanosoma brucei rhodesiense and T. b. gambiense are known causes of human Africantrypanosomiasis (HAT), or "sleeping sickness," which is deadly if untreated. We previously reported that a specific inhibitor of trypanosome alternative oxidase (TAO), ascofuranone, quickly kills African trypanosomes in vitro and cures mice infected with another subspecies, non-human infective T. b. brucei, in in vivo trials. As an essential factor for trypanosome survival, TAO is a promising drug targetdue to the absence of alternative oxidases in the mammalian host. This study found TAO expression in HAT-causing trypanosomes; its amino acid sequence was identical to that in non-human infective T. b. brucei. The biochemical understanding of the TAO including its 3 dimensional structure and inhibitory compounds against TAO could therefore be applied to all three T. brucei subspecies in search of a cure for HAT. Our in vitro study using T. b. rhodesiense confirmed the effectiveness of ascofuranone (IC50 value: 1 nM) to eliminate trypanosomes in human infective strain cultures.

Keywords: Human African Trypanosomiasis, Sleeping sickness

*1 The University of Tokyo
*2 Obihiro University of Agriculture and Veterinary

Medicine
*3 Nagoya City University
*4 Tottori University

Nakamura, K., Yamada, C.*1, Akiyama, H., Takabatake, R.*2, Kitagawa, M.*3, Kitta, K.*2, Kawakami, H.*1, Teshima, R.: **Evaluation of tomato DNA fragmentation and PCR amplicon size for detection of tomato DNA in processed products**

Jpn. J. of Food Chem. and Safety, **17**, 123-129 (2010)

The degree of DNA fragmentation in various commercially processed tomato products was investigated using polymerase chain reaction (PCR) with primers designed to amplify amplicons of different lengths. From the low-processed tomato products like tomato paste, fragments up to 284 bp could be amplified, while from highly processed products, such as ketchup, sauce, chili sauce and juice, fragments of only 92 bp could be amplified. We detected, for the first time, tomato DNA from commercially processed tomato products by amplifying a 92 bp target amplicon using conventional PCR. In addition, tomato DNA could be detected in all processed tomato products using qualitative real-time PCR with specific primers and fluorescently labeled probes, targeting the 92 bp amplicon. Thus, primers and probes designed to target the approximately 90 bp amplicon are essential for the detection of unauthorized genetically modified tomato contamination in processed tomato products.

Keywords: processed tomato products, amplifiable amplicon length, qualitative real-time PCR

*1 Department of Food Science & Nutrition, Kyoritsu Women's University
*2 National Food Research Institute
*3 Research Institute, Kagome Company, Limited

Sakai, S., Adachi, R., Akiyama, H., Teshima, R., Doi, H.*1, Shibata, H.*1, Urisu, A.*2: **Determination of Walnut Protein in Processed Foods by Enzyme-Linked Immunosorbent Assay: Interlaboratory Study**

J. AOAC Int., **93**, 1255-1261 (2010)

Because food allergens from tree nuts, including walnuts, are a frequent cause of adverse food reactions for allergic patients, the labeling of foods containing ingredients derived from tree nuts is required in numerous countries. According to Japanese regulations, the labeling of food products containing walnuts is recommended. To ensure proper labeling, a novel sandwich ELISA kit for the determination of walnut protein in processed foods (Walnut Protein [2S-Albumin] Kit; Morinaga Institute of Biological Science, Inc.; "walnut kit") has been developed. We prepared seven types of incurred samples (model processed foods: biscuits, bread, sponge cake, orange juice, jelly, chicken meatballs, and rice gruel) containing 10 μg walnut soluble protein/g of food for use in interlaboratory evaluations of the walnut kit. The walnut kit displayed sufficient reproducibility relative standard deviations (interlaboratory precisions: 5.8-9.9% RSD_R) and a high level of recovery (81-119%) for all the incurred samples. All the repeatability relative standard deviation (RSD_r) values for the incurred samples that were interlaboratory evaluation suggested that the walnut kit could be used as a precise and reliable tool for determination of walnut protein in precessed foods.

Keywords: food allergen, interlaboratory studies, ELISA, walnut

*1 Morinaga Institute of Biological Science, Inc.
*2 Fujita Health University

中村　厚，酒井信夫，川浦知子*，小林政人*，安達玲子，穐山　浩，手島玲子：**すり身およびその加工食品中に自然混入する甲殻類の実態調査**

日本食品化学学会誌，**17**，213-220（2010）

2008年6月，厚生労働省は我が国の食品表示制度を改正し，えびおよびかにを特定原材料に指定した．それに伴い，魚肉製品77検体（すり身29検体およびその加工食品48検体）に甲殻類由来のタンパク質およびDNAが含まれているか，ELISA法およびPCR法を用いて実態調査を行った．その結果，ELISA法において定量下限値以上の甲殻類タンパク質が検出されたすり身検体は75.9%（Mキット）または79.3%（Nキット）であった．また，えびPCR法では62.1%，かにPCR法では3.4%が陽性であった．すり身加工食品では，56.3%（Mキット）または72.9%（Nキット）の検体が陽性となり，えびPCR法，かにPCR法の陽性率はそれぞれ72.9%，2.1%であった．このように，魚肉すり身およびその加工食品には，意図しない甲殻類の自然混入が高頻度に認められた．特に，小さい魚体を原料とするすり身から高濃度の甲殻類タンパク質が検出される場合が多く見受けられた．

Keywords：crustacean soluble protein，allergen，processed food

*（財）食品分析開発センター　SUNATEC

Ishii-Watabe, A., Saito, Y., Suzuki, T., Tada, M., Ukaji, M., Maekawa, K., Kurose, K., Kaniwa, N., Sawada, J., Kawasaki, N., Yamaguchi, T., Nakajima, T. E.*1, Kato, K.*1, Yamada, Y.*1, Shimada, Y.*1, Yoshida, T.*1, Ura, T.*2, Saito, M.*2, Muro, K.*2, Doi, T.*1, Fuse, N.*1, Yoshino, T.*1, Ohtsu, A.*1, Saijo, N.*1, Hamaguchi, T.*1, Okuda, H. and Matsumura, Y.*1：**Genetic polymorphisms of *FCGRT* encoding FcRn in a Japanese population and their functional analysis**

Drug Metab. Pharmacokinet., **25**, 578-587（2010）

Neonatal Fc receptor（FcRn）plays an important role in regulating IgG homeostasis in the body. Changes in FcRn expression levels or activity caused by genetic polymorphisms of *FCGRT*, which encodes FcRn, may lead to interindividual differences in pharmacokinetics of therapeutic antibodies. In this study, we sequenced the 5'-flanking region, all exons and their flanking regions of *FCGRT* from 126 Japanese subjects. Thirty-three genetic variations, including 17 novel ones, were found. Of these, two novel non-synonymous variations, 629G>A（R210Q）and 889T>A（S297T）, were found as heterozygous variations. We next assessed the functional significance of the two novel non-synonymous variations by expressing wild-type and variant proteins in HeLa cells. Both variant proteins showed similar intracellular localization as well as antibody recycling efficiencies. These results suggested that at least no common functional polymorphic site with amino acid change was present in the *FCGRT* of our Japanese population.

Keywords：genetic polymorphisms, FcRn, monoclonal antibody

*1（独）国立がん研究センター
*2愛知がんセンター

Saito, Y., Yamamoto, N.*, Katori, N., Maekawa, K., Fukushima-Uesaka, H., Sugimoto, D., Kurose, K., Sai, K., Kaniwa, N., Sawada, J., Kunitoh, H.*, Ohe, Y.*, Yoshida, T.*, Matsumura, Y.*, Saijo, N.*, Okuda, H. and Tamura, T.*：**Genetic polymorphisms and haplotypes of por, encoding cytochrome p450 oxidoreductase, in a Japanese population**

Drug Metab. Pharmacokinet., **26**, 107-116（2011）

Cytochrome P450 oxidoreductase（POR）transfers electrons from NADPH to all microsomal cytochrome P450（CYP）enzymes and is necessary for microsomal CYP activities. In this study, to find genetic variations and to elucidate the haplotype structures of *POR*, we comprehensively screened the genetic variations in the 5'-flanking region, all the exons and their flanking introns of *POR* for 235 Japanese subjects. Seventy-five genetic variations including 26 novel ones were found：7 were in the 5'-flanking region, 2 in the 5'-untranslated region（5'-UTR, non-coding exon 1）, 16 in the coding exons（10 nonsynonymous and 6 synonymous）, 45 in the introns, 4 in the 3'-UTR and 1 in the 3'-flanking region. Of these, 4 novel nonsynonymous variations, 86C>T（T29M）, 1648C>T（R550W）, 1708C>T（R570C）and 1975G>A（A659T）, were detected with allele frequencies of 0.002. We also detected known nonsynonymous SNPs 683C>T（P228L）, 1237G>A（G413S）, 1453G>A（A485T）, 1508C>T（A503V）, 1510G>A（G504R）and 1738G>C（E580Q）with frequencies of 0.002, 0.009, 0.002, 0.434, 0.002 and 0.002, respectively. Based on the linkage disequilibrium（LD）profiles, the analyzed region could be divided into two LD blocks. For Blocks 1 and 2, 14 and 46 haplotypes were inferred, respectively, and 2 and 6 common haplotypes found in more than 0.03 frequencies accounted for more than 81% of the inferred haplotypes. This study provides fundamental and useful information for the pharmacogenetic studies of drugs metabolized by CYPs in the Japanese population.

Keywords：genetic polymorphisms, haplotype, POR

*国立がん研究センター

Saeki, M., Kurose, K., Hasegawa, R. and Tohkin, M.：**Functional analysis of genetic variations in the 5'-flanking region of the human MDR1 gene**

Mol. Genet. Metab., **102**, 91-98（2011）

薬物間相互作用の発症に関与しているP-糖タンパク質の発現量に個人差が生じる要因を明らかにすることを目的とした．その結果，P-糖タンパク質の転写調節領域に存在する一塩基置換によって転写活性が低下することがわかった．以上の結果より，転写調節領域の遺伝子多型がP-糖タンパク質の発現量に個人差が生じる要因の一つであることが明らかになった．

Keywords：P-glycoprotein，gene transcription，individ-

ual difference

Maekawa, K., Harakawa, N., Yoshimura, T.*, Kim, S. R., Fujimura, Y.*, Aohara, F.*, Sai, K., Katori, N., Tohkin, M., Naito, M., Hasegawa, R., Okuda, H., Sawada, J., Niwa, T.* and Saito, Y.: **CYP3A4*16 and CYP3A4*18 Alleles Found in East Asians Exhibit Differential Catalytic Activities for Seven CYP3A4 Substrate Drugs**

Drug Metab. Dispos., **38**, 2100-2104 (2010)

CYP3A4, the major form of cytochrome P450 (P450) expressed in the adult human liver, is involved in the metabolism of approximately 50% of commonly prescribed drugs. Several genetic polymorphisms in CYP3A4 are known to affect its catalytic activity and to contribute in part to interindividual differences in the pharmacokinetics and pharmacodynamics of CYP3A4 substrate drugs. In this study, catalytic activities of the two alleles found in East Asians, CYP3A4*16 (T185S) and CYP3A4*18 (L293P), were assessed using the following seven substrates: midazolam, carbamazepine, atorvastatin, paclitaxel, docetaxel, irinotecan, and terfenadine. The holoprotein levels of CYP3A4.16 and CYP3A4.18 were significantly higher and lower, respectively, than that of CYP3A4.1 when expressed in Sf21 insect cell microsomes together with human NADPH-P450 reductase. CYP3A4.16 exhibited intrinsic clearances (V(max)/K(m)) that were lowered considerably (by 84-60%) for metabolism of midazolam, carbamazepine, atorvastatin, paclitaxel, and irinotecan compared with CYP3A4.1 due to increased K(m) with or without decreased V(max) values, whereas no apparent decrease in intrinsic clearance was observed for docetaxel. On the other hand, K(m) values for CYP3A4.18 were comparable to those for CYP3A4.1 for all substrates except terfenadine; but V(max) values were lower for midazolam, paclitaxel, docetaxel, and irinotecan, resulting in partially reduced intrinsic clearance values (by 34-52%). These results demonstrated that the impacts of both alleles on CYP3A4 catalytic activities depend on the substrates used. Thus, to evaluate the influences of both alleles on the pharmacokinetics of CYP3A4-metabolized drugs and their drug-drug interactions, substrate drug-dependent characteristics should be considered for each drug.

Keywords: genetic polymorphism, CYP3A4, function

* 田辺三菱製薬（株）

Gay, S. C.*1, Roberts, A. G.*1, Maekawa, K., Talakad, J. C.*1, Hong, W. X.*2, Zhang, Q.*2, Stout, C. D.*2 and Halpert, J. R.*1: **Structures of Cytochrome P450 2B4 Complexed with the Antiplatelet Drugs Ticlopidine and Clopidogrel**

Biochemistry, **49**, 8709-8720 (2010)

Prior X-ray crystal structures of rabbit cytochrome P450 2B4 (2B4) in complexes with various imidazoles have demonstrated markedly different enzyme conformations depending on the size of the inhibitor occupying the active site. In this study, structures of 2B4 were determined with the antiplatelet drugs clopidogrel and ticlopidine, which were expected to have greater freedom of movement in the binding pocket. Ticlopidine could be modeled into the electron density maps in two distinct orientations, both of which are consistent with metabolic data gathered with other mammalian P450 enzymes. Results of ligand docking and heme-induced NMR relaxation of drug protons showed that ticlopidine was preferentially oriented with the chlorophenyl group closest to the heme. Because of its stereocenter, clopidogrel was easier to fit in the electron density and exhibited a single orientation, which points the chlorophenyl ring toward the heme. The C (α) traces of both complexes aligned very well with each other and revealed a compact, closed structure that resembles the conformation observed in two previously determined 2B4 structures with the small molecule inhibitors 4-(4-chlorophenyl) imidazole and 1-(4-chlorophenyl)imidazole. The 2B4 active site is able to accommodate small ligands by moving only a small number of side chains, suggesting that ligand reorientation is energetically favored over protein conformational changes for binding of these similarly sized molecules. Adjusting both protein conformation and ligand orientation in the active site gives 2B4 the flexibility to bind to the widest range of molecules, while also being energetically favorable.

Keywords: X-ray crystallography, CYP2B4, ticlopidine, clopidogrel

*1 University of California, San Diego

*2 The Scripps Research Institute

Kaniwa, N., Saito, Y., Aihara, M.*1, Matsunaga, K.*2, Tohkin, M., Kurose, K., Furuya, H.*3, Takahashi, Y.*4,

Muramatsu, M.[*5], Kinoshita, S.[*6], Abe, M.[*2], Ikeda, H.[*4], Kashiwagi, M.[*5], Song, Y.[*5], Ueta, M.[*6], Sotozono, C.[*6], Ikezawa, Z.[*1] and Hasegawa, R. for JSAR research group.: **HLA-B*1511 is a risk factor for carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Japanese patients**

Epilepsia, **51**, 2461-2465（2010）

Stevens-Johnson syndrome（SJS）and toxic epidermal necrolysis（TEN）are rare but life-threatening severe cutaneous adverse reactions. Recently, strong associations of HLA-B*1502 with carbamazepine-induced SJS/TEN have been found in Han Chinese patients. These associations have been confirmed in several Asian populations, excluding Japanese. SJS patients carrying HLA-B*1508, HLA-B*1511, or HLA-B*1521, which are members of the HLA-B75 type along with HLA-B*1502, were detected in studies in India and Thailand. In the current study, we genotyped the HLA-B locus from 14 Japanese typical and atypical SJS/TEN patients in whom carbamazepine was considered to be involved in the onset of adverse reactions. Although there were no HLA-B*1502 carriers, four patients had HLA-B*1511. Our data suggest that HLA-B*1511, a member of HLA-B 75, is a risk factor for carbamazepine-induced SJS/TEN in Japanese.

Keywords: HLA-B*1502, HLA-B75, serotype

*[1] Department of Environmental Immunodermatology, Yokohama City University Graduate School of Medicine
*[2] Department of Dermatology, Fujita Health University School of Medicine
*[3] Department of Neurology, Neuro-Muscular Center, National Oomuta Hospital
*[4] Shizuoka Institute of Epilepsy and Neurological Disorders, National Epilepsy Center
*[5] Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental University
*[6] Department of Ophthalmology, Kyoto Prefectural University of Medicine

Sugiyama, E., Kaniwa, N., Kim, S. R., Hasegawa, R., Saito, Y., Ueno, H.[*1], Okusaka, T.[*1], Ikeda, M.[*1], Morizane, C.[*1], Kondo, S.[*1], Yamamoto, N.[*1], Tamura, T.[*1], Furuse, J.[*2], Ishii, H.[*2], Yoshida, T.[*3], Saijo, N.[*2] and Sawada, J.[*4]: **Population pharmacokinetics of gemcitabine and its metabolite in Japanese cancer patients: impact of genetic polymorphisms**

Clin. Pharmacokinet., **49**, 549-558（2010）

Gemcitabine is an anticancer drug, which is effective against solid tumours, including non-small-cell lung cancer and pancreatic cancer. The aim of this study was to determine the factors, including genetic polymorphisms of cytidine deaminase（*CDA*）, deoxycytidine kinase（*DCK*）and solute carrier family 29A1（*SLC29A1 [hENT1]*）, that alter the pharmacokinetics of gemcitabine and its inactive metabolite（dFdU）in Japanese cancer patients.

Two hundred fourty-six Japanese cancer patients who received 30-minute intravenous infusions of gemcitabine at 800 or 1000 mg/m^2 in the period between September 2002 and July 2004 were recruited for this study. Two patients who experienced gemcitabine-derived life-threatening toxicities in October 2006 and January 2008 were added to this analysis. Plasma concentrations of gemcitabine and dFdU were measured by HPLC. In total, 1973 and 1975 plasma concentrations of gemcitabine and dFdU, respectively, were used to build population pharmacokinetic models using nonlinear mixed-effects modelling software（NONMEM version V level 1.1）.

Two-compartment models fitted well to plasma concentration-time curves for both gemcitabine and dFdU. Major contributing factors for gemcitabine clearance were genetic polymorphisms of *CDA*, including homozygous *CDA*3*［208G>A（Ala70Thr）］（64% decrease）, heterozygous *3（17% decrease）and *CDA* -31delC（an approximate 7% increase per deletion）, which has a strong association with *CDA*2*［79A>C（Lys27Gln）］, and coadministered S-1（an approximate 19% increase）. Genetic polymorphisms of *DCK* and *SLC29A1*（*hENT1*）had no significant correlation with gemcitabine pharmacokinetic parameters. Aging and increased serum creatinine levels correlated with decreased dFdU clearance.

A population pharmacokinetic model that included *CDA* genotypes as a covariate for gemcitabine and dFdU in Japanese cancer patients was successfully constructed. The model confirms the clinical importance of the *CDA*3* genotype.

Keywords: population pharmacokinetics, gemcitabine, *CDA*3*

*[1] National Cancer Center Hospital
*[2] National Cancer Hospital East

*3 National Cancer Center Research Institute
*4 Pharmaceuticals and Medical Devices Agency

Komeiji, Y.*1, Mochizuki, Y.*2 and Nakano, T.: **Three-body the fragment molecular orbital-based molecular dynamics (FMO-MD)**
Chem. Phys. Lett., **484**, 380-386 (2010)

The fragment molecular orbital-based molecular dynamics (FMO-MD) was improved by the introduction of the three-body extension (FMO3) and the generalized dynamic fragmentation. An analytical energy gradient was derived for FMO3 to realize FMO3-MD. An algorithm of generalized dynamic fragmentation was devised to treat each covalent-bonded and, optionally, hydrogen-bonded atom cluster as a fragment in the course of FMO-MD. The new algorithms were tested by performing conventional MO-MD, FMO2- MD, based on two-body extension, and FMO3-MD simulations of $(H_2O)_{32}$ and $H^+(H_2O)_{32}$. FMO2-MD resulted in lower precision, especially in $H^+(H_2O)_{32}$, while FMO3-MD gave a precision comparable to that of MO-MD.
Keywords: FMO3, FMO-MD

---

*1 (独) 産業技術総合研究所
*2 立教大学

Fujiwara, T.*1, Mochizuki, Y.*1, Komeiji, Y.*2, Okiyama, Y.*3, Mori, H.*4, Nakano, T. and Miyoshi, E.*5: **Fragment molecular orbital-based molecular dynamics (FMO-MD) simulations on hydrated Zn (II) ion**
Chem. Phys. Lett., **490**, 41-45 (2010)

Recently, the method of fragment molecular orbital-based molecular dynamics (FMO-MD) was enhanced by including the three-body corrections (FMO3) [Y. Komeiji, Y. Mochizuki, T. Nakano, Chem. Phys. Lett. 484 (2010) 380]. This simulation protocol was applied to a droplet model consisting of a divalent zinc ion and 64 water molecules, in order to investigate the hydration structure in ab initio fashion. The first peak position of the Zn-O radial distribution function (RDF) was evaluated to be 2.05 Å at the FMO3-HF/6-31G level of theory, which was in agreement with an X-ray value of 2.06 ± 0.02 -. The coordination number was evaluated to be 6, corresponding to an octahedral coordination.
Keywords: FMO-MD, Zn (II) ion

---

*1 立教大学
*2 (独) 産業技術総合研究所
*3 東京大学
*4 お茶の水女子大学
*5 九州大学

Okiyama, Y.*1, Nakano, T., Yamashita, K.*2, Mochizuki, Y.*3, Taguchi, N.*3 and Tanaka, S.*4: **Acceleration of fragment molecular orbital calculations with Cholesky decomposition approach**
Chem. Phys. Lett., **490**, 84-89 (2010)

A novel method, Cholesky decomposition with adaptive metric (CDAM), is applied to the two-electron integral calculations in the fragment molecular orbital (FMO) method. We thus accelerate the Hartree-Fock and the second-order M-ller-Plesset perturbation (MP2) energy calculations substantially. Especially, the MP2 part for fragment dimers, which is computationally expensive, is accelerated by a factor of about 10. The CDAM approximations would enable FMO-MP2 calculations to easily process multiple structure samples even including dynamics of large molecular systems and lead to next-generation high-performance computations where statistical samplings or free energy estimates would be important.
Keywords: FMO, Cholesky decomposition, CDAM

---

*1 東京大学
*2 NEC ソフト (株)
*3 立教大学
*4 神戸大学

Yamagishi, K.*1, Yamamoto, K.*2, Mochizuki, Y.*1, Nakano, T., Yamada, S.*3 and Tokiwa, H.*1: **Flexible ligand recognition of peroxisome proliferator-activated receptor-gamma (PPARgamma)**
Bioorg. Med. Chem. Lett., **20**, 3344-3347 (2010)

The peroxisome proliferator-activated receptor-γ (PPARγ) is a direct pharmacological target for drugs that enhance insulin sensitivity and are used clinically for the treatment of type II diabetes. Because the specificity of ligand recognition is lower for PPAR- than for other nuclear receptors, PPAR- can bind a larger variety of ligand types. In order to elucidate why the ligand recognition of PPAR- is so flexible, we performed correlated fragment molecular orbital calculations for complexes of PPAR- and each of two distinctive ligands, rosiglitazone and farglitazar. We found quite different

patterns of ligand binding for these two ligands. The ligand-binding system of rosiglitazone, a drug in common clinical use, is based mainly on local electrostatic interactions around the thiazolidine ring, whereas both electrostatic interactions and van der Waals dispersion interactions with hydrophobic residues are required for the binding of farglitazar to PPARγ. We suggest that the development of novel ligands will require adequately hydrophobic pharmacophores.

Keywords: FMO, PPARγ

*1 立教大学
*2 昭和薬科大学
*3 日本大学

Sato, M.*1, Yamataka, H.*1, Komeiji, Y.*2, Mochizuki, Y.*1 and Nakano, T.: **Does amination of formaldehyde proceed through a zwitterionic intermediate in water- Fragment molecular orbital molecular dynamics simulations by using constraint dynamics**

Chem. Eur. J., **16**, 6430-6433 (2010)

The present FMO-MD simulations clearly show that the reaction of ammonia and formaldehyde produces carbinolamine by the stepwise mechanism through the zwitterionic intermediate, not by the concerted mechanism. Although there are many subjects to be refined, such as calculation levels, boundary conditions and relaxation time, simulations including hundreds of explicit water molecules at a full QM level as presented here will be a promising method to provide molecular level information in both organic and biological chemistry.

Keywords: FMO-MD, amination of formaldehyde, zwitterionic intermediate

*1 立教大学
*2 (独) 産業技術総合研究所

Mochizuki, Y.*1, Yamashita, K.*2, Fukuzawa, K.*3, Takematsu, K.*4, Watanabe, H.*4, Taguchi, N.*1, Okiyama, Y.*5, Tsuboi, M.*1, Nakano, T. and Tanaka, S.*4: **Large-scale FMO-MP3 calculations on the surface proteins of influenza virus, hemagglutinin (HA) and neuraminidase (NA)**

Chem. Phys. Lett., **493**, 346-352 (2010)

Two proteins on the influenza virus surface have been well known. One is hemagglutinin (HA) associated with the infection to cells. The fragment molecular orbital (FMO) calculations were performed on a complex consisting of HA trimer and two Fab-fragments at the third-order M-ller-Plesset perturbation (MP3) level. The numbers of residues and 6-31G basis functions were 2351 and 201276, and thus a massively parallel-vector computer was utilized to accelerate the processing. This FMO-MP3 job was completed in 5.8 h with 1024 processors. Another protein is neuraminidase (NA) involved in the escape from infected cells. The FMO-MP3 calculation was also applied to analyze the interactions between oseltamivir and surrounding residues in pharmacophore.

Keywords: FMO3, MP3, hemagglutinin

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

Watanabe, H.*1, Okiyama, Y.*2, Nakano, T. and Tanaka, S.*1: **Incorporation of solvation effects into the fragment molecular orbital calculations with the Poisson-Boltzmann equation**

Chem. Phys. Lett., **500**, 116-119 (2010)

We developed FMO-PB method, which incorporates solvation effects into the Fragment Molecular Orbital calculation with the Poisson-Boltzmann equation. This method retains good accuracy in energy calculations with reduced computational time. We calculated the solvation free energies for polyalanines, Alpha-1 peptide, tryptophan cage, and complex of estrogen receptor and 17β-estradiol to show the applicability of this method for practical systems. From the calculated results, it has been confirmed that the FMO-PB method is useful for large biomolecules in solution. We also discussed the electric charges which are used in solving the Poisson-Boltzmann equation.

Keywords: FMO, Poisson-Boltzmann equation

*1 神戸大学
*2 東京大学

Kurisaki, I.*1, Fukuzawa, K.*2, Nakano, T., Mochizuki, Y.*3, Watanabe, H.*1 and Tanaka, S.*1: **Fragment molecular orbital (FMO) study on stabilization**

mechanism of neuro-oncological ventral antigen (NOVA)-RNA complex system

J. Mol. Str. (*THEOCHEM*), **962**, 45-55 (2010)

We report the molecular mechanism of protein-RNA complex stabilization based on the electronic state calculation. Fragment molecular orbital (FMO) method based quantum mechanical calculations were performed for neuro-oncological ventral antigen (NOVA)-RNA complex system. The inter-molecular interactions and their effects on the electronic state of NOVA were examined in the framework of ab initio quantum calculation. The strength of inter-molecular interactions was evaluated using inter-fragment interaction energies (IFIEs) associated with residue-RNA base and residue-RNA backbone interactions. Under the influence of inter-molecular interactions, the change of electronic state of NOVA upon the complex formation was examined based on IFIE values associated with intra-NOVA residue-residue interactions and the change of atomic charges by each residue. The results indicated that non-specifically recognized bases contributed to the stability of the complex as well as specifically recognized bases and that the secondary structure of NOVA was remarkably associated with the change of electronic state upon the complex formation.

Keywords: FMO, IFIE, NOVA

*1 神戸大学
*2 みずほ情報総研（株）
*3 立教大学

Taguchi, N.*, Mochizuki, Y.* and Nakano, T.: **Fragment molecular orbital calculations for excitation energies of blue- and yellow-fluorescent proteins**

Chem. Phys. Lett., **504**, 76-82 (2010)

The excitation energies of blue- and yellow-fluorescent proteins (BFP and YFP) were evaluated by the method of configuration interaction singles and perturbative doubles CIS (D) in conjunction with the fragment molecular orbital (FMO) scheme. Three amino acid residues were identified to be contributive to the excitation energy by the so-called pairwise procedure. Under the multilayer treatment (MFMO) with these selected residues, the best estimates for BFP and YFP were obtained as 3.36 and 2.53 eV, respectively, where the second-order self-energy shift was utilized to modify the CIS (D) expression [Mochizuki, Chem. Phys. Lett. 472 (2009) 143]. These values were comparable to the respective experimental values of 3.21/3.25 and 2.41 eV.

Keywords: FMO, BFP, YFP

* 立教大学

Mochizuki, Y.*1, Nakano, T., Komeiji, Y.*2, Yamashita, K.*3, Okiyama, Y.*4, Yoshikawa, H.*1 and Yamataka, H.*1: **Fragment molecular orbital-based molecular dynamics (FMO-MD) method with MP2 gradient**

Chem. Phys. Lett., **504**, 95-99 (2010)

The energy gradient of the second-order Møller-Plesset perturbation theory (MP2) has been implemented in conjunction with the fragment molecular orbital-based molecular dynamics (FMO-MD) method including up to three-body correction (FMO3). A hybrid integral-direct approach of both atomic and molecular orbital indices was utilized with parallelism for the gradient calculations. A droplet model consisting of 64 water molecules was then simulated with the 6-31G* basis set. The first peak position of O-O radial distribution function was evaluated to be 2.75 Å at the FMO3-MP2 level, whereas the corresponding Hartree-Fock (FMO3-HF) value was 2.89 Å. Comparison with an X-ray value of 2.73 Å indicated better reliability of the MP2 gradient for FMO-MD.

Keywords: FMO3, MP2, MD

*1 立教大学
*2 (独) 産業技術総合研究所
*3 NEC ソフト（株）
*4 東京大学

Fujita, T.*, Nakano, T. and Tanaka, S.*: **Fragment molecular orbital calculations under periodic boundary condition**

Chem. Phys. Lett., **506**, 112-116 (2011)

The periodic boundary condition (PBC) is incorporated in the fragment molecular orbital (FMO) method to appropriately describe systems with aqueous solutions. We present benchmark calculations for $(H_2O)_{64}$ and show that this PBC-FMO method can eliminate artificial surface effects. An application to molecular dynamics simulation for liquid water is also shown, and calculated radial distribution functions are in reasonable agreement with those obtained from experiments. It is thus confirmed that the present PBC-FMO method is

useful for ab initio simulations in aqueous solution.
Keywords: PBC, FMO, MD

* 神戸大学

Hirabayashi, Y. and Inoue, T.: **The low-dose issue and stochastic responses to endocrine disruptors**
J. App. Toxicol., **31**, 84-88（2011）

The impact of endocrine disruptors, and specifically the low-dose issue, involves interdisciplinary sciences. Thus, in the past these topics have been published widely in the toxicology area. Owing to recent developments in biology, including the whole-genome reading program, the mechanisms underlying the low-dose issue have been clarified. These mechanisms have been found to involve stochastic and probabilistic receptor-mediated adverse effects induced by endocrine disruptors. The effects thought to be induced by low doses of endocrine-disrupting chemicals remain disputed, and the underlying mechanisms remain poorly understood. Three independent factors, each only recently identified and never before encountered in the history of toxicological studies, are associated with what is termed the 'low-dose issue'. First, toxicological risk has been estimated only by extrapolation of adverse phenotypes from high-dose effects and thus provides no reliable information on low-dose effects observed at the right time under experimental paradigm with sufficient sensitivity. Second, toxicity is based on disturbances of homeostatic regulation, a largely unexplored area in toxicology. Third, toxicity is based on stochastic and probabilistic xenobiotic response, a new field of toxicology that is specifically linked to low-dose and less-frequent events. To resolve the low-dose issue whether it causes effects or whether effects observed at low-doses should be considered 'adverse'- or both, each of these factors needs to be addressed.
Keywords: computational toxicology, deterministic xenobiotic responses, systems toxicology

Otsuka, K.*, Hirabayashi, Y., Tsuboi, I. and Inoue, T.: **Regeneration capability of Lin-/c-Kit+/Sca-1+ cells with or without radiation exposure for repopulation of peripheral blood in lethally irradiated mice monitored using Ly5.1 isotype on days 35, 90, and 270 after transplantation**
Exp. Hematol., **38**, 417-425（2010）

OBJECTIVE: Hematopoietic stem cells are supposed to repopulate and maintain long-term regeneration of the recipient's bone marrow and peripheral blood. In this study, we evaluated the regeneration capability of Lin(−)/c-Kit(+)/Sca-1(+)(LKS) cells, the putative hematopoietic stem cells, after radiation exposure at graded doses, for long-term regeneration of peripheral blood in lethally irradiated recipients. MATERIALS AND METHODS: LKS primitive progenitor cells, collected from the bone marrow of Ly5.1 mice that had been irradiated at graded increased doses (0.5, 1, 1.5, and 2 Gy) were transfused into lethally irradiated (9.5 Gy) Ly5.2 mice. Then, the Ly5.1 chimeric ratio in repopulated peripheral blood cells in the recipients was monitored. A reactive oxygen species (ROS)-reacting CM-H(2)DCFDA dye was used to evaluate the amount of ROS in LKS primitive progenitor cells with/without irradiation. Moreover, the amount of intracytoplasmic ROS generated after irradiation was estimated in terms of percent attenuation of cellular increase in number by the treatment with 100 microM N-acetyl-L-cysteine before irradiation. RESULTS: Differential regeneration capability of LKS cells irradiated at graded increased doses showed a dose-dependent suppression of regeneration of peripheral blood in the recipient mice as compared with LKS cells without radiation exposure. The amount of intracytoplasmic ROS in LKS cells was much smaller than that in mature bone marrow cells, and that of ROS in LKS increased slightly after radiation exposure, as evaluated by CM-H(2)DCFDA dye fluorescence analysis. The estimated amount of ROS generated in LKS cells after radiation exposure was different between progenitor cells for early regeneration and those for late regeneration; namely, the amount of ROS in progenitors on day 270 were estimated to be smaller than that in progenitors for day 35 or day 90. CONCLUSIONS: Because of the small amount of generated radiation-induced ROS calculated in terms of attenuation rate after N-acetyl-L-cysteine treatment, progenitor cells regenerating peripheral blood cells 270 days after transfusion were assumed to be anaerobic and more immature and radioresistant than those on day 35 or day 90. However, limited long-term regeneration capability (up to 270 days) of steady-state LKS cells than that of unfractionated rescue bone marrow cells suggests that LKS cells do not seem to be true hematopoietic stem cells.

Keywords : long-term regeneration capability, reactive oxygen species

* Central Research Institute of Electric Power Industry

Tsuboi, I., Harada, T.*, Hirabayashi, Y., Kanno, J., Inoue, T. and Aizawa, S.* : **Inflammatory biomarker, neopterin, predominantly enhances myelopoiesis, which suppresses erythropoiesis via activated stromal cells**

Immunobiology, **215**, 348-355 (2010)

Neopterin is produced by monocytes and is a useful biomarker for inflammation. We found previously that neopterin enhanced myelopoiesis but suppressed B-lymphopoiesis triggered by the positive and negative regulations of cytokines produced by stromal cells in mice. The effects of neopterin on erythropoiesis during the enhancement of myelopoiesis were determined in the present study using C57BL/6J mice. The intravenous injection of neopterin into mice resulted in a prolonged decrease in the number of femoral erythroid progenitor cells (BFU-Es and CFU-Es), whereas the number of femoral myeloid progenitor cells (CFU-GMs) was increased. Interestingly, the oscillatory changes in the number of erythroid progenitor cells were reciprocal to those of myeloid progenitor cells. The expression of Cdc42, a regulator of the balance between erythropoiesis and myelopoiesis, was down-regulated, implying that the suppression of erythropoiesis is due to myelopoietic predominance. Furthermore, the expression of SDF-1 in stromal cells, a negative regulator of erythropoiesis, was up-regulated. These results suggest that neopterin facilitates myelopoiesis in the bone marrow by suppressing erythropoiesis, thereby contributing to the potential up-regulation of inflammatory process.

Keywords : Cdc42, hematopoietic progenitor cells, SDF-1

* Nihon University

Oginuma, M.*[1], Takahashi, Y., Kitajima, S., Kiso, M.*[1], Kanno, J., Kimura, A.*[1,2] and Saga, Y.*[1] : **The oscillation of Notch activation, but not its boundary, is required for somite border formation and rostral-caudal patterning within a somite**

Development, **137**, 1515-1522 (2010)

Notch signaling exerts multiple roles during different steps of mouse somitogenesis. We have previously shown that segmental boundaries are formed at the interface of the Notch activity boundary, suggesting the importance of the Notch on/off state for boundary formation. However, a recent study has shown that mouse embryos expressing Notch-intracellular domain (NICD) throughout the presomitic mesoderm (PSM) can still form more than ten somites, indicating that the NICD on/off state is dispensable for boundary formation. To clarify this discrepancy in our current study, we created a transgenic mouse lacking NICD boundaries in the anterior PSM but retaining Notch signal oscillation in the posterior PSM by manipulating the expression pattern of a Notch modulator, lunatic fringe. In this mouse, clearly segmented somites are continuously generated, indicating that the NICD on/off state is unnecessary for somite boundary formation. Surprisingly, this mouse also showed a normal rostral-caudal compartment within a somite, conferred by a normal Mesp2 expression pattern with a rostral-caudal gradient. To explore the establishment of normal Mesp2 expression, we performed computer simulations, which revealed that oscillating Notch signaling induces not only the periodic activation of Mesp2 but also a rostral-caudal gradient of Mesp2 in the absence of striped Notch activity in the anterior PSM. In conclusion, we propose a novel function of Notch signaling, in which a progressive oscillating wave of Notch activity is translated into the rostral-caudal polarity of a somite by regulating Mesp2 expression in the anterior PSM. This indicates that the initial somite pattern can be defined as a direct output of the segmentation clock.

Keywords : notch signaling, mesp2, somitogenesis

*[1] Division of Mammalian Development, National Institute of Genetics

*[2] Cell Architecture Laboratory, National Institute of Genetics

Arase, S.*[1], Ishii, K.*[1], Igarashi, K., Aisaki, K., Yoshio, Y.*[1], Matsushima, A.*[2], Shimohigashi, Y.*[2], Arima, K.*[1], Kanno, J., Sugimura, Y.*[1].: **Endocrine disrupter bisphenol A increases in situ estrogen production in the mouse urogenital sinus**

Biol. Reprod., **84**, 734-42 (2011)

The balance between androgens and estrogens is very important in the development of the prostate, and even small changes in estrogen levels, including those

of estrogen-mimicking chemicals, can lead to serious changes. Bisphenol A (BPA), an endocrine-disrupting chemical, is a well-known, ubiquitous, estrogenic chemical. To investigate the effects of fetal exposure to low-dose BPA on the development of the prostate, we examined alterations of the in situ sex steroid hormonal environment in the mouse urogenital sinus (UGS). In the BPA-treated UGS, estradiol (E(2)) levels and CYP19A1 (cytochrome P450 aromatase) activity were significantly increased compared with those of the untreated and diethylstilbestrol (DES)-treated UGS. The mRNAs of steroidogenic enzymes, Cyp19a1 and Cyp11a1, and the sex-determining gene, Nr5a1, were upregulated specifically in the BPA-treated group. The upregulation of mRNAs was observed in the mesenchymal component of the UGS as well as in the cerebellum, heart, kidney, and ovary but not in the testis. The number of aromatase-expressing mesenchymal cells in the BPA-treated UGS was approximately twice that in the untreated and DES-treated UGS. The upregulation of Esrrg mRNA was observed in organs for which mRNAs of steroidogenic enzymes were also upregulated. We demonstrate here that fetal exposure to low-dose BPA has the unique action of increasing in situ E(2) levels and CYP19A1 (aromatase) activity in the mouse UGS. Our data suggest that BPA might interact with in situ steroidogenesis by altering tissue components, such as the accumulation of aromatase-expressing mesenchymal cells, in particular organs.

Keywords: bisphenol A, in situ estrogen production, urogenital sinus

---

*1 Department of Nephro-Urologic Surgery and Andrology, Mie University Graduate School of Medicine
*2 Laboratory of Structure-Function Biochemistry, Department of Chemistry, Faculty of Sciences, Kyushu University

Ikegami, D.*1, Narita, M.*1, Imai, S.*1, Miyashita, K.*1, Tamura, R.*1, Narita, M.*1, Takagi, S.*1, Yokomizo, A.*1, Takeshima, H.*1, Ando, T.*1, Igarashi, K., Kanno, J., Kuzumaki, N.*1, Ushijima, T.*2, Suzuki, T.*1: **Epigenetic modulation at the CCR2 gene correlates with the maintenance of behavioral sensitization to methamphetamine**

Addic. Biol., **15**, 358-361 (2010)

The intermittent administration of methamphetamine produces behavioral sensitization to methamphetamine. In the limbic forebrain, mainly including the nucleus accumbens, of mice that had been intermittently treated with methamphetamine, we found a significant increase in mRNA of a chemokine, CCR2. This increase was accompanied by a significant increase in histone H3 lysine 4 (H3K4) trimethylation at its promoter. Interestingly, the maintenance of sensitization to methamphetamine-induced hyperlocomotion was significantly decreased in CCR2 knockout mice. These findings suggest that increased CCR2 associated with epigenetic modification after the intermittent administration of methamphetamine may be associated with the maintenance of sensitization to methamphetamine-induced hyperlocomotion.

Keywords: epigenetic modulation, behavioral sensitization, CCR2

---

*1 Department of Toxicology, Hoshi University School of Pharmacy and Pharmaceutical Sciences
*2 Carcinogenesis Division, National Cancer Center Research Institute

Yoshida, T.*, Sekine, T.*, Aisaki, K., Mikami, T.*, Kanno, J., Okayasu, I.*: **CITED2 is activated in ulcerative colitis and induces p53-dependent apoptosis in response to butyric acid**

J. Gastroenterol, **46**, 339-349 (2011)

BACKGROUND: In ulcerative colitis (UC), Fusobacterium varium is significantly detected in patients' mucosa, and butyric acid (BA), abundantly produced by the bacterium, activates the p53 system and induces epithelial apoptosis, as we previously reported. However, factors active in the link between BA and p53 have yet to be clarified. Here, we identified a gene activated by BA specifically in UC-associated cancer cell lines and ascertained the mechanism of its activation of p53.

METHODS: cDNA microarray analysis based on the Percellome (per cell normalization) method was performed on BA-stimulated UC-associated cancers and sporadic colorectal cancer cell lines under conditions mimicking colonic epithelium UC. For validation of microarray results, molecular, biochemical, and histopathological analyses were performed.

RESULTS: We found the CBP/p300-interacting transactivator with glutamic acid/asparagine-rich carboxy-terminal domain 2 (CITED2) to be specifically upregulated in UC-associated cancer cell lines by BA treatment,

at both mRNA and protein expression levels. CITED2 could be shown to induce p53 acetylation and p53-dependent apoptosis, accompanied by binding of CBP/p300. BA-dependent apoptosis was suppressed by an inhibitor of monocarboxylate transporter-1 and an siRNA for p53. In inflammatory foci of UC, histologically evident inflammatory activity and CITED2 expression were significantly correlated.
CONCLUSIONS: CITED2 was identified as UC-associated protein by cDNA microarray based on the Percellome method under UC-mimicking conditions in vitro. CITED2 activation may induce mucosal apoptosis and erosion by activating p53 and thus play a critical role in linking enteric bacteria with mucosal inflammation in UC.
Keywords: ulcerative colitis, p53-dependent apoptosis, CITED2

---

* Department of Pathology, Kitasato University School of Medicine

Hirata, N., Sekino, Y. and Kanda, Y.: **Nicotine increases cancer stem cell population in MCF-7 cells**
Biochem. Biophys. Res. Commun., **403**, 138-143 (2010)

Epidemiological studies have suggested that cigarette smoking is related to increased breast cancer risk. Nicotine is most likely related to the risk in cigarette smoking. However, the mechanisms by which nicotine promotes cancer development are not fully understood. It has recently been suggested that development of breast cancer are originated from cancer stem cells, which are a minor population of breast cancer. In the present study, we investigated the effects of nicotine on the population of cancer stem cells in MCF-7 human breast cancer cells, using flow cytometry with a cancer stem cell marker aldehyde dehydrogenase (ALDH). We found that nicotine increased ALDH-positive cell population in a dose-dependent manner. We further demonstrated that a PKC-Notch pathway is involved in the effect of nicotine. In addition, the effect of nicotine was blocked by treatment with the α7 subunit-selective antagonist of nicotinic acetylcholine receptors (nAChR) α-Bungarotoxin. These data suggest that nicotine increases the stem cell population via α7-nAChR and the PKC-Notch dependent pathway in MCF-7 cells. These findings reveal a relationship between nicotine and the cancer stem cells in human breast cancer.
Keywords: cancer stem cells, nicotine, notch

Usami, M., Mitsunaga, K.[*1], Miyajima, A., Sunouchi, M. and Doi, O.[*2]: **Complement component C3 functions as an embryotrophic factor in early postimplantation rat embryos**
Int. J. Dev. Biol., **54**, 1277-1285 (2010)

A presumed embryotrophic factor for early postimplantation rat embryos, partially purified from rat serum, was identified as complement component C3 (C3), the central component of the complement system, by sequence analysis of its N-terminal. Purified rat C3 showed embryotrophic activity for rat embryos cultured from day 9.5 of gestation for 48 h in the culture medium composed of rabbit serum. The maximum embryotrophic activity of C3 was observed around 0.5 mg/ml, a level which is lower than rat serum C3 levels. In the culture medium composed of rat serum, cultured rat embryos selectively consumed C3, and C3-depletion by cobra venom factor affected embryonic growth. Inactivation of the internal thiolester bond of C3, the critical functional site for its activity in the complement system, by methylamine had no effects on its embryotrophic activity. Purified rabbit C3 had only weak embryotrophic activity for cultured rat embryos, suggesting species specificity of the embryotrophic activity of C3. Immunochemical analyses showed the specific presence of C3 on the visceral yolk sac, but not on the embryo proper of day 9.5 or 10.5 rat embryos both in utero and in vitro. In analysis using fluorescein-labeled rat C3, unfragmented C3s bound to the visceral yolk sac stronger than C3b, the primary active fragment of C3 in the complement system. These results indicate that C3, which has always been considered to be detrimental to embryos, functions as an embryotrophic factor by novel mechanisms probably through the visceral yolk sac. The present study thus provides new insights into functions of C3 and postimplantation embryonic growth.
Keywords: complement component C3, embryo trophic factor, rat

---

[*1] 東邦大学薬学部
[*2] 岐阜大学応用生物科学部

Kojima, H., Takeyoshi, M.[*1], Sozu, T.[*2], Awogi, T.[*3], Arima, K.[*4], Idehara, K.[*5], Ikarashi, Y., Kanazawa, Y.[*6], Maki, E.[*7], Omori, T.[*8], Yuasa, A.[*9], Yoshimura, I.[*10]:

Inter-laboratory validation of the modified murine local lymph node assay based on 5-bromo-2'-deoxyuridine incorporation

J. Appl. Toxicol., **31**(1), 63-74(2011)

The murine local lymph node assay (LLNA) is a well-established alternative to the guinea pig maximization test (GPMT) or Buehler test (BT) for the assessment of the skin sensitizing ability of drugs and chemicals. Instead of radioisotope using in this method, Chemicals Evaluation and Research Institute, Japan (CERI) has developed a modified LLNA based on the 5-bromo-2'-deoxyuridine (BrdU) incorporation (LLNA-BrdU ELISA). We conducted the validation study to evaluate the reliability and relevance of LLNA-BrdU ELISA.

The experiment involved 7 laboratories, wherein 10 chemicals were examined under blinded conditions. In this study, 3 chemicals were examined in all laboratories and the remaining 7 were examined in 3 laboratories. The data were expressed as the BrdU incorporation using ELISA method for each chemical-treated group, and the stimulation index (SI) for each chemical-treated group was determined as the increase in the BrdU incorporation relative to the concurrent vehicle control group. An SI of 2 was set as the cut-off value for exhibiting skin sensitization activity.

The results of this study obtained in the experiments conducted for the 3 chemicals that were examined in all the 7 laboratories and all the 7 chemicals were sufficiently consistent with small variations in their SI values. The sensitivity, specificity, and accuracy of LLNA-BrdU ELISA against those of GPMT/BT were 7/7(100%), 3/3(100%), and 10/10(100%), respectively.

Keywords: Inter-laboratory validation, Local lymph node assay, Skin sensitization

*1 (財) 化学物質評価研究機構
*2 大阪大学
*3 大塚製薬（株）
*4 大正製薬（株）
*5 ダイセル化学工業（株）
*6 (財) 食品薬品安全センター
*7 (財) 食品農医薬品安全性評価センター
*8 京都大学
*9 富士フィルム（株）
*10 東京大学

Yamamoto, N.*1, Hirano, K.*1, Kojima, H., Sumitomo, M.*1, Yamashita, H.*1, Ayaki, M.*2, Taniguchi, K.*1, Tanikawa, A.*1, Horiguchi, M.*1: **Cultured human corneal epithelial stem/progenitor cells derived from the corneal limbus**

In Vitro Cell Dev. Biol. Anim., **46**(9), 774-780(2010)

Stem/progenitor cells of the human corneal epithelium are present in the human corneal limbus, and several corneal epithelial stem/progenitor cell markers have been reported. Recently, the neurotrophin family receptors were reported to be useful markers of corneal epithelial stem/progenitor cells. Therefore, we examined an enzymatic separation method for obtaining corneal epithelial stem/progenitor cells and measuring the change in the expression of low-affinity neurotrophin receptor p75 (p75NTR), a receptor belonging to the neurotrophin family. As a result, it was found that our separation method preserved cell viability. Furthermore, p75NTR was mainly observed in epithelial basal cells as were the corneal epithelial stem/progenitor markers p63 and integrin β1. p75NTR was also observed in the cultured cells, but its frequency decreased with passage. In conclusion, we propose that our culture method will enable the culture of corneal stem cells and that it is a useful tool for elucidating the molecular basis of the niche that is necessary for the maintenance of epithelial stem cells in the corneal limbus. Furthermore, we conclude that p75NTR is a useful cell marker for evaluating the characteristics of stem/progenitor cells in culture.

Keywords: human corneal epithelium, corneal limbus, low-affinity neurotrophin receptor p75 (p75NTR)

*1 藤田保健衛生大学
*2 昭和大学藤が丘リハビリテーション病院

柘植英哉*1，森　充生*1，大庭澄明*1，大内　正*1，寺田三郎*1，五島隆志*2，田邊豊重*2，山影康次*3，田中憲穂*3，渡辺美香*3，畔上二郎*3，大向英夫*3，小島肇： **平成21年度「日本薬局方の試験法に関する研究」研究報告，輸液用ゴム栓試験法の見直し（第3報）－細胞毒性試験法の検討－**

医薬品医療機器レギュラトリーサイエンス，**42**(3), 258-271（2011）

注射剤等の「直接の容器」に使用されているプラスチック製医薬品容器については，その品質を規定する試験法が日局に「プラスチック製医薬品容器試験法」として

規定されている．この一般試験法は，日局13（1996年4月施行）において，「輸液用プラスチック容器試験法」から「プラスチック製医薬品容器試験法」へ名称が変更されるとともに，動物を用いる試験（急性毒性試験，皮内反応試験，発熱性物質試験，溶血性試験及び埋植試験）が削除され，in vitroの細胞毒性試験が導入された．一方，同じ注射剤等の「直接の容器」として使用されるゴム栓については，日局に「輸液用ゴム栓試験法」が規定されているが，日局9（1976年4月施行）以来30年間以上，見直しがなされず今日に至っており，動物を用いた急性毒性試験，発熱性物質試験及び溶血性試験は現在も規定され実施されている．しかし，2005年6月に「動物の愛護及び管理に関する法律」が改正され，動物実験における動物使用数の削減，動物に与える苦痛の低減，及び動物を使用しない方法への置き換えの努力が義務付けられ，法律遵守の観点から，日局「輸液用ゴム栓試験法」の再評価が必要な状況にある．

以上の背景から，動物を用いる急性毒性試験，発熱性物質試験及び溶血性試験のうち，急性毒性試験法を細胞毒性試験法へ切り替えるため，その基礎データを取得することを目的に本研究を行った．

Keywords：日局，細胞毒性試験法，輸液用ゴム栓

*1（社）東京医薬品工業協会
*2大阪医薬品協会技術研究委員会
*3（財）食品薬品安全センター

Saegusa, Y.*1,2, Woo, G. H., Fujimoto, H., Inoue, K., Takahashi, M., Hirose, M.*3, Igarashi, K., Kanno, J., Mitsumori, K.*1, Nishikawa, A. and Shibutani, M.*1:
Gene expression profiling and cellular distribution of molecules with altered expression in the hippocampal CA1 region after developmental exposure to anti-thyroid agents in rats
J. Vet. Med. Sci., **72**, 187-195 (2010)

To determine whether developmental hypothyroidism causes permanent disruption of neuronal development, we first performed a global gene expression profiling study targeting hippocampal CA1 neurons in male rats at the end of maternal exposure to anti-thyroid agents on weaning (postnatal day 20). As a result, genes associated with nervous system development, zinc ion binding, apoptosis and cell adhesion were commonly up- or down-regulated. Genes related to calcium ion binding were up-regulated and those for myelination were often down-regulated. We, then, examined immunohistochemical cellular distribution of Ephrin type A receptor 5 (EphA5) and Tachykinin receptor (Tacr)-3, those selected based on the gene expression profiles, in the hippocampal formation at the adult stage (11-week-old) as well as at the end of exposure. At weaning, both EphA5- and Tacr3-immunoreactive cells with strong intensities appeared in the pyramidal cell layer or stratum oriens of the hippocampal CA1 region. Although the magnitude of the change was decreased at the adult stage, Tacr3 in the CA1 region showed a sustained increase in expressing cells until the adult stage after developmental hypothyroidism. On the other hand, EphA5-expressing cells did not show sustained increase at the adult stage. The results suggest that developmental hypothyroidism caused sustained neuronal expression of Tacr3 in the hippocampal CA1 region, probably reflecting a neuroprotective mechanism for mismigration.

Keywords: Developmental hypothyroidism, Hippocampal CA1 region, Tacr3

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

Ishii, Y., Okamura, T., Inoue, T., Fukuhara, K., Umemura, T. and Nishikawa, A.:
Chemical structure determination of DNA bases modified by active metabolites of lucidin-3-O-primeveroside
Chem. Res. Toxicol., **23**, 134-141 (2010)

Lucidin-3-O- primeveroside (LuP) is one of the components of madder root (Rubia tinctorum L.; MR) which is reported to be carcinogenic in the kidney and liver of rats. Since metabolism of LuP generates genotoxic compounds such as lucidin (Luc) and rubiadin (Rub), it is likely that LuP plays a key role in MR carcinogenesis. In the present study, the chemical structures of Luc-specific 2'-deoxyguanosine (dG) and 2'-deoxyadenosine (dA) adducts following the reactions of dG and dA with a Luc carbocation or quinone methide intermediate derived from Acetoxy-Luc were determined by liquid chromatography with photodiode array and electron spray ionizaion-mass spectrometry (LC-PDA-ESI/MS). The identification of the two measurable adducts as Luc-N(2)-dG and Luc-N(6)-dA was confirmed by NMR analysis. Subsequently, using a newly developed quantitative analytical method using LC-ESI/MS, the formation of Luc-N(2)-dG and Luc-N

(6)-dA from the reaction of calf thymus DNA with Luc in the presence of S9 mixture was observed. The fact that this reaction with Rub also gave rise to the same dG and dA adducts strongly suggests that Rub genotoxicity involves a metabolic conversion to Luc. The precise determination of the modified DNA bases generated by LuP and the method for their analysis may contribute to further comprehension of the mode of action underlying carcinogenesis by MR and related anthraquinones.

Keywords: DNA adduct, Lucidin-3-O-primeveroside

Takami, S., Imai, T., Cho, Y. M., Hirose, M. and Nishikawa, A.: **Lack of modifying effects of prepubertal exposure to acrylamide (AA) on *N*-methyl-*N*-nitrosourea (MNU)-induced multi-organ carcinogenesis in F344 rats**

J. Toxicol. Sci., **35**, 57-68 (2010)

Acrylamide (AA) has been reported to be formed in fried and baked foods with various concentrations, and exposure levels to AA from cooked foods in children are estimated to be higher than those in adults. In order to evaluate the carcinogenicity of AA exposure during childhood, we conducted a medium-term carcinogenicity study with prepubertal administration of AA followed by treatments of a multi-organ-targeted genotoxic carcinogen and a promoting agent for thyroid carcinogenesis in rats. A total of 36 postpartum F344 rats were given drinking water containing AA at 0, 20, 40 or 80 ppm for 3 weeks during the lactation period, and their weaned offspring received the same AA-containing water for 3 more weeks. Offspring were then injected with N-methyl-N-nitrosourea (MNU; 40 mg/kg body weight, i.p.) once at week 7 after birth. Half the animals of the 0 and 40 ppm groups were additionally treated with the anti-thyroid agent sulfadimethoxine (SDM; 125 ppm) in the drinking water thereafter. Offspring were subjected to complete necropsy at week 50. All the major organs and macroscopic abnormalities were excised and examined histopathologically. There was no significant difference in the incidences of hyperplastic and neoplastic lesions in the target organs of AA and/or MNU, such as the brain, spinal cord, pituitary gland, thyroid, adrenal glands, uterus, mammary glands, clitoral gland and tunica vaginalis. In conclusion, no significant modifying actions of AA on MNU-induced multi-organ carcinogenesis were exhibited in any organs of rats when exposed prepubertally under the present experimental conditions.

Keywords: Acrylamide, Carcinogenesis, Prepubertal exposure

Jin, M., Dewa, Y.[*1], Kawai, M.[*1,2], Nishimura, J.[*1], Saegusa, Y.[*1,2], Kemmochi, S.[*1,2], Harada, T.[*1], Shibutani, M.[*1] and Mitsumori, K.[*1]: **The threshold dose for liver tumor promoting effects of dicyclanil in ICR mice**

J. Toxicol. Sci., **35**, 69-78 (2010)

To determine the threshold dose of dicyclanil (DC) that induces hepatocellular tumor-promoting effects associated with reactive oxygen species (ROS) generation via their metabolic pathways, partial hepatectomized ICR male mice were fed diets containing 0, 187.5, 375 or 750 ppm DC after an intraperitoneal injection of N-diethylnitrosamine (DEN) to initiate hepatocarcinogenesis. Immunohistochemically, the proliferating cell nuclear antigen (PCNA)-positive cell ratio was significantly increased in the DEN + 750 ppm DC group compared with the DEN alone group. However, significant increases in the number of gamma-glutamyltranspeptidase (GGT)-positive cells and formation of microsomal ROS were not observed in the DEN + DC groups compared with the DEN alone group. Real-time polymerase chain reaction (RT-PCR) showed that the expression of Cyp1a1, Cyp1a2, and OGG1genes was significantly up-regulated in mice given diets containing 375 ppm DC or more, 187.5 ppm DC or more, and 750 ppm DC, respectively. These results suggest that the threshold dose of DC that induces ROS-mediated liver tumor promotion in mice is more than 750 ppm, although expression of the Cyp1a2 gene, which is related to ROS generation, was up-regulated in the liver of mice, even at a DC dose of 187.5 ppm.

Keywords: Dicyclanil, ROS generation, Threshold dose

[*1] Tokyo University of Agriculture and Technology
[*2] Gifu University

Takahashi, M., Yoshida, M., Inoue, K., Morikawa, T. and Nishikawa, A.: **Age-related susceptibility to induction of osteochondral and vascular lesions by semicarbazide hydrochloride in rats**

Toxicol. Pathol., **38**, 598-605 (2010)

To compare the susceptibility to toxicity of semicarbazide hydrochloride (SEM-HCl) between young and

adult rats, 3- and 20-week-old female SD rats were given a diet containing SEM-HCl at 0, 500, or 1,000 ppm and 0 or 1,000 ppm, respectively, for 4 weeks. Half of the animals were then maintained on basal diet for a further 2 weeks as recovery groups. Only in young rats was deformation of the knee joints as well as thorax and tail observed at 500 and 1,000 ppm. Histopathologically, severe osteochondral lesions, such as disarrangement and thickening of the epiphyseal cartilage and deformation of articular cartilage, were observed, but the severity of these lesions became reduced during the recovery period. In adult rats, osteochondral lesions were relatively mild. Fissures in the cartilage matrix of the tibia were characteristic of adult rats, and in these, reduction of severity was not obvious in the recovery group. In the thoracic aorta, the appearance of elastic laminae was altered only in young rats in both the 4-week treatment and recovery groups. These results suggest that growing animals are more susceptible to toxicity of SEM-HCl than adults are. Effects and the induced lesions link to the developing stage of the target organs.

Keywords: Semicarbazide hydrochloride, Osteolathyrism, Children

Saegusa, Y.[*1,2], Woo, G. H., Fujimoto, H., Kemmochi, S.[*1,2], Shimamoto, K.[*1,2], Hirose, M.[*3], Mitsumori, K.[*1], Nishikawa, A. and Shibutani, M[*1].: **Sustained production of Reelin-expressing interneurons in the hippocampal dentate hilus after developmental exposure to anti-thyroid agents in rats**

Reprod. Toxicol., **29**, 407-414 (2010)

To detect molecular evidence reflecting a permanent disruption of neuronal development due to hypothyroidism, distribution of Reelin-producing cells that function in neuronal migration and positioning was analyzed in the hippocampal dentate hilus using rats. From gestation day 10, maternal rats were administered either 6-propyl-2-thiouracil (PTU) at 3 or 12ppm (0.57 or 1.97 mg/kg body weight/day) or methimazole (MMI) at 200ppm (27.2mg/kg body weight/day) in the drinking water and male offspring were immunohistochemically examined at the end of exposure on weaning (postnatal day 20) and at the adult stage (11-week-old). Offspring with MMI and 12ppm PTU displayed evidence of growth retardation lasting into the adult stage. On the other hand, all exposure groups showed a sustained increase in Reelin-expressing cells in the dentate hilus until the adult stage in parallel with Calbindin-D-28K-expressing cells at weaning and with glutamic acid decarboxylase 67-positive cells in the adult stage, confirming an increase in gamma-aminobutyric acid (GABA) ergic interneurons. At the adult stage, NeuN-positive postmitotic mature neurons were also increased in the hilus in all exposure groups, however, the increased population of Reelin-producing cells at this stage was either weakly positive or negative for NeuN, indicative of immature neurons. At weaning, neuroblast-producing subgranular zone of the dentate gyrus showed increased apoptosis and decreased cell proliferation suggestive of impaired neurogenesis. The results suggest that sustained increases of immature GABAergic interneurons synthesizing Reelin in the hilus could be a signature of compensatory regulation for impaired neurogenesis and mismigration during the neuronal development as a hypothyroidism-related brain effect rather than that secondary to systemic growth retardation.

Keywords: Impaired brain development, Reelin, GABAergic interneuron

---

[*1] Tokyo University of Agriculture and Technology
[*2] Gifu University
[*3] Food Safety Commission

Pitchakarn, P.[*1,2], Ogawa, K., Suzuki, S.[*1], Takahashi, S.[*1], Asamoto, M.[*1], Chewonarin, T.[*2], Limtrakul, P.[*2] and Shirai, T.[*1]: ***Momordica charantia*** **leaf extract suppresses rat prostate cancer progression *in vitro* and *in vivo***

Cancer Sci., **101**, 2234-2240 (2010)

Cancer metastasis is a major cause of death in cancer patients, with invasion as a first step greatly contributing to the failure of clinical treatments. Any compounds with an inhibitory influence on this process are therefore of prime interest. *Momordica charantia* (bitter melon) is widely consumed as a vegetable and especially as a folk medicine in Asia. Here, we investigated the anti-invasive effects of bitter melon leaf extract (BMLE) on a rat prostate cancer cell line (PLS10) *in vitro* and in vivo. The results indicated that non-toxic concentrations of BMLE significantly inhibited the migration and invasion of cells *in vitro*. The results of zymography showed that BMLE inhibited the secretion of MMP-2, MMP-9

and urokinase plasminogen activator（uPA）from PLS10. Real-time RT-PCR revealed that BMLE not only significantly decreased gene expression of MMP-2 and MMP-9, but also markedly increased the mRNA level of TIMP-2, known to have inhibitory effects on the activity of MMP-2. An EnzChek gelatinase/collagenase assay showed that collagenase type IV activity was partially inhibited by BMLE. In the *in vivo* study, intravenous inoculation of PLS10 to nude mice resulted in a 100% survival rate in the mice given a BMLE-diet as compared with 80% in the controls. The incidence of lung metastasis did not show any difference, but the percentage lung area occupied by metastatic lesions was slightly decreased in the 0.1% BMLE treatment group and significantly decreased with 1% BMLE treatment as compared with the control. Thus, the results indicate for the first time an anti-metastatic effect of BMLE both *in vitro* and *in vivo*.

Keywords：Prostate cancer, Metastasis, Bitter melon

*1 Nagoya City University
*2 Chiang Mai University

Hibi, D., Imazawa, T.*, Kijima, A., Suzuki, Y., Ishii, Y., Jin, M., Umemura, T. and Nishikawa, A.: **Investigation of Carcinogenicity for Levamisole Administered in the Diet to F344 Rats**

Food Chem. Toxicol., **48**, 3321-3326（2010）

A two year carcinogenicity study of anthelmintic drug levamisole（LV）was performed using 50 male and 50 female F344 rats at dietary drug concentrations of 0, 60, or 300 ppm. The daily intakes of LV were calculated to be 2.6, 12.9 mg/kg b.w./day for males and 2.9, 14.1 mg/kg b.w./day for females, respectively. No significant differences in general condition and survival rate（82%, 74%, 80% in males and 84%, 84%, 84% in females, respectively）were observed. In the 300 ppm group, suppression of body weight gain was observed from the onset of treatment and reduction in final body weights was 6% in males and 11% in females. Significant increases in the absolute and/or relative weights of the lungs, heart, spleen, liver, kidneys, and adrenals were observed in males and/or females treated with 300 ppm. Some of high incidences neoplasms were observed, and there were also tendencies to increase for mammary gland fibroma and thoracic/abdominal cavity mesothelioma in males. However, there were no significant inter-group differences in incidences, histopathological types or differences compared with historical control data. Thus, it was concluded that LV was not carcinogenic to male and female F344 rats under the experimental conditions.

Keywords：Two year carcinogenicity study, Levamisole

* National Institute of Biomedical Innovation

Yahia, D.*1,2, El-Nasser, M. A.*1, Abedel-Latif, M.*1, Tsukuba, C.*2, Yoshida, M., Sato, I.*2 and Tsuda, S.*2: **Effects of perfluorooctanoic acid（PFOA）exposure to pregnant mice on reproduction**

J. Toxicol. Sci., **35**, 527-533（2010）

Perfluorooctanoic acid（PFOA）has similar characteristics to perfluorooctane sulfonate（PFOS）in reproduction toxicity featured by neonatal death. We found that PFOS exposure to mice during pregnancy led to intracranial blood vessel dilatation of fetuses accompanied by severe lung collapse which caused neonatal mortality. Thus, we adopted the corresponding experimental design to PFOS in order to characterize the neonatal death by PFOA. Pregnant ICR mice were given 1, 5 and 10 mg/kg PFOA daily by gavage from gestational day（GD）0 to 17 and 18 for prenatal and postnatal evaluations, respectively. Five to nine dams per group were sacrificed on GD 18 for prenatal evaluation；other 10 dams were left to give birth. No maternal death was observed. The liver weight increased dose-dependently, with hepatocellular hypertrophy, necrosis, increased mitosis and mild calcification at 10 mg/kg. PFOA at 10 mg/kg increased serum enzyme activities（GGT, ALT, AST and ALP）with hypoproteinemia and hypolipidemia. PFOA treatment reduced the fetal body weight at 5 and 10 mg/kg. Teratological evaluation showed delayed ossification of the sternum and phalanges and delayed eruption of incisors at 10 mg/kg, but did not show intracranial blood vessel dilatation. Postnatal evaluation revealed that PFOA reduced the neonatal survival rate at 5 and 10 mg/kg. At 5 mg/kg pups were born alive and active and 16% died within 4 days observation, while all died within 6 hr after birth at 10 mg/kg without showing intracranial blood vessel dilatation. The cause of neonatal death by PFOA may be different from PFOS.

Keywords：PFOA, Mice, Neonatal death

*1 Assiut University
*2 Iwate University

Okamura, T., Ishii, Y., Suzuki, Y., Inoue, T., Tasaki, M., Kodama, Y., Nohmi, T., Mitsumori, K.*, Umemura, T. and Nishikawa, A.: **Enhancing effects of carbon tetrachloride on in vivo mutagenicity in the liver of mice fed 2-amino-3,8-dimethylimidazo[4,5-f] quinoxaline (MeIQx)**

J. Toxicol. Sci., **35**, 709-720 (2010)

Chronic stimulus subsequent to cell injury plays an important role in cancer development, but the precise mechanisms remain unknown partly because appropriate animal models are lacking. In the present study, the effects of hepatotoxicant carbon tetrachloride (CCl(4)) on in vivo mutagenicity were investigated using gpt delta mice with or without p53. Female B6C3F(1) p53-proficient or -deficient gpt delta mice were given a diet containing 300 ppm of 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline (MeIQx) for 13 weeks, concurrently with intraperitoneal injection of 1 ml/kg CCl(4) solution once a week. Mutant frequencies of gpt and red/gam in p53-proficient mice fed MeIQx were both significantly elevated by CCl(4) co-treatment. Enhancing effects of CCl(4) treatment were also noted in p53-deficient mice. In the mutation spectra analysis of gpt mutant colonies, G : C to T : A transversions were predominantly observed regardless of CCl(4) injection, and clonal expansion of gpt colonies were increased in the co-treated group as compared with MeIQx alone group. The present data showing no significant changes in mRNA expression levels of CYP1A2 and GSTa4 between MeIQx-treated groups with and without CCl(4). In the Western blotting analysis, CYP1A2 protein levels were significantly decreased in the co-treated group as compared to MeIQx alone group, and GSTα protein levels were not changed among any groups. It is suggested that the mutant frequency by co-treatment with CCl(4) might result from some factors other than p53 or MeIQx metabolism /excretion. Thus, our data clearly demonstrate that this model could be a powerful tool for identifying the mechanisms underlying combinatorial effects on carcinogenesis.

Keywords: *In vivo* mutagenicity, Hepatotoxicant carbon tetrachloride, 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline

---

* Tokyo University of Agriculture and Technology

Okamura, T., Ishii, Y., Suzuki, Y., Inoue, T., Tasaki, M., Kodama, Y., Nohmi, T., Mitsumori, K.*, Umemura, T. and Nishikawa, A.: **Effects of co-treatment of dextran sulfate sodium and MeIQx on genotoxicity and possible carcinogenicity in the colon of p53-deficient mice**

J. Toxicol. Sci., **35**, 731-741 (2010)

To investigate the effects of dextran sulfate sodium (DSS) and/or 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) on in vivo genotoxicity in the colon, male C57BL/6 p53(+/+), p53(+/−) or p53(−/−) gpt delta mice were twice given 1-week treatment with DSS, 2 weeks apart, and then sacrificed after 2 and 14 weeks. Although colon length was significantly shortened after DSS treatment in all genotypes at each time point, no significant difference in gpt mutant frequency (MF) and tumorigenicity was found between DSS and control groups regardless of genotype. Then, male B6C3F(1) p53(+/+) or p53(+/−) gpt delta mice were given DSS as described above and/or fed 300 ppm MeIQx for 7 weeks. Colon length was significantly shortened with DSS in either genotype at weeks 7 and 26, but no effects of co-treatment with MeIQx or p53 deficiency were evident. MeIQx showed a tendency to increase gpt MF in the colon of mice with either genotype, but co-treatment with DSS did not affect these increments. Appreciable incidences of colonic aberrant crypt foci (ACFs) were reported in DSS as well as co-treatment groups of each genotype. Colonic adenomas were observed in co-treatment groups of both genotypes as well as the DSS-only group of p53(+/+). No effects of the combination of DSS and MeIQx on colon pre- and neoplastic lesions were reported. Our results indicate that MeIQx may take more than 7 weeks to induce genotoxicity in the colon and that the co-treatment of mice did not enhance colon tumorigenicity even in p53-deficient mice.

Keywords: *p53*, Dextran sulfate sodium, 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline

---

* Tokyo University of Agriculture and Technology

Tasaki, M., Umemura, T., Suzuki, Y., Hibi, D., Inoue, T., Okamura, T., Ishii, Y., Maruyama, S.*, Nohmi, T. and Nishikawa, A.: **Oxidative DNA damage and reporter**

gene mutation in the livers of *gpt* delta rats given non-genotoxic hepatocarcinogens with cytochrome P450-inducible potency

Cancer Sci., **101**, 2525-2530（2010）

Previous reports have proposed that reactive oxygen species resulting from induction of cytochrome P450（CYP）isozymes might be involved in the modes of action of hepatocarcinogens with CYP-inducible potency. In the present study, we investigated 8-hydroxydeoxyguanosine（8-OHdG）levels, in vivo mutagenicity and glutathione S-transferase placental form（GST-P）-positive foci in the livers of gpt delta rats treated with piperonyl butoxide（PBO）or phenobarbital（PhB）for 4 and 13 weeks. Significant elevations in Cyp 1A1 and Cyp 1A2 mRNA levels after PBO treatment, and in Cyp 2B1 mRNA levels after PBO or PhB treatment, appeared together with remarkable hepatomegaly through the experimental period. Time-dependent and statistically significant increases in 8-OHdG levels were observed in the PBO treatment group along with significant increases in proliferating cell nuclear antigen（PCNA）-positive hepatocytes at 4 weeks, while no increase in 8-OHdG levels was found in PhB-treated rats. No changes in mutant frequencies of gpt and red/gam（Spi（−））genes in liver DNA from PBO- or PhB-treated rats were observed at 4 or 13 weeks. A 13-week exposure to either PBO or PhB did not affect the number and area of GST-P-positive hepatocytes. CYP 1A1 and 1A2 induction may be responsible for elevated levels of 8-OHdG in PBO-treated rats. However, neither GC：TA transversions nor deletion mutations, typically regarded as 8-OHdG-related mutations, were observed in any of the treated rats. We conclude that reactive oxygen species, possibly produced through CYP catalytic pathways, likely induced genomic DNA damage but did not give rise to permanent gene mutation.

Keywords：Oxidative DNA damage, *In vivo* mutagenicity, Cytochrome P450

---

* Nihon University

Kawamoto, K.*, Sato, I.*, Yoshida, M. and Tsuda, S.*：**Air purifiers that diffuse reactive oxygen species potentially cause DNA damage in the lung**

J. Toxicol. Sci., **35**, 929-933（2010）

Several appliance manufacturers have recently released new type air purifiers that can disinfect bacteria, fungi and viruses by diffusing reactive oxygen species（ROS）into the air. In this study, mice were exposed to the outlet air from each of 3 air purifiers from different manufacturers（A, B, C）, and the lung was examined for DNA damage, lipid peroxidation and histopathology to confirm the safety of these air purifiers. Neither abnormal behavior during exposure nor gross abnormality at necropsy was observed. No histopathological changes were also observed in the lung. However, significant increase of DNA damage was detected by the comet assay in the lung immediately after the direct exposure for 48 hr to models A and B, and for 16 hr to model B. As for model B, DNA migration was also increased by 2 hr exposure in a 1 m（3）plastic chamber but not by 48 hr exposure in a room（12.6 m（3））. Model C did not cause DNA damage. Lipid peroxidation and 8-hydroxy deoxyguanosine（8-OH-dG）was not increased under the conditions DNA damage was detected by the comet assay. The present results revealed that some models of air purifiers that diffuse ROS potentially cause DNA damage in the lung although the mechanism was left unsolved.

Keywords：Reactive oxygen species, Air ion, Lung

---

* Iwate University

Kawai, M.*, Saegusa, Y.*, Dewa, Y.*, Nishimura, J.*, Kemmochi, S.*, Harada, T.*, Ishii, Y., Umemura, T., Shibutani, M.* and Mitsumori, K.*：**Elevation of cell proliferation via generation of reactive oxygen species by piperonyl butoxide contributes to its liver tumor-promoting effects in mice**

Arch. Toxicol., **84**, 155-164（2010）

Piperonyl butoxide（PBO）is a pesticide synergist used with pyrethroids as a domestic insecticide, and it acts as a non-genotoxic hepatocarcinogen in rats and mice. To clarify whether oxidative stress is involved in the liver tumor-promoting effect of PBO in mice, male mice were subjected to two-thirds partial hepatectomy, followed by N-diethylnitrosamine（DEN）treatment, and given a diet containing 0.6% PBO for 25 weeks. The incidences of cytokeratin（CK）8/18-positive foci, adenomas, and carcinomas significantly increased in the DEN + PBO group compared with the DEN-alone group. The PCNA-positive ratio significantly increased in non-tumor hepatocytes, CK8/18-positive foci and adenomas in the DEN + PBO group compared with the

DEN-alone group. PBO increased reactive oxygen species (ROS) production in microsomes but did not change oxidative DNA damage as assessed by 8-hydroxydeoxyguanosine (8-OHdG). In real-time RT-PCR, PBO upregulated the expression of genes related to metabolism, such as Cytochrome P450 1a1, 2a5, and 2b10, and metabolic stress, such as Por and Nqo1, but downregulated Egfr and Ogg1. PBO also increased early response genes downstream of mitogen-activated protein kinase (MAPK), such as c-Myc that is induced by excessive ROS production, and G1/S transition-related genes, such as E2f1 and Ccnd1. Thus, PBO can generate ROS via the metabolic pathway without any induction of oxidative DNA damage, activate cell growth, increase c-Myc- and E2F1-related pathways, and act as a liver tumor promoter of DEN-induced hepatocarcinogenesis in mice.

Keywords: Piperonyl butoxide, Oxidative stress, Hepatocarcinogenesis

---

* Tokyo University of Agriculture and Technology

Mizukami, S.[*1], Ichimura, R.[*1], Kemmochi, S.[*1,2], Taniai, E.[*1], Shimamoto, K.[*1,2], Ohishi, T.[*1], Takahashi, M., Mitsumori, K.[*1] and Shibutani, M.[*1]: **Induction of GST-P-positive proliferative lesions facilitating lipid peroxidation with possible involvement of transferrin receptor up-regulation and ceruloplasmin down-regulation from the early stage of liver tumor promotion in rats**

Arch. Toxicol., **84**, 319-331 (2010)

To elucidate the role of metal-related molecules in hepatocarcinogenesis, we examined immunolocalization of transferrin receptor (Tfrc), ceruloplasmin (Cp) and metallothionein (MT)-1/2 in relation to liver cell foci positive for glutathione-S-transferase placental form (GST-P) in the early stage of tumor promotion by fenbendazole (FB), phenobarbital, piperonyl butoxide or thioacetamide in a rat two-stage hepatocarcinogenesis model. To estimate the involvement of oxidative stress responses to the promotion, immunolocalization of 4-hydroxy-2-nonenal, malondialdehyde and acrolein was similarly examined. Our findings showed that MT-1/2 immunoreactivity was not associated with the cellular distribution of GST-P and proliferating cell nuclear antigen, suggesting no role of MT-1/2 in hepatocarcinogenesis. We also found enhanced expression of Tfrc after treatment with strong tumor-promoting chemicals. With regard to Cp, the population showing down-regulation was increased in the GST-P-positive foci in relation to tumor promotion. Up-regulation of Tfrc and down-regulation of Cp was maintained in GST-P-positive neoplastic lesions induced after long-term promotion with FB, suggesting the expression changes occurring downstream of the signaling pathway involved in the formation of GST-P-positive lesions. Furthermore, enhanced accumulation of lipid peroxidation end products was observed in the GST-P-positive foci by promotion. Post-initiation treatment with peroxisome proliferator-activated receptor alpha agonists did not enhance any such distribution changes in GST-P-negative foci. The results thus suggest that facilitation of lipid peroxidation is involved in the induction of GST-P-positive lesions by tumor promotion from an early stage, and up-regulation of Tfrc and down-regulation of Cp may be a signature of enhanced oxidative cellular stress in these lesions.

Keywords: Transferrin receptor, Ceruloplasmin, Hepatocarcinogenesis

---

[*1] Tokyo University of Agriculture and Technology
[*2] Gifu University

Suzuki, T.*, Jin, M., Dewa, Y.*, Ichimura, R.*, Shimada, Y.*, Mizukami, S.*, Shibutani, M.* and Mitsumori, K.*: **Evaluation of *in vivo* liver genotoxic potential of Wy-14,643 and piperonyl butoxide in rats subjected to two-week repeated oral administration**

Arch. Toxicol., **84**, 493-500 (2010)

Wy-14,643 (WY), a peroxisome proliferator-activated receptor-alpha agonist, and piperonyl butoxide (PBO), a pesticide synergist, induce oxidative stress and promote hepatocarcinogenesis in the liver of rodents. These chemicals belong to a class of non-genotoxic carcinogens, but DNA damage secondary to the oxidative stress resulting from reactive oxygen species generation is suspected in rodents given these chemicals. To examine whether WY or PBO have DNA-damaging potential in livers of rats subjected to repeated oral administration for 14 days, the in vivo liver comet assay was performed in partially hepatectomized rats, and the expression of some DNA-repair genes was examined. Then, to examine whether they have genotoxic potential, the in vivo liver initiation assay was performed in rats. In the comet

assay, positive results were obtained at 3 h after the last treatment of WY, and some DNA-repair genes such as Apex1, Mlh1, Xrcc5, and Gadd45 were up-regulated in the liver. In the liver initiation assay, negative results were obtained for both WY and PBO. The results of the present study suggest that WY, but not PBO, causes some DNA damage in livers of rats, but such DNA damage was repaired by the increased activity of some DNA repair genes and may not lead to a DNA mutation.
Keywords: Wy-14,643, Piperonyl butoxide, Comet assay

---

* Tokyo University of Agriculture and Technology

Sheh, A.*, Lee, C. W.*, Masumura, K., Rickman, B. H.*, Nohmi, T., Wogan, G. N.*, Fox, J. G.*, Schauer, D. B.*: **Mutagenic potency of *Helicobacter pylori* in the gastric mucosa of mice is determined by sex and duration of infection**

Proc. Natl. Acad. Sci. USA., **107**, 15217-15222（2010）

Helicobacter pylori is a human carcinogen, but the mechanisms evoked in carcinogenesis during this chronic inflammatory disease remain incompletely characterized. We determined whether chronic *H. pylori* infection induced mutations in the gastric mucosa of male and female *gpt* delta C57BL/6 mice infected for 6 or 12 mo. Point mutations were increased in females infected for 12 mo. The mutation frequency in this group was 1.6-fold higher than in uninfected mice of both sexes ($P < 0.05$). A : T-to-G : C transitions and G : C-to-T : A transversions were 3.8 and 2.0 times, respectively, more frequent in this group than in controls. Both mutations are consistent with DNA damage induced by oxidative stress. No increase in the frequency of deletions was observed. Females had more severe gastric lesions than males at 6 mo postinfection (MPI; $P < 0.05$), but this difference was absent at 12 MPI. In all mice, infection significantly increased expression of IFNgamma, IL-17, TNFalpha, and iNOS at 6 and 12 mo, as well as *H. pylori*-specific IgG1 levels at 12 MPI ($P < 0.05$) and IgG2c levels at 6 and 12 MPI ($P < 0.01$ and $P < 0.001$). At 12 MPI, IgG2c levels in infected females were higher than at 6 MPI ($P < 0.05$) and also than those in infected males at 12 MPI ($P < 0.05$). Intensity of responses was mediated by sex and duration of infection. Lower *H. pylori* colonization indicated a more robust host response in females than in males. Earlier onset of severe gastric lesions and proinflammatory, Th1-biased responses in female C57BL/6 mice may have promoted mutagenesis by exposing the stomach to prolonged oxidative stress.
Keywords: *Helicobacter pylori*, chronic inflammatory, point mutations

---

* Massachusetts Institute of Technology

Kimura, A.*, Torigoe, N.*, Miyata, A.*, Honma, M.: **Validation of a simple *in vitro* comet assay method using CHL cells**

Genes and Environ., **32**, 61-65（2010）

The comet assay has been widely used as a genotoxicity test in *vitro*/*vivo* for detecting initial DNA damage in individual cells. One of the difficulties of the assay is slide preparation, for which agarose top and bottom layers and a cell-containing middle layer are needed to immobilize the cells. To establish a practical methodology while maintaining sensitivity and reproducibility, we assessed a simple comet assay method with a hydrophilic slide glass (MAS-coat type, Matsunami glass Ind., Ltd.) instead of an agarose bottom layer. Ethyl methanesulfonate (EMS), mitomycin C (MMC), and *N*-nitroso dimethylamine (DMN) as genotoxic chemicals and triton X-100 (TRX) as a non-genotoxic chemical were used for validation of this method. Chinese hamster lung (CHL) cells were used. The results showed that EMS and DMN induced a significant increase in tail intensity. However, MMC, a known interstrand cross-linker, did not increase tail intensity, and it was considered that this was because MMC-induced DNA-DNA crosslinks prevent separation of the DNA duplex. TRX did not increase tail intensity. These results are consistent with previous reports and demonstrate that the simple comet assay can clearly detect genotoxicity of chemicals other than interstrand cross-linkers.
Keywords: comet assay, genotoxicity test, validation

---

* 新日本科学（株）

Yasui, M., Koyama, N.*[1], Koizumi, T., Senda-Murata, K.*[2], Takashima, Y.*[3], Hayashi, M.*[4], Sugimoto, K.*[2], Honma, M.: **Live cell imaging of micronucleus formation and development**

Mutat. Res., **692**, 12-18（2010）

The micronucleus (MN) test is widely used to biomonitor humans exposed to clastogens and aneugens, but little is known about MN development. Here we

used confocal time-lapse imaging and a fluorescent human lymphoblastoid cell line (T105GTCH), in which histone H3 and α-tubulin stained differentially, to record the emergence and behavior of micronuclei (MNi) in cells exposed to MN-inducing agents. In mitomycin C (MMC)-treated cells, MNi originated in early anaphase from lagging chromosome fragments just after chromosome segregation. In γ-ray-treated cells showing multipolar cell division, MN originated in late anaphase from lagging chromosome fragments generated by the abnormal cell division associated with supernumerary centrosomes. In vincristine (VC)-treated cells, MN formation was similar to that in MMC-treated cells, but MNi were also derived from whole chromosomes that did not align properly on the metaphase plate. Thus, the MN formation process induced by MMC, γ-rays, and VC, were strikingly different, suggesting that different mechanisms were involved. MN stability, however, was similar regardless of the treatment and unrelated to MN formation mechanisms. MNi were stable in daughter cells, and MN-harboring cells tended to die during cell cycle progression with greater frequency than cells without MN. Because of their persistence, MN may have significant impact on cells, causing genomic instability and abnormally transcribed genes.

Keywords: micronucleus test, micronuclei, chromosome aberratioin

[*1] 静岡県立大学大学院生活健康科学研究科
[*2] 大阪府立大学大学院生命環境科学研究科
[*3] (独) 放射線医学総合研究所
[*4] (財) 食品農医薬品安全性評価センター

Wong, J. H. Y.[*1], Brown, J. A.[*2], Suo, Z.[*2], Blum, P.[*3], Nohmi, T., Ling, H.[*1]: **Structural insight into dynamic bypass of the major cisplatin-DNA adduct by Y-family polymerase Dpo4**

EMBO J., **29**, 2059-2069 (2010)

Y-family DNA polymerases bypass Pt-GG, the cisplatin-DNA double-base lesion, contributing to the cisplatin resistance in tumour cells. To reveal the mechanism, we determined three structures of the Y-family DNA polymerase, Dpo4, in complex with Pt-GG DNA. The crystallographic snapshots show three stages of lesion bypass: the nucleotide insertions opposite the 3'G (first insertion) and 5'G (second insertion) of Pt-GG, and the primer extension beyond the lesion site. We observed a dynamic process, in which the lesion was converted from an open and angular conformation at the first insertion to a depressed and nearly parallel conformation at the subsequent reaction stages to fit into the active site of Dpo4. The DNA translocation-coupled conformational change may account for additional inhibition on the second insertion reaction. The structures illustrate that Pt-GG disturbs the replicating base pair in the active site, which reduces the catalytic efficiency and fidelity. The *in vivo* relevance of Dpo4-mediated Pt-GG bypass was addressed by a dpo-4 knockout strain of *Sulfolobus solfataricus*, which exhibits enhanced sensitivity to cisplatin and proteomic alterations consistent with genomic stress.

Keywords: cis-platin DNA adducts, Y-family DNA polymerase, Dpo4

[*1] University of Western Ontario,
[*2] The Ohio State University
[*3] University of Nebraska

Horibata, K., Saijo, M.[*1], Bay, M. N.[*1], Lan, L.[*2], Kuraoka, I.[*1], Brooks, P. J.[*3], Honma, M., Nohmi, T., Yasui, A.[*2], Tanaka, K.[*1]: **Mutant Cockayne syndrome group B protein inhibits repair of DNA topoisomerase I-DNA covalent complex**

Genes to Cells, **16**, 101-14 (2011)

Two UV-sensitive syndrome patients who have mild photosensitivity without detectable somatic abnormalities, lack detectable Cockayne syndrome group B (CSB) protein due to a homozygous null mutation in the CSB gene. In contrast, mutant CSB proteins are produced in CS-B patients with the severe somatic abnormalities of Cockayne syndrome and photosensitivity. It is known that the piggyBac transposable element derived 3 is integrated within the CSB intron 5, and that CSB-piggy Bac transposable element derived 3 fusion (CPFP) mRNA is produced by alternative splicing. We found that CPFP or truncated CSB protein derived from CPFP mRNA was stably produced in CS-B patients, and that wild-type CSB, CPFP, and truncated CSB protein interacted with DNA topoisomerase I. We also found that CPFP inhibited repair of a camptothecin-induced topoisomerase I-DNA covalent complex. The inhibition was suppressed by the presence of wild-type CSB, consistent with the autosomal recessive inheritance of Cockayne syndrome. These results suggested that

reduced repair of a DNA topoisomerase I-DNA covalent complex due to truncated CSB proteins is involved in the pathogenesis of CS-B.

Keywords : Cockayne syndrome, topoisomerase, aging

*1 大阪大学大学院生命機能研究科
*2 東北大学加齢医学研究所
*3 National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health

Horibata, K., Ukai, A., Koyama, N., Takagi, A., Kanno, J., Kimoto, T.*, Miura, D.*, Hirose, A., Honma, M. : **Fullerene (C_{60}) is negative in the in vivo *Pig-A* gene mutation assay**

Genes and Environ., **33**, 27-31 (2011)

Carbon nanoparticles, such as carbon nanotubes and fullerene (C_{60}), are potential candidates as leading substances in nanotechnological fields, but little is known about their safety. Here we examined the *in vivo* genotoxicity of fullerene C_{60} by performing the *Pig-A* gene mutation assay in the peripheral blood of male C57 BL/6 Cr mice. Mice were given single intraperitoneal injection of 3 mg of C_{60} particles in 0.5 mL suspension containing 0.1%-Tween80-saline. As a positive control for *Pig-A* gene mutation assay, mice were given single oral administration of *N*-nitroso-*N*-ethylurea. At 2 and 8 weeks after treatments, we analyzed CD24-negative and -positive red blood cells in peripheral blood and calculated *Pig-A* mutant frequencies. As a result, we detected no significant differences in the mutant frequencies between C_{60} treated and non-treated mice, indicating that C_{60} is negative for genotoxicity *in vivo* in the limited target tissues assessed in this study. For the full assessment, we need comprehensive whole body survey on the genotoxicity of C_{60}.

Keywords : carbon nanoparticle, *in vivo* genotoxicity, *Pig-A* gene mutation assay

* 帝人ファーマ（株）

Sassa, A.*, Ohta, T.*, Nohmi, T., Honma, M. and Yasui, M. : **Mutational specificities of brominated DNA adducts catalyzed by human DNA polymerases**

J. Mol. Biol., **406**, 679-686 (2011)

Chronic inflammation is known to lead to an increased risk for the development of cancer. Under inflammatory condition, cellular DNA is damaged by hypobromous acid, which is generated by myeloperoxidase and eosinophil peroxidase. The reactive brominating species induced brominated DNA adducts such as 8-bromo-2'-deoxyguanosine (8-Br-dG), 8-bromo-2'-deoxyadenosine (8-Br-dA), and 5-bromo-2'-deoxycytidine (5-Br-dC). These DNA lesions may be implicated in carcinogenesis. In this study, we analyzed the miscoding properties of the brominated DNA adducts generated by human DNA polymerases (pols). Site-specifically modified oligodeoxynucleotides containing a single 8-Br-dG, 8-Br-dA, or 5-Br-dC were used as a template in primer extension reactions catalyzed by human pols α, κ, and η. When 8-Br-dG-modified template was used, pol α primarily incorporated dCMP, the correct base, opposite the lesion, along with a small amount of one-base deletion (4.8%). Pol κ also promoted one-base deletion (14.2%), accompanied by misincorporation of dGMP (9.5%), dAMP (8.0%), and dTMP (6.1%) opposite the lesion. Pol η, on the other hand, readily bypassed the 8-Br-dG lesion in an error-free manner. As for 8-Br-dA and 5-Br-dC, all the pols bypassed the lesions and no miscoding events were observed. These results indicate that only 8-Br-dG, and not 5-Br-dC and 8-Br-dA, is a mutagenic lesion ; the miscoding frequency and specificity vary depending on the DNA pol used. Thus, hypobromous acid-induced 8-Br-dG adduct may increase mutagenic potential at the site of inflammation.

Keywords : inflammation, hypobromous acid, mutagenesis

* 東京薬科大学生命科学部

Sassa, A.*1, Niimi, N., Fujimoto, H.*2, Katafuchi, A., Gruz, P., Yasui, M., Gupta, R. C.*3, Johnson, F.*3, Ohta, T.*1 and Nohmi, T. : **Phenylalanine 171 is a molecular brake for translesion synthesis across benzo [*a*] pyrene-guanine adducts by human DNA polymerase kappa**

Mutat. Res., **718**, 10-17 (2011)

Human cells possess multiple specialized DNA polymerases (Pols) that bypass a variety of DNA lesions which otherwise would block chromosome replication. Human polymerase kappa (Pol κ) bypasses benzo [*a*] pyrene diolepoxide-N^2-deoxyguanine (BPDE-N^2-dG) DNA adducts in an almost error-free manner. To better understand the relationship between the structural features in the active site and lesion bypass by Pol κ, we

mutated codons corresponding to amino acids appearing close to the adducts in the active site, and compared bypass efficiencies. Remarkably, the substitution of alanine for phenylalanine 171 (F171), an amino acid conserved between Pol κ and its bacterial counterpart *Escherichia coli* DinB, enhanced the efficiencies of dCMP incorporation opposite (−)- and (+)-*trans-anti*-BPDE-N^2-dG 18-fold. This substitution affected neither the fidelity of TLS nor the efficiency of dCMP incorporation opposite normal guanine. This amino acid change also enhanced the binding affinity of Pol κ- to template/primer DNA containing (−)-*trans-anti*-BPDE-N^2-dG. These results suggest that F171 functions as a molecular brake for TLS across BPDE-N^2-dG by Pol κ and that the F171A derivative of Pol κ bypasses these DNA lesions more actively than does the wild-type enzyme.

Keywords: translesion DNA synthesis, DNA polymerase kappa, benzo[*a*]pyrene diolepoxide-N^2-deoxyguanine

*1 東京薬科大学生命科学部
*2 国立感染症研究所放射能管理室
*3 Department of Pharmacological Sciences, Stony Brook University

Hori, M.*1, Yonekura, S.*1,2, Nohmi, T., Gruz, P., Sugiyama, H.*3, Yonei, S.*1 and Zhang-Akiyama, Q. M.*1: **Error-prone translesion DNA synthesis by *Escherichia coli* DNA polymerase IV (DinB) on templates containing 1,2-dihydro-2-oxoadenine**

J. Nucleic Acids, **2010**, 807579 (2010)

Escherichia coli DNA polymerase IV (Pol IV) is involved in bypass replication of damaged bases in DNA. Reactive oxygen species (ROS) are generated continuously during normal metabolism and as a result of exogenous stress such as ionizing radiation. ROS induce various kinds of base damage in DNA. It is important to examine whether Pol IV is able to bypass oxidatively damaged bases. In this study, recombinant Pol IV was incubated with oligonucleotides containing thymine glycol (dTg), 5-formyluracil (5-fodU), 5-hydroxymethyluracil (5-hmdU), 7,8-dihydro-8-oxoguanine (8-oxodG) and 1,2-dihydro-2-oxoadenine (2-oxodA). Primer extension assays revealed that Pol IV preferred to insert dATP opposite 5-fodU and 5-hmdU, while it inefficiently inserted nucleotides opposite dTg. Pol IV inserted dCTP and dATP opposite 8-oxodG, while the ability was low. It inserted dCTP more effectively than dTTP opposite 2-oxodA. Pol IV's ability to bypass these lesions decreased in the order: 2-oxodA > 5-fodU～5-hmdU > 8-oxodG > dTg. The fact that Pol IV preferred to insert dCTP opposite 2-oxodA suggests the mutagenic potential of 2-oxodA leading to A : T→G : C transitions. Hydrogen peroxide caused an ～2-fold increase in A : T→G : C mutations in *E. coli*, while the increase was significantly greater in *E. coli* overexpressing Pol IV. These results indicate that Pol IV may be involved in ROS-enhanced A : T→G : C mutations.

Keywords: translesion DNA synthesis, DinB, oxidative DNA damage

*1 京都大学大学院理学研究科ストレス反応生物学教室
*2 東京大学分子細胞生物学研究所
*3 京都大学大学院理学研究科生物化学教室

Koyama, N.*1, Yasui, M., Oda, Y.*2, Suzuki, S.*3, Satoh, T.*3, Suzuki, T.*4, Matsuda, T.*4, Masuda, S.*1, Kinae, N.*1 and Honma, M.: **Genotoxicity of acrylamide *in vitro*: Acrylamide is not metabolically activated in standard *in vitro* systems**

Environ. Mol. Mutagen., **52**, 11-19 (2011)

The recent finding that acrylamide (AA), a genotoxic rodent carcinogen, is formed during the frying or baking of a variety of foods raises human health concerns. AA is known to be metabolized by cytochrome P450 2E1 (CYP2E1) to glycidamide (GA), which is responsible for AA's *in vivo* genotoxicity and probable carcinogenicity. In *in-vitro* mammalian cell tests, however, AA genotoxicity is not enhanced by rat liver S9 or a human liver microsomal fraction. In an attempt to demonstrate the *in vitro* expression of AA genotoxicity, we employed *Salmonella* strains and human cell lines that overexpress human CYP2E1. In the umu test, however, AA was not genotoxic in the CYP2E1-expressing *Salmonella* strain or its parental strain. Moreover, a transgenic human lymphoblastoid cell line overexpressing CYP2E1 (h2E1 v2) and its parental cell line (AHH-1) both showed equally weak cytotoxic and genotoxic responses to high (>1 mM) AA concentrations. The DNA adduct N7-GA-Gua, which is detected in liver following AA treatment *in vivo*, was not substantially formed in the *in vitro* system. These results indicate that AA was not metabolically activated to GA *in vitro*. Thus, AA is not relevantly genotoxic *in vitro*, although its *in vivo* genotoxicity was clearly demonstrated.

Keywords : acrylamide, glycidamide, genotoxicity

*1 静岡県立大学大学院生活健康科学研究科
*2 近畿大学理工学部
*3 HAB 研究機構附属研究所
*4 京都大学大学院工学研究科

Yatagai, F.*1, Honma, M., Takahashi, A.*3, Omori, K.*2, Suzuki, H.*4, Shimazu, T.*4, Seki, M.*5, Hashizume, T.*4, Ukai, A., Sugasawa, K.*6, Abe, T.*1, Dohmae, N.*1, Enomoto, S.*1, Ohnishi, T.*3, Gordon, A.*7 and Ishioka, N.*2 : **Frozen human cells can record radiation damage accumulated during space flight : mutation induction and radioadaptation**

Radiat. Environ. Biophys., **50**(1), 125-34(2011)

To estimate the space-radiation effects separately from other space-environmental effects such as microgravity, frozen human lymphoblastoid TK6 cells were sent to the "Kibo" module of the International Space Station (ISS), preserved under frozen condition during the mission and finally recovered to Earth (after a total of 134 days flight, 72 mSv). Biological assays were performed on the cells recovered to Earth. We observed a tendency of increase (2.3-fold) in thymidine kinase deficient (TK(−)) mutations over the ground control. Loss of heterozygosity (LOH) analysis on the mutants also demonstrated a tendency of increase in proportion of the large deletion (beyond the TK locus) events, 6/41 in the in-flight samples and 1/17 in the ground control. Furthermore, in-flight samples exhibited 48% of the ground-control level in TK (−) mutation frequency upon exposure to a subsequent 2 Gy dose of X-rays, suggesting a tendency of radioadaptation when compared with the ground-control samples. The tendency of radioadaptation was also supported by the post-flight assays on DNA double-strand break repair : a 1.8- and 1.7-fold higher efficiency of in-flight samples compared to ground control via non-homologous end-joining and homologous recombination, respectively. These observations suggest that this system can be used as a biodosimeter, because DNA damage generated by space radiation is considered to be accumulated in the cells preserved frozen during the mission, Furthermore, this system is also suggested to be applicable for evaluating various cellular responses to low-dose space radiation, providing a better understanding of biological space-radiation effects as well as estimation of health influences of future space explores.

Keywords : space-environmental effects, International Space Station (ISS), DNA damage

*1 (独) 理化学研究所基幹研究所
*2 JAXA・ISAS
*3 奈良県立医科大学医学部
*4 (財) 日本宇宙フォーラム
*5 エイ・イー・エス (株)
*6 神戸大学バイオシグナル研究センター
*7 Baylor College of Medicine

Matsumoto, M., Fujii, S.*, Hirose, A. and Ema, M. : **Prenatal developmental toxicity of gavage or feeding doses of 2-sec-butyl-4,6-dinitrophenol in rats**

Reprod. Toxcol., **29**, 292-297(2010)

This study evaluated the prenatal developmental toxicity of the pesticide 2-sec-butyl-4,6-dinitrophenol (dinoseb). Pregnant rats were given dinoseb by gavage at 0, 8.0 or 10 mg/kg bw/day on days 6-15 of gestation, or in the diet at 0, 120 or 200 ppm (0, 6.52 or 8.50 mg/kg bw/day) on days 6-16 of gestation, and litters were evaluated on day 20 of gestation. Maternal toxicity was observed as evidenced by significantly decreased body weight gain and reduced food consumption during the administration period in all the dinoseb-treated groups, and two dams died at 10 mg/kg bw/day. Significantly lower fetal weights and delayed skeletal ossification was observed in the dinoseb-treated groups except for the group fed dinoseb at 120 ppm. The teratogenic potential of the gavage dose of dinoseb was confirmed as evidenced by increased incidences of fetuses with external and skeletal malformations at 10 mg/kg bw/day. The incidence of fetuses with microphthalmia was significantly increased at this dose. On the other hand, feeding doses of dinoseb up to 200 ppm did not induce teratogenicity in this study. These data indicate that dinoseb is teratogenic at maternally toxic doses, but the exposure range of dinoseb at which malformations occur seems to be narrow.

Keywords : dinoseb, nitrophenolic herbicide, teratogenicity

* (株) 化合物安全性研究所

Hasegawa, R., Hirata-Koizumi, M., Dourson, M. L.*, Parker, A.*, Sweeney, L. M.*, Nishikawa, A., Yoshida,

M., Ono, A. and Hirose, A.: **Proposal of new uncertainty factor application to derive tolerable daily intake**

Regul. Toxicol. Pharmacol., **58**, 237-242 (2010)

We propose new uncertainty factors (UFs) and a new subdivision of default factors in chemical risk assessment using a probabilistic approach based on the latest applicable information. Rounded values of 150 for mice, 100 for hamsters and rats, and 40 for rabbits, monkeys and dogs for inter- and intra-species differences (UF_{AH}) were derived from the probabilistic combination of two log-normal distributions. Further calculation of additional UFs when chronic data (UF_S) or NOAEL (UF_L) are lacking was conducted using available log-normal distribution information. The alternative UF_S and UF_L values of 4 are considered to be appropriate for both cases where data are lacking. The default contributions of inter-species difference (UF_A) and intra-species difference (UF_H) to the UF_{AH} of 100 for hamsters and rats as an example are considered to be 25 and 4, respectively. The UF_A of 25 was subdivided into $25^{0.6}$ (i.e., 7.0) for pharmacokinetics (PK) ($UF_{A,PK}$) and $25^{0.4}$ (i.e., 3.6) for pharmacodynamics (PD) ($UF_{A,PD}$), and the UF_H of 4 was evenly subdivided into $4^{0.5}$ (i.e., 2) ($UF_{H,PK}$ and $UF_{H,PD}$), to account for chemical-specific difference data between humans and laboratory animals for PK and/or PD. These default UFs, which come from actual experimental data, may be more appropriate than previous default UFs to derive tolerable daily intake values.

Keywords: uncertainty factor, probabilistic approach, subdivision of UF

---

* Toxicology Excellence for Risk Assessment

Wako, K.*, Kotani, Y.*, Hirose, A., Doi, T.* and Hamada, S.*: **Effects of preparation methods for multi-wall carbon nanotube (MWCNT) suspensions on MWCNT induced rat pulmonary toxicity**

J. Toxicol. Sci., **35**, 437-446 (2010)

Since there is a possibility of inhaling the fibers of multi-wall carbon nanotube (MWCNT) without any agglomeration, it is important that the pulmonary toxicity is evaluated by intratracheal instillation without agglomeration. MWCNT suspended in an artificial lung surfactant (ALS) with or without grinding in an agate mortar was instilled once intratracheally to rats to determine whether differences of the effects to pulmonary toxicity by different amounts of agglomerated MWCNT particle. The MWCNT suspension preparation method with grinding was effective at reducing agglomerates and in increasing uniform dispersion of the fibers. The ground MWCNT induced higher LDH levels and neutrophil ratios in the bronchoalveolar lavage fluid (BALF). There were no remarkable responses in rats in the non-ground MWCNT group, with the exception of inflammatory responses in the early phase. Some histopathological findings varied between rats given the ground MWCNT and non-ground MWCNT. A major difference was an MWCNT-laden macrophage infiltration site in the lung, which were in the alveolus in the ground MWCNT group, and in the interstitium in non-ground MWCNT group. Accordingly, the preparation method with grinding is considered to be effective at reducing agglomerates and ensuring uniform dispersion of the fibers. These findings lead us to conclude that the amount of agglomerates in the suspension is an important factor affecting the pulmonary toxicity of MWCNT.

Keywords: multi-wall carbon nanotube (MWCNT), lung toxicity, inflammation

---

* 三菱化学メディエンス（株）

広瀬明彦，高木篤也，西村哲治，津田洋幸*1，坂本義光*2，小縣昭夫*2，中江　大*2，樋野興夫*3，菅野　純：**ナノマテリアルの慢性影響研究の重要性**

薬学雑誌，**131**，195-201（2011）

Manufactured nanomaterials are the most important substances for the nanotechnology. The nanomaterials possess different physico-chemical properties from bulk materials. The new properties may lead to biologically beneficial effects and/or adverse effects. However, there are no standardized evaluation methods at present. Some domestic research projects and international OECD programs are ongoing, in order to share the health impact information of nanomaterails or to standardize the evaluation methods. From 2005, our institutes have been conducting the research on the establishment of health risk assessment methodology of manufactured nanomaterials. In the course of the research project, we revealed that the nanomaterials were competent to cause chronic effects, by analyzing the intraperitoneal administration studies and carcinogenic promotion studies. These studies suggested that even aggregated

nanomaterials were crumbled into nano-sized particles inside the body during the long-term, and the particles were transferred to other organs. Also investigations of the toxicokinetic properties of nanomaterials after exposure are important to predict the chronically targeted tissues. The long lasting particles/fibers in the particular tissues may cause chronic adverse effects. Therefore, focusing on the toxicological characterization of chronic effects was considered to be most appropriate approach for establishing the risk assessment methods of nanomaterials.

Keywords: chronic toxicity, multi-wall carbon nanotube (MWCNT), fullerene

*1 名古屋市立大学
*2 東京都健康安全研究センター
*3 順天堂大学

Kobayashi, K.*1, Sakuratani, Y.*1, Abe, T.*1, Yamazaki, K.*1, Nishikawa, S.*1, Yamada, J.*1, Hirose, A., Kamata, E. and Hayashi, M.*1,2: **Influence of coefficient of variation in determining significant difference of quantitative values obtained from 28-day repeated-dose toxicity studies in rats**

J. Toxicol. Sci., **36**, 63-71 (2011)

In order to understand the influence of coefficient of variation (CV) in determining significant difference of quantitative values of 28-day repeated-dose toxicity studies, we examined 59 parameters of 153 studies conducted in accordance with Chemical Substance Control Law in 12 test facilities. Sex difference was observed in 12 parameters and 10 parameters showed large CV in females. The minimum CV was 0.74% for sodium. CV of electrolytes was comparatively small, whereas enzymes had large CV. Large differences in CV were observed for major parameters among 7-8 test facilities. The changes in CV were grossly classified into 11. Our study revealed that a statistical significant difference is usually detected if there is a difference of 7% in mean values between the groups and the groups have a CV of about 7%. A parameter with a CV as high as 30% may be significantly different, if the difference of the mean between the groups is 30%. It would be ideal to use median value to assess the treatment-related effect, rather than mean, when the CV is very high. We recommend using CV of the body weight as a standard to judge the adverse effect level.

Keywords: coefficients of variation, repeated-dose study, quantitative value

*1 (独) 製品評価技術基盤機構
*2 (財) 食品農医薬品安全性評価センター

Hirata-Koizumi, M., Fujii, S.*1, Ono, A., Hirose, A., Imai, T.*2, Ogawa, K., Ema, M. and Nishikawa, A.: **Two-generation reproductive toxicity study of aluminium sulfate in rats**

Reprod. Toxicol., **31**, 219-230 (2011)

In a two-generation reproductive toxicity study, male and female rats were given aluminium sulfate (AS) in drinking water at 0, 120, 600 or 3000 ppm. AS reduced water consumption in all treatment groups, and body weight was transiently decreased in the 3000ppm group. In the F1 and F2 pups, preweaning body weight gain was inhibited at 3000 ppm, and the liver and spleen weight was decreased at weaning. At this dose, vaginal opening was slightly delayed. There were no compound-related changes in other reproductive/developmental parameters, including developmental neurobehavioral endpoints. The data indicated that the NOAEL of AS in this two-generation study is 600ppm for parental systemic toxicity and reproductive/developmental toxicity. The total ingested dose of aluminium from drinking water and food (standard rat diet, containing 25-29ppm of aluminium) combined for this 600ppm group was calculated to be 8.06mg Al/kg bw/day.

Keywords: aluminium sulfate, two-generation reproductive toxicity, developmental toxicity

*1 (株) 化合物安全性研究所
*2 (独) 国立がん研究センター研究所