Katori, N., Aoyagi, N., Kojima, S.: **The Study of the Applicability of Content Uniformity and Weight Variation Test-The State of Commercial Tablets and Capsules in Japan-**

Chem. Pharm. Bull., **49**, 1412-1419 (2001)

This study intends to determine the rational criteria (e.g., threshold value) for applying the weight variation test and to investigate the adequacy of the acceptance value for existing commercial products in Japan. The studied products were 489 lots (3 lots3163 products) of compressed tablets (plain, film-coated, sugar-coated) and 42 lots (3 lots314 products) of hard capsules marketed in Japan. Product-specific intra-lot relative standard deviation of content (RSDD), weight (RSDW) and concentration (RSDC) were calculated. A good correlation was found between RSDD and RSDC but not between RSDD and RSDW. These findings indicate that 1) it is difficult to rationally set the threshold level for weight variation, especiallly regarding the dosage forms except for plain tablets, 2) the application of weight variation tests should, in principle, be decided on the mixing homogeneity that is RSDC. 3) Most (99.6%) of the tablets and all the capsules investigated met the requirement of content uniformity test of JP13.

Keywords: content uniformity; mixing homogeneity; Japanese Pharmacopoeia; International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)

Anchordoquy, TJ.*, Izutsu, K., Randolph, TW.*[1], Carpenter, JF.*[1]: **Maintenance of quaternary structure in the frozen state stabilizes lactate dehydrogenase during freeze-drying**

Arch. Biochem. Biophys. **390**, 35-41 (2001)

Polymers often fail to inhibit protein unfolding during lyophilization because steric hindrance prevents effective hydrogen bonding of the polymer to the protein's surface. However, in certain cases, polymers have been shown to stabilize multimeric enzymes during lyophilization. Here we test the hypothesis that this protection is due to inhibition of dissociation into subunits during freezing using mixtures of lactate dehydrogenase isozymes that form electrophoretically distinguishable hybrid tetramers during reversible dissociation. We examined hybridization and recovery of catalytic activity during freeze-thawing and freeze-drying in the presence of polymers (dextran, Ficoll, and polyethylene glycol), sugars, and surfactants. The surfactants did not protect LDH during freeze-thawing or freeze-drying. Polymers and sugars prevented dissociation of LDH during the freezing step of lyophilization, resulting in greater recovery of enzyme activity after lyophilization and rehydration. This beneficial effect was observed even in systems that do not form glassy solids during freezing and drying. We suggest that stabilization during drying results in part from greater inherent stability of the assembled holoenzyme relative to that of the dissociated monomers. Polymers inhibit freezing-induced dissociation thermodynamically because they are preferentially excluded from the surface of proteins, which increases the free energy of dissociation and denaturation.

Key words: freeze-drying, protein formulation, stabilization

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伊豆津健一, 小嶋茂雄:
各種タンパク質と二糖類の凍結溶液中における混合性
低温生物工学会誌, **47**, 106-108 (2001).

Miscibility of proteins and saccharides (monosaccharides to oligosaccharides) in aqueous frozen solutions was studied through thermal analysis to model freeze-dried protein formulations. Thermal transitions (glass transition temperature of maximally freeze-concentrated solutions: Tg's) of the frozen solutions showed varied solute miscibility depending on the combinations and concentration ratios. Many protein and saccharide combinations were freeze-concentrated into amorphous mixture phase in protein-rich to moderately saccharide-rich concentration ratios, whereas saccharide phase appeared besides the mixture phase above certain saccharide/protein concentration ratios. Some saccharide-rich combinations separated into individual phases in the freeze-concentrates.

Key words: phase separation, freeze-drying, protein formulation

Yoshioka S., Aso Y. and Kojima S.: **Usefulness of Kohlraush-Williams-Watts Stretched Exponential Function to Describe Protein Aggregation in Lyophilized Formulations and Temperature Dependence Near the Glass Transition Temperature**

Pharm. Res., **18**, 256-260 (2001)

We studied the feasibility of using the Kohlrausch-

Williams-Watts stretched exponential function (KWW equation) to describe protein aggregation in lyophilized formulations during storage. Parameters representing "mean aggregation time" (τ_a) and stretched exponential constant (β_a) were calculated according to the KWW equation by assuming that the time required for protein molecules to aggregate (τ) varies due to the fact that protein aggregation occurs at a rate which depends on the degree of protein deformation resulting from stresses created during freeze-drying. The results indicate that the parameter β_a is reflective of physical changes within lyophilized formulations. The parameter τ_f was found to be useful in comparing the protein aggregation behavior of formulations having different τ_a and β_a values.
Key words: protein aggregation, KWW function

Aso, Y., Yoshioka S. and Kojima S.: Explanation of the Crystallization Rate of Amorphous Nifedipine and Phenobarbital from Their Molecular Mobility as Measured by ^{13}C NMR Relaxation Time and the Relaxation Time Obtained from the Heating Rate Dependence of T_g
J. Pharm. Sci., **90**, 798-806 (2001)

In order to gain further insight into the effect of molecular mobility on the crystallization rate of amorphous drugs, the mean relaxation time of amorphous nifedipine and phenobarbital was calculated based on the Adam-Gibbs-Vogel (AGV) equation, using the parameters D and T_0, and T_f, derived from the heating rate dependence of the glass transition temperature (T_g) of the amorphous drugs and heat capacity of the drugs in the amorphous and crystalline states. These relaxation times were compared with the crystallization rate of amorphous nifedipine and phenobarbital reported previously. The spin-lattice relaxation time (T_1) and the spin-lattice relaxation time in the rotating frame ($T_{1\rho}$) of phenobarbital and nifedipine carbons were also determined. The temperature dependence of the crystallization rate of nifedipine and phenobarbital around the Tg was coincident with that of the mean relaxation time calculated according to the AGV equation within experimental error, indicating that the crystallization of nifedipine and phenobarbital is largely correlated with molecular mobility at the temperatures studied. A ^{13}C NMR relaxation study indicated that the molecular motion of nifedipine and phenobarbital in the mid-kHz frequency range became significant at temperatures higher than T_g-20 and T_g, respectively.
Keywords: Crystallization, Mobility, Relaxation Time

Aso Y., Yoshioka S. and Kojima S.: **Feasibility of Using Isothermal Microcalorimetry to Evaluate the Physical Stability of Amorphous Nifedipine and Phenobarbital**
Thermochimica Acta, **380**, 199-204 (2001).

Feasibility of microclorimetry to evaluate the physical stability of amorphous drugs was studied. Amorphous forms of nifedipine and phenobarbital were prepared by melting and subsequent cooling in a differential scanning calorimetry (DSC) sample pan, and their heats of crystallization were monitored by isothermal microcalorimetry. The time required for 10% of the amorphous drug to crystallize (t_{90}), a direct measure of the crystallization rate, could be obtained from a single microcalorimetric trace of the amorphous nifedipine or phenobarbital. The t_{90} values were also determined by conventional storage studies in which the heat of crystallization was determined by DSC. The t_{90} values obtained by microcalorimetry were consistent with those obtained by DSC, within experimental error, indicating that microcalorimetry is a useful method for evaluating the physical stability of amorphous drugs.
Keywords: Microcalorimetry, Crystallization, Stability

Toyo'oka, T.*, Yano, M.*, Kato, M.* and Nakahara, Y.: **Simultaneous determination of morphine and its glucuronides in rat hair and rat plasma by reversed-phase liquid chromatography with electrospray ionization mass spectrometry**
Analyst, **126(8)**, 1339-1345 (2001)

The simultaneous determination of morphine and the glucuronide metabolites [morphine-3-beta-D-glucuronide (M3G) and morphine-6-beta-D-glucuronide (M6G)] in rat hair and rat plasma was carried out using reversed-phase high-performance liquid chromatography (HPLC) coupled with electrospray ionization mass spectrometry (ESI-MS). The chromatographic separation of the analytes was achieved using a semi-micro-HPLC column (3 microm particle size; 100 x 2.0 mm id) by gradient elution with 50 mM ammonium acetate and acetonitrile as eluents. After separation, morphine and the glucuronides were determined by selected ion monitoring (SIM) of ESI-MS using the quasi-molecular ions $[M+H]^+$ at m/z = 286 and 462, respectively. The calibration curves were linear between the concentration of the analytes and the deuterium-labelled morphine (M-d3) selected as internal standard. The method was applied for

the determination of the incorporation of morphine and the glucuronides into the hair shafts and hair roots of Dark Agouti rats after single intraperitoneal administration of morphine hydrochloride. Plasma concentrations of morphine and glucuronides were simultaneously determined after administration. Morphine and M3G were detected in the hair shafts and the hair roots. The concentrations of M3G in the hair root were lower than those of morphine in all sampling periods. In contrast, M3G concentrations in plasma were relatively higher at each sampling time. Small quantities of M6G were also identified in the plasma up to 4 h after administration. The concentration difference between the hair root and plasma seems to be due to the incorporation ratio of morphine and glucuronide into hair. As M3G was also identified in the hair shaft 1 week after administration, the incorporation of glucuronide metabolites into hair is obvious. This is the first report of the identification of morphine glucuronide in hair samples without the use of acid hydrolysis or enzyme digestion.

Keywords: Hair analysis, LC-ESI-MS, Morphine

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Toyo'oka, T.*, Kanbori, M.*, Kumaki, Y.* and Nakahara, Y. : Determination of triazolam involving its hydroxy metabolites in hair shaft and hair root by reversed-phase liquid chromatography with electrospray ionization mass spectrometry and application to human hair analysis

Anal. Biochem., **295(2)**, 172-179 (2001)

A sensitive method using reversed-phase liquid chromatography coupled with electrospray ionization mass spectrometry has been developed for simultaneous determination of triazolam and its hydroxy metabolites in hair. After the addition of deuterium-labeled 1-hydroxymethyltriazolam as an internal standard, the analytes in hair shaft and hair root samples were extracted with a basic medium, CH_2Cl_2:MeOH:28% NH_4OH (20:80:2) at room temperature overnight. The chromatographic separation of the analytes was achieved using a semimicro HPLC column (3-microm particle size; 100 x 2.0-mm i.d.) by gradient elution with acetonitrile in water containing 1% acetic acid as eluent. The mass spectrometer was operated in selected-ion monitoring mode at quasi-molecular ions $[M+H]^+$ of triazolam and its metabolites. The method has been applied to determine the incorporation of triazolam and its metabolites into the hair shafts and hair roots of Dark Agouti rats administered 3 or 6 mg/kg triazolam intraperitoneally twice a day for 5 days. Triazolam, 1-hydroxymethyltriazolam, and 4-hydroxytriazolam were incorporated into the hair shafts and the hair roots. The concentration of 4-hydroxytriazolam was the highest of all compounds detected. An unknown substance considered to be 1,4-dihydroxytriazolam also appeared in the hair samples. The structural elucidation was performed with online HPLC-MS after acetylation of the substance with acetic anhydride and pyridine. The time course studies of triazolam and the metabolites in both rat hair roots and plasma were carried out after single intraperitoneal administration of triazolam. The concentrations of triazolam and the metabolites in the hair roots reflected those in the plasma. The proposed method using selected-reaction monitoring was applied to the determination of triazolam and the metabolites in human hairs of a triazolam addict. Triazolam, 1-hydroxymethyltriazolam, and 4-hydroxytriazolam were identified in the black hair shafts, whereas only triazolam was detected in the hair roots and the white hair shafts. This is the first report on the detection of triazolam and its metabolites in human hairs.

Keywords: Hair analysis, LC-ESI-MS, Triazolam

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Kuroda, N.*, Sato, D.*, Ohyama, K.*, Wada, M.*, Nakahara, Y. and Nakashima, K.* : **Separation of sympathomimetic amines of abuse and related compounds by micellar electrokinetic chromatography**

Chem. Pharm. Bull., **49(7)**, 905-908 (2001)

Separation of twelve sympathomimetic amines and related compounds by micellar electrokinetic chromatography (MEKC) with UV absorbance detection is described. These amines were well separated within 25 min using 50 mM sodium tetraborate solution containing 15 mM sodium dodecylsulfate (SDS) of pH 9.3 as a running solution and detected at 210 nm. MEKC was performed with an applied voltage of 13 kV at 25 degrees C using a fused-silica capillary (50 cm x 75 mm i.d.) with effective length of 37.5 cm. The detection limits of these compounds were in the range from 4 to 97 fmol/injection at a signal-to-noise ratio (S/N) of 3. The reproducibility of the method expressed as relative standard deviation (RSD) for within-day (n=6) and between-day (n=5) assays was less than 4.8 and 8.8%, respectively. The proposed method could be applied to the determination of an anorectic drug, phentermine, in Chinese tea with a

detection limit of 99 microg/g (105 fmol/injection, S/N=3).

Keywords: Micellar electrokinetic chromatography, Sympathomimetic amines

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Saisho, K., Scott, K. S., Morimoto, S.* and Nakahara, Y. : **Hair analysis for pharmaceutical drugs. II. Effective extraction and determination of sildenafil (Viagra) and its N-desmethyl metabolite in rat and human hair by GC-MS**

Biol. Pharm. Bull., **24(12)**, 1384-1388 (2001)

In order to study the incorporation of sildenafil (SDF) and its N-demethylated metabolite (norSDF) into hair, animal model experiments were carried out. After shaving the back hair, SDF was dosed to two sets of three male dark-agouti pigmented rats (5 weeks old) per each group at 25 mg/kg once a day for 5 successive days with intraperitoneal (i.p.) (set1) and oral administration (set2). The regrown back hair was collected 14 d after the first administration. Three typical extraction methods, using methanol-5 M hydrochloric acid, methanol-trifluoroacetic acid and 1 M sodium hydroxide, were evaluated using the rat hair samples containing SDF and norSDF. Methanol-5 M hydrochloric acid was the best extraction method in terms of high efficiency and reproducibility. The extract was purified using Bond Elut Certify columns and was derivatized with trimethylsilylimidazole: N,O-bis(trimethylsilyltrifluoroacetamide): trimethylchlorosilane (3: 3: 2) at 90 degrees C for 30 min. The trimethylsilylated products were analyzed by GC-MS using selected ion monitoring. SDF and norSDF were simultaneously detected in the rat hair. The hair concentrations were 4.9-6.3 (av. 5.8) ng/mg and 15.6-20.3 (av. 17.6) ng/mg for SDF and norSDF, respectively, with i.p. administration, and 2.6-4.1 (av. 3.6) ng/mg and 8.1-10.4 (av. 9.1) ng/mg with oral administration. The hair concentrations of norSDF were about three times higher than those of SDF, and the ratios of both compounds showed no significant difference between i.p. and oral administrations. This method was applied to the scalp hair of two patients who orally took SDF at regular intervals for the treatment of penile erectile dysfunction. The hair concentrations of SDF and norSDF in the two patients were 19.8 and 55.9 ng/mg, and 1.7 and 5.6 ng/mg, respectively.

Keywords: Hair, Sildenafil, GC-MS

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Eguchi, A*[1], Akura, T.*[2,3], Okuyama, H.*[2,4], Senda T.*[5], Yokoi, H.*[2,6], Inokuchi, H.*[7], Fujita, S.*[1,8], Hayakawa, T., Takeda, K.*[2], Hasegawa, M.*[2], and Nakanishi, M*[1]: **Protein transduction domain of HIV-1 Tat protein promotes efficient delivery of DNA into mammalian cells**

J. Biol, Chem., **276**, 26204-26210 (2001)

The plasma membrane of mammalian cells is one of the tight barriers against gene transfer by synthetic delivery systems. Various agents have been used to facilitate gene transfer by destabilizing the endosomal membrane under acidic conditions, but their utility is limited, especially for gene transfer in vivo. In this article, we report that the protein transduction domain of human immunodeficiency virus type 1 Tat protein (Tat peptide) greatly facilitates gene transfer via membrane destabilization. We constructed recombinant lambda phage particles displaying Tat peptide on their surfaces and carrying mammalian marker genes as part of their genomes (Tat-phage). We demonstrate that, when animal cells are briefly exposed to Tat-phage, significant expression of phage marker genes is induced with no harmful effects to the cells. In contrast, recombinant phage displaying other functional peptides, such as the integrin-binding domain or a nuclear localization signal, could not induce detectable marker gene expression. The expression of marker genes induced by Tat-phage is not affected by endosomotropic agents but is partially impaired by inhibitors of caveolae formation. These data suggest that Tat peptide will become a useful component of synthetic delivery vehicles that promote gene transfer independently of the classical endocytic pathway.

Keywords: gene transfer, Tat peptide, Tat-phage

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Hyuga, S., Kawasaki, N., Hyuga, M., Ohta, M., Shibayama, R., Kawanishi, T., Yamagata, S.*, Yamagata, T.* and Hayakawa, T.: **Ganglyoside GD1a inhibits HGF-induced motility and scattering of cancer cells through suppression of tyrosine phospholylation of**

c-MET

Int. J. Cancer, **94**, 328-334 (2001)

We previously reported that ganglioside GD1a, which is highly expressed in poorly metastatic FBJ-S1 cells, inhibits the serum-induced motility of FBJ-LL cells and that the metastatic potential of FBJ-LL cells is completely suppressed by enforced GD1a expression (Hyuga et al., *Int J Cancer* 1999; **83**: 685-91). We recently discovered that hepatocyte growth factor (HGF) induces FBJ-LL cell motility. In the present study, the HGF-induced motility of FBJ-S1 cells was found to be one-thirtieth that of FBJ-LL cells. This motility of GD1a-expressing transfectants, which were produced by transfection of FBJ-LL cells with GM2/GD2 synthase cDNA, decreased with increases in their GD1a expression and HGF induced almost no motility in GD1a-pretreated FBJ-LL cells, indicating that GD1a inhibits the HGF-induced motility of FBJ-LL cells. The expression of the HGF receptor c-Met on FBJ-S1 cells, FBJ-LL cells, transfectants and a mock-transfectant was almost the same. The level of tyrosine phosphorylation of c-Met after HGF stimulation in FBJ-S1 cells, GD1a-pretreated FBJ-LL cells and a GD1a-expressing transfectant was significantly lower than in FBJ-LL cells and a mock-transfectant. These findings suggested that GD1a inhibits the HGF-induced motility of FBJ-LL cells through suppression of tyrosine phosphorylation of c-Met. HepG2 cells, a human hepatoma cell line, were used to investigate whether GD1a interferes with other cancer cells expressing c-Met. HepG2 cells did not express GD1a. HGF induced cell scattering of HepG2 cells and the scattering was inhibited by pretreating the cells with GD1a. The c-Met in the cells was autophosphorylated by stimulation with HGF, but after treating the cells with GD1a, the HGF-induced autophosphorylation of c-Met was suppressed. These results suggest that GD1a acts as a negative regulator of c-Met in cancer cells.

Keywords: GD1a, HGF, c-Met

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Kawasaki, N., Haishima, Y., Ohta, M., Itoh, S., Hyuga, M., Hyuga, S. and Hayakawa, T.: **Structural analysis of sulfated N-linked oligosaccharides in erythropoietin**

Glycobiology, **11**, 1043-1049 (2001)

We previously demonstrated that high-performance liquid chromatography with electrospray ionization mass spectrometry (LC/MS) equipped with a graphitized carbon column (GCC) is useful for the structural analysis of carbohydrates in glycoproteins. Using LC/MS with GCC, sulfated N-linked oligosaccharides were found in erythropoietin (EPO) expressed in baby hamster kidney cells. Sulfation occurs in a part of the N-linked oligosaccharides in the EPO. Sulfated monosaccharide residue in the sulfated N-linked oligosaccharide was determined by exoglycosidase digestion followed by sugar mapping by LC/MS. The linkage position and branch-location of the sulfate group in the tetraantennary oligosaccharide were analyzed by ^{1}H-nuclear magnetic resonance. It was suggested that sulfation occurs on the C-6 position of GlcNAc located in the GlcNAcbeta1-4Manalpha1-3 branch.

Keywords: LC/MS, Sulfated oligosaccharide, erythropoietin

豊田淑江, 山口照英, 押澤 正, 内田恵理子, 早川堯夫：**好中球の機能分化と増殖の制御**

炎症・再生, **21(3)**, 199-207(2001)

好中球は炎症の場で殺菌作用を示し，重要な役割を演じている．この好中球分化の制御機構をHL-60細胞を用いて解析した．HL-60細胞をDMSOで分化すると増殖型トランスフェリン受容体陽性（Trf-R^{+}）細胞と分化型の陰性（Trf-R^{-}）細胞が出現し，顆粒球コロニー刺激因子（G-CSF）はTrf-R^{+}細胞の増殖を，Trf-R^{-}細胞の分化をそれぞれ促進した．これらのシグナルを解析したところ，Trf-R^{+}細胞の増殖にはp70 S6キナーゼの活性化が，Trf-R^{-}細胞の分化にはSTAT3のチロシンリン酸化が重要な役割を果たしていることが示唆された．また，Trf-R^{+}細胞のp70 S6キナーゼをラパマイシンで阻害すると分化型のTrf-R^{-}細胞と同様の分化能を示すことから，p70 S6キナーゼが分化や増殖の方向性を決める重要な役割をもっていると考えられた．またp70 S6キナーゼの上流はフォスファチジルイノシトール3キナーゼであることを示した．一方，顆粒球・単球コロニー刺激因子（GM-CSF）はHL-60細胞のG-CSFによる好中球分化の促進作用を阻害した．このシグナル伝達の解析より，G-CSFによりチロシンリン酸化されたSTAT3の核移行が，GM-CSFによって活性化されるMAPキナーゼにより阻害されると考えられた．

Keywords: G-CSF, granulopoiesis, signal transduction

Kawanishi T, Kiuchi T*, Asoh H*, Shibayama R, Kawai H, Ohata H*, Momose K*, Hayakawa T : **Effect of Tributyltin Chloride on Release of Calcium Ion from Intracellular Calcium Stores in Rat Hepatocytes.**

Biochem. Pharmacol., **62**, 863-872 (2001)

The effects of tri-n-butyltin chloride (TBT) on release

of Ca^{2+} from intracellular stores were investigated in isolated rat hepatocytes. Images of Ca^{2+} concentration in the intracellular stores of primary cultured hepatocytes loaded with fura-2 was obtained after digitonin-permeabilization using digitalized fluorescence microscopy. The permeabilized hepatocytes that had been preincubated with 4.0 μM TBT for 30 min showed a very low fluorescence ratio of 340nm/380nm, suggesting that the stored Ca^{2+} was released. When the hepatocytes were treated with 4.0 μM TBT after digitonin-permeabilization, the decrease in the fluorescence ratio was very small. However, when the permeabilized hepatocytes were incubated with 4.0 μM TBT and 2.0 μM NADPH, the decrease was enhanced, raising the possibility that TBT might be metabolized to the active form(s) releasing Ca^{2+} from the stores. When the hepatocytes were preincubated with 0.1 μM TBT for 30 min and then permeabilized, the fluorescence ratio was almost the same as that in the control permeabilized hepatocytes. However, the InsP3-induced decrease in the fluorescence ratio was significantly suppressed in the permeabilized hepatocytes. These results suggest that TBT released Ca^{2+} from the intracellular stores at high concentrations, and suppressed the $Ins(1,4,5)P_3$-induced Ca^{2+} release at non-toxic low concentrations. It is probable that the latter effect was responsible for the previously reported suppression of Ca^{2+} response induced by hormonal stimulations.

Keywords: Hepatocyte, Tributyltin, Calcium

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Hisamitsua T*[1], Ohata H*[1], Kawanishi T, Iwamoto T*[2], Shigekawa M*[2], Amano H*[3], Yamada S*[3], Momose K: **A mechanism of Ca^{2+} release from Ca^{2+} stores coupling to the Na^+/Ca^{2+} exchanger in cultured smooth muscle cells.**

Life Sciences, **69**, 2775-2787 (2001)

We previously observed Ca^{2+} release from intracellular Ca^{2+} stores caused by reduction in extracellular Na^+ concentration ($[Na^+]_0$). The purpose of this study was to determine whether lowering $[Na^+]_0$ can elicit Ca^{2+} release from Ca^{2+} stores via the Na^+/Ca^{2+} exchanger and to elucidate the mechanisms related to the Ca^{2+} release pathway in cultured longitudinal smooth muscle cells obtained from guinea pig ileum. Low $[Na^+]_0$-induced Ca^{2+} release was inhibited by antisense oligodeoxynucleotides for Na^+/Ca^{2+} exchanger type 1 (anti-NCX). Application of anti-NCX to cells attenuated both the number of Ca^{2+} responding cells and the expression of the exchanger. Moreover, microinjection of heparin, a blocker of inositol I, 4,5-trisphosphate (IP_3) receptors, into the cells inhibited low $[Na^+]_0$-induced Ca^{2+} release. These findings suggest that low $[Na^+]_0$-induced Ca^{2+} release occurs through an IP_3-induced Ca^{2+} release mechanism due to changes in the Ca^{2+} flux regulated by the Na^+/Ca^{2+} exchanger.

Keywords: Antisense, Sodium/calcium exchange, ileum

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Tanaka H*[1], Masumiya H*[1], Sekine T*[1], Kase J*[1], Kawanishi T, Hayakawa T, Miyata S*[2], Sato Y*[2], Nakamura R*[3], Shigenobu K: **Involvement of Ca^{2+} waves in excitation-contraction coupling of rat atrial cardiomyocytes.**

Life Sciencs, **70**, 715-726 (2001)

Two-dimensional and line-scan analyses of the early phase Ca^{2+} transients in rat cardiomyocytes were performed with a rapid-scanning laser confocal microscope and fluo-3 to elucidate the mechanism of activation of Ca^{2+} release from the sarcoplasmic reticulum in atrial myocytes which lack a well developed T-tubular network. On electrical stimulation of ventricular myocytes, Ca^{2+} concentration began to rise earliest at the Z-line level and became uniform throughout the cytoplasm within about l0msec. In contrast, on stimulation of atrial myocytes, the earliest rise in Ca^{2+} occurred at the cell periphery and then spread to the cell interior; cytoplasmic Ca^{2+} became uniform after more than 30msec. The velocity of the propagation of rise in Ca^{2+} was 112 $\pm$ 5.1 μm/sec (n=10), which was similar to that of spontaneous Ca^{2+} waves observed in atrial and ventricular myocytes. No difference in frequency, amplitude and kinetics of spontaneous Ca^{2+} sparks was observed between the subsarcolemmal and central regions of atrial myocytes. Ryanodine concentration-dependently decreased the contractile force of isolated rat atrial and ventricular tissue preparations; the sensitivity was higher in atrial myocytes. The present study visualized the involvement of a propagated Ca^{2+}-induced-Ca^{2+} release mechanism in atrial but not ventricular myocytes. This difference may underlie some of the atrio-ventricular difference in response to physiological and pharmacological stimuli.

Keywords: Cardiomyocyte, Calcium, Atria

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Masumiya H*1, Kase J*1, Kawanishi T, Hayakawa T, Miyata S*2, Sato Y*2, Nakamura R*3, Tanaka H*1, Shigenobu K*1 : **Effect of T-type and L-type Ca^{2+} Channel Blockade on Early Phase Ca^{2+} Transients in Rat Atrial and Ventricular Cardioiomyocytes.**

Bioimages, **9**, 87-93 (2001)

Effects of L-type and T-type Ca^{2+} channel blockade on the early phase Ca^{2+} transients in rat cardiomyocytes were examined with a rapid-scanning laser confocal microscope and fluo-3. On electrical stimulation of ventricular myocytes, Ca^{2+} concentration was elevated uniformly throughout the cytoplasm within 8 to 12 msec. In contrast, on stimulation of atrial myocytes, the earliest rise in Ca^{2+} occurred at the cell periphery and then spread to the cell interior. T-type Ca^{2+} channel blockade by Ni^{2+} or mibefradil had no effect while L-type Ca^{2+} channel blockade by Cd^{2+} greatly inhibited the Ca^{2+} transient in both ventricular and atrial cells. The present study suggested the involvement of a propagated Ca^{2+}-induced-Ca^{2+} release mechanism in atrial but not ventricular excitation-contraction coupling. In both the ventricle and atrium, the Ca^{2+} influx triggering Ca^{2+} release from the sarcoplasmic reticulum was shown to flow through L-type but not T-type Ca^{2+} channels.

Key Words: Cardiomyocyte, Calcium, T-type Ca^{2+} channel

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Tanaka H*, Ishii T*, Fujisaki R*, Moiyamoto Y*, Tanaka Y*, Aikawa T*, Hirayama W*, Kawanishi T, Shigenobu K* : **Effect of Manganese on Guinea Pig Ventricle: Initial Depression and Late Augmentation of Contractile Force**

Biol. Pharm. Bull., **25**, 323-326 (2002)

Effects of Mn^{2+} on isolated guinea pig ventricular myocardia were examined. In isolated papillary muscles, Mn^{2+} produced a transient decrease in contractile force followed by a late sustained augmentation. Mn^{2+} markedly increased the amplitude of post-rest contractions; the time course of potentiation was almost the same as that of the late augmentation of contractile force after Mn^{2+} application. Mn^{2+} also increased the amplitude of rapid-cooling contractures. The negative inotropic effect of diltiazem and nicardipine was not affected by the presence of Mn^{2+}. Mn^{2+} shortened the action potential duration under normal condition whereas it prolonged the duration under Ca^{2+} free conditions. Mn^{2+}, when applied to fura-2-loaded ventricular myocytes, markedly quenched the cytoplasmic fluorescence excited at 360 nm wavelength. We concluded that Mn^{2+} not only causes a decrease in contractile force by blocking the L-type Ca^{2+} channel, but also enters the cytoplasm through the channel and produces late augmentation of the contractile force through enhancement of sarcoplasmic reticulum function.

Key words : manganese, myocardium, inotropism

*東邦大学薬学部

Niimi, S., Horikawa, M.*, Seki, T.*, Ariga, T.*, Kobayashi, T., Hayakawa, T.: **Effect of activins AB and B on DNA synthesis stimulated by epidermal growth factor in primary cultured rat hepatocytes.**

Biol. Pharm. Bull., **25**, 437-440 (2002)

The effect of activins AB and B on DNA synthesis stimulated by epidermal growth factor (EGF) was studied in primary cultured rat hepatocytes and compared with the effect of activin A, a suppressor of DNA synthesis. Activin AB inhibited DNA synthesis as assessed by [^{3}H] thymidine incorporation. The inhibition by activin AB was detected at 6 ng/ml, and the 12.5 ng/ml concentration produced almost maximal inhibition, approximately 40%, almost the same as that produced by activin A. Inhibition by activin A was detected at 3 ng/ml, and the 6 ng/ml concentration produced almost maximal inhibition. Activin B, on the other hand, had no effect on DNA synthesis up to 50 ng/ml. The increase in labeling index by EGF was also reduced to about 20% by 25 ng/ml activin A and activin AB, but not by activin B. Activin B, however, inhibited the binding of [^{125}I] activin A to hepatocytes, but had no effect on the inhibition of DNA synthesis by activin A, even at 3-fold excess concentrations. These findings suggest that activin AB may act in the same manner as activin A does in terms EGF's inhibitory effect on DNA synthesis, although the effective concentration is higher than that of activin A. The findings also suggest that activin B receptors are present in hepatocytes but that they do not mediate signal transduction leading to the inhibition of DNA synthesis.

Key words: activin; cultured rat hepatocytes; DNA synthesis

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Niimi, S., Oshizawa, T., Naotsuka, M.*1, Ohba, S.*1, Yokozawa, A.*2, Murata, T.*2, Hayakawa, T.: **Establishment of a standard assay method for human thrombomodulin and determination of the activity of the Japanese reference standard**

Biologicals **30**, 69-76 (2002)

This study was undertaken to establish a standard method for determination of the activity of human(h) thrombomodulin (TM). The reactions mainly consisted of formation of h-TM and h-thrombin complex, activation of h-protein C by the complex, and digestion of substrate by activated h-protein C. Linear time-dependent formation of *p*-nitroaniline from the substrate, S-2366, was observed up to 12 min during measurement of the activity of urinary h-TM (uh-TM) reference material by the standard method. Therefore, 10 min was established as the reaction time in the standard method. In the standard method, we defined the activity of h-TM forming 0.1 μmol of *p*-nitroaniline per min in the reaction as 1 JRS Unit. Recombinant h-TM (rh-TM) and uh-TM reference material gave rectilinear dose-response curves within a certain range of specific activities by their original methods in the standard method. The validity of the standard method was assessed based on the coefficients of variation (CV) obtained in the various measurements of h-TM. Intra-batch precision (CV) of h-thrombin and h protein C was 2.90% and 6.57%, respectively, in the measurement of uh-TM activity. The intra-sample, inter-day, and inter-laboratory precision (CV) was 1.30%, 1.63%, and 5.02%, respectively, in the measurement of the first Japanese reference standard for h-TM coded TJRS1. In these assays, the activity of the first Japanese standard was also determined and found to be 205 JRS units per ampoule. When the stability of the Japanese reference standard was assessed by measuring of the standard stored under various thermal conditions, the predicted loss of activity assuming monomolecular degradation according to the Arrhenius equation was less than 3.0% during 103 years at -20℃. These results indicate that the standard method is appropriate for determination of the activity of h-TM and that the Japanese reference standard for h-TM, whose activity was determined in this study, can be stored at -20℃ for long periods without loss of activity.

Key words: thrombomodulin, standard method, reference standard

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Mizuguchi, H., Hayakawa, T.: **Enhanced anti-tumor effect and reduced vector dissemination with fiber-modified adenovirus vectors expressing herpes simplex virus thymidine kinase**

Cancer Gene Ther., **9**, 236-242 (2002)

There are at least two hurdles confronting use of the adenovirus (Ad)-mediated herpes simplex virus thymidine kinase (HSVtk)/ganciclovir (GCV) system for the treatment of cancer. One is inefficient Ad vector-mediated gene transfer into tumor cells lacking the primary receptor, i.e., the coxsackievirus and adenovirus receptor (CAR). The other is hepatotoxicity due to unwanted vector spread into the liver, even when Ad vectors are injected intratumorally. Herein, we present an attractive strategy for overcoming such limitations based on use of a fiber-modified Ad vector containing an RGD peptide motif in the fiber knob. Combination of fiber-modified vectors and a HSVtk/GCV system is a potentially useful and safe approach for the treatment of tumors lacking CAR expression, and that fiber-modified vectors could be of great utility for gene therapy and gene transfer experiments.

Keywords: adenovirus vector, gene therapy, cancer

Mizuguchi, H., Hayakawa, T.: **Adenovirus vectors containing chimeric type 5 and type 35 fiber proteins exhibit altered and expanded tropism and increase the size limit of foreign genes**

Gene, **285**, 69-77 (2002)

Adenovirus (Ad) fiber proteins are responsible for the initial attachment of the virion to the cell membrane. Most Ad vectors currently in use are based on the Ad type 5 (Ad5), which belong to subgroup C, and use the coxsackievirus and adenovirus receptors (CAR) as the initial receptor. Ad35, which belong to subgroup B, recognizes unknown receptor(s) other than CAR. In this study, the feasibility of the Ad vector containing Ad5/35 chimeric fiber protein was examined in a wide variety of cell types, such as CAR positive or negative human tumor cells, rodent cells, and blood cells (a total of 20 cell types), and in mice in vivo. Iinclusion of the Ad35 fiber protein into the Ad5-based vector could lead to an improved efficiency in gene therapy and in gene transfer experiments, especially for the cells lacking in sufficient CAR expression.

Keywords: adenovirus vector, gene therapy, targeting

Okada, Y.*1, Okada, N.*2, Nakagawa, S.*3, Mizuguchi, H., Takahashi, K.*1, Mizuno, N.*1, Fujita,T.*2, Yamamoto, A.*2, Hayakawa, T., Mayumi, T.*3: **Tumor necrosis factor α-gene therapy for an established murine melanoma using RGD (Arg-Gly-Asp) fiber-mutant adenovirus vectors**

Jpn. J. Cancer Res., **93**, 436-444 (2002)

Although adenovirus vectors (Ad) provide high-level transduction efficacy to many cell types, extremely high doses of Ad are required for sufficient gene transduction into several tumors, including melanoma. Here, we demonstrated that the expression of coxsackie-adenovirus receptor, a primitive Ad-receptor, was very low in murine and human melanoma cells. We also found that fiber-mutant Ad containing the Arg-Gly-Asp (RGD) sequence in the fiber knob remarkably augmented gene transduction efficacy in melanoma cells by targeting αv-integrins. In addition, intratumoral injection of RGD fiber-mutant Ad containing the tumor necrosis factor α gene (Ad-RGD-TNFα) revealed dramatic anti-tumor efficacy through hemolytic necrosis in an established murine B16 BL6 melanoma model. These results suggest that αv-integrin-targeted Ad will be a very powerful tool for the advancement of melanoma gene therapy.

Keywords: adenovirus vector, gene therapy, cancer

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Nagayama, Y.*, Kita-Furuyama, M.*, Ando, T.*, Nakao, K.*, Mizuguchi, H., Hayakawa, T., Eguchi, K.*, Niwa, M.*: **A Novel murine model of graves' hyperthyroidism with intramuscular injection of adenovirus expressing the thyrotropin receptor**

J. Immunol., **168**, 2789-2794 (2002)

In this work we report a novel method to efficiently induce a murine model of Graves' hyperthyroidism. Inbred mice of different strains were immunized by i.m. injection with adenovirus expressing thyrotropin receptor (TSHR) or beta-galactosidase and followed up to 8 wk after the third immunization. Fifty-five percent of female and 33% of male BALB/c (H-2d) and 25% of female C57BL/6 (H-2b) mice developed Graves'-like hyperthyroidism with elevated serum thyroxine (T4) levels and positive anti-TSHR autoantibodies with thyroid-stimulating Ig (TSI) and TSH-binding inhibiting Ig (TBII) activities. The highly efficient means that we now report to induce Graves' hyperthyroidism in mice will be very useful for studying the pathogenesis of autoimmunity in Graves' disease.

Keywords: adenovirus vector, graves' disease

*長崎大学医学部

Omori, M.*1, Mizuguchi, H., Ohsawa, K.*2, Kohsaka, S.*2, Hayakawa, T., Abe, K.*1, Shibasaki, F.*3: **Modification of a fiber protein in an adenovirus vector improves in vitro gene transfer efficiency to microglial cell line**

Neurosci. Lett., **324**, 145-148 (2002)

In microglia, it is difficult to introduce exogenous genes of interest even by recombinant adenovirus vectors (Ad) which can infect with high efficiency only to the cells expressing coxackievirus and adenovirus receptors (CAR). We found a lack of CAR expression in primary cultured murine microglia (PCMG) and its immortalized cell line MG5 by reverse transcription-polymerase chain reaction. In order to improve the efficiency of gene transfer, we generated a novel Ad (Ad-RGD) by an incorporation of the Arg-Gly-Asp motif (RGD) containing peptide in the HI loop of the viral fiber knob domain, which enables the virus to contact target cells through V integrins which are known to be ubiquitously expressed on the surface of mammalian cells. Ad-RGD showed a remarkable improvement in the delivery of Escherichia coli LacZ gene in MG5 cells and a moderate increase in PCMG cells under the treatment with granulocyte/macrophage colony stimulating factor.

Keywords: adenovirus vector, gene therapy, targeting

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Takahashi, M.*1, Seki, N.*3, Ozaki, T.*1, Kato, M.*1, Kuno, T.*1, Nakagawa, T.*1, Watanabe, K.*1, Miyazaki, K.*2, Ohira, M.*1, Hayashi, S.*1, Hosoda, M.*1, Tokita, H.*1, Mizuguchi, H., Hayakawa, T., Todo, S.*3, Nakagawara, A.*1: **Identification of the p33ING1-regulated genes which include cyclin B1 and proto-oncogene DEK by using cDNA microarray in a mouse mammary epithelial cell line NmuMG**

Cancer Res., **62**, 2203-2209 (2002)

The candidate tumor suppressor p33(ING1) plays an important role in inducinggrowth arrest at G0-G1 phase of the cell cycle and/or promoting apoptosis in cancerous cells. Here we analyzed expression profiles in mouse

mammary epithelial cells (NMuMG) by using a cDNA microarray consisting of 2304 mouse cDNAs after inducing transformation with antisense inhibitor of growth 1 (ING1) in retrovirus vector. The subsequent confirmation of the altered expression levels of the selected genes demonstrated that overexpression of the antisense ING1 stimulated expression of 14 genes, whereas we have detected transcriptional repression of 5 genes. In addition, adenovirus-mediated overexpression of ING1 in NMuMG cells resulted in down-regulation of cyclin B1, 12-O-tetradecanoylphorbol-13-acetate-inducible sequence 11, DEK, and osteopontin, whereas the levels of TPT1 expression were increased. Thus, our cDNA microarray analysis suggested that p33(ING1) targets the multiple genes, including proto-oncogene DEK and cyclin B1, at least some of which are regulated in a p53-dependent manner, in the cells undergoing cell growth or apoptosis.

Keywords: microarray, tumor suppressor, oncogene

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Nakagawa, T.*1, Takahashi, M.*1, Ozaki, T.*1, Watanabe, K.*1, Todo, S.*2, Mizuguchi, H., Hayakawa, T., Nakagawara, A.*1: **Autoinhibitory regulation of p73 by DNp73 to modulate cell survival and death through p73-specific target element within the DNp73 promoter**

Mol. Cell. Biol., **22**, 2575-2585 (2002)

p73 is a p53-related tumor suppressor but is also induced by oncogene products such as E2F-1, raising a question as to whether p73 is a tumor suppressor gene or oncogene. Unlike p53, p73 has several variants, including Delta Np73, which lacks the NH2- terminal transactivation domain. Although, in developing neurons, Delta Np73 is expressed abundantly and seems to inhibit the proapoptotic function of p53, the role of p73 and Delta Np73 and their regulatory mechanism in cell growth and differentiation are poorly understood. Here we report that p73, but not p53, directly activates the transcription of endogenous Delta Np73 by binding to the p73-specific target element located at positions -76 to -57 within the Delta Np73 promoter region. The negative feedback regulation of p73 by its target Delta Np73 is a novel autoregulatory system for modulating cell survival and death.

Keywords: tumor suppressor, oncogene

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Okada, Y.*1, Okada, N.*2, Nakagawa, S.*3, Mizuguchi, H., Kanehira, M.*1, Nishino, N.*1, Takahashi, K.*1, Mizuno, N.*1, Hayakawa, T., Mayumi, T.*3: **Fiber-mutant technique can augment gene transduction efficacy and anti-tumor effects against established murine melanoma by cytokine-gene therapy using adenovirus vectors**

Cancer Lett., **177**, 57-63 (2002)

In the present study, we investigated whether fiber-mutant Ad containing the Arg-Gly-Asp (RGD) sequence in the fiber knob could promote gene delivery and anti-tumor effects in the murine B16 BL6 tumor model. B16 BL6 cells (in vitro) and tumors (in vivo) infected with RGD fiber-mutant Ad containing a tumor necrosis factor α gene (Ad-RGD-TNFα) produced more TNFα than those infected with conventional Ad-TNFα. In addition, Ad-RGD-TNFα required about one-tenth the dosage of Ad-TNFα for induction of equal therapeutic effects upon intratumoral injection into established B16 BL6 tumors. Furthermore, the combination of both TNF-α and interleukin 12-expressing RGD fiber-mutant Ads exhibited more effective tumor regression than the Ad expressing each alone. These results suggested that the fiber-mutant for altering Ad-tropism is a very potent technology for advancing gene therapy for melanoma.

Keywords: adenovirus vector, gene therapy, cancer

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Maruyama, K.*, Akiyama, Y.*, Nara-Ashizawa, N.*, Hojo, T.*, Cheng, J. Y.*, Mizuguchi, H., Hayakawa, T., Yamaguchi, K.*: **Adenovirus-mediated MUC1 gene transduction into human blood-derived dendritic cells**

J. Immunother., **24**, 345-353 (2001)

In the current study, we made MUC1-expressing human dendritic cells (DCs) using recombinant adenovirus vector. Flow cytometric analysis showed that 30% to 40% of the transduced DCs expressed MUC I protein. Adenovirus-mediated MUC1 gene transduction into DCs had no significant effect on DC surface marker expressions or functions such as mixed leukocyte reaction. Furthermore, MUCI-specific CD8+ CTLs could be induced from healthy donor blood lymphocytes using MUC1-expressing DCs as stimulators. These results suggested that MUC1 gene-transduced DCs are a

functional and potent tool for triggering a CTL response against MUC1 cancer cells.

Keywords: adenovirus vector, gene therapy, cancer

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Okada, N.*[1], Saito, T.*[1], Masunaga, Y.*[1], Tsukada, Y.*[2], Nakagawa, S.*[2], Mizuguchi, H., Mori, K.*[1], Okada, Y.*[3], Fujita, T.*[1], Hayakawa, T., Mayumi, T.*[2], Yamamoto, A.*[1]: **Efficient antigen gene transduction using Arg-Gly-Asp fiber-mutant adenovirus vectors can potentiate anti-tumor vaccine efficacy and maturation of murine dendritic cells**

Cancer Res., **61**, 7913-7919 (2001)

In the present study, we compared immunological properties and vaccine efficacy of DC2.4 cells, an immature murine dendritic cells (DCs) line, transduced with an ovalbumin (OVA) gene by fiber-mutant Ad (Ad-RGD-OVA) or conventional Ad (Ad-OVA). Ad-RGD-OVA-infected DC2.4 cells could more efficiently present OVA peptides via MHC class I molecules in a vector particle-dependent manner and induce OVA-specific CTL response by vaccination than Ad-OVA-infected DC2.4 cells. Moreover, vaccination with Ad-RGD-OVA-infected DC2.4 cells could achieve an equal or greater antitumor effect against challenge with E.G7-OVA tumor cells with lower doses of Ad on infection or fewer cells for immunization than the vaccination procedure using Ad-OVA-infected DC2.4 cells. Flow cytometric analysis indicated enhanced expression of MHC class I and II molecules as well as CD80, CD86, CD40, and CD54. We propose that DC manipulation using the Arg-Gly-Asp fiber-mutant Ad system could advance the development of more effective vaccines and allow for more convenient administration of DC-based gene immunotherapy.

Keywords: adenovirus vector, gene therapy, cancer

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Mizuguchi, H., Hayakawa, T.: **Characteristics of adenovirus-mediated tetracycline controllable expression system**

Biochim. Biophys. Acta, **1568**, 21-29 (2001)

Combination of recombinant adenovirus (Ad) vectors and tetracycline-controllable expression system is clearly advantage in gene therapy and gene transfer experiment. In this study, we examined the characteristics of Ad vector containing the tet-off or tet-on system. The Ad vector containing the tet-off system showed tightly regulatable transgene expression even at low MOI (multiplicity of infection). In contrast, regulation of gene expression by the Ad vector containing the tet-on system was not tight at low MOI, while it showed moderate regulation at high MOI (MOI=100). The Ad vector-mediated tet-on system showed lower inducible and higher background (basal) luciferase production than that of the Ad vector-mediated tet-off system. Moreover, the former system required a concentration of doxycycline, a derivative of tetracycline, approximately 2-to 3-log orders higher than that of the latter system to switch the luciferase expression. These results suggest that the Ad vector containing the tet-off system is considered to be functionally superior to the vector containing the tet-on system.

Keywords: adenovirus vector, gene therapy, regulation

Koizumi, N.*, Mizuguchi, H., Hosono, T., Ishii-Watabe, A., Uchida, E., Utoguchi, N.*, Watanabe, Y.*, Hayakawa, T.: **Efficient gene transfer by fiber-mutant adenoviral vectors containing RGD peptide**

Biochim. Biophys. Acta, **1568**, 13-20 (2001)

One of the hurdles to adenovirus (Ad)-mediated gene transfer is that Ad vectors mediate inefficient gene transfer into cells lacking in the primary receptors, Coxsackievirus and adenovirus receptor (CAR). We previously developed a fiber-mutant Ad vector containing the Arg-Gly-Asp (RGD)-containing peptide motif on the HI loop of the fiber knob, and showed that the mutant vector had enhanced gene transfer activity to human glioma cells, which showed little CAR expression, compared to the vector containing wild type fiber. In this study, the feasibility of the Ad vector containing RGD peptide on the fiber knob was examined in a wide variety of cell types: CAR-positive or-negative human tumor cells, mouse cells, and leukemia cells. The mutant vector infected the cells, which lacked CAR expression but showed αv integrin expression, about 10-1000 times more efficiently than the vector containing wild type fiber via an RGD-integrin ($\alpha v\beta 3$ and $\alpha v\beta 5$)-dependent, CAR-independent cell entry pathway. The results of this study indicate that Ad vector containing RGD peptide on the fiber knob could be of great utility for gene therapy and gene transfer experiments.

Keywords: adenovirus vector, gene therapy, targeting

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Xu, Z.L.*, Mizuguchi, H., Ishii-Watabe, A., Uchida, E., Mayumi, T.*, Hayakawa, T.: **Optimization of transcriptional regulatory elements for constructing plasmid vectors**

Gene, **272**, 149-156 (2001)

In studies regarding both gene therapy and gene function, transgene expression by plasmid vectors benefits from the use of transcriptional regulatory elements which permit high-level gene expression. Therefore, with respect to transgene (luciferase) expression activity both in vitro (using HeLa, HepG2, and ECV304 cells) and in vivo (mouse liver and skeletal muscle), we investigated the effective combination of commonly-used regulatory elements, such as the promoter/enhancer, intron, and polyadenylation signal (P(A)) sequence by constructing a series of plasmids that differed only in the particular sequence element being evaluated. Of the several promoter/enhancers that were tested, hybrid CA promoter/enhancer containing human cytomegalovirus immediate-early 1 gene (CMV) enhancer and chicken beta-actin promoter with the beta-actin intron sequence, and the improved CMV promoter/enhancer containing the largest intron of CMV (intron A) produced the highest levels of expression both in vitro and in vivo. P(A) sequences were found to have significant effects on transgene expression. The effect of a multiple enhancer was also examined. Optimized plasmids of this study were pCASL3 (composed of CMV enhancer, beta-actin promoter, beta-actin intron, Simian virus (SV40) P(A) sequence and SV40 enhancer) and pCMVSL3 (composed of CMV enhancer, CMV promoter, intron A, SV40 P(A) sequence and SV40 enhancer). These comparative analyses could provide a systematic reference for the development of vector construction for gene therapy, vaccine development, and gene transfer experiments.

Keywords: gene therapy, transcriptional regulatory elements, plasmid

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Xu, Z.L.*, Mizuguchi, H., Ishii-Watabe, A., Uchida, E., Mayumi, T.*, Hayakawa, T.: **Strength evaluation of transcriptional regulatory elements for transgene expression by adenovirus vector**

J. Control. Release, **81**, 155-63 (2002)

In studies of both gene function and gene therapy, transgene expression may be assisted considerably through the use of transcriptional regulatory elements with high activity. In this study, we evaluated the strength of various transcriptional regulatory elements both in vitro (six types of cell line) and in vivo (mouse heart, lung, kidney, spleen, and liver) by adenovirus-mediated gene transfer. In the case of the promoter/enhancer (P/E), the activity of CMV P/E (from the human cytomegalovirus immediate-early 1 gene) and hybrid CA P/E (composed of the CMV enhancer and chicken beta-actin promoter) were investigated, both of which are known to be strong and widely used. While hybrid CA P/E showed a higher transgene expression activity than CMV P/E, the addition of the intron A sequence (the largest intron of CMV) to CMV P/E increased the activity of CMV P/E to the same or higher level than that of hybrid CA P/E. Concerning the polyadenylation signal (P(A)) sequence, one from the bovine growth hormone (BGH) gene was about two times more efficient than that from the Simian virus 40 (SV40) late gene, both in vitro and in vivo. In the context of the CMV P/E containing the intron A sequence, a further increase in transgene expression was obtained by the addition of a SV40 enhancer downstream from the P(A) sequence. The combination of the SV40 P(A) and a SV40 enhancer showed almost comparable activity to BGH P(A). This information would be helpful for the construction of adenovirus vectors for studies regarding both gene function and gene therapy.

Keywords: adenovirus vector, gene therapy, transcriptional regulatory elements

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Ishii-Watabe, A., Uchida, E., Mizuguchi, H., Hayakawa, T.: **Involvement of a calcium-independent pathway in plasmin-induced platelet shape change**

Life Sci. **69**, 945-960 (2001)

Plasmin-induced platelet activation is considered to be a cause of reocclusion after thrombolytic therapy with plasminogen activators. However, little is known regarding its mechanism and regulation, particularly with respect to the initial step shape change. We here demonstrate that a Ca^{2+}-independent pathway is involved in plasmin-induced human platelet shape change, and that Rho-kinase plays an important role in this pathway. When the increase in cytosolic Ca^{2+} was prevented by an intracellular Ca^{2+} chelator, 5,5'-dimethyl-BAPTA, plasmin-induced platelet shape change was partially inhibited but still occurred. In the presence of 5,5'-dimethyl-BAPTA, a specific Rho-kinase inhibitor, Y-27632, completely inhibited the shape change.

Phosphorylation of myosin light chain, a key regulator of platelet shape change, was completely inhibited by Y-27632 in 5,5'-dimethyl-BAPTA-treated platelets. Although plasmin caused tyrosine phosphorylation of the 80 kDa protein during the shape change, it did not seem to have a critical role. cAMP-elevating agents inhibited plasmin-induced shape change in 5,5'-dimethyl-BAPTA- or Y-27632-treated platelets with similar efficiency. These results indicated that plasmin causes platelet shape change by activating Ca^{2+}-dependent and Ca^{2+}-independent-Rho-kinase-dependent pathways, both of which are sensitive to cAMP.

Keywords: platelet, plasmin, shape change

Matsuoka, T.*, Kuribara, H.*, Suefuji S.*, Akiyama, H., Miura, H.*, Kusakabe, Y.*, Goda, Y., Isshiki, K.*, Toyoda, M., Hino, A.*: **A detection method for recombinant DNA from genetically modified maize CBH351**

J. Food Hygienic Soc. Japan, **42**, 197-201 (2001)

A method using polymerase chain reaction (PCR) was designed for the detection of genetically modified maize CBH351, which has not authorized as safe for use in foods and feeds in Japan yet. We analyzed a recombinant DNA (r-DNA) sequence introduced into CBH-351 maize and designed specific primer pairs to amplify a segment including part of the r-DNA. The PCR products obtained by using the designed primer pairs are specific for CBH351 and should prevent false positive results caused by other maizes and other main cereal crops. The r-DNA introduced into CBH351 could be detected from maize samples containing 0.05~0.1% CBH351 maize. this sensitivity is theoretically equivalent to a level of several genome copies and so this technique is a very efficient means to detect CBH351 maize.

Keywords: genetically modified maize CBH351, detection method, PCR

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Ngang, E.*, Matsufuji, H.*, Chino, M.*, Goda, Y., Toyoda, M., Takeda, M.*: **Structural determination of subsidiary color in commercial food green No.3 (Fast Green FCF, FD&D Green No.3)**

J. Food Hyg. Soc. Japan, **42**, 298-303 (2001)

HPLC analysis revealed that eight subsidiary colors existed in commercial Food Green No.3 (fast green FCF, FD & C Green No. 3). Among them, four subsidiary colors C, F, G and H were isolated by using preparative HPLC and their structures were determined by MS and NMR. They were the disodium salt of 2-[[4-[N-ethyl-N-(3-sulfophenylmethyl)amino]phenyl][4-[N-ethyl-N-(3-sulfophenylmethyl)amino]phenyl]methylio]-4-hydroybenzenesulfonic acid (abbrebiated as m-p-G-3), the sodium salt of 2-[(4-N-ethyamino)phenyl][4-[N-ethyl-N-(4-sulfophenylmethyl)amino]phenyl]methylio]-4-hydroybenzenesulfonic acid [abbrebiated as HSBA-(EA)(m-EBASA)], the sodium sailt of 2-[[(4-N-diethyamino)phenyl][4-[N-ethyl-N-(3-sulfophenylmethyl)amino]phenyl]methylio]-4-hydroybenzenesulfonic acid [abbrebiated as HSBA-(di-EA)(m-EBASA)], and the sodium sailt of 2-[[4-[N-ethyl-N-(phenylmethyl)amino]phenyl][4-[N-ethyl-N-(3-sulfophenylmethyl)amino]phenyl]methylio]-4-hydroybenzenesulfonic acid [abbrebiated as HSBA-(EBA)(m-EBASA)], respectively. HSBA-(di-EA)(m-EBASA) was a subsidiary color newly found in commercial Food Green No. 3.

Keywords: food green No.3, subsidiary color, structural determination

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山田真紀子*1, 森本隆司*1, 中村幹雄*1, 合田幸広, 中澤裕之*2： **食用黄色5号製造時における副成色素 Trisodium salt of 6-hydroxy-7-(4-sulfophenyl)-5-(4-sulfophenylazo)-2-naphthalenesulfonic acid の生成条件**

日食化誌, **8**, 73-77 (2001)

Y5製造時における副成色素Y5-SAの生成条件を検討した．Y5-SAの生成実験より，以下の点が判明した．1) スルファニル酸のジアゾニウム塩とシェファー塩の比を変えて反応させたとき，Y5-SAの副成はスルファニル酸のジアゾニウム塩の量に依存して増加した．2) Y5にスルファニル酸のジアゾニウム塩を加えると，時間とスルファニル酸のジアゾニウム塩の量に依存してY5-SAが生成されることが明らかとなった．3) pHの影響について検討したところ，pH8.0～pH10.0では少なく，pH12.0のとき最も多く，pH 13.0では減少した．以上の結果より，Y5を製造する際は，スルファニル酸のジアゾニウム塩とシェファー塩を正確に等量でカップリング反応させるとともに，Y5-SAの副成を抑制し，Y5の生成時のカップリング反応が速やかであるpH10.0で反応を行う必要がある．また，Y5-SAはY5製造時のGMPマーカーとして利用可能であると考えられる．

Keywords: food yellow No.5, subsidiary color, Sunset Yellow FCF

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合田幸広，浅野卓哉，渋谷雅明*1，日野明寛*2，豊田正武：**遺伝子組換えパパイヤからの組換え遺伝子の検知**

食衛誌 **42**, 231-236 (2001)

日本で安全性審査未終了のGMパパイヤ（55-1系統，Sunup）について，ポリメラーゼ連鎖反応（PCR）を用いた検知法を検討した．Papain遺伝子を検知するプライマー対を陽性対照とした結果，生食用パパイヤでは，シリカゲル膜タイプのキットで，缶詰由来のパパイヤでは，イオン交換樹脂タイプのキットで，パパイヤ由来の遺伝子が良好に抽出精製され，検知されることが明らかとなった．さらに，生食用のものでは，GUS遺伝子，NPTII遺伝子のみを検知するプライマー対を含め5種のプライマー対で特異的にGMパパイヤが検知されることが判明した．他方，缶詰由来のパパイヤでは，2種類の生物に由来する連続したDNA領域を増幅するプライマー対を用いた場合，良好な検知結果が得られた．

Keywords: genetically modified papaya, detection method, PCR

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合田幸広，柿原芳輝，穐山 浩，松岡 猛*，日野明寛*，豊田正武：**トウモロコシ穀粒における非意図的組換え遺伝子の検知**

食衛誌 **43**, 74-79 (2002)

品種（系統）が明確な遺伝子組換えトウモロコシの穀粒及び非組換え穀粒について，PCRを用いた品種（系統）特定型の検知法を利用し，当該穀粒で表示されている品種（系統）と，実際の検知結果の差違について検討した．その結果，Bt11では100粒中11粒で，Event176では，30粒中5粒で表示と異なった組換え品種（系統）の遺伝子が検知された．また，非組み換え品種では，30粒中4粒から組換え品種の遺伝子が検知された．従って，他殖性のトウモロコシでは，穀粒では表示と異なった非意図的組換え遺伝子が検知される可能性があることが明らかとなった．また，定量PCRの結果より，GM遺伝子がホモで存在する粒の遺伝子型はすべて表示の組換え品種（系統）のもので，他の組換え品種（系統）の遺伝子はすべてヘテロな遺伝子型として観察され，これらの遺伝子が風媒により導入された可能性が非常に高いことが示された．

Keywords: genetically modified maize grain, unexpected recombinant DNA, quantitative-PCR

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Ohtsuki, T.*, Matsufuji, H.*, Toyoda, M., Goda, Y.,: **Acylated anthocyanins from red radish (*Raphanus sativus* L.)**

Phytochemistry, **60**, 79-87 (2002).

Twelve acylated anthocyanins were isolated from the red radish (Raphanus sativus L.) and their structures were determined by spectroscopic analyses. Six of the anthocyanins were novel compounds, which were identified as pelargonidin 3-*O*-[6-*O*-(*E*)-feruloyl-2-*O*-β-D-glucopyranosyl]-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), pelargonidin 3-*O*-[6-*O*-(*E*)-caffeoyl-2-*O*-(6-(*E*)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), pelargonidin 3-*O*-[6-*O*-(*E*)-p-coumaroyl-2-*O*-(6-(*E*)-caffeoyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), pelargonidin 3-*O*-[6-*O*-(*E*)-feruloyl-2-*O*-(6-(*E*)-caffeoyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), pelargonidin 3-*O*-[6-*O*-(*E*)-p-coumaroyl-2-*O*-(6-(*E*)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside), and pelargonidin 3-*O*-[6-*O*-(*E*)-feruloyl-2-*O*-(2-(*E*)-feruloyl-β-D-glucopyranosyl)-(1→2)-β-D-glucopyranoside]-5-*O*-(β-D-glucopyranoside).

Keywords: red radish, acylated anthocyanin, structural determination

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Shoji, T.*, Goda, Y., Toyoda, M., Yanagida, A.*, Kanda, T.*: **Characterization and structures of novel anthocyanin pigments generated in rose cider during vinification.**

Phytochemistry, **59**, 183-189 (2002)

Anthocyanin pigments, which are not found in apple juice, were detected in rosé cider. We confirmed by HPLC/DAD and LC/ESI-MS analyses that some of these anthocyanin pigments generated in rosé cider during the vinification corresponded to those formed in model cider containing anthocyanin and flavan-3-ol in the presence of acetaldehyde. To confirm their structures, two anthocyanin pigments formed in a model cider containing cyanidin-3-galactoside and (-)-epicatechin in the presence of acetaldehyde were isolated, and purified, and their structures were elucidated by high resolution FAB-MS and ^{1}H and ^{13}C NMR analyses. These two pigments

were found to consisit of cyanidin-3-galactoside and (-)-epicatechin linked by a CH_3-CH bridge at the 8-position. They were diastereomers that differed in the configuration of the asymmetric methine carbon.

Keywords: rosé cider, anthocyanin pigments, structural determination

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Kato, Y.*, Sato, H.*, Aoki, H.*, Goda, Y.: **Chemical strucutre of an anthocyanain pigment isolated from "Myoga"** (*Zingiber mioga* Rosc.)
Food and Food Ingredients Journal of Japan, **197**, 28-33 (2002)

Two anthocyanins were isolated from young inflorescence of "Myouga" (*Zingiber mioga* ROSC.), and the structure of the major anthocyanin was elucidated by means of spectroscopic analyses and chemical degradation techniques. The major pigment was malvidin 3-rutinoside (malvidin 3-*O*-(6-*O*-(α-rhamnopyranosyl)-β-D-glucopyranoside)).

Keywords: anthocyanain pigment, Zingiber mioga, structural determination

* San-Ei Gen FFI. Inc.

Hada, N.*1, Sato, K.*1, Sakushima, J.-i., Goda, Y., Sugita, M.*2, Takeda, T.*1 : **Synthetic Studies on Glycosphingolipids from Protostomia Phyla: Synthesis of amphoteric glycolipid analogues containing a phosphocholine residue from the earthworm *Pheretima hilgendorfi***
Chem. Pharm. Bull., **49**, 1464-1467 (2001).

Two kinds of amphoteric glycosphingolipid analogues from the earthworm *Pheretima hilgendorfi* were synthesized as follows: The key reaction is a coupling of a phosphocholine group at the position C-6 of 1 and 6 which was attempted using 2-chloro-2-oxo-1,3,2-dioxaphospholane, followed by reaction of the resulting cyclic phosphate intermediate with anhydrous trimetjhylamine to give the compounds **2** and **7**. Subsequent debenzaylation afforded target compounds (**3**, **8**). Their ability to inhibit the histamine release in vitro was examined.

Keywords: amphoteric glycosphingolipid, chemical synthesis, histamine release

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Kawahara, N., Masuda, K.*, Sekita, S., Satake, M.: **A new Secoiridoid Glucoside, Amaronitidin, from the Peruvian Folk Medicine "Hercampuri"** (*Gentianella nitida*)
Chem. Pharm. Bull., **49 (6)**, 771-772 (2001).

A new secoiridoid glucoside designated amaronitidin was isolated from the Peruvian folk medicine "Hercampuri" (*Gentianella nitida*) along with three known secoiridoid glucosides. Their structures were determined by extensive spectroscopic investigation.

Key words : amaronitidin, *Gentianella nitida*, Hercampuri, Gentianaceae, secoiridoid glucoside

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Komatsu, H.*, Watanabe, M.*, Ohyama, M.*, Enya, T.*, Koyama, K.*, Kanazawa, T.*, Kawahara, N., Sugimura, T.*, Wakabayashi, K.* : **Phenanthroindolizidine Alkaloids as Cytotoxic Substances in a Danaid Butterfly, *Ideopsis similis*, against Human Cancer Cells**
Medicinal Chemistry **44 (11)**, 1833-1836 (2001).

In the present study, we isolated cytotoxic substances against a human gastric cancer cell line, TMK-1, in Ideopsis similis pupae, with an activity similar to that of the adult butterfly. The basic fraction, prepared from a methanol extract, accounted for 83% of the cytotoxic activity. Two major cytotoxic substances were purified by HPLC, and one was determined to be a new phenanthroindolizidine alkaloid, *trans*-(+)-3,14α-dihydroxy-6,7-dimethoxyphenanthroindolizidine (**1**), and the other a known compound, *trans*-(+)-3,14α-dihydroxy-4,6,7-trimethoxyphenanthroindolizidine (**2**). The IC_{50} values for TMK-1 cells were 0.5 ng/mL and 0.7 ng/mL, respectively. These two compounds showed similar cytotoxic potential with four other cancer cell lines including cervical, lung, and colon carcinomas and leukemia. Quantitative analyses indicated the presence of each of the two phenanthroindolizidine alkaloids at levels of 11-74 μg in each larva, pupa, or adult of *I. similis*. However, **1** was not detected in the leaves of Tylophora tanakae, a host plant for larvae of *I. similis*, and the level of **2** (2 μg per gram of leaves) was far less than that in the larvae. Since the leaves of *T. tanakae* are known to contain various phenanthroindolizidines, compounds **1** and **2** are presumably metabolically converted from such alkaloids in larvae of *I. similis*.

Key words : phenanthroindolizidine alkaloid, cytotoxic

substance

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Kuroyanagi, M.*1, Seki, T.*2, Hayashi, T.*3, Nagashima, Y.*3, Kawahara, N., Sekita, S., Satake, M.: **Anti-androgenic Triterpenoids from the Brazilian Medicinal Plant, *Cordia multispicata***

Chem. Pharm. Bull., **49 (8)**, 954-957 (2001).

6 compounds were isolated from the AcOEt soluble fraction of leaves of the Brazilian medicinal plant, *Cordia multispicata*, and their structures were elucidated to be 3β, 25-epoxy-21β-acetoxy-3α, 22β-dihydroxyurs-12-en-28-al, 3β, 25-epoxy-28-acetoxy-3α, 21β, 22β-trihydroxyurs-12-ene, 21β-acetoxy-22β-hydroxy-3-oxours-12-en-28-al, 28-acetoxy-6β, 21β, 22β-trihydroxy-3-oxours-12-ene, 21β, 22β-dihydroxy-3-oxours-12-en-28-al and 3β, 21β, 22β-trihydroxyurs-12-en-28-al, respectively, by means of spectral data, especially two dimensional NMR techniques. Triterpenes having the hemiketal structure at the A-ring, an acyloxy group at C-22 and/or ketone at C-3 showed potent anti-androgenic activity.

Key words : *Cordia multispicata*, ursan-type triterpene, anti-androgenic activity, hemiketal

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Hada, N.*1, Totsuka, Y.*2, Enya, T.*2, Tsurumaki, K.*2, Nakazawa, M.*2, Kawahara, N., Murakami, Y.*3, Yokoyama, Y.*3, Sugimura, T.*2, Wakabayashi, K.*2: **Structures of mutagens produced by the co-mutagen norharman with *o*-and *m*-toluidine isomers**

Mutation Research, 493, 115-126 (2001).

Norharman, abundantly present in cigarette smoke and cooked foods, is not mutagenic to *Salmonella typhimurium* strains. However, norharman shows mutagenicity to *S. typhimurium* TA98 and YG1024 in the presence of S9 mix when coexisting with aromatic amines, including aniline, *o*-and *m*-toluidines. We previously reported that the mutagenicity from norharman and aniline in the presence of S9 mix was due to the formation of a mutagenic compound, 9-(4'-aminophenyl)-9*H*-pyrido[3,4-*b*]indole (aminophenylnorharman). In the present study, we analyzed the mutagens produced by norharman with *o*-or *m*-toluidine in the presence of S9 mix. When norharman and *o*-toluidine were reacted at 37℃ for 20 min, two mutagenic compounds, which were mutagenic with and without S9 mix, respectively, were produced, and these were isolated by HPLC. The former mutagen was deduced to be 9-(4'-amino-3'-methylphenyl)-9*H*-pyrido[3,4-*b*]indole (amino-3'-methylphenylnorharman) on the basis of various spectral data, and this new heterocyclic amine was confirmed by its chemical synthesis. The latter mutagen was identified to be the hydroxyamino derivative. Amino-3'-methylphenylnorharman induced 41,000 revertants of TA98, and 698,000 revertants of YG1024 per μg with S9 mix. Formation of the same DNA adducts was observed in YG1024 when amino-3'-methylphenylnorharman or a mixture of norharman plus *o*-toluidine was incubated with S9 mix. These observations suggest that norharman reacts with *o*-toluidine in the presence of S9 mix to produce amino-3'-methylphenylnorharman, and this compound is metabolically activated to yield its hydroxyamino derivative. After activation by *O*-acetyltransferase, it might bind to DNA and exert mutagenicity in S. typhimurium TA98 and YG1024. When norharman and *m*-toluidine were reacted in the presence of S9 mix, 9-(4'-amino-2'-methylphenyl)-9*H*-pyrido[3,4-*b*]indole (amino-2'-methylphenylnorharman) was identified as a mutagen. Thus, the mutagenicity of norharman with *m*-toluidine may follow a mechanism similar to that with *o*-toluidine.

Key words : norharman, toluidine isomers, amino-3'-methylphenylnorharman, amino-2'-methylphenylnorharman

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Kawahara, N., Kurata, A.*, Hakamatsuka, T.*, Sekita, S., Satake, M.: **Two Novel Cucurbitacins, Neocucurbitacins A and B, from theBrazilian Folk Medicine "Buchinha" (*Luffa operculata*) and their Effect onPEBP2αA and OCIF Gene Expression in a Human Osteoblast-like Saos-2 Cell Line**

Chem. Pharm. Bull., **49 (10)**, 1377-1379 (2001).

Two novel cucurbitacins designated as neocucurbitacins A, possessing inhibitory activity of polyoma enhancer binding protein 2αA (PEBP2αA) and osteoclastogenesis-inhibitory factor (OCIF) gene expression in human osteoblast-like cells, and B were isolated from the fruit of *Luffa operculata*. Their structures have been determined

by extensive spectroscopic investigation.

Key words : neocucurbitacin, *Luffa operculata*, Buchinha, cucurbitacin, bone formation

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Chakravarty, A. K.*1, Sarkar, T.*1, Masuda, K.*2, Shiojima, K.*2, Nakane, T., Kawahara, N.: **Bacopaside I and II: two pseudojujubogenin glycosides from *Bacopa monniera***
Phytochemistry, **58 (4)** 553-556 (2001).

Two saponins, designated as bacopaside I and II, have been isolated from *Bacopa monniera* Wettst. and their structures have been elucidated as 3-*O*-α-L-arabinofuranosyl-(1→2)-[6-*O*-sulphonyl-β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyl pseudojujubogenin (**1**) and 3-*O*-α-L-arabinofuranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]β-D-glucopyranosyl pseudojujubogenin (**2**) mainly on the basis of 2D NMR and other spectral analyses.

Key words : *Bacopa monniera*, Scrophulariaceae, Saponins, bacopaside I and II

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Xiao, D.*1, Kuroyanagi, M.*1, Itani, T.*2, Matsuura, H.*2, Udayama, M.*2, Murakami, M.*3, Umehara, K.*3, Kawahara, N.: **Studies on Constituents from *Chamaecyparis pisifera* and Antibacterial Activity of Diterpenes**
Chem. Pharm. Bull., **49 (11)**, 1479-1481 (2001)

In the course of our research for biologically active constituents from coniferous plants, a chromone derivative and an abietane derivative were isolated along with several diterpenes from *Chamaecyparis pisifera*. Structures of the new compounds were determined to be 5,7-dihydroxy-2-(1-acety-1-2-methoxycarbonylethyl) chromone and *rel*-(8*R*, 10*R*, 20*S*)-8,10,20-trihydroxy-9(10→20)-*abeo*-abieta-9,13-dien-12-one by means of spectral methods including two-dimensional NMR experiments. Some of these abietane-type compounds isolated from this plants showed antibacterial activity against the gram-positive bacteria *Staphylococcus aureus and Bacillus subtilis*.

Key words : 2-substituted chromone, 9(10→20)-*abeo*-abietane, *Chamaecyparis pisifera*, antibacterial activity, diterpene

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Wang, T.*1, Shirota, O., Nakanishi, K.*1, Berova, N.*1, McDonald, L. A.*2, Barbieri, L. R.*2, and Carter, G. T.*2: **Absolute stereochemistry of the Spiroxins**
Can. J. Chem., **79**, 1786-1791 (2001)

The absolute configuration of spiroxin A has been determined by exciton-coupled circular dichroism (CD). Namely, the difference CD between spiroxin A bis-methoxycinnamate, as well as spiroxin A bis-retinoate, both exhibit negative exciton couplings between the phenolic hydroxyl group chromophores. This establishes the absolute configuration as 2S, 3R, 4S, 2'S, 3'R, 4'S.

Keywords: circular dichroism, exciton coupling, spiroxins

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Ozaki Y., Ono K., Sakaguchi I.,* Kato Y.* : **Histological Study of the Accelerating Effect of Shikonin and Alkannin on the Proliferation of Granulation Tissue in Rats**
Natural Mediciens, **56**, 29-33(2002)

紫根の成分で光学異性の関係にあるシコニンとアルカニンの肉芽組織増殖促進作用の作用発現機序をラット綿球法を用いて組織学的に解明し，さらに，その効力を比較検討した．シコニン及びアルカニンの局所投与は，綿球をラット背部に埋め込んだ後，5日目ごろから肉芽組織増殖を促進する傾向にあり，10日後には有意に促進作用を示した．この促進作用を示している時の肉芽組織周辺部を蛍光染色して毛細血管の状態を観察したところ肉芽組織周辺部には新生毛細血管の増殖の促進作用が観察された．これらのことから，シコニン及びアルカニンは肉芽組織周辺部の新生毛細血管の増殖を促進して，肉芽組織増殖の促進作用を発現していることが明らかとなった．

Keywords : shikonin, alkannin, granulation tissue

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Tsuchiya, T., Itahashi, Y., Ichikawa, T., Ichikawa, A.*: **Studies on the biocompatibility of artificial organs and tissue engineered products**
Animal Cell Technology: Basic & Applied Aspects, **12**, 253-256 (2002)

Representative biodegradable-polymers are assayed to clarify the property when used as the materials for the nerve regeneration using the midbrain cell differentiation systems. As for polyglycolic acid [PGA], both the

proliferation and differentiation of the midbrain cells were does-dependently inhibited by PGA3000 (Mw=3000). Poly(L-lactic acid)[PLLA]5000 (Mw=5000) showed slightly inhibitory effects on the proliferation and differentiation. In the case of the poly (L-lactic acid-co-ε-caprolactone)$_{25}$ 10000 [P(LA-CL)$_{25}$ 10000] (Mw =10000), the differentiation was inhibited at 7.5 μg/ml to the levels of about 50% of the controls. But, P(LA-CA)$_{50}$ 18000 (Mw=18000) showed weakly inhibitory action on the differentiation of midbrain cells in comparison with P(LA-CA)$_{25}$ 10000. The inhibitory activity of these polymers on neuronal cell differentiation were in the following order: P(LA-CA)$_{25}$ 10000 > PGA3000 > P(LA-CA)$_{50}$ 18000 = PLLA5000. The catalyst of $SnCl_2$ showed the strongest inhibitory action on the midbrain cell differentiation among test chemicals.

Keywords: midbrain, neuronal cell differentiation, biodegradable polymer

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Ichikawa, A.*, Tsuchiya, T.: **Reversion of transformed phenotype of polyetherurethane-induced tumor cells by Cx43 transfection**

Animal Cell Technology: Basic & Applied Aspects, **12**, 269-273 (2002)

Rat tumor cell line U41 is derived from rat malignant fibrous histiocytoma (MFHC) developed in the part of subcutaneous implantation of polyetherurethane (PEU) films. U41 cells is suppressed in gap junctional intercellular communication (GJIC). Western blot and RT-PCR analysis indicate that the expression levels of the gap junction protein connexin43 (Cx43) are decreased in this cell line. Rat Cx43 cDNA was transfected into U41 derived subclone U41-22. A stable Cx43 transfectant clone, which acquired high levels of Cx43 expression and the capacity of GJIC, was established and compared with mock transfectant control clone. Cx43 transfected clone U41-22/Cx43 showed reduced growth rate, recovery of contact inhibition and loss of colony formation ability in soft agar. These results strongly suggests that suppression of Cx43 expression play an important role in development of rat MFHC caused by PEU implantation, and Cx43 acts as tumor suppressor to PEU induced tumor.

Keywords: polyurethane, tumorigenesis, gap-junctional intercellular communication

* Kyoto Institute of Technology

Rahman, M.S., Tsuchiya, T.: **Enhancement of chondrogenic differentiation of human articular chondrocytes by biodegradable polymers**

Tissue Engineering, **7**, 781-790 (2001)

Biodegradable polymers are attractive candidates for chondrocyte embedding and transplantation in cartilage tissue engineering. In an attempt to determine the effects of a variety of biodegradable materials on cartilage proliferation and extracellular matrix production, poly-L-lactic acid (PLLA) with a molecular weight of 5000, polyglycolic acid (PGA) with a molecular weight of 3000, and copolymer of poly (L-lactic acid-glycolic acid) 50:50 (PLGA) with a molecular weight of 5000, were dissolved in DMSO and added into the medium for 4 weeks in in vitro high-density micromass culture of multiplied human articular chondrocytes (HAC). PLLA with a molecular weight of 270000 (PLA03) was used as thin film. Cell proliferation and differentiation in these biomaterials were compared with tissue culture polystyrene (TCPS) as a control. Alamar blue and alcian blue staining were carried out to determine the chondrocyte proliferation and differentiation, respectively. Samples exposed to these biomaterials promoted cell proliferation in the range of 86-105% of the control proliferation, and a slight but significant increase in cell proliferation was noted only in the culture exposed to PLGA. The sample exposed to PGA elicited a significant 3.7-fold higher ($p < 0.01$) cell differentiation than controls and was significantly higher than that of the samples exposed to PLLA, PLA03, and PLGA. After 4 weeks of culture, the cell differentiation from most to least was in the following order PGA > PLA03 > PLGA = PLLA > Cont. = DMSO. Chondrocyte differentiation of the samples exposed to various biomaterials were significantly higher compared with controls. Thus, serially passage chondrocytes are competent for cell growth and quantifiable matrix production, and biodegradable polymers, especially PGA, hold promise as suitable substrates for scaffolding materials for human cartilage tissue engineering.

Keywords: human articular chondrocytes, chondrogenesis, biodegradable polymer

Park, J.U., Tsuchiya, T.: **Increase in gap-junctional intercellular communications (GJIC) of normal human dermal fibroblasts (NHDF) on surfaces coated with high-molecular-weight hyaluronic acid (HMW HA)**

J. Biomed. Mater. Res., **60**, 541-547 (2002)

Normal human dermal fibroblast (NHDF) cells were used to detect differences in gap-junctional intercellular communication (GJIC) by hyaluronic acid (HA), a linear polymer built from repeating disaccharide units that consist of N-acetyl-D-glucosamine (GlcNa) and D-glucuronic acid (GlcA) linked by a b1-4 glycosidic bond. The NHDF cells were cultured with different molecular weights (MW) of HA for 4 days. The rates of cell attachment in dishes coated with high-molecular-weight (HMW; 310 kDa or 800 kDa) HA at 2 mg/dish were significantly reduced at an early time point compared with low-molecular-weight (LMW; 4.8 kDa or 48 kDa) HA with the same coating amounts. HA-coated surfaces were observed by atomic force microscopy (AFM) under air and showed that HA molecules ran parallel in the dish coated with LMW HA and had an aggregated island structure in the dish coated with HMW HA surfaces. The cell functions of GJIC were assayed by a scrape-loading dye transfer (SLDT) method using a dye solution of Lucifer yellow. Promotion of the dye transfer was clearly obtained in the cell monolayer grown on the surface coated with HMW HA. These results suggest that HMW HA promotes the function of GJIC in NHDF cells. In contrast, when HMW HA was added to the monolayer of NHDF cells, the functions of GJIC clearly were lowered in comparison with the cells grown in the control dish or with those grown on the surface of HMW HA. Therefore it is concluded that the MW size of HA and its application method are important factors for generating biocompatible tissue-engineered products because of the manner in which the GJIC participates in cell differentiation and cell growth rate.

Keywords: normal human dermal fibroblasts, gap-junctional intercellular communication, hyaluronic acid

Park, J.U., Tsuchiya, T.: **Increase in gap junctional intercellular communication by high molecular weight hyaluronic acid associated with fibroblast growth factor 2 and keratinocyte growth factor production in normal human dermal fibroblasts**

Tissue Engineering, **8**, 419-427 (2002)

The effects of the different molecular weights of hyaluronic acid (HA), a major component of extracellular matrix, on gap junctional intercellular communication (GJIC) in normal human dermal fibroblasts (NHDF cells) were investigated. NHDF cells were cultured for 4 days with different molecular weights of HA and then the extents of GJIC was assessed by the scrape-loading dye transfer method, using Lucifer yellow. The area of dye transfer was greater in the dishes coated with HA than in those to which HA was added. Thus, NHDF cells cultured on surfaces coated with high molecular weight (HMW) HA (MW, 800 kDa) showed greatly enhanced GJIC. Furthermore, another aim of this study was to evaluate the effects of different molecular weights of HA on the production of FGF-2 and KGF, because both are important cytokines produced by NHDF cells. When FGF-2 and KGF protein levels of cell extracts and media were determined by ELISA, both levels were significantly enhanced when cells were grown on plates coated with HMW HA. This finding indicated that the function of gap junction channels in NHDF cells grown on plates coated with HMW HA may promote the biosynthesis of growth factors such as FGF-2 and KGF.

Keywords: bFGF, KGF, normal human dermal fibroloblast

Rahman, M.S., Tsuchiya, T.: **Effects of biomaterials and nutrient factors on chondrogenesis of human chondrocytes**

Animal Cell Technology: Basic & Applied Aspects, **12**, 235-239 (2002)

The biocompatibility of poly-L-lactic acid (PLLA) and copolymer of poly (DL-lactic-co-glycolic acid) 50:50 (PLGA), and the effects of basic fibroblast growth factor (bFGF) and ferrous sulfate ($FeSO_4$) on human articular chondrocytes (HAC) proliferation and differentiation were investigated in a 4 weeks micromass culture system. Aliquots of 20 μl (per well) containing 4 x 10^5 cells were spotted onto 24-well tissue culture plates and supplemented with the media containing dimethyl sulphoxide (as a vehicle), PLLA, PLGA, bFGF, or $FeSO_4$, respectively. Cells cultured with medium only was used as a control. Alamar blue and alcian blue staining were done to determine the chondrocyte proliferation close to control level. The cultures exposed to PLLA, PLGA, bFGF and $FeSO_4$ increased HAC differentiations up to the levels of 1.2-, 1.6-, 1.3- and 2-fold of the control, respectively. These results suggested that both the PLLA and PLGA are suitable as scaffold for tissue-engineered cartilage, and bFGF and $FeSO_4$ also possessed the stimulatory activities on HAC chondrogenesis

Keywords: human articular chondrocytes, chondrogenesis, nutrient factors

Miura, T.*[1], Katakura, Y.*[1], Yamamoto, K.*[1], Uehara,

N.*1, Tsuchiya, T., Kim, E.H.*2, Shirahata, S.*1: **Neural stem cells lose telomerase activity upon differentiating into astrocytes**

Cytotechnology, **36**, 137-144 (2001)

Serum-free mouse embryo (SFME) cells were established by D. Barnes et al., and are known to be a neural stem cell line, which differentiate into astrocytes upon treatment with TGF-β. Therefore, SFME cells are thought to be a model well suited to analyze the differentiation mechanism of neural stem cells. Until now, we have investigated the regulation mechanisms of telomerase activity and telomere length in human cancer and normal cells. Telomerase is the enzyme responsible for the synthesis and maintenance of telomere repeats located at chromosomal ends and is normally expressed in embryonic and germline cells, but not in most normal cells. Here, using SFME cells, we attempted to analyze the regulation mechanism of telomerase activity in neural stem cells and to detect a change upon differentiation into astrocytes. When SFME cells were cultured in the presence of TGF-β, cells showed an elongated morphology and decreased its growth to 50% of control culture. Cells also expressed the glial fibrillary acidic protein (GFAP), a marker for astrocytes, indicating that TGF-β induced differentiation in SFME cells from neural stem cells into astrocytes. At the same time, TGF-β also inhibited telomerase activity and repressed the expression of the mouse telomerase reverse transcriptase (*mTERT*), demonstrating that SFME cells was vested with a finite replicative life span upon treatment with TGF-β. To understand the mechanisms regulating *mTERT* levels during differentiation into astrocytes, we have estimated the expression level of *c-myc*, which is known to be a key molecule in activating the *TERT* promoter. As a result, TGF-β-treated SFME cells were shown to repress the expression of *c-myc*. Furthermore, promoter analysis, using the 5'-region of the *mTERT* gene, which possess two E-box elements bound to c-Myc/Max, demonstrated that *mTERT* promoter activity greatly decreased in TGF-β-treated SFME cells as compared to non-treated SFME cells. These suggest that *c-myc* might play a critical role in the expression of *mTERT*, and that down-regulation of *c-myc* dependent upon the astrocytic differentiation in SFME cells might cause the repression of *mTERT* in TGF-β-treated SFME cells.

Keywords: astrocytes, neural stem cells, telomerase

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Hayashi, Y., Matsuda, R., Haishima, Y., Yagami, T., Nakamura, A.: **Validation of HPLC and GC-MS systems for bisphenol-A leached from hemodialyzers on the basis of FUMI theory**

J. Pharm. Biomed. Anal., **28**, 421-429 (2002)

ポリカーボネートおよびポリスルホン製血液透析器からのビスフェノールA溶出量をGC-MSおよびHPLC分析により評価し，両測定系における分析精度をFUMI理論により解析した．その結果，同理論を使用して予測した両分析系の精度は実測値と良く一致し，分析法バリデーションの概念に基づく繰り返し測定を行わなくとも，精度管理が可能であることが明らかになった．

Keywords: bisphenol-A, FUMI theory, validation

Kitagawa, K.*1, Aida, C.*1, Fujiwara, H.*1, Yagami, T., Futaki, S.*2: **Facile solid-phase synthesis of sulfated tyrosine-containing peptides: Part II. Total synthesis of human big gastrin-II and its C-terminal glycine-extended peptide (G34-Gly sulfate) by the solid-phase segment condensation approach**

Chem. Pharm. Bull., **49**, 958-963 (2001)

Fmoc型固相合成を基盤とするセグメント縮合法を，硫酸化チロシン残基を含む大分子型ペプチドに適用できるかどうか，ヒトビッグガストリンIIの合成を通して検討した．硫酸化チロシン残基を有するC-末端フラグメントは，穏和な酸条件で切断できる2-クロロトリチル樹脂を用いて合成した．そして，各保護セグメントペプチドを，樹脂上のC-末端フラグメントにPyBOP法で順次導入した．最終的に得られた保護ビッグガストリンIIを硫酸化チロシン残基の分解が最小限に抑制される酸条件で処理し，脱保護と樹脂から遊離を同時に行い，14％の全収率で高純度の目的物を得た．この結果は，セグメント縮合法が大分子型硫酸化ペプチドの合成にも有効であることを示すものであった．

Keywords: peptide synthesis, tyrosine O-sulfate, segment condensation

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Futaki, S.*1, Fukuda, M.*2, Omote, M.*1, Yamauchi, K.*1, Yagami, T., Niwa, M.*2, Sugiura, Y.*1: **Alamethicin-leucine zipper hybrid peptide: A prototype for the design of artificial receptors and ion channels**

J. Am. Chem. Soc., **123**, 12127-12134 (2001)

チャネル形成能を持つペプチドの膜内会合状態とそれ

に伴うチャネル電流の変動を，膜外ドメインにより調節するという概念について検討した．イオンチャネル形成性ペプチドであるアラメシチンは，膜と相互作用しつつ幾つかの会合状態をとる．安定なホモダイマーを形成することで知られるロイシンジッパーペプチドを，各アラメシチン単量体に膜外ドメインとして結合させた結果，チャネルが単一の開口状態で安定化するようになった．このハイブリットペプチド会合体は，テンプレートに固定化したアラメシチン-ロイシンジッパー四量体のチャネル電流との比較から，四量体であると推察された．ランダムな立体構造をとるペプチドを膜外ドメインとして結合させた場合は，より多くの電流を通す開口状態が安定化した．一連の結果は，膜外ドメインの立体構造を転換させることにより，チャネル電流を調節できる可能性があることを示すものであった．

Keywords: peptide synthesis, artificial protein, ion channel

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Yagami, T., Ballard, B.T.*1, Padovan, J.C.*2, Chait, B.T.*2, Popowicz, A.M.*2, Manning, J.M.*1: **N-terminal contributions of the γ-subunit of fetal hemoglobin to its tetramer strength: Remote effects at subunit contacts**
Protein Sci., **11**, 27-35 (2002)

胎児型ヘモグロビン四量体（$\alpha 2\gamma 2$）の二量体（$\alpha\gamma$）への解離定数は，成人型ヘモグロビン（$\alpha 2\beta 2$）の70分の1ほどでしかない．この差異は，γ鎖とβ鎖のN-末端領域の違いによりもたらされることが既に明らかにされている．著者らは，具体的にγ鎖のどのアミノ酸残基が安定化に寄与しているのかを調べるため，対応する成人型ヘモグロビンのβ鎖遺伝子に様々な部位特異的変異を導入し，得られた変異体の解離定数を測定した．その結果，β鎖1位のVal，5位のProおよび7位のGlu残基を，相当するγ鎖のGly，GluおよびAspにそれぞれ置換すると，成人型ヘモグロビンの解離定数が胎児型に近くなることがわかった．この結果は，各サブユニットが実際に接触する部位ではなく，遠く離れたN-末端のアミノ酸残基により相互作用の強さが調節されているという考えを実証するものであった．

Keywords: hemoglobin, long-range interaction, site-directed mutagenesis

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入江和夫*1，前田典子*1，吉田啓子*1，河野泰子*2，鹿庭正昭：**ペンキ中の鉛から子供を守るための実験教材と授業実践**
山口大学教育学部研究論叢，**51**（第3部），161-174 (2001)

日本の家庭科教科書において，ペンキ中の鉛による中毒から子どもを守ることを目的として，鉛中毒について記載されたものはほとんどなかった．そこで，ペンキ中の鉛の簡易確認法を開発して，実際に授業において実験教材として実践した結果，実際にペンキ中の鉛を簡便に確認できることが証明できた．身の回りのペンキ中の鉛の実態を的確に把握することによって，鉛フリーのペンキでの塗装に切り替えたり，手洗いなどを励行させることによって，塗装された金属製遊具などで遊んだ際に子どもが受ける鉛への曝露量，翻って子どもの健康リスクを低減化させることへの関心が高められることが確認できた．

Keywords: lead, paint, experimental teaching material

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山崎典子*，中田良子*，上出良一*，新村眞人*，鹿庭正昭：**眼鏡フレームの先セルによる接触皮膚炎の1例**
日本皮膚アレルギー学会雑誌，**10**，8-12 (2002)

眼鏡フレームのプラスチック製の先セル部分による両耳介後部に発生した接触皮膚炎事例についてパッチテスト，化学分析を併用して検討した結果，先セルに配合されていた3種の着色剤に共通して配合されていたペリノン系油溶性染料 Solvent Orange 60が原因化学物質となっていたことを明らかにした．

Keywords: spectacle frame, contact dermatis, Solvent Orange 60

*東京慈恵医科大学皮膚科

Mi, H.*, Hiramoto, K.*, Kujirai, K.*, Ando, K.*, Ikarashi, Y., Kikugawa, K.*: **Effect of food reductones, 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) and hydroxy hydroquinone (HHQ), on lipid peroxidation and type IV and I allergy responses of mouse**
J. Agric. Food Chem., **49**, 4950-4995 (2001)

The effect of long-term supplementation of food reductones, DMHF (2%), detected in many foodstuffs including soy sauce, and HHQ (1.2%), detected in coffee, on mouse lipid peroxidation and allergy responses was investigated. Levels of thiobarbituric acid-reactive substances in lung were remarkably increased, and those in kidney and liver were slightly decreased by supplementation of DMHF or HHQ. The 2,4-dinitrochlorobenzene-induced lymph node cell proliferation was

remarkably enhanced by supplementation of DMHF or HHQ. Allergen-induced increases in IgE levels of mice were enhanced to greater extent by DMHF or HHQ. No additive effect of nitrogen oxide inhalation was observed. Both type IV and I allergy responses of mice may be enhanced by supplementation of food reductones, DMHF or HHQ.

Keywords: food reductone, nitrogen oxide, allergy

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Mi, H.*, Hiramoto, K.*, Kujirai, K.*, Ando, K.*, Ikarashi, Y., Kikugawa, K.*: **Effects of vitamin E-deficiency and/or nitrogen dioxide inhalation on allergen-sensitized type IV and type I allergy responses of mice**

J. Health Sci., **48**, 22-29 (2002)

Mice were fed a vitamin E-adequate diet (C group) or vitamin E-deficient diet (-E group), and exposed to air or NO_2 (5-6 ppm) (C+NO_2 and -E+NO_2 groups) for 1-2 weeks. The degree of lymph node cell proliferation induced by 2,4-dinitrochlorobenzene of mice of the C+NO_2 group was similar to that of mice of the C groups. While the proliferation of the -E group was lower than that of the C group, the response of the -E+NO_2 group was higher than that of C and C+NO_2 groups. While the trimellitic anhyderide-sensitized IgE levels of mice of the -E group were similar to those of mice of the C group, the level of the C+NO_2 and -E+NO_2 groups were much higher. These suggest that allergen-sensitized type IV and I allergy responses of mice are enhanced by oxidative stress induced by vitamin E-deficiency and/or NO_2 inhalation.

Keywords: vitamin E-deficiency, nitrogen oxide, allergy

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Matsuoka, A., Tada, A.*1, Terao, Y.*2, Nukaya, H.*2, Onefelt, A.*3, Wakabayashi, K.*1: **Chromosomal effects of newly identified water pollutants PBTA-1 and PBTA-2 and their possible mother compounds (AZO DYEs) and intermediates (non-ClPBTAs) in two Chinese hamster cell lines**

Mutat. Res., **493**, 75-85 (2001)

We performed the in vitro micronucleus (MN) test on PBTA-1 and PBTA-2, which are newly identified water pollutants from the Nishitakase river in Kyoto, Japan, and on their possible mother compounds and intermediates, in two Chinese hamster cell lines CHL and V79-MZ. PBTA-2 induced the strongest responses among chemicals tested. It was also a strong inducer of binucleate cells, which suggested that it induced polyploidy. PBTA-1 showed clear positive results only in V79-MZ cells, inducing aneuploidy. In CHL cells AZO DYE-1 significantly induced MN cells in the presence of S9 mix, and AZO DYE-2 induced MN and polynuclear cells. In V79-MZ cells, AZO DYE-1 and -2 induced primarily mitotic cells in the presence of S9 mix. The non-ClPBTAs were a bit more cytotoxic than the other compounds and induced a slight increase in MN cells in both cell lines. Structure-activity relationships have been discussed.

Keywords: AZO DYE, benzotriazole derivatives, in vitro micronucleus test

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Matsuoka, A., Furuta, A.*1, Ozaki, M.*1, Fukuhara, K., Miyata, N.: **Resveratrol, a naturally occurring polyphenol, induces sister chromatid exchanges in a Chinese hamster lung (CHL) cell line**

Mutat. Res., **494**, 107-113 (2001)

We tested the genotoxicity of resveratrol, a polyphenolic phytoalexin found in grapes. Resveratrol was negative in the bacterial reverse mutation assay. It induced structural chromosome aberrations and showed weak aneuploidy induction in CHL cells. It induced micronucleated cells after 48 h treatment. In the sister chromatid exchange (SCE) test, resveratrol caused a clear cell-cycle delay. Resveratrol induced SCEs dose-dependently at up to 10 μg/ml and the number was almost as large as mitomycin C, a strong SCE inducer. Cell cycle analysis by FACScan indicated that resveratrol caused S phase arrest, and 48 h treatment induced apoptosis. Our results suggest that resveratrol may preferentially induce SCE but not CA, that is, it may cause S phase arrest only when SCEs are induced.

Keywords: resveratrol, polyphenol, sister chromatid exchanges

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Isama, K., Tsuchiya, T.: **Effect of γ-ray irradiated poly(L-lactide) on the differentiation of mouse osteoblast-like MC3T3-E1 cells**

J. Biomater. Sci. Polymer Edn., **13**, 153-166 (2002)

マウス骨芽細胞様MC3T3-E1細胞の増殖及び分化に及ぼす，ガンマ線照射したポリL-乳酸（PLLA）の影響

を検討するために，微小集積培養を用いた評価法を確立した．ガンマ線照射によって，PLLAの分子量は低下した．また，ガンマ線照射したPLLAの上で培養したMC3T3-E1細胞は，増殖には影響がなく，分化が促進した．ガンマ線照射によるPLLAの分子量の低下が，MC3T3-E1細胞の分化促進をもたらすことが示唆された．さらに，低分子量PLLAも，MC3T3-E1細胞の増殖には影響せず，分化を促進させた．

Keywords: poly(L-lactide), γ-ray irradiation, osteoblastic MC3T3-E1 cells

Nakaoka, R., Tsuchiya, T., Sakaguchi, K., Nakamura, A.: **Studies on in vitro evaluation for the biocompatibility of various biomaterials: Inhibitory activity of various kinds of polymer microspheres on metabolic cooperation**

J. Biomed. Mater. Res., **57**, 279-284 (2001)

Gap junctional intercellular communication is a function that plays an important role in maintaining cell and tissue homeostasis and in regulating cell growth, development and differentiation. Change in this function when contacting fibroblasts with various polymer microspheres was estimated using the metabolic cooperation assay system. When the cells were in contact with the microspheres after their adhesion onto a substrate, the function did not alter. However, when they were in contact with pre-coated microspheres on test dishes, the function was inhibited as the quantity of microspheres increased. Moreover, the inhibition level increased as the diameters of polyethylene and polystyrene microspheres decreased. However, no inhibition was observed if pre-coated microspheres were composed from poly(L-lactic acid). These findings suggest that the size and the material of microspheres, and how cells recognize the microspheres are factors affecting cell function of gap junctional intercellular communication. Therefore, estimating this function may provide valuable information about the biocompatibility of many kinds of materials even in the form of particles.

Keywords: gap junctional intercellular communication, polymer microspheres, metabolic cooperation assay

Nakaoka, R., Tsuchiya, T., Nakamura, A.: **The inhibitory mechanisms of gap junctional intercellular communication induced by polyethylene and the restorative effects by surface modification with various proteins**

J. Biomed. Mater. Res., **57**, 567-574 (2001)

Gap junctional intercellular communication (GJIC) is a function that plays an important role in maintaining cell and tissue homeostasis and in regulating cell growth, development and differentiation. Change in this function of V79 fibroblasts cultured on polyethylene films modified with albumin or collagen was estimated using fluorescence re-distribution after photobleaching (FRAP) analysis. The GJIC function of V79 cells on non-treated polyethylene was strongly inhibited in comparison with those on a glass coverslip. When the cells were culture on collagen-immobilized polyethylene film, this function was recovered to about 70% of the cells cultured on the coverslip. However, albumin immobilization did not recover the function as much as collagen immobilization. Western blotting analysis and immunostaining of connexin 43, which is a major protein constituting gap junctional channel of these cells, revealed its abnormal expression and distribution in the cells on non-treated PE, whereas its almost normal distribution was observed in the cells on collagen-immobilized polyethylene. This abnormal expression and distribution of connexin 43 induced by the surface of polyethylene may be ascribed to a strong inhibition of GJIC of V79 fibroblasts.

Keywords: gap junctional intercellular communication, surface modification, connexin 43

Nakaoka, R., Tsuchiya, T.: **Studies on the biocompatibility of biomaterials: Effect of various types of biomaterial particles**

Proc. Fourth Pacific Rim Int. Conference on Advanced Materials and Processing (PRICM4), The Japan Institute of Metals, 189-191 (2001)

Gap junctional intercellular communication (GJIC) is a function that plays an important role in maintaining cell and tissue homeostasis and in regulating cell growth and differentiation. In this study, change in this GJIC function of cells contacting with various microspheres prepared from biomaterials was estimated using different methods. It was observed that microspheres pre-coated before test cell adhesion inhibited the function more than those added after cell adhesion. In addition, the inhibition levels were affected by amounts, diameter, composition and time period of interaction of microspheres as well as cell type used in the study. These findings suggest that not only the way for cells to recognize the microspheres but also characteristics of the microspheres are important factors affecting the GJIC function. Interaction of microspheres with osteoblasts resulted in inhibition of

their differentiation, indicating microspheres are probable to disturb both cell differentiation and the GJIC function of the cells. These findings suggest the possibility that GJIC function can be used as an index that gives information of biocompatibility of many kinds of materials even in the forms of particles.
Keywords: gap junctional intercellular communication, biomaterial microspheres, cell function

Shintani, H., Hayashi, F.*: **HPLC validation of ameziniummetilsulfate in human plasma of uremia patient treated with dialysis**
Chromatographia, **53**, 574-578 (2001)

A high performance liquid chromatographic method using internal standard is validated for the determination of 4-amino-6-methoxy-1-phenyl-pyridazinium methyl sulfate (ameziniummetilsalfate, AM) in uremia patient treated with artificial dialysis. The method involves liquid-liquid extraction and ion suppression reverse phase high performance liquid chromatography (HPLC) combined with endocapped C-18 column. There is no interference by plasma components or AM metabolites in HPLC analysis. Average recovery rate using liquid-liquid extraction was 88.7% and limit of determination was 2 ng mL^{-1}. This concentration was sufficient to determine AM in human plasma. Accuracy and precision was within 15% at 2 ng mL^{-1} of limit of determination.
Keywords: column liquid chromatography, ameziniummetilsulfate, uremia
* Health care facility of Namiki hospital

Oishi, S., Nakagawa, J., Ando M. : **Effects of Ingestion of Cadmium-Polluted Rice or Low-Dose Cadmium-Supplemented Diet on the Endogenous Metal Balance in Female Rats**
Biological Trace Element Research, **84**, 155-167 (2001)

The concentrations of endogenous metal ions in liver, kidney, and bone tissues of female rats were measured after ingestion of cadmium polluted rice (1.24ppm as Cd) or cadmium-supplemented rice (1.24 and 4.96ppm) for 2 or 4 mo. The metal accumulated mainly in the kidneys of rats feda 1.24-ppm Cd-supplemented diet was significantly higher than in the Cd-polluted rice group.

After 2 months, the levels of iron and sodium in the liver were elevated in the Cd-polluted rice group, but not in the 1.24-ppm Cd-supplemented group, as compared to controls. The zinc concentration in the Cd-polluted rice group was decreased. The concentration of copper in the kidneys was increased for all Cd-containing diet groups. After 4 months, the effects of Cd on essential metals in the Cd-polluted and 1.24-ppm Cd-supplemented groups had almost disappeared, although several metal ions in selected organs in the 4.96-ppm Cd-supplemented group showed more prominent changes than in the group exposed for 2 months.
Keywords: Cadmium-polluted rice, female rats

Hanioka, N., Jinno, H., Nishimura, T., Ando, M., Ozawa, S., Sawada, J.: **High-performance liquid chromatographic assay for glucuronidation activity of 7-ethyl-10-hydroxycamptothecin (SN-38), the active metabolite of irinotecan (CPT-11), in human liver microsomes**
Biomed. Chromatogr., **15**, 328-333 (2001)

A simple and sensitive assay for glucuronidation activity of 7-ethyl-10-hydroxycamptothecin (SN-38), the active metabolite of irinotecan (CPT-11), in human liver microsomes by high-performance liquid chromatography (HPLC) with fluorescence detection is reported. The method was validated for the determination of SN-38 glucuronide (SN-38G) with respect to specificity, linearity, recovery, stability, precision, accuracy, and limits of detection and quantitation. The limit of quantitation for SN-38G was 5 nM (2.5 pmol/assay). The intra-and inter-day precision and accuracy were less than 7 and 4%, respectively. With this improved sensitivity, the kinetics of SN-38 glucuronidation in human liver microsomes could be determined more precisely. Therefore, this method is applicable to *in vitro* study on the side effects and drug interactlons of CPT-11 using small amounts of biological sample.
Keywords: Irinotecan, SN-38, Glucuronidation

Hanioka, N., Ozawa, S., Jinno, H., Ando, M., Saito, Y., Sawada, J.: **Human liver UDP-glucuronosyltransferase isoforms involved in the glucuronidation of 7-ethyl-10-hydroxycamptothecin**
Xenobiotica, **31**, 687-699 (2001)

The human liver UDP-glucuronosyltransferase (UGT) isoforms involved in the glucuronidation of 7-ethyl-10-hydroxycamptothecin (SN-38), the active metabolite of irinotecan (CPT-11), have been studied using microsomes from human liver and insect cells expressing human UGTs. The glucuronidation of SN-38 was catalyzed by UGT1A1, UGT1A3, UGT1A6 and UGT1A9 as well as by liver microsomes. The ranking in order of

activity at low and high substrate concentrations was UGT1A1 > UGT1A9 > UGT1A6 > UGT1A3 and UGT1A1 > UGT1A3 > UGT1A6 > or = UGT1A9, respectively. The UGT isoforms involved in SN-38 glucuronidation could be classified into two types: low-K_m types such as UGT1A1 and UGT1A9, and high-K_m types such as UGT1A3 and UGT1A6, in terms of affinity toward substrate. These results demonstrate that at least four UGT1A isoforms are responsible for SN-38 glucuronidation in human livers, and suggest that the role and contribution of each differ substantially.

Keywords: Irinotecan, SN-38, UDP-glucuronosyltransferase

Hanioka, N., Watanabe, K., Yoda, R., Ando, M.: **Effect of alachlor on hepatic cytochrome P450 enzymes in rats**
Drug Chem. Toxicol., **25**, 25-37 (2002)

We examined the effects of alachlor on cytochrome P450 enzymes in rat liver microsomes. Among the cytochrome P450-dependent monooxygenase activities, 7-pentoxyresorufin O-depentylase, which is associated with CYP2B1, was dose-dependently increased by alachlor. The induction relative to control activity was 1.7-4.2-fold. The activities of CYP1A-dependent monooxygenases such as 7-ethoxyresorufin O-deethylase and acetanilide 4-hydroxylase were also significantly increased by alachlor at doses of 50 and 100 mg/kg (1.7-2.1-fold). Furthermore, immunoblotting showed that alachlor significantly increased CYP2B1/2 and CYP1A1/2 protein levels by 4.2-6.3-and 1.8-fold, respectively. However, there was no significant change in the protein levels of CYP2C11/6, CYP2D1, CYP2E1, CYP3A2/1 and CYP4A1/2/3. These results suggest that alachlor selectively induces cytochrome P450 isoforms of the CYP1A and CYP2B subfamilies in rat liver microsomes, and that the expression of these isoforms is closely related to the toxicity of alachlor.

Keywords: Alachlor, Cytochrome P450, Rat liver microsomes

Hanioka, N., Ozawa, S., Jinno, H., Tanaka-Kagawa, T., Nishimura, T., Ando, M., Sawada, J.: **Interaction of irinotecan (CPT-11) and its active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) with human cytochrome P450 enzymes**
Drug Metab. Dispos., **30**, 391-396 (2002)

The inhibition and mechanism-based inactivation potencies of irinotecan (CPT-11) and its active metabolite (SN-38) for human cytochrome P450 (P450) enzymes were investigated to evaluate the potential for drug interactions involving CPT-11 using microsomes from insect cells expressing specific human P450 isoforms. CPT-11 and SN-38 competitively inhibited CYP3A4 activity with K_i values of 129 and 121 μM, respectively. CYP2A6 and CYP2C9 activities exhibited a mixed type of inhibition comprising competitive and noncompetitive components in response to SN-38, the K_i values being 181 and 156 μM, respectively. However, no inactivation of CYP2A6 and CYP2C9 by SN-38 was observed. These results mean that CPT-11 and SN-38 interact with human P450 isoforms, such as CYP2A6, CYP2C9, and CYP3A4, in vitro and imply that the significant drug interactions involving CPT-11 may be caused by a mechanism-based inactivation of CYP3A4 by SN-38 as an active metabolite of CPT-11 rather than competitive inhibition.

Keywords: Irinotecan, SN-38, Cytochrome P450

徳永裕司, 鄭 然孫, 内野 正, 安藤正典：**フタル酸ジエチルの*In Vitro*経皮吸収に関する研究**
日本化粧品技術者会誌, **35**, 312-316(2001)

内分泌攪乱化学物質の一種として化粧品に含まれると想定されるフタル酸ジエチル（DEP）の経皮吸収的な試験をモルモットの腹部の剥離皮膚を用いて検討した．Franz型拡散セルに装着した後，10 mMドデシル硫酸ナトリウム（SDS），10 mM塩化ベンザルコニウム（BK）あるいは0.5%ポリオキシエチレン（10）オレイルエーテル（POE.OE）溶液をdonor側に加え，32℃で2時間放置した．donor側の界面活性剤を除き，0.05％DEP溶液をdonor側に加えた．32℃で16～24時間でのDEPのreceptor側への透過量がHPLCで測定された．HPLC Unisil Q C_{18}（4.6 mm×150 mm）カラム，移動相として，水／アセトニトリル混液（1：1）及び255 nmの分光光度計を用いた．皮膚をSDS，BKあるいはPOE.OEで処理したとき，DEPの透過速度は，controlに対して190，174あるいは110％と増加し，使用された界面活性剤が皮膚角質層に影響を与えることが示唆された．また，フタル酸ジエチル溶液中にSDS，BKあるいはPOE.OEを共存させた場合，DEPのFluxは，controlに対して，それぞれ，82.0，105.7及び99.5％を示し，SDSが共存した場合，DEPの透過量が低下することが示唆された．24時間でのDEPの累積透過量は21.8 g/cm^{2}であった．

Keywords: diethyl phthalate, skin permeation, Franz type diffusion cell

内野 正，徳永裕司，安藤正典：**化粧品に配合が禁止**

されている成分の分析法に関する研究：ビタミンL1および塩酸ピロカルピンについて

粧技誌, **35**, 305-311 (2001)

ビタミンL1および塩酸ピロカルピンは，昭和61年の厚生省通知（昭和61年3月12日薬審2第100号）により化粧品への配合禁止成分となっている．我々は配合禁止成分の有無を効率よく確認し，化粧品の安全性を確保するためにビタミンL1および塩酸ピロカルピンの高速液体クロマトグラフィーによる定量法を確立し，化粧品への応用について検討した．化粧水及び乳液中のビタミンL1及び塩酸ピロカルピンをメタノールで抽出し，ODSカラム（Shiseido CAPCELL PACKC_{18}, 4.6×250 mm），移動相として50 mMリン酸塩緩衝液（pH 5）／アセトニトリル混液（37:3）又は（19:1），検出器として紫外吸光光度計（測定波長244 nm又は214 nm）を用い，高速液体クロマトグラフィーで分析した．この方法を用いることにより，化粧水及び乳液中のビタミンL1又は塩酸ピロカルピンを原料の影響もなく定量することが出来ることを明らかにした．

Keywords: vitamine L1, pilocarpine hydrochloride, HPLC

Uchino T., Tokunaga H., Onodera H. and Ando M.: **Effect of squalene monohydroperoxide on cytotoxicity and cytokine release in a three-dimensional human skin model and human epidermal keratinocytes**

Biol. Pharm. Bull., **25**, 605-610 (2002)

In order to clarify that squalene monohydroperoxide (SQOOH) correlates with changes in morphology through cytotoxicity and establish in vitro evaluation of the cytotoxicity of lipid hydroperoxide, the effect of SQOOH on cytotoxicity and morphology in normal human epidermal keratinocytes and the Gunze three-dimensional cultured human skin model was investigated. A concentration-dependent and protective effect on the increase in cytotoxicity and PCOOH content was observed. IL-2 release from the cells was enhanced by SQOOH and increased at a non-cytotoxic dose.

These results suggest that the increase in lipid hydroperoxides resulting from the auto-oxidation of lipids within cellular membranes in the presence of SQOOH correlates with changes in morphology due to cytotoxicity. SQOOH enhanced the IL-2 release from the cells at a non-cytotoxic dose. A method for assessing the protective effect on the cytotoxicity of lipid hydroperoxides using cells would be useful for in vitro evaluation of the cytotoxicity.

Key words: squalene monohydroperoxide, cytotoxicity, IL-2

Toyo'oka, T.*, Tanabe, J.*, Jinno, H.: **Determination of rat hepatocellular glutathione by reversed-phase liquid chromatography with fluorescence detection and cytotoxicity evaluation of environmental pollutants based on the concentration change.**

Biomed. Chromatogr. **15**, 240-247 (2001)

Three methods for the determination of rat hepatocellular thiols by high-performance liquid chromatography (HPLC) with fluorescence detection have been developed. The thiols in the cells were tagged with three fluorogenic reagents, SBD-F, ABD-F and DBD-F. These reagents could permeate into cells and effectively reacted with thiols to produce highly fluorescent derivatives. The five biological thiols tagged were perfectly separated by reversed-phase liquid chromatography and were sensitively and selectively detected without any interference from endogenous substances. The proposed procedures were applied to the determination of hepatocellular GSH after treatment of environmental pollutants such as volatile organic compounds and endocrine disrupting chemicals.

Keywords: Rat hepatocellular glutathione, HPLC with fluorescence detection, Environmental pollutants

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佐々木久美子，辰濃　隆*1，中村宗知*2，金子正堅*3，後藤修宏*4，近藤安昭*1，高畑　薫*5，三浦嘉巳*6，豊田正武：**食品衛生法告示酸化フェンブタスズ及びシヘキサチン試験法の評価**

食品衛生学雑誌，**42**, 210-214(2001)

殺ダニ剤酸化フェンブタスズ（FBTO）及びシヘキサチン（CHT）の告示試験法評価のために6分析機関で共同実験を行った．玄米等6作物からのFBTO回収率の平均値は85.2～96.5％，CHTのそれは大豆を除いて83.5～89.2％であった．FBTO回収率の併行再現性及び室間再現性の相対標準偏差はそれぞれ2.3～9.4％，3.9～12.6％，CHTのそれらは3.2～6.3％，8.3～12.9％であった．検出限界は0.015～0.05 μg/g（FBTO），0.005～0.02 μg/g（CHT）であった．

Keywords: fenbutatin oxide, cyhexatin, method-performance study

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食品衛生学雑誌，**42**, 269-272(2001)

植物成長調整剤イナベンフィドの告示試験法評価のために共同実験を行った．6分析機関でイナベンフィドを添加した玄米を分析したときの平均回収率は85.0％，併行再現性及び室間再現性の相対標準偏差はそれぞれ4.2％，8.1％と良好な結果が得られた．検出限界は0.002～0.01 μg/gであった．

Keywords: inabenfide, method-performance study

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食品衛生学雑誌，**42**, 273-277(2001)

殺虫殺菌剤キノメチオネートの告示試験法評価のために共同実験を行った．6分析機関でキノメチオネートを添加した玄米，キャベツ等5作物を分析したときの各作物における平均回収率は90.2～100.5％，併行再現性及び室間再現性の相対標準偏差はそれぞれ4.4～7.7％，10.9～17.1％であった．検出限界は0.003～0.012 μg/gであった．

Keywords: chinomethionat, method-performance study

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食品衛生学雑誌，**42**, 278-282(2001)

殺ダニ剤クロフェンテジンの告示試験法評価のために共同実験を行った．6分析機関でクロフェンテジンを添加した小豆，りんご等7作物を分析したときの回収率の平均値は78.4～85.2％，併行再現性及び室間再現性の相対標準偏差はそれぞれ2.2～4.6％，4.8～10.3％と良好な結果が得られた．検出限界は0.005～0.01 μg/gであった．

Keywords: clofentezine, method-performance study

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食品衛生研究，**52(4)**, 7-26(2002)

食品衛生法に基づく残留農薬の検査に使用する標準品は，法律で純度が定められている．検査機関は各メーカーの提供する市販の標準品を購入して検査に使用している．しかし，市販の標準品には定められた純度基準を逸脱しているものがあり，また，純度は各メーカー独自の方法でそれぞれ算出されており，品質比較が出来ない．そこで3メーカーから市販されている標準品29種についてHPLC及びGCを用いた同一の方法で品質評価を行い，その結果について報告した．

Keywords: pesticide, standard compounds

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根本 了，小村麻子，高附 巧，佐々木久美子，豊田正武：**食品中の2,4,6-トリ-*tert*-ブチルフェノール及び関連化合物の分析**

食品衛生学雑誌，**42**, 359-366(2001)

第一種特定化学物質である2,4,6-tri-*tert*-butylphenol（TTBP）について，食品中から水蒸気蒸留で抽出しGC/MS（SIM）で測定する分析法を開発した．開発した方法は，di-*tert*-butylphenol（DTBP）の3種の異性体（2,4-DTBP，2,6-DTBP及び3,5-DTBP）及び2,4-di-*tert*-pentylphenol（2,4-DTPP）の同時分析が可能であった．市販の食品101検体について汚染実態調査を行った結果，TTBPは肉類，レバー及び魚介類（筋肉）からそれぞれ痕跡量(tr)～0.50 ng/g，痕跡量及びtr～1.83 ng/g検出された．2,4-DTBPは野菜・精白米，肉類，レ

バー，魚介類（筋肉）及び魚介類（内臓）からそれぞれ1.4～10.6 ng/g，2.7～26.4 ng/g，tr～34.2 ng/g，tr～21.6 ng/g及び痕跡量検出された．2,6-DTBPは魚介類（筋肉）及び魚介類（内臓）からそれぞれtr～3.9 ng/g及び痕跡量検出された．3,5-DTBP及び2,4-DTPPは分析したいずれの食品からも検出されなかった．

Keywords: 2,4,6-tri-*tert*-butylphenol, steam distillation, GC/MS

高附 巧，根本 了，佐々木久美子，豊田正武：**HPLC及びLC/MSによる果実中のエチクロゼート及び分解物の分析法**

食品衛生学雑誌，**43**, 30-34 (2002)

果実中のエチクロゼート（CIE）及び分解物の5-chloro-3(1H)-indazolylacetic acid (CIA)のHPLC及びLC/MSによる分析法を確立した．試料から塩酸酸性下，CIE及びCIAをアセトンで抽出後，エーテル－ヘキサン（2:1）で再抽出し，メタノール－4mol/L水酸化カリウム溶液（1:1）でCIEをCIAに加水分解した後，塩酸酸性としてCIAをエーテル－ヘキサン（2:1）で抽出した．これをシリカゲルカラムで精製し，HPLC-UV及びLC/MSで測定した．

4種の果実にCIE又はCIAを0.5 μg/g添加しHPLCで測定したときの平均回収率は，それぞれ77.2～83.2％，71.2～89.2％であった．LC/MSで定量した時の値は，これらより10～25％高かった．CIA標準液の検出限界は，試料中のCIEに換算して0.015 μg/g（HPLC），0.009 μg/g（LC/MS:SIR）であった．

Keywords: ethychlozate, pesticide residue, LC/MS

Hori, T.*, Nakagawa, R.*, Tobiishi, K.*, Iida, T.*, Tsutsumi, T., Sasaki, K., Toyoda, M.: **Effects of cooking on concentrations of polychlorinated dibenzo-*p*-dioxins and related compounds in green leafy vegetable 'Komatsuna'**

J. Food Hyg. Soc. Japan, **42**, 339-342 (2001)

The effects of ordinary household cooking processes on concentrations of PCDD/Fs and dioxin-like PCBs (dioxins) were investigated in 'Komatsuna', a green leafy vegetable popular in Japan. The concentrations of dioxins were compared using isomer-specific analyses of both uncooked and cooked edible parts of the plant. The mean total 2,3,7,8-chlorine substituted PCDD and PCDF concentrations were reduced from 46.53 pg/g and 0.714 pg/g to 8.301 pg/g and 0.210 pg/g by washing with tap water, and further reduced to 6.054 pg/g and 0.148 pg/g by subsequent boiling, respectively. The cooking processes markedly decreased the concentrations of PCDD/Fs, while having little effect on those of dioxin-like PCBs. The mean total TEQ concentration was reduced from 0.058 pgTEQ/g to 0.026 pgTEQ/g by washing with tap water and further reduced to 0.019 pgTEQ/g by subsequent boiling. These results suggest that ordinary cooking processes provide a means of reducing the level of dioxins in green leafy vegetables.

Keywords: dioxin(s); vegetable; cooking

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Tsutsumi, T., Yanagi, T.*[1], Nakamura, M.*[1], Kono, Y.*[1], Uchibe, H.*[1], Iida, T.*[2], Hori, T.*[2], Nakagawa, R.*[2], Tobiishi, K.*[2], Matsuda, R., Sasaki, K., Toyoda, M.: **Update of daily intake of PCDDs, PCDFs, and Dioxin-like PCBs from food in JAPAN**

Chemosphere, **45**, 1129-1137 (2001)

Total diet study samples of fourteen food groups from sixteen locations in Japan, collected in 1999 and 2000, were analyzed for PCDD/Fs and dioxin-like PCBs to estimate of update of daily intake of these contaminants from food. The mean daily intake was estimated to be 2.25 pg TEQ/kg b.w./day (3.22 pg TEQ/kg b.w./day) calculated at ND=0 (ND=1/2 LOD). In the both estimates, the mean daily intakes were highest from fish and shellfish (76.9% at ND=0 and 53.9% at ND=1/2 LOD of the total TEQs), followed by that from meat and eggs (15.5% at ND=0 and 11.7% at ND=1/2 LOD of the total TEQs). Congener specific data revealed that these total TEQ levels were dominated by 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF and 3,3', 4,4', 5-PeCB in each case (71.7% at ND=0 and 63.1% at ND=1/2LOD of the total TEQs). The dioxin-like PCBs accounted for about 50% of these total TEQs. These data will be very useful in risk assessment of PCDD/Fs and dioxin-like PCBs from food in Japan.

Keywords: dioxins, total diet study, dietary intake

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Tsutsumi, T., Iida, T.*[1], Hori, T.*[1], Nakagawa, R.*[1], Tobiishi, K.*[1], Yanagi, T.*[2], Kono, Y.*[2], Uchibe, H.*[2], Matsuda, R., Sasaki, K., Toyoda, M.: **Recent survey and effects of cooking processes on levels of PCDDs, PCDFs and Co-PCBs in leafy vegetables in JAPAN**

Chemosphere, **46**, 1443-1449 (2002)

We report here the latest levels of PCDD/Fs and Co-PCBs in leafy vegetables in Japan as well as the effect of cooking processes on the reduction of these

contaminants. Three kinds of leafy vegetables from seven districts in Japan in 1998 were analyzed for PCDD/Fs and non-ortho PCBs. The mean total TEQ levels in the "komatsuna", lettuce and spinach were 0.094, 0.025 and 0.196 pg/g fresh weight, respectively. The TEQ levels are dominated by 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF and 3,3', 4,4', 5-PeCB in many of the samples. For one of these isomers, the 2,3,4,7,8-PeCDF TEQ levels showed good correlation with the total TEQ levels in the samples (r=0.957). This suggests that 2,3,4,7,8-PeCDF may be an indicator for dioxin contamination in the analysis of the leafy vegetables. Also, the effects of two cooking processes (washing and washing followed by boiling) on the dioxin levels in two types of spinach samples were investigated. On average, in both samples, the total concentrations of the PCDDs, PCDFs and Co-PCB were reduced to about 38%, 73% and 88% of the initial concentrations by washing, and to 21%, 35% and 61% of the initial concentrations by washing followed by boiling. The total TEQ levels were reduced to about 30% of the initial TEQ levels by washing followed by boiling. Thus, the cooking processes may reduce the risk of dioxin intake from the leafy vegetables.

Keywords: dioxins, cooking processes, vegetables

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Amakura, Y., Lou, H.*2, and Yoshida, T*1.: **Tannins and flavonol glycosides from the leaves of *Euphorbia pekinensis***

Natural Medicines, **55**, 313 (2001)

The dried leaves of *E. pekinensis* collected in China were homogenized in 70% acetone and filtered. A concentrated solution was extracted successively with Et_2O, AcOEt and *n*-BuOH. Each extract was subjected to a combination of chromatography to give four flavonols and thirteen hydrolyzable tannins (7 monomers and 6 dimers). All of the dimeric tannins isolated had a characteristic feature of the Euphorbiaceae, which contains geraniin and related unit possessing a dehydrohexahydrodiphenoyl (DHHDP) group, as a partial structure.

Keywords: *Euphorbia pekinensis*, Euphorbiaceae, tannins

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Amakura, Y., Tsutsumi, T., Nakamura, M.*1, Kitagawa, H.*1, Fujino, J.*1, Sasaki, K., Yoshida, T.*2, and Toyoda, M.: **Preliminary screening of the inhibitory effects of food extracts on activation of the aryl hydrocarbon receptor induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin**

Biol. Pharm. Bull., **25**, 272-274(2002)

A preliminary screening for the inhibitory effects on the activation of the aryl hydrocarbon receptor (AhR) by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) by applying AhR-based bioassays for dioxins, the Ah-Immunoassay and CALUX assay, was attempted. Thirty-nine food extracts including vegetables, fruits, herbs, and teas were initially screened *in vitro*. We first examined the application of both bioassay methods using green tea extracts and (-)-epigallocatechin gallate, reported antagonists of the AhR, since the results could reveal an inhibitory effect versus the control in both assays. Food extracts were then tested. Among the herbs, extracts of sage, among the vegetables, green leafy ones such as spinach, and among the fruit, citrus showed inhibitory effects on AhR activation by TCDD, although some tested samples did not show parallel behavior in both assays. Sage had a remarkable inhibitory effect (83% in the CALUX assay and 79% in the Ah-Immunoassay compared with control) and its effects were dose dependent. The results suggest that these assays might be applicable to the preliminary screening of antagonist activity against the AhR. Moreover, based on these results, the potential benefit of factors that function as dietary ligands of the AhR and are present in several foodstuffs is indicated.

Keywords: foods, aryl hydrocarbon receptor, dioxins

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岩木和夫*, 大羽宏*, 勝峰万里*, 小澤さやか*, 松田りえ子, 林譲：**ダイオキシン類のGC/HRMS測定における検出下限の推定法の比較**

環境化学, **11**, 173-180 (2001)

GC／HRMSによるダイオキシン類の検出下限を，くり返し測定に基づく方法，S/N比に基づく方法，FUMI理論による方法により求め，比較した．ピーク高さのくり返し再現性から求めた検出下限は特に大きい値となったが，他の方法による値はほぼ同程度であった．また，検出限界を数回求めたとき，FUMI理論による方法が最も変動の少ない結果となった．

Keywords: limit of detection, dioxins, FUMI theory

*荏原製作所

Akiyama,H., Goda,Y., Tanaka,T.,*, Toyoda,M.: **Determination of aflatoxins B1, B2, G1 and G2 in spices using a multifunctional column clean-up**

J. Chromatogr. A, **932**, 153-157 (2001)

A rapid and simple method using a multifunctional column, which contains lipophilic and charged active sites, was developed to analyse aflatoxins B1, B2, G1 and G2 in various spices, such as red pepper and nutmeg. After extraction by acetonitrile: water (9:1) and clean-up using MultiSep #228 column, the aflatoxins and aflatoxin-TFA derivatives are determined using LC with fluorescence detection. Recoveries of each aflatoxin B1, B2, G1 and G2 spiked to red pepper, white pepper, black pepper, nutmeg and tear grass at the level of 10 ng/g were over 80-85% in all instances. The minimum detectable concentration for aflatoxins in red pepper was 0.5 ng/g

Keywords: Spices, Multifunctional column, Aflatoxins

* Kobe Institute of Health

Akiyama, H., Teshima, R., Sakushima, J., Okunuki, H., Goda, Y., Sawada, J., Toyoda, M.: **Examination of oral sensitization with ovalbumin in Brown Norway rats and thress strains of mice**

Immunology Letters, 78, 1-5 (2001)

We studied the oral feeding of food allergens conditions needed to sensitize animals without induction of tolerance in order to investigate the allergenicity of orally ingested food proteins. BN rats were sensitized by daily OVA (ovalbumin)-gavage or by drinking water ad libitum and the ASA (active systemic anaphylaxis) response, as the immediate hypersensitivity response to antigen stimulation after oral sensitization, was examined. The oral administration of OVA by gavage produced a higher OVA-specific IgE response and an increase in serum histamine after antigen challenge, as compared to those produced by drinking water. Next, we examined the effect of murine age, the oral feeding technique and the oral feeding dose on sensitization using BALB/c, B10A and ASK mice. Twenty-week old mice show the strongest OVA-specific IgE and IgG1 responses and ASA-associated serum histamine contents increased with gavage in the three different age groups of BALB/c mice. Administering 0.1 mg of OVA by gavage daily for 9 weeks appeared to induce a higher response than administering 1 mg of OVA in terms of OVA-specific IgE and IgG1 antibody response and ASA responses. Among the three strains of mice, B10A mice exhibited the highest response in terms of OVA-specific IgE and IgG1 antibody and ASA responses. These findings suggested BN rats and B10A mice to be suitable models for oral sensitization with antigen protein, and that oral sensitization in mice requires low dose, intermittent antigen intakes.

Keywords: ovalbumin, oral sensitization, B10A mice

穐山浩，杉本和恵，松本美佐緒，五十鈴川和人，渋谷雅明*，合田幸広，豊田正武：**遺伝子組換えジャガイモ（NewLeaf Plus Potato）からの組換え遺伝子検知法の確立及びスナック菓子からの検知**

食品衛生学雑誌 **43**, 24-29 (2002)

日本で安全性審査未終了の遺伝子組換えジャガイモ（NewLeaf Plus potato；NL-P）について，ポリメラーゼ連鎖反応（PCR）を用いた検知法を検討した．陽性対照プライマー対は，Potato sucrose synthase遺伝子を認識するものを用いた．ジャガイモ葉巻ウイルスのreplicase（PLRV-rep）遺伝子を認識するプライマー対によりPCRを行った結果，NL-Pに特異的なバンドが検出された．さらに擬陽性を避けるために，2生物種由来の連続した遺伝子領域を増幅するプライマー対を設計し，特異的にNL-Pが検知されることが明らかとなった．確立したPCR検知法をジャガイモ加工品25検体に応用したところ，スナック菓子3検体からNL-Pが検出された．

Keyword:：genetically modified potato, recombinant DNA, NewLeaf Plus potato

*東京大学大学院薬学系研究科

後藤典子*，田辺　寛子*，宮原　誠：**電子線照射牛生挽肉の炭化水素法による検知**

食品照射，2001，**36**，13-22

円柱状の牛挽肉に5MeVの電子線を片面照射し，中心軸に近い部分を深度方向に従って1gずつ試料を採取し，それぞれの深度のおける炭化水素の量を測定した．炭化水素の量は表面から7-10mmのところが最大になり，深さ25から30mmで不検出となった．この深度-生成量の関係は理論計算による深度－線量分布の関係に類似していた．しかし，この円柱の中心軸からはずれた，外縁部においては，深度20から30mmにおいても炭化水素の生成が認められた．

Keywords: identification by hydrocarbon detection, irradiated food, electron beam irradiation

*東京都産業技術研究所　駒沢放射線利用施設

田辺　寛子*，後藤典子*，宮原　誠：**2-アルキルシクロブタノン法による照射鶏肉の検知**

食品照射，2001，**36**，26-32

定法により，照射鶏肉より2-アルキルシクロブタノンを定量し，照射の有無を検知した．2-ドデシルシクロブタノン，2-テトラデシルシクロブタノンを指標とした場合は3kGy，2-(5-テトラデセニル) シクロブタノンを指標とした場合1kGyから検知が可能であった．

Key words: 1-cyclobutanones, identification, irradiated food

*東京都産業技術研究所 駒沢放射線利用施設

Makoto Miyahara, Hitoshi Ito*, Kouji Ueno**, Yutaka Yamase**, Masatake Toyoda : **Evaluation of Several Dosimeters for Identification of Irradiated Foods Using a 5 MeV Electron Beam.**

J. Health Sci., **48**, 37-41(2002)

Electron-beam irradiation facilities are commercially utilized for food irradiation. This study investigates whether dosimeters used to detect gamma-rays can also be used to accurately detect electron-beam irradiation. The irradiation souses for gamma-ray at the Takasaki Institute were used throughout this study. Four kinds of dosimeters, RadiaChromic(RC), GammaChromic(GC), Amber3042 (AM), Radix(RX), and alanine(AL) were irradiated at doses from 0.1 to 60 kGy using a 5 MeV electron-beam(EB) at the Japan Irradiation Service Co. LTD. AL was submitted to the National Physics Laboratory in order to evaluate the doses that AL were absorbed. At low-level dose range, all dosimeters indicated correct values. This means those dosimeters were usable to accurately detect electron beam irradiation. But at higher range they all required correction due to either temperature effects or varying sensitivity to electron-beam exposure. Dose-depth profiles were obtained using ham and cheese samples, in order to check penetration of EB by the calibrated machine.

Keywords: electron-beam irradiation, dosimeter, food irradiation

*Japan Atomic Energy Research Institute Takasaki Establishment
**Japan Irradiation Service Co.

Makoto Miyahara, Taeko Nagasawa*, Tomomi Kamimura*, Hitoshi Ito**, Masatake Toyoda, and Yukio Saito : **Identification of irradiation of boned chicken by determination of o-tyrosine and electron spin resonance spectrometry**

J. Health Sci., **48**, 79-82(2002)

Ortho-tyrosine detection methods is used for detection of irradiation of protein-rich foods. A new procedure determining o-tyrosine was examined in regard to the identification of food having undergone ionization treatment. A new fluorometric HPLC method allows the detection of irradiated foods at 10k Gy. This method was compared with an ESR method recommended by European countries recommended for the detection of irradiated foods. The method is applied only few food samples that contain bone or cellulose.
The dose response of the o-tyrosine production was linearly increased upto 30 kGy. ESR signals in chicken bone were recorded after being dried in desiccator. The dose response of the ESR signal was observed at two weeks after irradiation, and a linear relationship was observed. ESR signal intensity was correlated with the logarithm of o-tyrosine production to confirm the performance and a good relationship was observed.

Keywords: ESR, o-tyrosine detection, identification of irradiated foods

* School of Allied Health Sciences Kitasato University
** Japan Atomic Energy Research Institute Takasaki Establishment

Makoto Miyahara, Akiko Saito*, Hitoshi Ito**, and Masatake Toyoda: **Identification of Low-level gamma Irradiation of Meats by High Sensitiveity Comet Assay**

Radia. Phys. Chem. **63**, 451-454 (2002)

Detection of low level irradiation in meats (pork, beef, and chicken) using a new comet assay was investigated in order to assess the capability of the procedure. The new assay includes a process to make it sensitive to irradiation and a novel evaluation system for each slide (influence score and distribution of comet type). Samples used were purchased at retailers and were irradiated at 0.5 and 2kGy at 0 oC. The samples were processed to obtain comets. Slides were evaluated by typing comets, and calculating the influence score and analyzing distribution chart of comet type shown on the slide. Influence scores of beef, pork, and chicken at 0 kGy were 287(SD=8.0), 305 (SD=12.9), and 320 (SD=21.0), respectively. Those at 500Gy, were 305 (SD=5.3), 347 (SD=10.6), and 364 (12.6), respectively. Irradiation levels in food were successfully determined. Sensitivity of the samples to irradiation was differed among them(chicken > pork >beef).

Keywords: comet assay, irradiated food, low level dose

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HirokoTanabe*, Michiko Goto*, Makoto Miyahra
:Detection method of irradaited chicken by GC analysis of 2-alkylcyclobutanones and hydrocarbons using Soxhlet extraction and Florisil chromatography
Radioisotoopes, **51**, 109-119(2002)

A procedure was established for the detection of irradiated chicken by GC/FID analysis of 2-alkylcyclobutanones(RCB) and hydrocarbons (HC) using simultaneous cleanup by soxhlet extraction and florisil chromatography as stated following.

Fat extract was obtained from irradiated chicken by soxhlet apparatus. The concentrated fat extract which contains 200 mg of fat with 2 μg of n-eicosane(IS) was applied to florisil column deactivated by adding 17 parts of water to 100 parts of adsorbent, and HC and RCB were separated from fat.

The HC were eluted in 60mL of the eluate. This eluate was concentrated and analyzed by GC/FID. After 150mL of n-hexane eluted from the same column, RCB were eluted with 120mL of 1% dietylether solution in n-hexane. This eluate was concentrated to 0.2mL and was added 0.1 μg of 2-cyclohexylcyclohexanone (IS)and analyzed by GC/FID. This process made it possible to detect HC and RCB efficiently and simultaneously by one time operation of analysis.

In this study, HC and RCB were detected from chicken irradiated at 0.5kGy by GC/FID.

KeyWord: food irradiation, 2-alkylcyclobutanones(RCB), hydrocarbons (HC)

*Tokyo Metropolitan Industrial Technology Research Institute

田邊寛子*, 後藤典子*, 宮原 誠：**2-アルキルシクロブタノンおよび炭化水素の同時分析（GC/FID）による照射鶏肉の検知に関する考察**
Radioisotoopes, **51**, 157-166(2002)

照射鶏肉の的確な検知のために脂肪の放射線分解によって生成する2-アルキルシクロブタノンと炭化水素の同時分析を試みた．検出はGC/FIDで行った．0.5kGyから10kGy 0度，照射において，これらの化合物の生成量は吸収線量に依存していた．しかし，照射の判定基準として，従来掲げられている方法に問題点があり，新しい基準を提案した．

Keywords: food irradiation, 2-alkylcycrobutanone, radiolytic product

*東京都産業技術研究所 駒沢放射線利用施設

六鹿元雄，太田久恵，豊田正武，合田幸広：**遺伝子組換え及び非組換えパパイヤ中のカロテノイド成分の比較**
食衛誌，**42**，367-373 (2001)

ウイルス抵抗性遺伝子組換えパパイヤ及び，非組換えパパイヤに含有されるカロテノイド成分の構造決定とその含有量についての比較をおこなった．フォトダイオードアレイ検出LC/MSによる解析の結果，主カロテノイドはb-カロテン，リコペン，b-クリプトキサンチン及び，そのカプロイル（C10），ラウロイル（C12）エステル体であった．これら5種の化合物について，両パパイヤの含有量を比較検討した．果実の中央より可食部を取り出し，凍結乾燥後，メタノールで抽出，その後，ヘキサンとメタノールで分配抽出し，ヘキサン抽出物をHPLCで分析した．その結果，総カロテノイド量，総b-クリプトキサンチン量に有意な差は観察されなかった．

Keywords: genetic modified, papaya, carotenoid

Sugimoto N., Kikuchi H., Yamazaki T., Maitani T. : **Polyphenolic Constituents from Leaves of *Rubus suavissimus***
Natural Medicines, **55**, 219 (2001).

The hot water extract from the leaves of *R. suavissimus*, named "tenryocha extract", is used as a natural sweetener. Seven peaks of compounds **1**-**7** were mainly observed in tenryocha extract by HPLC over SP-120-5-ODS-BP eluting with 2% HCO_2H/MeOH. The extract was chromatographed by prep. HPLC under the same conditions as described above, affording gallic acid (**1**), ellagic acid (**2**), 2-pyrone-4,6-dicarboxylic acid (**3**), brevifolin carboxylic acid (**4**), sanguisorbic acid dilactone (**5**), caffeic acid (**6**), and 1-α-galloyl-2,3-(*S*)-hexahydroxy-diphenoyl-D-glucose (sanguiin H-4) (**7**). This is the first report on the isolation and characterization of compounds **3**, **4**, and **7** from *R. suavissimus*.

Keywords: *Rubus suavissimus* S. LEE, 2-pyrone-4,6-dicarboxylic acid, Sanguiin H-4

Yun, Y. S., Sugimoto, N., Sekita, S., Maitani, T., Satake, M.: **A Cembrane-type diterpen from flue-cured burly tabacco (*Nicotiana tabacum* L.) leaves**
Natural Medicines, **55**, 262-264 (2001)

A new diterpene, named (1*E*, 3*Z*, 5*E*, 7*Z*, 11*E*)-1-isopropyl-4,8,12-trimethyl-1,3,5,7,11-cyclotetradecapentaene was isolated from an AcOEt extract of flue-cured burley tobacco (*Nicotiana tabacum* L.) leaves, and the structure was determined on the basis of spectral data.

Keywords: *Nicotiana tabacum* L., diterpene, cembrane

Sugimoto, N., Fukuda, J., Takatori, K., Yamada, T., Maitani, T.: **Identification of Principal Constituents in Enzymatically Hydrolyzed Coix Extract**
J. Food Hyg. Soc. Japan, **42**, 309-315 (2001)

The structural elucidation of main constituents in enzymatically hydrolyzed coix extract, a natural food preservative, was carried out. After peracetylation, five compounds, namely peracetylated forms of glucose, maltose, maltotriose, maltotetraose, and maltopentaose were isolated. These structures were determined by PFG HMQC and HMBC experiments. In addition, by using HPLC with an RI detector, the main components of this coix extract were identified as a mixture of oligosaccharides having one to seven glucose units conjugated by α-(1→4) linkages. Since this extract showed no antimicrobial activity, its preservative effect may be caused by its covering of the food surface, which blocks the contact with air.

Keywords: enzymatically hydrolyzed coix extract, *Coix lachryma-jobi* L. *var.* ma-yuen STAPF, natural preservative

Sugimoto, N., Kawasaki, Y., Sato, K., Aoki, H.*, Ichi, T.*, Koda, T.*, Yamazaki, T., Maitani, T.: **Structure of Acid-Stable Carmine**
J. Food Hyg. Soc. Japan, **43**, 18-23 (2002)

Acid-stable carmine has recently been distributed in the U. S. market because of the acid stability. But it is not permitted in Japan. We analyzed and determined the structure of the major pigment in acid-stable carmine, in order to establish the analytical method for it. Carminic acid was transformed into a different type of pigment, named acid-stable carmine, through amination when heated in ammonia solution. The features of the structure were clarified using a model compound purpurin, whose orientation of hydroxyl groups on the A ring of an anthraquinone skeleton is the same as carminic acid. By spectroscopic means and the synthesis of acid-stable carmine and purpurin derivatives, the structure of the major pigment in acid-stable carmine was established as 4-aminocarminic acid, a novel compound.

Keywords: acid-stable carmine, carminic acid, 4-aminocarminic acid

* San-Ei Gen F. F. I. Inc.

Uno, Y.*, Omoto, T.*, Goto, Y.*, Asai, I*, Nakamura, M*, Maitani, T.: **Molecular weight distribution of carrageenans studied by a combined gel permeation/inductively coupled plasma (GPC/ICP) method**
Food Additives and Contaminants, **18**, 763-772 (2001)

Degraded carrageenan (poligeenan, 20-30 kDa) causes ulcerative colitis in experimental animals. The molecular weight (MW) distributions of 29 samples of refined carrageenans were studied by GPC/ICP method as well as GPC/refractive index (RI) detection. All samples had a major broad peak of high MW which eluted at around 6.5 min in both RI and ICP mode (sulphur and carbon). No obvious peak of poligeenan was detected (the detection limit was about 5%). The number average MWs of these carrageenans ranged from 193 to 324 kDa, and the weight average MWs from 453 to 652 kDa. Some samples had a few minor peaks at 10-12 min. These peaks came from ionic sulphate, sucrose or glucose. It was considered that if the data-sampling programme was improved, the GPC/ICP system would become a more powerful technique for evaluation of carrageenan samples containing ionic substances and sugar.

Keywords: carrageenan, GPC/ICP, molecular weight distribution

* San-Ei Gen F. F. I., Inc.

宇野喜貴*, 大本俊郎*, 後藤康慶*, 浅井以和夫*, 中村幹雄*, 米谷民雄：**ラットに混餌投与されたカラギナンの糞中への排泄量と分子量**
日本食品化学学会誌, **8**, 83-93 (2001)

ラットにλタイプ精製カラギナンを混餌投与し，糞中カラギナンの分子量と含量をGPC/ICP-AES法で測定した．7週齢SD系ラット雌雄各1匹に，一夜絶食後，5％のλタイプ精製カラギナンを含む混餌飼料を1日間自由に摂取させ，その後2日間基礎飼料を摂取させた．投与開始1日後までに糞中排泄されたカラギナンの平均分子量は，雌雄平均でMn = 355 kDa，Mw = 782 kDa，投与翌日の糞では，Mn = 300 kDa，Mw = 718 kDaであった．検体カラギナンの平均分子量はMn = 308 kDa，Mw = 832 kDaであり，カラギナンはラット消化管中でほとんど分解されないと考えられた．糞中カラギナン量は，混餌投与当日で雄659 mg，雌466 mg，翌日で377 mgと329 mg，3日後で10 mgと6.0 mgであった．投与と採糞のスケジュールを考慮すると，投与カラギナンは，ほぼ1日以内に糞中に排泄されると考えられた．一方，投与カラギナンの糞中からの回収率は3日間の合計で約90%であり，これには糞中からの抽出率が影響していることも考えられた．

Keywords: carrageenan, GPC/ICP, molecular weight distribution

*三栄源エフ・エフ・アイ㈱

河村葉子，中島明子，山田　隆：**食品用天然ゴム製品中の残存化学物質**

食衛誌，**42**, 179-184 (2001)

天然ゴム製の乳首，パッキング，手袋，ハム用ネットなど12検体中の残存化学物質を検索したところ，加硫促進剤のジメチルジチオカルバミン酸亜鉛，ジエチルジチオカルバミン酸亜鉛（EZ），ジ-*n*-ブチルジチオカルバミン酸亜鉛（BZ）及び2-メルカプトベンゾチアゾールが各数千 μg/g検出されたほか，酸化防止剤のBHT, Irganox 1076, Yoshinox 2246R，可塑剤のフタル酸ジブチル，フタル酸ジ(2-エチルヘキシル)，滑剤のパルミチン酸，ステアリン酸，さらに植物ステロイドのスチグマステロール，β-シトステロールなども検出された．水，4％酢酸，20％エタノールではいずれの溶出もみられなかったが，*n*-ヘプタンではBHT, Yoshinox 2246R, EZ, BZ，ステロール類などの溶出がみられた．

Keywords: natural rubber, vulcanization accelerator, β-sitosterol

河村葉子，中島明子，六鹿元雄，山田 隆，米谷民雄：**食品用シリコーンゴム製品中の残存化学物質**

食衛誌，**42**, 316-321 (2001)

ゴム製品のうち食品用に最も広範に使用されているシリコーンゴム製のほ乳びんなどの乳首，密閉容器や魔法びんなどのパッキング，調理用ヘラの合計23検体について残存化学物質を検討した．材質中には酸化防止剤のBHT，可塑剤のフタル酸ジブチル及びフタル酸ジ(2-エチルヘキシル)（DEHP）が検出されたが，検出頻度，検出量ともにそれほど高くはなく，乳首からはいずれも検出されなかった．一方，全検体からシロキサンが6～25個程度結合した環状ポリジメチルシロキサン群が検出された．残存量の合計は3,310～14,690 μg/gと概算され，主に未反応原料または副生成物由来と推定された．溶出試験において，20％エタノールではいずれの溶出も認められなかったが，*n*-ヘプタンではDEHP及び環状ポリジメチルシロキサンの溶出がみられた．

Keywords: silicone rubber, food contact use, polydimethylcyclosiloxane

和久井千世子，河村葉子，米谷民雄：**使い捨て手袋における溶出物及び残存アクリロニトリルの分析**

食衛誌，**42**，322-328 (2001)

各種素材の使い捨て手袋について，蒸発残留物，溶出金属等，溶出化学物質及び材質中のアクリロニトリルを分析した．フタル酸エステル含有及び非含有の塩化ビニル製手袋では，蒸発残留物が*n*-ヘプタン溶出で870～1,300ppmと極めて高く可塑剤由来と考えられた．後者の可塑剤の中には，従来食品用途では使用されていないものがあった．ポリエチレン製手袋では溶出物は少なかったが，抗菌表示手袋から銅及び亜鉛の溶出が見られた．一方，天然ゴム及びニトリルゴム製手袋は，製品毎に溶出物の種類や量に大きな差異が見られた．約半数では4％酢酸溶出時に蒸発残留物と共に亜鉛やカルシウムの溶出量も高く，ジチオカーバメイト系加硫促進剤も検出された．ニトリルゴム手袋のアクリロニトリル残存量は0.40～0.94 μg/gであった．

Keywords: disposable glove, polyvinyl chloride, nitrile-butadiene rubber

馬場二夫[*1]，渡辺悠二[*2]，河村葉子，山田耕平[*3]，藤井正美[*4]：**ポリカーボネート製食器から高濃度のビスフェノールAが検出された原因の解明に関する研究**

日本食品化学学会誌，**8**, 121-127 (2001)

1997年抗菌剤を含有するポリカーボネート製幼児用食器から高濃度のビスフェノールAが検出され問題となった．そこで，ポリカーボネートに6種類の酸化金属と当該抗菌剤を添加しペレットを調製したところ，添加量に比例してビスフェノールAの増加がみられ，特に酸化亜鉛で顕著であった．酸化防止剤を添加すると，Irgafos 168ではあまり変化がみられなかったが，P-EPQでは明らかに生成抑制がみられた．以上から，高濃度ビスフェノールAの生成原因は，食器に添加された抗菌剤中の酸化亜鉛によるポリカーボネートの酸化分解促進および酸化防止剤の選択ミスによると結論された．

Keywords: bisphenol A, polycarbonate, anti-bacterial agent

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新野竜大[*1]，石橋　亨[*1]，伊藤　武[*1]，坂井千三[*1]，杉田たき子，石綿　肇，山田　隆[*2]，小野寺祐夫[*3]：**ポリ塩化ビニル製玩具中の可塑剤フタル酸エステルの分析：1998年以降の市販品含有量調査**

日本食品化学学会誌，**8**，194-199 (2001)

ポリ塩化ビニル（PVC）製玩具中のフタル酸エステル類（PAEs）の分析を行った．玩具からのPAEsの抽出はアセトンを用い，室温，3時間，回転振とうした．定量はHPLCで行い，GC/MSで確認した．フタル酸ジイソノニル（DINP）500 mg/g含有のPVCプレートからの回収率は92%であった．1999年11月～2000年2月に購入した玩具22製品からは，DINPが196-449mg/g（7製品），フタル酸ジ(2-エチルヘキシル）が63-453mg/g（5製品）

検出された．また，フタル酸ジブチル及びアジピン酸ジ(2-エチルヘキシル）が39-118mg/g（3製品）及び63-254mg/g（3製品）それぞれ検出された．乳幼児が口に入れるのを目的とした玩具からはPAEsは検出されなかった．

Keywords: phthalate ester, plasticizer, polyvinyl chloride toy

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Niino, T.*1, Ishibashi, T.*1, Itho, T.*1, Sakai, S.*1, Ishiwata, H., Yamada, T.*2, Onodera, S.*3 : **Monoester Formation by Hydrolysis of Dialkyl Phthalate Migrating from Polyvinyl Chloride Products in Human Saliva**

J. Health Sci., **47**, 318-322 (2001).

ポリ塩化ビニル製玩具のチューイングにより溶出したジアルキルフタル酸エステルについて検討した．フタル酸ジ-*n*-ブチル（DBP）100 mg/g，及びフタル酸ジ(2-エチルヘキシル)（DEHP）185 mg/gを含有するボールAからの溶出量は人工唾液による振とうでは，339及び315 μg/10 cm^2/hr，ヒトチューイングでは11.7，44.4 μg/10 cm^2/hr，フタル酸ジイソノニル256 mg/gを含有するボールBでは人工唾液による振とうで535 μg/10 cm^2/hr，ヒトチューイングでは78.0 μg/10 cm^2/hrであった．チューイングしたヒト唾液中にはフタル酸モノ-*n*-ブチル及びフタル酸モノ(2-エチルヘキシル）が存在することをGC/MSにより確認した．また，ヒト唾液にDBP及びDEHPを添加し，37℃で60分間インキュベートしたところ，これらのフタル酸ジエステル類は加水分解され対応するフタル酸モノエステルを生成した．

Keywords: dialkyl phthalate, monoalkyl phthalate, PVC

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*2 Japan Food Additives Association
*3 Tokyo University of Science

佐々木次雄*，棚元憲一：**「ろ過滅菌法」の日局導入に関する研究**

医薬品研究，**32**, 814-819 (2001)

無菌操作法で製される医薬品の工程で，ろ過滅菌工程は最も重要なものである．本研究では日局「最終滅菌法及び滅菌指標体」から「ろ過滅菌法」を切り離し，国際規格（ISO/DIS 13408-2）を反映した「ろ過滅菌法」を日本薬局方参考情報に導入するための調査研究を行った．特にフィルターの孔径については，*B. diminuta*のように0.45 μmはもちろん，条件によっては0.20/0.22 μmフィルターを通過する可能性が指摘される一方，小孔になるほど圧力損失も大きくなりろ過が困難になる製品も出てくることから問題となっている．この選択に関して品質保証，工程特性，日常管理等の観点から調査研究を行い，さらにろ過前液に対するバイオバーデン試験や測定頻度，疎水フィルターの規格等について検討を行った．これらの結果を元に「ろ過滅菌法」の日本薬局方参考情報導入を計る予定である．

Keywords: Filtration, Japanese Pharmacopoeia, Microbiological tests

*国立感染症研究所

Ohnishi, T., Muroi, M., Tanamoto, K.: ***N*-linked glycosylations at Asn^{26} and Asn^{114} of human MD-2 are required for Toll-like receptor 4-mediated activation of NF-κB by lipopolysaccharide.**

J. Immunol., 167, 3354-3359 (2001)

MD-2 is physically associated with Toll-like receptor 4 (TLR4) and is required for TLR4-mediated LPS signaling. Western blotting analysis revealed the presence of three forms of human (h)MD-2 with different electrophoretic mobilities. After *N*-glycosidase treatment of the cellular extract prepared from cells expressing hMD-2, only a single form with the fastest mobility was detected. Mutation of either one of two potential glycosylation sites Asn^{26} and Asn^{114} of MD-2 resulted in the disappearance of the slowest mobility form, and only the fastest form was detected in hMD-2 carrying mutations at both Asn^{26} and Asn^{114}. Although these mutants were expressed on the cell surface and maintained its ability to associate with human TLR4, these mutations or tunicamycin treatment substantially impaired the ability of MD-2 to complement TLR4-mediated activation of NF-κB by LPS. LPS binding to cells expressing CD14, TLR4, and MD-2 was unaffected by these mutations. These results demonstrate that hMD-2 undergoes *N*-linked glycosylation at Asn^{26} and Asn^{114}, and that these glycosylations are crucial for TLR4-mediated signal transduction of LPS.

Keywords: MD-2, Toll-like receptor 4, NF-κB

宮原美知子*，小沼博隆：**輸入冷凍生カキより*Shigella sonnei*赤痢菌の検出**

防菌防黴，**30**, 299-302 (2002)

2001年11月下旬より，西日本では赤痢患者が急増した．各自治体の疫学調査等により，生カキが原因と考えられた．厚生労働省は，全国の自治体に対し，生カキの流通状況の調査などを通知した．2001年12月27日までに30都府県で159人の感染者が届け出られた．そのうち110人の便と，今回報告する方法により，検出された

*Shigella sonnei*のPFGE（パルスフィールドゲル電気泳動）でのDNAパターンが国立感染症研究所の細菌部によって一致することが確認された．MPN法では0.2 cfu/gの赤痢菌汚染であった．日本では，現在までに自然汚染した赤痢菌が食品から検出された報告はないことから，食品からの赤痢菌の検出は難しいことと思われていた．今回の検査で，凍結生ガキより*Shigella sonnei*を分離検出することができたので，その概要を報告する．

Keywords: frozen oyster, *Shigella sonnei*, two-stepwise enrichment

村瀬 稔[*1]，木股祐子[*1]，仲西寿男[*1]，小澤一弘[*2]，赤羽荘資[*2]，浅川 豊[*2]，南沢仁志[*3]，上條茂徳[*3]，小沼博隆：**腸管出血性大腸菌O157分離培地BD CHROM agarO157の評価**

日本食品微生物学会誌，**18**, 75-81(2001)

現在，腸管出血性大腸菌O157H：7の分離培地としては，ソルビトールの分解能あるいは合成酵素基質による発色能を鑑別に利用したものが開発されている．しかしながら，両方法とも検出分離には満足のいくものではなかった．そこで，著者らは発色酵素基質を応用したBD CHROMagarO157を改良した培地と改良前のBD CHROMagarO157およびCT-SMACを比較検討した結果，改良BD CHROMagarO157は，食品中の雑菌を抑制し，腸管出血性大腸菌O157：H7を効率よく検出分離することができた．

Keywords：*Escherichia coli* O157:H7, chromogenic substrate, ground beef

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齋藤章暢[*1]，小野冷子[*1]，柴田 譲[*1]，濱田佳子[*1]，山口正則[*1]，小沼博隆：**炭疽菌芽胞に対する各種殺菌剤の有効性**

感染症学雑誌，**76**, 291-292(2002)

米国において炭疽菌がバイオテロリズムに利用され，多くの人々が死亡するなど重大な被害を受けた．そこで，著者らは炭疽菌によるテロに対する対応として適切な消毒方法を模索するため，炭疽菌芽胞を用いて5種薬剤（グルタルアルデヒド，過酢酸，次亜塩素酸ナトリウム，過酸化水素およびホルムアルデヒド）の殺菌効果を検討した．その結果，最も強い殺菌効果の認められた薬剤は，10％ホルムアルデヒド，次いで0.3％過酢酸，2％グルタルアルデヒド等の順であった．しかし，各種薬剤はヒトに対する毒性や施設，器具・機材の腐食性などの問題点を持つものが多いことから，使用に際しては目的に適した薬剤を適切な濃度で使用することが望ましいと考えられた．

Keywords：*Bacillus anthracis*, disinfectant, spore,

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Hara-Kudo, Y., Sakakibara, Y.[*1], Konuma, H. Sawada, T.[*1] and Kumagai, S.[*2] :**Laying season and shell eggcracks on the growth of *Salmonella* Enteritidis in the egg albumen during storage**

J. Food Prot., **64**, 1134-1137 (2001)

We studied the effects of laying seasons and egg shell cracks on the ability of the egg albumen to support the growth of Salmonella Enteritidis (SE) in eggs. Hens eggs used were those laid in February, June and October in a farm in Japan and stored at 10℃, 20℃ and 30℃, and at 30℃ after storage at 10℃, immediately after receipt or after cracking the shell. At several days intervals during storage, the egg contents were poured into a dish and SE was inoculated into albumen, and then the growth of SE during 3 days incubation at 18℃ was measured. The results demonstrated that storage temperature and laying season affected the growth of SE in the egg albumen. The proportion of eggs which albumen allowed the growth of SE was higher in the eggs stored at 30℃ than those stored at 10℃. The growth of SE in eggs was lowest in the following order of laying; February, October and June. SE grew preferably in albumen of cracked eggs than intact eggs.

Keywords: Salmonella Enteritidis, egg, storage

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Hara-Kudo, Y., Okubo, T.[*1], Tanaka, S.[*1], Chu, D.[*1], Juneja, L[*1]. R. Saito, N.[*2] and Sugita-Konishi, Y.:**Bactericidal Action of Green Tea Extract and Damage to the Membrane of *Escherichia coli* O157:H7.**

Biocontrol Science, **6**: 58-61 (2001)

The antibacterial activity of green tea extract was investigated on 36 isolates of pathogens. The minimum inhibitory concentration of the extract against some isolates such as *Escherichia coli* O157:H7 was less than 250 μg/ml. The growth of the organism in culture broth was completely inhibited with 500 μg/ml of the extract. The organism was not detected by culturing on agar plates after 6 h incubation in phosphate buffered saline with 1,000 μg/ml of the extract. It was observed by fluorescence microscopy and scanning electric microscopy that the cell membranes were damaged with 1,000 μg/ml of the extract.

Key words: *E. coli* O157:H7, Green tea, chatechin
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Hara-Kudo Y., Nishina, T.*1, Nakagawa, H.*2, Konuma, H. and Kumagai, S.*3: **Improved method for detection of *Vibrio parahaemolyticus* in seafoods.**
Appl. Environ. Microbiol., **67**, 5819-5823 (2001)

We have developed a new effective procedure for detecting *Vibrio parahaemolyticus* in seafoods using enrichment and plating onto a chromogenic agar medium. Samples were cultured in salt trypticase soy broth, which is a nonselective medium, and then a portion of the culture was cultured with salt polymyxin broth, which is a selective medium for *V. parahaemolyticus*. This two-step enrichment was more effective than the one step enrichment in salt polymyxin broth alone. The enrichment cultures were then plated onto a new chromogenic agar containing substrates for beta-galactosidase. The *V. parahaemolyticus* colonies developed a purple color on this growth medium that distinguished it from other related bacterial strains. *V. parahaemolyticus* was isolated more frequently from naturally contaminated seafood samples using the chromogenic agar than thiosulfate citrate bile salts sucrose (TCBS) agar medium which is currently used for the isolation of *V. parahaemolyticus*. Our findings suggest that this new enrichment and isolation scheme is more sensitive and accurate for identifying *V. parahaemolyticus* in seafood samples.
Keywords: seafood, *Vibrio parahaemolyticu*, detection
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工藤由起子，杉山寛治*1，斎藤章暢*2，仁科徳啓*3，長谷川順子*3，中川弘*4，市原智子*5，小沼博隆，熊谷進*6：**免疫磁気ビーズ法および酵素基質培地を用いたTDH産生性腸炎ビブリオO3:K6の自然汚染貝からの検出**
感染症学雑誌，**75**, 955-960 (2001)

日本において主要な食中毒の原因である腸炎ビブリオの検出を，1999年までにTDH産生性腸炎ビブリオ血清型O3:K6が海水またはアサリから分離されたことのある海域のアサリについて行った．方法は2％食塩加TSBでの6時間と食塩ポリミキシンブイヨンでの18時間の2段階増菌培養を行った．この培養液1 mlを用いて腸炎ビブリオK6抗原に対する免疫磁気ビーズ法を行い，特に血清型O3:K6の効率的分離を行った．また，従来のTCBS培地に加え腸炎ビブリオ特異的分離用に開発された酵素基質培地を用いた．この結果，TDH産生性腸炎ビブリオO3:K6の自然汚染貝からの分離は66ロット中3ロット（4.5％）で陽性であった．また，分離した腸炎ビブリオの4,265コロニー中6コロニーがTDH産生性O3:K6（0.14％）であった．
Keywords: seafood, *Vibrio parahaemolyticus*, detection
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長谷川順子*1，工藤由起子，仁科徳啓*1，小沼博隆，熊谷進*2：**酸性下における腸炎ビブリオO3:K6の生残性**
食品衛生学雑誌，**43**, 90-94 (2002)

腸炎ビブリオの血清型O3:K6株と他血清型株の酢酸，クエン酸および塩酸における生存性を研究した．その結果，これらの酸に対する耐性は血清型O3:K6株と他血清型株の間に差はなかった．クエン酸はpH5.6において酢酸よりも腸炎ビブリオの菌数減少に対してより効果的であったが，pH4.5においては酢酸はクエン酸よりも効果的であった．さらに，pH4.0であるワインビネガーと米酢において腸炎ビブリオの菌数変化を確認したところ，菌数は急速に減少した．
Keywords: survival, *Vibrio parahaemolyticus*, acid
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Sugita-Konishi, Y.,. Hara-Kudo, Y., Iwamoto T.* and Kondo, K.**: **Wine has activity against entero-pathogenic bacteria in vitro but not in vivo.**
Biosci. Biotech. Biochem., **65**, 954-957 (2001)

We studied the activity of wine against entero-pathogenic bacteria both in vitro and in vivo. The food-borne bacteria were killed in both red and white wine within 30min. However, the results of a *Salmonella* infection experiment using mice suggested that wine was not effective in preventing food-borne diseases in vivo.
Key words: polyphenol, Salmonella enteritidis,
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**お茶の水大学大学院

Sugita-Konishi, Y., and Pestka, J.J.*,: **Differential upregulation of TNF-alpha, IL-6, and IL-8**

production by deoxynivalenol (vomitoxin) and other 8-ketotrichothecenes in a human macrophage model.

J. Toxicol Environ Health A, **64**, 619-636 (2001)

The effects of deoxynivalenol (DON or vomitoxin) and four closely related 8- ketotrichothecenes on proinflammatory cytokine and chemokine production were evaluated in a clonal human macrophage model. U-937 cells, which represent a human monocytelike histocytic lymphoma, were differentiated into macrophages by preincubation with phorbol 12-myristate 13-acetate (PMA). Differentiated macrophages were incubated with DON in the absence or presence of lipopolysaccharide (LPS), and supernatant was analyzed by enzyme-linked immunosorbent assay (ELISA) for the proinflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha), and for the chemokine interleukin-8 (IL-8). In the absence of LPS, DON at 500 or 1,000 ng/ml upregulated TNF-alpha production as early as 3 h and up to 6 h, whereas 100 to 1,000 ng/ml of DON significantly increased production of IL-6 from 3 to 24 h and IL-8 from 6 to 48 h. In cells costimulated with 0.2 microg/ml LPS, DON at 500 or 1000 ng/ml markedly superinduced TNF-alpha and IL-8 production. Although 100 ng/ml of DON also potentiated LPS-induced IL-6 production, 500 or 1,000 ng/ ml of the toxin suppressed the LPS-induced IL-6 response. Four other 8-ketotrichothecenes, fusarenon X, nivalenol, 3-acetyl DON, and 15-acetyl DON, were also capable of upregulating or suppressing TNF-alpha, IL-6, and IL-8 production at concentrations similar to that of DON. In total, the results suggest that DON and other 8-ketotrichothecenes have the potential to both directly induce and superinduce proinflammatory cytokine and chemokine expression in human macrophages, even at toxin concentrations that are cytotoxic.

Keywords:, deoxynivalenol (vomitoxin), other 8-ketotrichothecenes, TNF-alpha, IL-6,

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Yamashita, S .,* Sugita-Konishi, Y. and Shimizu, M .* : **In Vitro Bacteriostatic Effects of Dietary Polysaccharides.**

Food Sci. Technol. Res., **7**, 262-264, (2001).

The antimicrobial action of dietary polysaccharides on eight food-borne pathogenic bacteria were examined. Among the polysaccharides, the carrageenans showed the most pronounced inhibitory effect, the growth of all the bacteria strains except *Listeria monocytogenes* being significantly inhibited by them, particularly by iota carrageenan. A growth-inhibition experiment using *Salmonella enteritidis* showed that the inhibitory effect of the carrageenans was not bactericidal but bacteriostatic. The removal of sulfate residues eliminated the bacteriostatic effect of iota-carrageenan, suggesting that the sulfate residue(s) in carrageenan plays an essential role in the effect. The results of the present study suggest that dietary polysaccharides, and particularly carrageenans, may act as effective preservatives in various types of processed food.

Keywords, carrageenan antimicrobiol activty, *Salmonella enteritidis*

*東京大学大学院

Sugita-Konishi, Y., Sakanaka, S.,* Sasaki, K.,* Juneja, L. R.,* Noda, T.** and Amano, F .**: **Inhibition of bacterial adhesion and Salmonella infection in BALB/c mice by sialyloligosaccharides and their derivatives from chicken egg yolk**

J. Agric. Food Chem., **50**, 3607-3613 (2002)

The effects of an egg yolk-derived sialyloligosaccharide (YDS), asialo YDS and a sialylglycopeptide of YDS (SGP) on bacterial adhesion to intestinal epithelial cells and on Salmonella infection in BALB/c mice were examined. YDS, it's derivative and SGP strongly inhibited the binding of *Salmonella enteritidis* but not *E. coli* K-88 to a human epithelial cell line Caco-2. In a *Salmonella* infection experiment using BALB/c mice, oral administration of these reagents effectively prevented the bacteria from proliferating in spleen as well as lethality. An experiment using radioactive SGP orally administered to mice revealed that the compound was absorbed from the intestine into blood and eliminated via urine within 8 hrs. However, these reagents did not influence the production of TNF-α or NO. in culture macrophages. The results suggest that they inhibit Salmonella infection not by activating macrophages but by inhibiting the entry of bacteria through the gut, suggesting that YDS and its derivatives are useful for preventing *Salmonella* infection when ingested continuously.

Key words: sialyloligosaccharides, sialyoligosaccharide-conjugated peptide, bacterial adhesion,

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Kanayama, A.,* Inoue, J.,* Sugita-Konishi, Y., Shimizu, M.* and Miyamoto, Y. *: **Oxidation of**

IkappaBalpha_at the 45th methionnine is one cause of taurine chloramines-induced inhibition of NFkappaB.
J. Biol. Chem., **277**, 24049-24056 (2002).

A band shift of IkappaBalpha was observed in Western blots with Jurkat cells treated with 1 mM taurine chloramines (Tau Cl) for 1 hour. TauCl treatment inhibited TNF-alpha-inhibited NFkappaB activation. TauCl did not inhibit either the upstream of IKK activition or IKK itself but NFkappaB activation induced by IKK overexpression. Deletion experiments showed that a TauCl modification site causing the band shift of IkappaBalpha is the 45th methionnine (Met45). HPLC and mass spectrometry analyses of a small peptide containing Met45 revealed that TauCl oxidizes Met45 . A mutant of IkappaBalpha whose Met45 was converted to alanime did not generate a band shift upon Tau Cl treatment and degraded in response to TNF-alpha stimulation. However, a reporter assay revealed that NFkappaB dependent inciferase expression was not fully recovered in cells transfer with this mutant. These results indicate that Met45 oxidation of IkappaBalpha is a molecular mechanism underlying in TauCl-induced inhibition of NFkappaB activation. A similar band shift was observed when HL-60 cells expressing myeloperoxidase were treated with 100 μM hydrogen peroxide for 5 min. When rat neurophils were incubated with bacteria, intracellular taurine decreased interleukin-8 production. Therefore, taurine may help suppress excessive inflammatory reaction in neutrophils.

Keywords: taurin, NFkappaB, neutrophils

*東京大学大学院 農学生命科学

Aihara, Maki, Tanaka, T.* and Takatori, K.: ***Cladosporium* as the main fungal contaminant of locations in dwelling environments.**
Biocontrol Science, **6**: 49-52 (2001)

A total of 75 locations in 26 houses were examined for fungal contamination. Sixteen genera from 68 locations were detected. *Cladosporium* was the dominant contaminant. A high frequency of contamination was seen to involve *Cladosporium*, of which *C. sphaerospermum* and *C. cladosporioides* were detected most frequently at rates of 63.6% and 14.6%,respectively.

Keywords: *Cladosporium*, fungal contamination, dwelling environments

* Ochanomizu University

Jong-Chul, Park*, Han D-W, Park*, B-J Lee D-H*, K. Takatori and Hwal Suh*: **Effective screening medium for the biodegradation of oleic acid by *Aspergillus niger*.**
Biocontrol Science, **6**: 37-41 (2001)

To investigate oleic acid biodegradation, 7 strains of *Aspergillus niger* were tested with 3 different types of Czapek-Dox broth (CzDB) medium containing oleic acid, and their metabolic abilities to decompose the fatty acid into carbon dioxide and water were compared. When the fungal strains were grown in the CzDB media with both 14C-labeled and non-labeled oleic acid, *A. niger* oxidized more than 58% of the supplied substrate within 72h. The addition of saccharose as an additional carbon source substantially reduced the biodegradation of oleic acid to the point that all the strains showed less than 4% degradation.

Keywords: oleic acid, biodegradation, saccharose

*韓国 延世大学

Makimura K*, Hanazawa R, Takatori K, Tamura Y*, Fujisaki R*, Nishiyama Y*, Abe S*, UchidaK*, Kawamura Y**, Ezaki T** and Yamaguchi H*: **Fungal flora on board Mir-space station, identification by morphological features and ribosomal DNA sequences.**
Microbiology and Immunology, **45(5)**: 357-363 (2001)

The morphological ad molecular biological identification, using 18S-and ITS1-rDNA sequences of the space fungi is discussed. The six fungal strains were isolated from air by using an air sampler. Strains were identified as *Penicillium chrysogenum*, *Aspergillus versicolor* or *Penicillium* sp by both methods. The species of space fungi were common saprophytic fungi in our living environments. This study concluded that the environment on board the space station Mir allows the growth of potentially pathogenic fungi as true in residential areas on the earth.

Keywords: fungal flora, DNA sequences, Mir-space station

*帝京大学
**岐阜大学

Kosuke Takatori, Akemi Saito*, Hiroshi Yasueda* and Kazuo Akiyama*: **The effect of house design and environment on fungal movement in homes of bronchial asthma patients.**
Mycopathologia, **152(1)**: 41-49 (2001)

The effect of house building design and environment on

the fungal movement in houses of 41 bronchial asthma(BA) patients has been investigated by examining house dust. The presence and composition of fungi were determined and compared in relation to building structure, house age, size of living room, main flooring material, presence of a living room rug or air purifier, and frequency of vacuum cleaning. Among these elements, fungal CFU apparently varied only between building structure. Classification of the fungal types in the house dust of BA patients showed that, regardless of the building designs, there were high levels of osmophilic fungi that survive at relatively dry conditions, whereas fungi that survive in very wet conditions were present at low frequency.

Keywords: allergy, bronchial asthma, house design

*国立相模原病院

李 憲俊*, 長峰英之*, 武井康裕*, 宮島千鶴*, 高鳥浩介：**CO_2測定による抗カビ試験の評価**

防菌防黴, **29**: 367-370 (2001)

通常抗カビ試験は培養法により判定することにあり，そのため培養日数を要すという欠点がある．培養法では液体法，寒天法があり，特にカビの場合両者によって判定するが，前者は菌糸発育，後者は胞子および菌糸発育によって評価される特徴を有している．そこで，迅速かつ客観的に評価できる試験法の改良が必要であり，ここでは培養により発生する微量のCO_2を指標として抗カビ試験の改良を試みたところ，CO_2の発生量で抗カビ活性の評価が可能であることを確認した．

Keywords: antifungal test, carbon dioxide, estimation

*衛生微生物研究センター

稲田知佳*, 工藤たか子*, 芳住邦雄*, 高鳥浩介, Alan Hedge**: **光照射による*Penicillium*不活化の波長依存性**

防菌防黴, **29(12)**: 757-762 (2001)

光照射による不活化特性を波長および照射エネルギーの観点から明らかにすることを目的として波長別光照射が可能な大型スペクトログラフおよびB領域紫外線，A領域紫外線蛍光灯による*Penicillium implicatum*不活化の作用スペクトルを検討した．波長別による試料被爆実験から波長260nm紫外線では50J/m^{2}の照射量で不活化した．また280nmでは300J/m^2，300nmでは800J/m^{2}であった．さらに長波長側では500nmまで不活化作用を認めた．真菌の不活化を認めるには64kJ/m^{2}程度の照射エネルギーを要すことがわかった．

Keywords: light irradiation, fungi, action spectrum

*共立女子大学

**Cornell University

菊池裕, 高鳥浩介, 伊藤均*1, 小沼博隆：**低線量放射線による微生物毒素産生能の変化に関する研究1 ベロ毒素を産生する腸管出血性大腸菌*E. coli* O157:H7に及ぼす影響**

食品照射, **36**, 23-25 (2001)

The effect of irradiation was examined on the sensitivity and verotoxin production of *Escherichia coli* O157:H7, using 13 clinical isolates. All isolates were sensitive to gamma irradiation compared with normal type *E. coli*. D10 values of 0.05-0.1 kGy were obtained in 0.067 M phosphate buffer at room temperature in an air-equilibrium atmosphere. Irradiation at 0.1 kGy produced no noticeable effect on verotoxin production in any isolates.

Keywords: irradiation, verotoxin, *Escherichia coli* O157:H7

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Hanna, A.N.*1, Berthiaume, L.G.*1, Kikuchi, Y., Begg, D.*1, Bourgoin, S.*2, Brindley, D.N.*1: **Tumor necrosis factor-alpha induces stress fiber formation through ceramide production: Role of sphingosine kinase**

Mol. Biol. Cell, **12**, 3618-3630 (2001)

Tumor necrosis factor-alpha (TNF-alpha) is a proinflammatory cytokine that activates several signaling cascades. We determined the extent to which ceramide is a second messenger for TNF-alpha-induced signaling leading to cytoskeletal rearrangement in Rat2 fibroblasts. TNF-alpha, sphingomyelinase, or C2-ceramide induced tyrosine phosphorylation of focal adhesion kinase (FAK) and paxillin, and stress fiber formation. Ly 294002, a phosphatidylinositol 3-kinase (PI 3-K) inhibitor, or expression of dominant/negative Ras (N17) completely blocked C2-ceramide-and sphingomyelinase-induced tyrosine phosphorylation of FAK and paxillin and severely decreased stress fiber formation. The TNF-alpha effects were only partially inhibited. Dimethylsphingosine, a sphingosine kinase (SK) inhibitor, blocked stress fiber formation by TNF-alpha and C2-ceramide. TNF-alpha, sphingomyelinase, and C2-ceramide translocated Cdc42, Rac, and RhoA to membranes, and stimulated p21-activated protein kinase downstream of Ras-GTP, PI 3-K, and SK. Transfection with inactive RhoA inhibited the TNF-alpha-and C2-ceramide-induced stress fiber formation. Our results demonstrate that stimulation by TNF-alpha, which increases sphingomyelinase activity and ceramide formation, activates sphingosine kinase,

Rho family GTPases, focal adhesion kinase, and paxillin. This novel pathway of ceramide signaling can account for approximately 70% of TNF-alpha-induced stress fiber formation and cytoskeletal reorganization.

Keywords: Tumor necrosis factor-alpha; Stress fibers; sphingosine kinase

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Sera, N.*1, Fukuhara, K., Miyata, N., Tokiwa, H.*2: **Micronucleus induction and chromosomal aberration of 1-and 3-nitroazabenzo[a]pyrene and their N-oxides**
Mutagenesis, **16**,183-187(2001)

Nitro-azabenzo[a]pyrenes, 1-or 3-nitro-azabenzo [a] pyrene and their N-oxides are nitrated derivatives of azabenzo[a]pyrene (ABP) containing nitrogen in the 6-position of benzo[a]pyrene (B[a]P). The nitro-ABP-N-oxides (ABPOs) were formed by reaction of ABP with excess HNO_3. These derivatives were noteworthy as potent mutagens for Salmonella strains, and were present in fine particles of diesel particulates. In this study, micronucleus induction in mice and chromosomal aberrations due to means of Chinese hamster lung fibroblast (CHL) cells were investigated to determine genotoxicity in order to define the relationship with the mutagenic potency of these derivatives. The induction of micronucleus polychromatic erythrocytes (MNPCEs) was dependent on the dose response of 10-40 mg for 3-N-6-ABP, and of 10-40 mg for 1-N-6-ABP, and in addition, 1-and 3-N-6-ABPOs markedly induced MNPCEs in a dose range of 10-400 mg and from 1 to 80 mg, respectively, when the compound was intraperitoneally administrated in two mice at each dose. The results show that of the four compounds, 3-N-6-ABPO demonstrated a marked increase in MNPCEs. On the other hand, chromosomal aberrations of the four compounds were investigated by the duplicate tests using CHLs. The results after a 48 h treatment induced aberrations of the chromatid type, chromatid breaks and exchanges for 1-and 3-N-6-ABP, and mainly chromatid exchanges for 1-and 3-N-6-ABPO. The frequency of chromosomal aberrations associated with nitro substitution on the ABPO structure. Chromosomal aberrations of nitro derivatives of ABPO substituted at the 3-position on the structure were more potent than those at the 1-position. N-oxide derivatives have been found to be reduced to anion radicals much more easily than azaB[a]P and its nitro derivatives. This suggest that the electrochemical reduction of the chemicals plays an important role in the metabolic activation of nitrated B[a]P derivatives.

Keywords: benzo[a]pyrene, micronucleus induction, chromosomal aberration

*1 Fukuoka Institute of Health and Environmental Science
*2 Kyushu Women's University

Fukuhara, K., Kurihara, M., Miyata, N.: **Photochemical generation of nitric oxide from 6-nitrobenzo[a]pyrene**
J. Am. Chem. Soc., **123**, 8662-8666 (2001)

Photolabile, 6-nitrobenzo[a]pyrene (6-nitroBaP) released nitric oxide (NO) under visible-light irradiation. The generation of NO and the concomitant formation of the 6-oxyBaP radical were confirmed by ESR. BaPquinones were also detected as further oxidized products of the 6-oxyBaP radical. No such photogeneration was observed with other nitrated BaPs, such as 1-nitroBaP and 3-nitroBaP. DNA-strand breakage, caused by photoexcited 6-nitroBaP, was closely related to its NO-releasing activity. MO calculations of nitrated BaP suggest that the perpendicular conformation of the nitro substituent to the aromatic ring is important for the release of NO with light. These finding may be useful for the development of a new type of NO donor.

Keywords: nitric oxide, nitrobenzo[a]pyrene, DNA scission

Nakanishi, I., Fukuhara, K., Ohkubo, K.*1, Shimada, T.*2, Kansui, H., Kurihara, M., Urano, S.*2, Fukuzumi, S.*1, Miyata, N.: **Superoxide anion generation via electron-transger oxidation of catechin dianion by molecular oxygen in an aprotic medium**
Chem. Lett. 1152-1153 (2001)

Superoxide anion ($O_2^{\cdot-}$) was generated via an electron transfer oxidation of catechin dianion, which was produced in the reaction of catechin with two equivalents of methoxide anion, by molecular oxygen in acetnitrile. From the detailed spectroscopic and kinetic analysis was determined the rate constant for the formation of $O_2^{\cdot-}$ to be 5.8×10^{-2} $mol^{-1}dm^3s^{-1}$.

Keywords: catechin, superoxide anion, ESR

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*2 Shibaura Institute of Technology

Nishio, T*1, Hakamata, W, Kimura, A*2, Chiba, S*2, Takatsuki, A*3, Kawachi, R*1, Oku, T*1: **Glycon specificity profiling of α-glucosidases using monodeoxy**

and mono-*O*-methyl derivatives of *p*-nitrophenyl α-D-glucopyranoside

Carbohydr. Res., **337** 629-634 (2002)

Hydrolysis of probe substrates, eight possible monodeoxy and mono-*O*-methyl analogs of *p*-nitrophenyl α-D-glucopyranoside (*p*NP α-D-Glc), modified at the C-2, C-3, C-4, and C-6 positions, was studied as part of investigations into the glycon specificities of seven α-glucosidases (EC 3.2.1.20) isolated from *Saccharomyces cerevisiae, Bacillus stearothermophilus*, honeybee (two enzymes), sugar beet, flint corn, and *Aspergillus niger*. The glucosidases from sugar beet, flint corn, and *A. niger* were found to hydrolyze the 2-deoxy analogs with substantially higher activities than against *p*NP α-D-Glc. Moreover, the flint corn and A. niger enzymes showed hydrolyzing activities, although low, for the 3-deoxy analog. The other four α-glucosidases did not exhibit any activities for either the 2- or the 3-deoxy analogs. None of the seven enzymes exhibited any activities toward the 4-deoxy, 6-deoxy, or any of the methoxy analogs. The hydrolysis results, with the deoxy substrate analogs, demonstrated that α-glucosidases having remarkably different glycon specificities exist in nature. Further insight into the hydrolysis of deoxyglycosides was obtained by determining the kinetic parameters (k_{cat} and K_m) for the reactions of sugar beet, flint corn, and A. niger enzymes.

Keywords: α-Glucosidase, Substrate specificity, Glycon specificity profiling

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Fujishima, T.*1, Konno, K.*1, Nakagawa, K.*2, Tanaka, M.*2, Okano, T.*2, Kurihara, M., Miyata, N., Takayama, H.*1: **Synthesis and Biological Evaluation of 5,6-trans-2-Methyl-1,25-dihydroxyvitamin D3 and their 20-Epimers: Possible Binding Modes of Potent A-Ring Analogues to Vitamin D Receptor**

Chem. Biol., **8**, 1011-1024 (2001)

The secosteroid 1alpha, 25-dihydroxyvitamin D(3) (1) has a wide variety of biological activities, which makes it a promising therapeutic agent for the treatment of cancer, psoriasis and osteoporosis. Insight into the structure-activity relationships of the A-ring of 1 is still needed to assist the development of more potent and selective analogues as candidate chemotherapeutic agents, as well as to define the molecular mode of action.Results: All possible A-ring stereoisomers of 5,6-trans-2-methyl-1, 25-dihydroxyvitamin D(3) (6a-h) and their 20-epimers (7a-h) were designed and efficiently synthesized. The dependence of the affinities for vitamin D receptor (VDR) and vitamin D binding protein (DBP), as well as the HL-60 cell differentiation-inducing activity, upon the stereochemistry of the A-ring and at C20 in the side chain was evaluated.Conclusions: The binding affinities and potency of the 5,6-trans and 5,6-cis analogues were enhanced by a 2-methyl substituent in a certain orientation. Molecular docking studies based upon the X-ray crystal structure of VDR suggested that the axial 2-methyl group would be accommodated in a pocket surrounded by hydrophobic amino acid residues in the ligand binding domain, resulting in enhanced interaction.

Keywords: 5,6-trans-2-methyl-1, 25-dihydroxyvitamin D3, vitamin D receptor, binding modes

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Suhara, Y.*1, Nihei, K.*1, Kurihara, M., Kittaka, A.*1, Fujishima, T.*1, Konno, K.*1, Miyata, N., Takayama, H.*1: **Efficient and Systematic Synthesis of Novel 2alpha-Substituted 1alpha,25-Dihydroxyvitamin D3 Analogues and Docking Analysis to Vitamin D Receptor**

J. Org. Chem., **66**, 8760-8771 (2001)

Novel 2alpha-substituted 1alpha, 25-dihydroxyvitamin D(3) analogues with 2alpha-alkyl and 2alpha-hydroxyalkyl groups were systematically synthesized from D-xylose. Their conformation on binding to the ligand binding domain (LBD) of the vitamin D receptor was analyzed. It has been found that the 2alpha-hydroxypropyl group best fits the cavity of the LBD, and the binding activity is three times higher than that for the natural hormone.

Keywords: 2alpha-substituted 1alpha, 25-dihydroxyvitamin D3 analogues, docking analysis, vitamin D receptor

*1 帝京大学薬学部

Kurihara, M., T. Hayashi, T., Miyata, N.: **Enantioselective Radical Cross-coupling Reactions of Silyl Enol Ethers Using Chiral Oxovanadium**

Chem. Lett., **2001**, 1324-1325

Asymmetric induction was observed in radical cross-coupling reactions of silyl enol ethers using chiral oxovanadium generated in situ from 8-phenylmenthol and vanadium oxytrichloride in the presence of

MS4A(molecular sieves-4A).
Keywords: asymmetric synthesis, enantioselective radical cross-coupling reactions, chiral oxovanadium

Kittaka, A.*1, Takayama, H.*1, Kurihara, M., Horii, C.*2, Tanaka, H.*2, Miyasaka, T.*2, J. Inoue, J.*3: **DNA Sequence Recognition by NFkB p50 Homodimer: Strict and Obscure Recognition Sites in the Binding Sequence**
Nucleosides Nucleotides & Nucleic Acids, **20**, 669-672 (2001)

5-Formyl-and 5-(formylmethyl)-2'-deoxyuridines are introduced into a kB site instead of thymidine(s) in order to understand target sequence specificity of NFkB. It was found that one thymidine in the kB site is particularly important for the sequence specific recognition by NFkB.
Keywords: NFkB, DNA sequence recognition
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*3 慶應義塾大学理工学部

Kurihara, M., Kondo, K., Toyoda, M., N. Miyata: **Antioxidation Mechanisms of Catechins: A Computational Study**
JCPE Journal, **13**, 255-262 (2001)

Catechins are a group of polyphenolic compounds abundantly contained in green tea. It is well known that catechins have multiple biological activities including anticarcinogenic and antiinflammatory effects. These protective effects are due to their antioxidative activities by scavenging free radicals. All C-H and O-H bond dissociation enthalpies (BDE's) in catechins ((-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate) were calculated by semiempirical molecular orbital method using SPARTAN program. The BDE's of benzyl hydrogens (C-2 position in catechins) are found to be quite low. This result suggests that abstraction of benzyl hydrogen is a crucial step for antioxidant activity. This is also supported by the reported results of LC/MS/MS and spectrophotometric analysis of reaction intermediate from catechins treated with AAPH.
Keywords: catechins, antioxidative activities, semiempirical molecular orbital calculation

Kurihara, M., Tanaka, M.*, Oba, M.*, Suemune, H.*, Miyata, N.: **Conformation of Oligopeptides Containing Chiral alpha,alpha-Disubstituted Amino Acids: Computational Study**
Peptide Science 2001, 287-288 (2002)

We have shown conformational search calculations of oligopeptides prepared from alpha, alpha-disubstituted amino acids were in agreement with their conformational properties in the solid state determined by X-ray crystallographic analysis of oligopeptides. In this paper we show it can be predicted the helical screw sense of oligopeptides containing a chiral alpha, alpha-disubstituted amino acid by computational study.
Keywords: oligopeptides, conformational search calculation, helical screw sense
*九州大学薬学部

Tanaka, M.*, Oba, M.*, Kurihara, M., Demizu, Y.*, Nishimura, S.*, Hayashida, K.*, Suemune, H.: **Asymmetric Synthesis and Conformational Analysis of alpha, alpha-Disubstituted alpha-Amino Acids and Their Peptides**
Peptide Science 2001, 263-266 (2002)

We have developed a practical procedure for the preparation of various optically active alpha, alpha-disubstituted alpha-amino acids using (S,S)-or (R,R)-cyclohexane-1,2-diol as a chiral auxiliary. Homo-and heteropeptides containing the chiral alpha, alpha-disubstituted alpha-amino acids, such as (S)-butylethylglycine (Beg) and (S)-ethylleucine (EtLeu), have been prepared by solution-phase methods. The preferred conformations of (S)-Beg homopeptides, and the heteropeptides containing (S)-Beg or (S)-EtLeu within the sequence of Aib residues have been studied using X-ray crystallographic analysis, IR, 1H NMR, and CD spectra.
Keywords: chiral auxiliary, alpha_alpha-disubstituted alpha-amino acid, asymmetric synthesis
*九州大学薬学部

Fujishima, T.*1, Konno, K.*1, Nakagawa, K.*2, Okano, T.*2, Kittaka, A.*1, Kurihara, M., Takayama, H*1.: **2-Methyl analogues of 1alpha, 25-dihydroxyvitamin D3, the potent inducers of cell differentiation and apoptosis: synthesis and biological evaluation**
Anti-Cancer Drugs, **13**, 10 (2002)

The hormonally active metabolite of vitamin D, 1alpha, 25-dihydroxyvitamin D_3 (1), has a wide variety of biological activities, which makes it a promising therapeutic agent for the treatment of cancer, psoriasis and osteoporosis. Insight into the structure-activity

relationships of the A-ring of 1 is needed to assist the development of more potent and selective analogues, as well as to define the molecular mode of action. We have synthesized all eight possible A-ring stereoisomers of 2-methyl-1, 25-dihydroxyvitamin D_3, demonstrating that the introduction of a simple methyl group to the A-ring of 1 yields the analogues with unique activity profiles. In particular, 2alpha-methyl-1alpha, 25-dihydroxyvitamin D_3 (2) showed 4-fold higher affinity for bovine thymus VDR and 2-fold higher cell differentiation-inducing activity towards HL-60 cells in comparison with 1. The eight 2-methyl analogues, which differ in stereochemistry of the methyl group on C2 and the hydroxyl groups on C1 and C3, exhibited cell differentiation-or apoptosis-inducing activity towards HL-60 cells, depending on the A-ring structure. Further 20-epimerization of the 2-methyl analogues enhanced the VDR binding affinity and cell differentiation-inducing potency. The 20-epimer of 2, 20-epi-2alpha-methyl-1alpha, 25-dihydroxyvitamin D_3 (3), proved to be a 12-fold better binder to VDR and its cell differentiation-inducing activity was 590-fold higher than 1. Molecular docking studies based upon the X-ray crystal structure of VDR suggested the importance of the 2-methyl substitution in a certain orientation. The detailed biological activities and molecular docking studies of the analogues will be presented, together with the results of the 5,6-trans-2-methyl analogues.

Keywords: vitamin D_3, cell differentiation-inducing activity, molecular docking study

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*2 神戸薬科大学

Kittaka, A.*, Suhara, Y.*, Fujishima, T.*, Kurihara, M., Konno, K.*, Takayama, H.*: **The 2alpha-positive motifs of 1alpha, 25-dihydroxyvitamin D_3 in VDR binding**

Anti-Cancer Drugs, **13**, 14-15(2002).

The seco steroid hormone 1alpha, 25-dihydroxyvitamin D_3 (1) is the most potent metabolite of vitamin D_3 and regulates primarily calcium and phosphorus homeostasis, as well as proliferation and differentiation of cells. Some of synthetic analogues of 1 have been clinically used in the treatment of calcium and bone diseases, secondary hyperparathyroidism, and skin disorder psoriasis. Current frontiers of vitamin D research efforts also include oncology, especially breast cancer, prostate cancer, colon cancer, and leukemia to develop new drugs for clinical applications.

Most of the biological actions of 1 are mediated through its specific receptor, the vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily acting as a ligand-dependent transcription factor with coactivators. We believe that studies of structure-activity relationships on binding affinity and binding mode of the newly synthetic ligands for VDR, the latter would alter a 3-dimensional shape of the ligand-VDR complex and the change would ultimately affect selectivity of coactivators, are essentially important for finding novel vitamin D_3 analogues that show desired biological actions.

Recently, we have found several structural modifications at the 2alpha-position on the A-ring of 1 that strengthen binding affinity for VDR. The representative positive motifs are 2 alpha-methyl (1a), 2alpha-(3-hydroxypropyl) (1b), and 2alpha-(3-hydroxypropoxy) (1c) groups.

Introduction of one of these motifs to 1 showed ca. 2-to 4-fold higher binding affinity for bovine thymus VDR than that of the natural hormone (1). We present effective synthetic routes to these vitamin D_3 analogues and binding affinity with computational binding modes for each derivative in the VDR ligand binding domain.

Keywords: vitamin D_3, vitamin D receptor, molecular docking study

*帝京大学薬学部

Yamakoshi, Y., Schlittler, R.R.*1, Gimzewski, J.K.*1 and Diederich, F.*2: **Mecanoreceptor: Synthesis of Molecular-Gripper-Type Dynamic Receptors and STM Imaging of Self-Assembled Monolayer on Gold**

J. Mat. Chem., **11**, 2895-2897(2001)

Dynamic receptors capable of undergoing large temperature or pH-dependent conformational change were functionalized with dialkyl thioether legs and adsorbed on Au(111) single crystal surfaces to give self-assembled monolayers(SAMs). The SAMs were characterized by ellipsometry and contact angle measurements. Imaging by scanning tunneling microscopy(ATM) revealed well-ordered monolayers at molecular resolution.

Keywords: molecular gripper, dynamic receptor, scanning tunneling microscopy(STM), self-assembled monolayer (SAM)

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Takahashi, T.*, Yamakoshi, Y., Ge, W.-Y.*, Sugita, J.*, Okayama, K.* and Koizumi, T.*: **High-Pressure Mediated Asymmetric Diels-Alder Reaction of Chiral Sulfinyl Acrylate Derivatives and Its Application to Chiral Synthesis of (-)-COTC and (-)-Gabosine C**

Heterocycles, **55**, 209-220(2002)

The asymmetric Diels-Alder reactions of chiral sulfinylacrylater derivatives with dienes were examined under high-pressure (1.2 Gpa) conditions. The *endo* cycloadduct obtained from sulfinyl acrylate and 2-mthoxyfuran was converted to (-)-COTC and (-)-gabosine C.

*富山医科薬科大学薬学部

Nakamura, T., Saito, Y., Murayama, N., Saeki, M., Soyama, A., Ozawa, S., Sawada, J.: **Apparent low frequency of sequence variability within the proximal promoter region of the cytochrome P450 (CYP) 3A5 gene in established cell lines from Japanese individuals**

Biol. Pharm. Bull., **24**, 954-957 (2001)

The members of the cytochrome P450 (CYP) 3A subfamily play an important role in the metabolism of more than 50% of the drugs metabolized by CYPs. Among the CYP3As, CYP3A5 is known to exhibit polymorphic expression. We hypothesized that this might be caused by single nucleotide polymorphisms in the promoter region of CYP3A5 gene. Due to the existence of highly homologous sequence, CYP3A5P1, we carefully amplified and sequenced the CYP3A5 promoter region from 86 established cell lines derived from Japanese individuals. However, no SNP was detected in the region. These results suggest that other factors is involved in the polymorphism of CYP3A5 expression.

Keywords: CYP3A5, Promoter, Single nucleotide polymorphisms

Soyama, A., Saito, Y., Hanioka, N., Murayama, N., Nakajima, O., Katori, N., Ishida, S., Sai, K., Ozawa, S., Sawada, J.: **Non-synonymous single nucleotide alterations found in the CYP2C8 gene result in reduced paclitaxel metabolism**

Biol. Pharm. Bull., **24**, 1427-1430 (2001)

By sequencing genomic DNA from 73 established cell lines derived from Japanese individuals, we detected 9 single nucleotide polymorphisms (SNPs) in the CYP2C8 gene. Of them, 3 exonic SNPs resulted in amino acid alterations (g416a, R139K; a1196g, K399R; c1210g, P404A). To examine the effects of these amino acid alterations on CYP2C8 function, wild-type and four types of variant CYP2C8 cDNA constructs (R139K, K399R, R139K/K399R and P404A) were transfected into Hep G2 cells and their paclitaxel 6α-hydroxylase activities were determined in vitro. The variant R139K/K399R showed reduced values for Vmax and clearance (Vmax/Km) similar to those of its single variant, R139K. The variant P404A also showed a significantly lowered clearance due to reduced level of protein expression. These results suggest that not only the double variant (R139K/K399R) but also our novel variant P404A in the CYP2C8 gene are less efficient in paclitaxel metabolism.

Keywords: CYP2C8, Paclitaxel, Single nucleotide polymorphism s

Stronge, V.*1, Saito, Y., Ihara, Y.*2, Williams, D.B.*1: **Relationship between calnexin and Bip in suppressing aggregation and promoting refolding of protein and glycoprotein substrates**

J. Biol. Chem., **276**, 39779-39787 (2001)

Calnexin (CNX) is a lectin and chaperone protein of endoplasmic reticulum. We assess the relative contributions of the oligosaccharide-and polypeptide-binding sites of CNX to its in vitro chaperone function by comparing it with Bip. Both proteins were equally effective in preventing the aggregation of non-glycosylated citrate synthase, indicating the polypeptide-binding site of CNX is capable of functioning at a similar to that of Hsp70. But when confronted with glycoprotein substrates, the lectin site of CNX provided a significant advantage over Bip in suppressing aggregation. CNX also cooperated with Bip and J domain of Sec63 in the ATP-dependent refolding of glycoprotein and non-glycoprotein. The lectin site of CNX was essential for refolding of the glycoprotein.

Keywords: Bip, Calnexin, Folding

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Nakamura, R., Teshima, R., Sawada, J.: **Effect of dialkyl phthalates on the degranulation and Ca^{2+} response of RBL-2H3 mast cells**

Immunol. Lett., **80**, 119-124 (2002)

We examined the effect of three dialkyl phthalates, di-n-butylphthalate (DBP), di-isobutylphthalate (DIBP) and di(2-ethylhexyl)phthalate (DEHP), on antigen-induced degranulation of RBL-2H3 mast cells. Exposure

to 50-500 μM DBP, 50-500 μM DIBP, and 500 μM DEHP significantly potentiated antigen-induced β-hexosaminidase release. Without antigen stimulation, the phthalates did not cause any significant increase in degranulation. Next, we examined the Ca^{2+} response of RBL-2H3 cells after exposure to these phthalates. The cytosolic calcium ion concentration ($[Ca^{2+}]_i$) of the cells clearly increased when the cells were stimulated with 50-500 μM and 50-500 μM DIBP, and increased slightly when stimulated with 50-500 μM DEHP. Digital imaging fluorescence microscope analysis showed that the addition of DBP evoked Ca^{2+} oscillation in individual mast cells. Finally, we investigated the relationship between the DBP-sensitive Ca^{2+} stores and thapsigargin (TG)-sensitive Ca^{2+} stores. A rise in $[Ca^{2+}]_i$ following challenge with DBP after TG was observed, and thus the DBP-sensitive and TG-sensitive Ca^{2+} stores in RBL-2H3 cells seem to be different. In conclusion, some dialkyl phthalates increase antigen-induced degranulation in RBL-2H3 cells dependent on the increase of $[Ca^{2+}]_i$.

Keywords: Dialkyl phthalates, Mast cells, Ca^{2+} response

Okunuki, H., Teshima, R., Shigeta, T., Sakushima, J., Akiyama, H., Goda, Y., Toyoda, M., Sawada, J.: **Increased digestibility of two products in genetically modified food (CP4-EPSPS and Cry1Ab) after preheating**

J. Food Hyg. Soc. Japan., **43**, 68-73 (2002)

We performed in vitro digestion experiments of newly expressed proteins by SGF (simulated gastric fluid) and SIF (simulated intestinal fluid) to assess the allergenicity of foods derived from biotechnology. For newly expressed proteins, we chose CP4-EPSPS (5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium sp.* strain CP4) and Cry1Ab derived from *Bacillus thuringiensis subsp. kurstaki* strain HD-1. The former is expressed in GM-soybeans and the latter is expressed in GM-corns. Both proteins were rapidly digested within 60 sec by SGF. By preheating, the digestibility by SGF was slightly increased. The digestion time of CP4-EPSPS and Cry1Ab by SIF was 240 min or more. However, digestibility of these proteins by SIF was dramatically increased by preheating and digestion time was less than 5 sec. Therefore, we suggest that the allergenicity of both proteins was extremely low because of the easy digestibility of these proteins by SGF and that by SIF by preheating.

Keywords: SIF, SGF, Digestibility

Takagi, K., Saito, Y., Sawada, J.: **Proteasomes are involved in the constitutive degradation of growth hormone receptors**

Biol. Pharm. Bull., **24**, 744-748 (2001)

In the mouse Ba/F3-hGHR cell line, which stably expresses human growth hormone receptors (hGHRs), constitutive degradation of hGHRs was inhibited by the proteasome inhibitors MG-132 and *clasto*-lactacystin β-lactone, or the vacuolar H^+-ATPase inhibitor, bafilomycin A_1. hGH-enhanced degradation and internalization of hGHRs were also inhibited by MG-132. Ubiquitinated hGHRs were detected in the cell lysate and increased by hGH-treatment. In the hGH-untreated cells, the ubiquitinated hGHRs were weakly detected. However, the ubiquitination of hGHR was not enhanced by MG-132. Thus, the ubiquitination of hGHR is unlikely to be involved, at least in the constitutive degradation.

Taken together, both the proteasome pathway and endosome/lysosome pathway are involved in the constitutive degradation of hGHRs.

Keywords: Human growth hormone receptor, Constitutive degradation, Proteasome inhibitor

Takagi, K., Saito, Y., Sawada, J.: **Proteasome inhibitor enhances growth hormone-binding protein release**

Mol. Cell. Endocrinol., **182**, 157-163 (2001)

We used murine Ba/F3 cells transfected with human growth hormone receptor (hGHR) cDNA to investigate the regulatory mechanisms of human growth hormone-binding protein (hGH-BP) release. The extracellular domain of hGHRs were cleaved and released as hGH-BPs. The hGH-BP release was enhanced by phorbol 12, 13-dibutyrate (PDBu), and suggested to be mediated by activation of PKC. The proteasome inhibitors MG-132 and *clasto*-lactacystin β-lactone also increased hGH-BP release from Ba/F3-hGHR cells, and MG-132 and PDBu synergistically increased hGH-BP release. The results obtained by using three PKC inhibitors Gö6976, GF109203X and Gö6983 suggest that the enhancement of hGH-BP release by MG-132 and PDBu is mediated by different mechanisms probably involving different PKC isozymes.

Keywords: Human growth hormone receptor, Human growth hormone-binding protein, Proteasome inhibitor

Prasad, S.S.[*1], Kojic, L.Z.[*1], Li, P.[*1], Mitchell, D.E.[*2], Hachisuka, A., Sawada, J., Gu, Q.[*1], Cynader, M.S.[*1]:

Gene expression patterns during enhanced periods of visual cortex plasticity

Neuroscience, **111**, 35-45 (2002)

Plasticity represents an integrated set of developmental processes that coordinates the action of many genes. We examined gene expression patterns of 18371 non-redundant cDNAs in the visual cortex of cats at birth, at eye opening, at the peak of eye dominance plasticity and in the adult cat using filter-based cDNA arrays and software-based hierarchical cluster analysis. We identified a small set of genes that were selectively expressed during the peak of the critical period for plasticity. These plasticity candidate genes that have characterized functions include participants in second messenger systems, in cell adhesion, in transmitter recycling and cytokines, among others. Comparison of cDNA array quantitation with RPC reaction showed almost identical expression profiles for three genes. The expression pattern of one identified gene, opioid binding cell adhesion molecule, is also in agreement with immunocytochemical results. We conclude that the approach of high-density cDNA array hybridization can be used as a useful tool for examining a complex phenomenon of developmental plasticity.

Keywords: Development, Plasticity, Gene expression

*1 University of British Columbia
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Furukawa, Y.*, Furuno, T.*, Teshima, R., Nakanishi, M.*: **Calcium signals in rat basophilic leukemia (RBL-2H3) cells primed with the neuropeptide substance P**

Biol. Pharm. Bull., **24**, 1060-1063 (2001)

Communication between nerves and mast cells is a prototypic demonstration of neuroimmune interaction. We have recently shown that direct nerve-mast cell cross-talk can occur in the absence of an intermediary transducing cell and that the neuropeptide substance P is an important mediator of this communication. Here we study the calcium signals in rat basophilic leukemia cells (RBL-2H3; mucosal-type mast cells) primed with substance P. RBL cells responded only slightly to stimulation with compound 48/80, however they responded to the stimulation when the cells had been primed with substance P (0.5 microM) for one week. The present results provide a foundation to study the neuroimmune cross-talk in a co-culture system.

Keywords: RBL-2H3 cells, Substance P, Calcium signals

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Yamaguchi, M.*1, Hirai, K.*1, Komiya, A.*1, Miyamasu, M.*1, Furumoto, Y.*1, Teshima, R., Ohta, K.*2, Morita, Y.*1, Galli, S.J.*3, Ra, C.*4, Yamamoto, K.*1 : **Regulation of mouse mast cell surface Fc epsilon RI expression by dexamethasone**

Int. Immunol. **13**, 843-851 (2001)

It is now clear that the mast cell's functional response to IgE-dependent stimulation can be influenced significantly by the level of expression of the high-affinity IgE receptor (Fc epsilon RI) on the cell's surface. Thus, modulation of Fc epsilon RI surface expression represents a potentially important mechanism for regulating mast cell activity in allergic reactions. In this study, we examined whether a glucocorticoid, dexamethasone (DEX), can influence levels of mast cell Fc epsilon RI expression either in the presence or absence of IgE, an up-regulator of the mast cell surface Fc epsilon RI level. In the absence of IgE, DEX decreased the surface Fc epsilon RI levels in mouse peritoneal mast cells, mouse bone marrow-derived cultured mast cells and a mouse mast cell line, Cl.MC/C57.1. Moreover, DEX also partially suppressed the ability of IgE to enhance surface expression of Fc epsilon RI in these cells. Three different glucocorticoids, DEX, methylprednisolone and hydrocortisone, suppressed Fc epsilon RI expression in mast cells. On the other hand, DEX did not affect levels of Fc epsilon RI alpha, beta or gamma mRNA, suggesting that its ability to decrease surface Fc epsilon RI reflects a post-transcriptional mechanism. These results show that mast cell surface Fc epsilon RI expression is suppressed by glucocorticoids in both the presence and absence of IgE, and suggest that reduction of mast cell surface Fc epsilon RI levels may be one of the favorable anti-allergic actions of glucocorticoids.

Keywords: Dexamethasone, IgE receptor, Mouse mast cell

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Matsui, S.*1, Matumoto, S.*1, Adachi, R., Kusui, K., Hirayama, A.*1, Watanabe, H.*1, Ohashi, K.*2, Mizuno, K.*2, Yamaguchi, T., Kasahara, T.*2, and Suzuki, K.: **LIM Kinase 1 Modulates Opsonized Zymosan-triggered Activation of Macrophage-like U937 Cells. Possible Involvement of Phosphorylation of Cofilin and**

Reorganization of Actin Cytoskeleton

J. Biol. Chem., **277**, 544-549 (2002)

In dormant phagocytes, the endogenous LIMK1 was diffusely distributed in the cytosol of macrophage-like U937 cells and, when activated by opsonized zymosan (OZ), it was translocated to plasma membranes. Green fluorescence protein (GFP)-conjugated LIMK was expressed in the phagocytes, and the GFP-positive cells were isolated by a fluorescence-activated cell sorter. The isolated wild-type LIMK-overexpressing cells produced superoxide at a rate that was 3.2-fold higher than that of only GFP-expressing control cells, while the respiratory burst of dominant negative LIMK1(D460A)-expressing cells decreased to 31% of that of the control cells. Phagocytic activity monitored by using Texas Red-labeled OZ was also decreased in the D460A-expressing cells. Phosphorylation of cofilin was dependent on the types of expressing LIMK1. Furthermore, in the wild-type LIMK 1-expressing cells, OZ-evoked increase in filamentous actin was markedly enhanced, while in the dominant negative LIMK 1-expressing cells, the total level of F-actin was strongly suppressed. These results suggest that LIMK1 regulates the functions of phagocytes through phosphorylation of cofilin and enhances the formation of filamentous actin.

Keywords: LIM kinase, Cofilin, Macrophage

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Ono, K., Masumiya, H.*1, Sakamoto, A.*2,3, Christe, G.*4, Shijuku, T.*1, Tanaka, H.*1, Shigenobu, K.*1 and Ozaki, Y.: **Electrophysiological analysis of the negative chronotropic effect of endothelin-1 in rabbit SA node cells.**

J. Physiol., **537**, 467-488 (2001)

Electrophysiological effects of endothelin-1 (ET-1) were studied in rabbit sinoatrial node (SAN) using conventional microelectrode and whole-cell voltage and current recordings. In rabbit SAN, RT-PCR detected ET_A endothelin receptor mRNA. ET-1 (100 nM) increased the cycle length of action potentials (APs) from 305 ± 15 to 388 ± 25 ms, which was antagonized by the ET_A receptor-selective antagonist BQ-123 (1 μM). ET-1 increased AP duration (APD_{50}) by 22 %, depolarized the maximum diastolic potential (MDP) by +6 mV, shifted the take-off potential by +5 mV and decreased the pacemaker potential (PMP) slope by 15 %. In the same experimental conditions, ET-1 caused a positive chronotropic effect in guinea-pig SAN with a 13 % decrease in APD_{50}, a-4 mV shift in the take-off potential and an 8 % increase in the PMP slope. Rabbit SAN exhibited two major cell types, distinguished by both their appearances and electrophysiological responses to ET-1. Whereas spontaneous pacing rate and the PMP slope were similarly decreased by ET-1 (10 nM) in both cell types, ET-1 depolarized MDP from by +5 mV in spindle-shaped cells but hyperpolarized it by -8 mV in rod-shaped cells. ET-1 decreased APD_{50} by 8 and 52 % and shifted the take-off potential by +5 and -9 mV in spindle- and rod-shaped cells, respectively. ET-1 decreased the high-threshold calcium current I_{CaL} by about 50 % in both cell types, without affecting its voltage-dependence, and decreased I_K, with significant shifts in its voltage-dependence by +4.7 and +14.0 mV in spindle- and rod-shaped cells, respectively. It was exclusively in rod-shaped cells that ET-1 activated a sizeable amount of time-independent inward-rectifying current. I_f, observed exclusively in spindle-shaped cells, was significantly increased by ET-1 at membrane potentials between -74.7 and -84.7 mV whereas it was significantly decreased at more negative potentials. ET-1 significantly decreased slope of the I-*V* relation of I_f tail without changing its half maximum voltage. The negative chronotropic response of the whole rabbit SAN to ET-1 would result from its diverse actions on spindle- and rod-shaped cells in this area.

Keywords: endothelin-1, sinoatrial node, chronotropic

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Sato, Y., Schmidt, A.G.*1, Kiriazis, H.*1, Hoit, B.D.*2, and Kranias, E.G.*1: **Re-evaluation of heart failure in transgenic mice with impaired SR Ca^{2+} release**

J. Mol. Cell. Cardiol. **33**, 1757-1759 (2001)

Impaired Ca^{2+} release from the sarcoplasmic reticulum (SR) has been associated with cardiac morbidity in cardiac hypertrophy and cardiomyopathy. However, it is not currently clear whether a primary defect in SR Ca^{2+} release drives the progressive deterioration of function, leading to heart failure. Recently, transgenic mice with cardiac specific overexpression of dog (dCSQOE) and mouse (mCSQOE) calsequestrin were created by Jones et al. and our laboratory, respectively. In both models, SR Ca^{2+} loading capacity is enhanced, but SR Ca^{2+} release is

depressed leading to attenuation of Ca^{2+} transients and contractile parameters, compared to isogenic wild-types. The dCSQOE model is also characterized by dilated cardiomyopathy and premature death, suggesting that an increase in cardiac calsequestrin and/or concomitant defects in SR Ca^{2+}-release develop dilated cardiomyopathy. However, our recent studies indicate that cardiac overexpression of calsequestrin per se does not result in heart failure. Therefore, the heart failure phenotype of the dCSQOE model may not be related to the primary action of calsequestrin overexpression, but could rather be consequences of distinct etiologies. Thus, in establishing a link between alterations in cellular function and geometry with transgenesis, one must carefully distinguish between primary and secondary effects of the transgene, and evaluate the modifier alleles of the strain used.

Keywords: cardiac hypertrophy, heart failure, sarcoplasmic reticulum

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Frank, K.F.*1, Mesnard-Rouiller, L.*1, Chu, G.*1, Young, K.B.*1, Zhao, W.*1, Haghighi, K.*1, Sato, Y., and Kranias, E.G.*1: **Structure and expression of the mouse cardiac calsequestrin gene**

Basic. Res. Cardiol. **96**, 636-644 (2001)

Calsequestrin (CSQ) is a sarcoplasmic reticulum protein, which plays a predominant role in diastolic Ca^{2+}-storage in the mammalian heart. The present study was designed to define the gene structure, developmental and tissue specific expression of the murine, cardiac isoform of CSQ. Two sets of genomic libraries (lambda phage and PAC) were screened using the mouse cardiac CSQ cDNA, and several overlapping clones were isolated. These clones were characterized using restriction enzyme digestion, Southern blotting and partial sequencing. The cardiac CSQ gene consists of 11 exons and its 5' flanking region is characterized by the presence of a TATA-like box, muscle specific promoter elements such as 7 E-boxes, 1 MEF-2, 1 MCBF and 1 Repeat (musS) motifs, as well as several muscle non-specific transcriptional elements (AP-2A, NRE1, NRE2, p53, Spel and TFI-IIA). Expression of the cardiac isoform of CSQ was first detected on day 11 pre-birth and approached adult levels by day 4 post-birth. Expression of cardiac CSQ was also detected in adult fast-twitch skeletal muscle, thyroid, testis and epididymis tissues. This genomic characterization of cardiac CSQ may form the basis for further evaluation of its regulatory role in Ca^{2+} homeostasis and contractility in the murine heart.

Keywords: calsequestrin, sarcoplasmic reticulum, promoter

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Kiriazis, H.*1, Sato, Y., Kadambi, V.J.*2, Schmidt, A.G.*1, Gerst, M.J.*1, Hoit, B.D.*3, and Kranias, E.G.*1: **Hypertrophy and functional alterations in hyperdynamic phospholamban-knockout mouse hearts under chronic aortic stenosis**

Cardiovasc. Res., **53**, 372-381 (2002)

Objective: To determine whether the hyperdynamic phospholamban-knockout hearts are capable of withstanding a chronic aortic stenosis. Methods: The transverse section of the aorta was banded in phospholamban-knockout (KO) and their isogenic wild-type (WT) mice, which were followed with echocardiography in parallel, along with sham-operated mice, before and at 2.5, 5 and 10 weeks after surgery. Results: Cardiac decompensation was evidenced by the presence of lung congestion in some banded KOs and WTs, giving rise to a subset of non-failing and failing hearts within each group. The incidence of heart failure was not genotype-dependent but rather associated with higher heart rates before surgery. The development of left ventricular hypertrophy was similar between KOs and WTs. Fractional shortening was reduced in failing KOs and WTs to a similar degree. In addition, fractional shortening was decreased in non-failing KOs but not WTs. Ejection times shortened after aortic banding particularly for failing hearts. Conclusion: The hyperdynamic phospholamban-knockout hearts are able to compensate against a sustained aortic stenosis similar to wild-types.

Keywords: cardiac hypertrophy, heart failure, sarcoplasmic reticulum

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Sai, K., Kanno, J., Hasegawa, R., Trosko, J.E.*, and Inoue, T.: **Prevention of the down-regulation of gap junctional intercellular communication by green tea in the liver of mice fed pentachlorophenol**

Carcinogenesis, **21**, 1671-1676(2000)

Much evidence has been documented supporting the hypothesis that the down-regulation of gap junctional intercellular communication (GJIC) is a cellular event underlining the tumor-promotion process, and that treatment to prevent the down-regulation or to up-regulate GJIC is important for preventing the tumor promotion process. We explored the potential preventive effects of green tea against the promoting action of pentachlorophenol (PCP) in mouse hepatocarcinogenesis, examining whether drinking green tea prevents the down-regulation of GJIC-inhibition in the liver caused by tumorigenic doses of PCP. We used our modified in vivo GJIC-assay, the incision loading/dye transfer (IL/DT) method. Male B6C3F1 mice were given a green-tea infusion for one week and then PCP was then fed at doses of 300 and 600 ppm in their diet for the following 2 weeks along with green tea-treatment. A dose-related inhibition of GJIC in the hepatocytes was evident in the mice treated with PCP alone that was associated with a reduction in connexin 32 (Cx32) plaques in the plasma membrane and an increase in the index for cell proliferation. Drinking green tea significantly protected mice against GJIC-inhibition, the reduction in Cx32, and elevation of the labeling index. These findings suggest that green tea might act as an anti-promoter against PCP-induced mouse hepatocarcinogenesis via its ability to prevent the down regulation of GJIC.

Keywords: GJIC, pentachlorophenol, green tea

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Sai, K., Kang, K.-S.*1, Hirose, A., Hasegawa, R., Trosko, J.*2, and Inoue, T.: **Inhibition of apoptosis by pentachlorophenol in v-myc-transfected rat liver epithelial cells: relationi to down-regulation of gap junctional intercellular communication**

Cancer Letters, **173**, 163-174 (2001)

Pentachlorophenol (PCP), a promoter of murine hepatocarcinogenesis, inhibits gap junctional intercellular communication (GJIC) in rat liver epithelial cells in vitro. To test the hypothesis that both inhibition of GJIC and apoptosis contribute to tumor promotion, we investigated the effect of PCP on both GJIC and serum deprivation-induced apoptosis in v-myc-transfected rat liver epithelial cells. The results showed that PCP-inhibited apoptosis, as measured by the TUNEL assay and DNA ladder formation. Inhibition of apoptosis was associated with a decrease in GJIC. The study demonstrated that PCP has a potential for inhibiting apoptosis and GJIC, supporting the hypothesis.

Key Words: Apoptosis, GJIC, pentachlorophenol

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Sai, K., Kaniwa, N., Ozawa, S., and Sawada, J.: **A new metabolite of irinotecan in which formation is mediated by human hepatic cytochrome P-450 3A4**

Drug Metabolism and Disposition, **29**, 1505-1513 (2001)

Irinotecan (CPT-11) is an anticancer prodrug. It is converted by carboxylesterase to yield an active metabolite, SN-38, that acts as a topoisomerase I inhibitor. Several oxidative metabolites of CPT-11 have been identified in humans, including APC and NPC, generated by CYP3A4. To further investigate the metabolism of CPT-11 in human liver, we analyzed metabolites of CPT-11 in human hepatic microsomes using an HPLC/MS system and detected a new metabolite that was the major one produced in the microsomal system. HPLC/MS/MS analysis indicated that this compound was an oxidation product formed by the loss of 2 hydrogen atoms from the terminal piperidine ring. Kinetic analyses indicated that the metabolite was generated by a single enzyme, and we have identified this enzyme in 2 *in vitro* systems. The formation of the new metabolite was significantly inhibited by SKF525A, ketoconazole, and an anti-CYP3A4 antibody and catalyzed specifically by CYP3A4 expressed in insect microsomes. A significant correlation was observed between the generation of this metabolite and the CYP3A4 content in individual human hepatic microsomes. These findings indicate that this newly detected metabolite is a CYP3A4-generated product that may be produced in hepatic microsomes of patients treated with CPT-11.

Keywords: CPT-11, CYP3A4, metabolites

Sai, K., Kaniwa, N., Ozawa, S., and Sawada, J.: **An analytical method for irinotecan (CPT-11) and its metabolites using a high-performance liquid chromatography: parallel detection with fluorescence and mass spectrometry.**

Biomedical Chromatography, **16**, 209-218 (2002)

Irinotecan (CPT-11) is an anticancer pro-drug used in the treatment of many types of cancer. We describe here the validation of an analytical method for CPT-11 and its metabolites, including an active metabolite, SN-38, its

glucuronidated form SN-38G, and several CYP3A-mediated products using an HPLC connected parallel to a fluorescence detector (FLD) and a mass selected detector (MSD). This method is characterized as follows: 1) simple extraction of the analytes from biomaterials with perchloric acid/methanol; 2) sensitive quantitation of major metabolites with FLD, where the limits of quantitation by FLD were 2.5 ng/ml for SN-38G and APC, 5 ng/ml for CPT-11 and 1 ng/ml for SN-38, respectively; 3) parallel selective monitoring of the metabolites including minor metabolites with MSD. There was no observed interference by other drugs expected to be co-administered. This method showed its usefulness by identifying a novel metabolite produced in human hepatic microsomes. The results indicate that this combination of FLD and MSD enables a highly selective analysis of CPT-11 and its metabolites, and is useful for studies both *in vivo* and *in vitro*.

Keywords: CPT-11, validation of analysis, HPLC/FLD & MSD

関澤 純：**内分泌攪乱化学物質のリスクアセスメントとリスクコミュニケーション**

最新医学, **57**, 273-278(2002)

内分泌かく乱化学物質によるリスクを検討する際の課題と，リスクコミュニケーションのあり方について，筆者自身が行った研究成果を基に論じた．すなわち，前者については大豆中のエストロゲン物質による影響の可能性と，低用量曝露による影響の可能性についてはポリカーボネート樹脂から成型された血液透析器からのビスフェノールA溶出によるリスクを取り上げ，リスク評価における不確実性の扱いについても述べた．後者については市民の不安の要因を分析し，O157病原性大腸菌事故時のリスク対応を事故時のコミュニケーション例として取り上げ，平常時におけるコミュニケーションの問題のひとつとしてコミュニケーションへの要求の多様性とそれへの的確な対応について論じた．

Nakata, K., Takai-Igarashi, T.[*1], Nakano, T. and Kaminuma, T.: **Extension of The Receptor Database (RDB)**

Chem-Bio Informatics Journal, **1**, 115-119 (2001)

Data on genetic polymorphisms particularly single nucleotide polymorphisms (SNPs) are now being rapidly accumulated and put on the Internet for public use. It is time consuming and cumbersome, however, for general researchers who are interested in certain groups of genes or proteins to collect and update genetic variation data for these genes and proteins. An agent system is developed to search for and fetch SNPs data related to those genes and proteins pre-registered in the system. The system is tested and improved for collecting SNPs of different types of proteins, including certain drug target proteins.

Keywords: receptor structure, binding affinity, cell signaling, SNP, drug

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Nakata, K., Tokunaga, M.[*1], Toda, K., Takai-Igarashi, T.[*2], and Kaminuma, T.: **An Agent System for Collecting SNPs Data on the Internet.**

Chem-Bio Informatics Journal, **1**, 120-123 (2001)

The Receptor Database (RDB, http://impact.nihs.go.jp/RDB.html) has been developed to help researchers retrieve various data related to receptors in a systematic manner. The system has been available on the Internet for public use since 1997. Recently, the RDB contents were updated and its links expanded to other Web sites and supplemented with additional database modules. The new RDB aims to support structural biologists and drug designers not only for the examination of receptor-ligand binding but also for the elucidation of post binding signal transduction pathways.

Keywords: Gene, nucleotide, polymorphisms, genetic variation

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Nakano T.[*1], Kaminuma T.[*1], Sato T.[*2], Fukuzawa K.[*2], Akiyama Y.[*3], Uebayasi M.[*3] and Kitaura K.[*3],: **Fragment molecular orbital method: use of approximate electrostatic potential**

Chem. Phys. Lett. **351**, 475-480 (2002).

Recently, we have proposed the fragment molecular orbital (FMO) method; an approximate MO method for calculating large molecules such as proteins. The method has been shown to reproduce ab initio total energies and geometries of molecules in good accuracy. The most time consuming part in the method, the calculations of environmental electrostatic potentials, were speeded up by employing the Mulliken approximation for two-electron integrals and a fractional point charge approximation. Numerical calculations on several polypeptides revealed that the approximations brought no significant loss of accuracy in the total energy of molecules and were of practical use.

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Yoon, B-I., Hirabayashi Y., Kaneko, T., Kodama, Y., Kanno, J., Yodoi J.*1, Kim, D-Y.*2 and Inoue, T.: **Transgene expression of thioredoxin (trx/adf) protects against 2,3,7,8-tetrachlorodibenzo-p-dioxin (tcdd)-induced hematotoxicity.**

Arch Environ Contam Toxicol. **41**, 232-236 (2001)

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) has a variety of toxic effects on a number of organs including hematopoietic system. The importance of TCDD-induced oxidative stress has been evaluated in several target organs. However, its role in hematotoxicity remains poorly understood, although bone marrow is an organ known to produce reactive oxygen species. The aim of this study is to evaluate not only the contribution of oxidative stress to TCDD-induced hematotoxicity but also evaluate the protective function of TRX/ADF, a known anti-oxidative stress agent, on the hematotoxicity of TCDD in ADF wild-type (WT) and transgenic (Tg) mice. WT and Tg mice received a single intraperitoneal injection of 20 mg TCDD/kg. One day after the treatment, blood and bone marrow cellularity was measured and bone marrow levels of granulotyce/macrophage colony-forming units (CFU-GM) were determined in the in vitro colony assay. The expression of human TRX (hTRX) transgene by their bone marrow cells was analyzed by Western blot electrophoresis. Our results showed that overexpression of TRX/ADF protects TCDD-induced hematotoxicity, indicating that induction of oxidative stress which results in disruption of redox regulation may be an important mechanism in TCDD-induced bone-marrow toxicity. Moreover, we detected a significant decrease of AhR mRNA levels in bone marrow cells of Tg mice following TCDD treatment, suggesting a biological role of TRX/ADF in the AhR-mediated pathway through which TCDD induces oxidative stress.

Keywords: Ah receptor, hematotoxicity, thioredoxin.

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Yoon, B-I, Hirabayashi, Y., Ogawa, Y., Kanno, J., Inoue, T. and Kaneko, T.: **Hemopoietic cell kinetics after intraperitoneal single injection of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice**

Chemosphere. **43**, 819-822(2001)

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a widely spread environmental pollutant. Homopoietic system is one of the targets of TCDD in laboratory animals including monkeys. The present study is the hemopoietic cell kinetics in mice, from the severe depression in cellularity of bone marrow and CFU-GM, to their recovery after the intraperitoneal injection of high dosage of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). The bone-marrow cellularity and CFU-GM were severely decreased to 37.8% and 48% of the control, respectively until day 1 after exposure to TCDD. They were, however, soon recovered, even overshot the control value. Subsequently, they tended to show decrease and oscillation again to and under the control value. In conclusion, our cell kinetic study has proven the oscillation in bone-marrow cellularity and CFU-GM during the recovery period, of which the observation seems to be useful to extend our understanding in the hematotoxicity of TCDD.

Keywords: hematotoxicity, mice, TCDD, granulocyte-macrophage.colony forming unit (CFU-GM)

Tanaka, K.*, Watanabe, K.*, Mori, M.*, Kamisaku, H.*, Tsuji, H.*, Hirabayashi, Y., Inoue, T., Yoshida, K.* and Aizawa, S.*: **Cytogenetic and cellular events during radiation-induced thymic lymphomagenesis in the p53 heterozygous (+/-) B10 mouse.**

Int J Radiat Biol., **78**, 165-172(2002)

PURPOSE: Cellular and cytogenetic events in radiation-induced thymic lymphomagenesis were investigated in the p53 heterozygous (+/-) mouse following a single dose of whole-body irradiation. MATERIALS AND METHODS: The loss of the wild-type p53 allele and microsatellite markers of chromosome 11 in thymic lymphomas that developed in the p53 heterozygous (+/-) mouse after irradiation, and the stage at which prelymphoma cells appeared were analysed. RESULTS: The p53 heterozygous mouse developed thymic lymphomas in a dose-dependent manner. The loss of the wild-type p53 allele (loss of heterozygosity; LOH) occurred in almost all thymic lymphomas induced in the irradiated p53 heterozygous mouse. Cytogenetic analysis for the mechanism of LOH strongly suggested that the loss of the wild-type p53 gene in the lymphomas was caused by duplication of the disrupted allele through either homologous recombination or non-disjunctional chromosome duplication. The assay for prelymphoma cells suggested that a critical event in the development of prelymphoma cells occurred at least 3 weeks after

irradiation. CONCLUSIONS: The loss of the wild-type p53 gene in thymocytes of the p53 heterozygous mouse may precede the development of prelymphoma cells after irradiation and be a valuable marker of radiation-induced leukemogenesis.

Keywords: loss of heterozygosity, microsatellite repeats, p53

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Kanno, J., Onyon, L.*1, Haseman, J.*2, Fenner-Crisp, P.*3, Ashby, J.*4 and Owens, W*5: **The OECD program to Validate the Rat Uterotrophic Bioassay to Screen Compounds for in Vivo Estrogenic Responses Phase 1**

Environmental Health Perspectives, **109**, 785-794(2001)

The OECD has completed the first phase of an international validation program for the rodent uterotrophic bioassay. It is intended to identify the in vivo activity of compounds that are suspected agonists or antagonists for further testing. We tested and compared two model systems, the immature female rat and the adult ovariectomized rat. Data from 19 participating laboratories using ethinyl estradiol(EE), and an antagonist, ZM189,154, indicate no substantive performance differences between models. All laboratories and all protocols successfully detected increases in uterine weights using EE in phase 1. For the next phase of the OECD validation program, both models will be tested against a battery of weak, partial estrogen agonists.

Keywords: endcrine disruption, estrogen, rat uterus

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Haraguchi, S.*1, Kitajima, S., Takagi, A., Takeda, H.*2, Inoue, T. and Saga Y.*2: **Transcriptional regulation of Mesp1 and Mesp2 genes: differential usage of enhancers during development**

Mech Dev., **108**, 59-69(2001)

Mesp1 and Mesp2 encode bHLH-type transcription factors, Mesp1 and Mesp2, respectively. The expression of both genes is observed in the nascent mesoderm, and subsequently in the rostral presomitic mesoderm. To determine the regulatory mechanism for gene expression, we attempted to identify enhancer elements by transient transgenic analysis. At least two enhancers were identified. Deletion studies indicate that either gene may use the same enhancer for early mesoderm development, whereas both genes may utilize separate enhancers to regulate their expression in the presomitic mesoderm.

Keywords: Mesp1, Mesp2, enhancer

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Nomura-Kitabayashi, A., Takahashi, Y., Kitajima, S., Inoue, T., Takeda, H.*1 and Saga, Y.*1: **Hypomorphic Mesp allele distinguishes establishment of rostrocaudal polarity and segment border formation in somitogenesis.**

Development **129**, 2473-2481 (2002).

A bHLH-type transcription factor, *Mesp2* plays an essential role on the somite segmentation in the mouse. To ask whether the zebrafish mesp-b represents functional homologue of the mouse *Mesp2*, zebrafish *mesp-b* was introduced into the mouse *Mesp2* locus by homologous recombination. Introduced *mesp-b* almost rescued the *Mesp2* deficiency in the homozygous *mesp-b* knockin mouse, indicating that *mesp-b* is a functional homologue of mouse *Mesp2*. Interestingly, however, the nature and dosage of *mespb* gene affected the rescue event. A mouse line, which has a hypomorphic *Mesp2* allele, gave rise to an epithelial somite, without normal rostro-caudal polarity within a somite. These results suggest that Mesp family transcription factor is involved in both segment border formation and establishment of RC polarity through different genetic cascades.

Keywords: somitogenesis, *Mesp2*, *Mespb*

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Ueno, M.*, Igarashi, K., Kimura, N., Okita, K.*, Takizawa, M.*, Nobuhisa, I.*, Kojima, T., Kitamura, T., Samulowitz, U, Vestweber, D, Shimomura, T.*, Suda, T.*, Nakashima, K.* and Taga, T.*: **Endomucin is expressed in embryonic dorsal aorta and is able to inhibit cell adhesion**

Biochem Biophys Res Commun, **287**, 501-6 (2001)

Recent studies have suggested the existence of progenitors common to hematopoietic and endothelial cells, called hemangioblasts, in, for instance, embryonic dorsal aorta. To identify a membrane-bound or secretory molecule regulating early hematopoiesis, we screened a cDNA library from dorsal aortas of embryonic day (E) 10.5 mice by a signal sequence trap method and obtained a clone encoding a sialoprotein, endomucin-1. Immunohistochemistry revealed that the endomucin-1

transcript was specifically expressed in the endothelial cells of dorsal aorta of E10.5 mouse embryo. Overexpression of endomucin-1 strongly inhibited adhesion and aggregation of cells, including cultured endothelial cells from E10.5 dorsal aorta. These data suggest that endomucin-1 may play a role in detachment of hematopoietic cells from endothelium during early hematopoiesis.

Kewords: endomucin, hematopoiesis

* Kumamoto University

Hikima, T.*, Ohno, Y., Maibach HI: **Metabolism of prednisolone 21-acetate in hairless mouse skin**

Skin Pharmacol. Appl. Skin Physiol., **14**, 203-209 (2001)

皮膚における21-酢酸プレドニゾロンの加水分解活性をヘアレスマウス皮膚を用いて検討し，ヒトでの結果と比較した．Km値は市販のエステラーゼとほぼ同じで14.2 μMであった．Cmax値は0.67nmol/mion/mg proteinであった．この活性は酵素阻害剤である3,4-dichloroisocoumarine（DCIC）で3割りほどしか抑制されなかった．この活性の66％はミクロソームに11％は可溶性分画に存在した．DCICは主にミクロソーム分画の活性を抑制したが，p-hydroxymercuribenzoic acid（HMBA）は可溶性分画の活性を主に阻害した．これらの結果から両分画に存在するエステラーゼは異なる分子種であることが示唆された．

Keywords：skin metabolism, prednisolone 21 acetate, hairless mouse, esterase

*九州工業大学

Tsuda, M., Koizumi, S. and Inoue, M.: **Role of endogenous ATP at the incision area in a rat model of postoperative pain**

Neuroreport, **12**, 1701-1704 (2001)

The aim of the present study is to characterize the role of endogenous ATP leaked from damaged cells in a rat model of postoperative pain. We found that systemic and local administration of a P2 receptor antagonist, PPADS before surgery significantly attenuated mechanical allodynia caused by an incision of the plantar surface of the hindpaw. Furthermore, PPADS significantly reduced the incision-evoked c-Fos protein expression in the dorsal horn of the spinal cord. The present findings suggest that excitatory signaling by endogenous ATP leaked from damaged cells via PPADS-sensitive P2 receptors is necessary for the induction of the postoperative pain characterized by mechanical allodynia.

Keywords: ATP, postoperative pain, PPADS

Inoue, K. and Koizumi, S.: **Mechanism of the inhibitory action of ATP in rat hippocampus**

Drug Development Research, **52**, 95-103 (2001)

We have already shown that ATP inhibits synaptic transmission in cultured rat hippocampal neurons. Using FM1-43 method and Ca^{2+} imaging technique, we show here that ATP acting on presynaptic P2Y receptors inhibits the bleaching of FM1-43 signals in the hippocampal neurons. This inhibition was presumably due to reduction of presynaptic N-or P/Q-type Ca^{2+} channels. Meanwhile, ATP stimulates GABA release and inhibits the excitable function of glutamate. Besides these actions, ATP is known to activate microglia to release some neuroprotective cytokines. Taken together, these data suggest that ATP may protect the hippocampal function from excess excitation.

Keywords: ATP, hippocampus, brain damage

Shigemoto-Mogami, Y., Koizumi, S., Tsuda, M., Ohsawa, K.*, Kohsaka, S.* and Inoue, K.: **Mechanisms undelying extracellular ATP-evoked interleukin-6 release in mouse microglial cell line, MG-5**

J. Neurochem., **78**, 1339-1349 (2001)

The ATP-evoked production of interleukin-6 (IL-6) and its intracellular signals were examined using a mouse microglial cell line, MG-5. ATP but not its metabolites produced IL-6. Although ATP-activated p38 was involved in the IL-6 induction, its activation was not sufficient for the IL-6 induction. An agonist to P2X7 receptors failed to produce IL-6 in spite of the fact that it activated p38. Unlike in other cytokines in microglial cells, P2Y rather than P2X7 receptors seem to have a major role in the IL-6 production by the cells. The ATP-evoked IL-6 production was attenuated by G_6976, an inhibitor of Ca^{2+}-dependent protein kinase C (PKC). The P2Y receptor responsible for these responses was insensitive to pertussis toxin (PTX) and was linked to phospholipase C. Taken together, ATP acting on PTX-insensitive P2Y receptors activates p38 and Ca^{2+}-dependent PKC, thereby resulting in the mRNA expression and release of IL-6 in MG-5.

Keywords: microglia, ATP, interleukin-6

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Inoue, K.*, Koizumi, S., Fujiwara, S.*, Denda, S.*, Inoue, K. and Denda, M.*: **Functional vanilloid receptors in cultured normal human epidermal**

keratinocytes

Biochem. Biophys. Res. Commun., **291**, 124-129 (2002)

Capsaicin receptor subtype 1, VR1, is an ion channel that serves as a polymodal detector of pain-producing chemicals such as capsaicin and protons in primary afferent neurons. Here we showed that both capsaicin and H^+ produced elevations in $[Ca^{2+}]i$ in cultured human epidermal keratinocytes (NHEK). The capsaicin-and acidification-evoked increases in $[Ca^{2+}]i$ were inhibited by capsazepine, an antagonist to VR1. Expression of VR1 mRNA and protein were observed in the cells. VR1 functions as a sensor against noxious chemical stimuli such as capsaicin or H^+ in NHEK.

Keywords: VR1, capsaicin, NHEK

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Scalettar,B.A.*1, Rosa, P.*2, Taverna, E.*2, Maura Francolini, M.*2, Tsuboi, T.*3, Terakawa, S.*3, Koizumi, S., Roder J.*4, and Jeromin, A.*4 : **Real-time imaging of neuronal calcium sensor-1 as a component of synaptic-like microvesicles in PC12 cells**

J. Cell Sci., **115**, 2399-2412 (2002)

The distribution, dynamics, and function of neuronal calcium sensor-1 (NCS-1) were studied using PC12. NCS-1 was associated with SLMVs in growth cones, enhanced recycling of regulated secretory organelles and increased levels of phosphoinositides. NCS-1 upregulated a population of PI4K that is associated with SLMVs, and influenced regulated exocytosis by enhancing phosphoinositide signaling. These results reveal novel attributes of SLMV dynamics in growth cones and interesting similarities and differences between protein trafficking to neurotransmitter-containing vesicles in neuroendocrine cells.

Keywords: NCS-1, PI4K, PC12 cells,

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Nakazawa, K., Ohno, Y.: **Modulations by Estrogens and Xenoestrogens of Recombinant Human Neuronal Nicotinic Receptors**

Eur. J. Pharmacol., **430**, 175-183 (2001)

The effects of estrogens and xenoestrogens on human neuronal nicotinic acetylcholine receptor/channels were examined by expressing recombinant channels in Xenopus oocytes. When functional channels were expressed with $\alpha 3$ and $\beta 4$ subuints, estrogens (17β-estradiol, 17α-estradiol, 17β-ethynylestradiol and diethylstilbestrol) and xenoestrogens (bisphenol A, p-nonylphenol and p-octylphenol) inhibited an ionic current activated by acetylcholine at concentrations up to 100 μM. When changed to $\alpha 4\beta 2$, diethystilbestrol and the xenoestrogens inhibited the acetylcholine-activated current, but 17β-estradiol or 17β-estradiol did not inhibit the current. For 17α-ethynylestradiol, the current through $\alpha 4\beta 2$ receptor/channel was inhibited at 1 μM, but it was markedly enhanced at 10 and 100 μM. Tamoxifen (10 μM), an antiestrogen, itself inhibited the acetylcholine-activated current, but did not antagonize the current modulations induced by the estrogens and the xenoestrogens. These and additional results suggest that human neuronal nicotinic acetylcholine receptors are the targets of non-genomic actions of estrogens and xenoestrogens.

Keywords: human nicotinic receptor, estrogen, xenoestrogen

Sato, K., and Matsuki, N*.: **A72 kilodalton-heat shock protein is protective against the selective vulnerability of CA1 neurons and is essential for the tolerance exhibitod by CA3 neurons in the hippocampus**

Neuroscience, **109**, 746-756 (2002)

The correlation between the expression of 72 kilodalton-heat shock protein and vulnerability of hippocampal CA1, CA3 and dentate gyrus regions to glutamate toxicity was investigated using a highly specific antisense oligonucleotide technique. Glutamate (1 mM, 15 min) caused region-dependent neuronal damage in cultured hippocampal slices 24 hr after the exposure and the most severe damage was observed in CA1. When slices were heat-shocked (43.5℃, 30 min) before the exposure to glutamate, the neuronal damage in CA1 was attenuated. The strongest protection was observed when the interval between the heat shock and the exposure to glutamate was 3 days, which coincided with that for the maximal induction of 72 kilodalton-heat shock protein in neurons. When the expression of 72 kilodalton-heat shock protein was suppressed by the antisense oligonucleotide, the protective effect of the heat shock was inhibited completely. Glutamate itself also induced 72 kilodalton-heat shock protein in neurons region-dependently 24 hr after the exposure. The signal of 72 kilodalton-heat shock protein in CA3 and dantate gyrus was significantly stronger than that in CA1. When the antisense

oligonucleotide was applied, the damage in CA3 and dentate gyrus was exaggerated dose-dependently and this effect was more remarkable in CA3 than in dentate gyrus. We concluded that: (i) HSP70 has a protective effect against the selective vulnerability of CA1 neurons, (ii) HSP70 is one of the essential factors for the tolerance of CA3 neurons, (iii) DG has some mechanism other than those mediated through HSP70.

Keywords : HSP70, hippocampal slice culture, antisense oligonucleotide

*東京大学

Murayama, N., Sai, K., Nakajima, Y., Kaniwa, N., Ozawa, S., Ohno, Y. and Sawada, J.: **Expression of CYP2A6 in tumor cells augments cellular sensitivity to tegafur**

Jpn.J.Cancer Res., **92**, 524-528 (2001)

To examine the role of cytochrome P4502A6 (CYP2A6) in the cellular sensitivity to an anti-tumor prodrug, tegafur (FT), a CYP2A6 cDNA construct was transfected into cells of a colon cancer cell line, DLD-1 (DLD/CYP2A6 cells). The extent of growth inhibition of the DLD-1/CYP2A6 cells by FT was greater than that of DLD-1/null cells. Thus, intracellular expression of CYP2A6 can sensitize cells to FT.

Keywords: tegafur, CYP2A6, prodrug

Ui, A*., Satoh, Y*., Onoda, F*., Miyajima, A., Seki, M*. and Enomoto T*. : **The N-terminal region of Sgs1, which interacts with Top3, is required for complementation of MMS sensitivity and suppression of hyper-recombination in *sgs1* disruptants.**

Mol Genet Genomics., **265**, 837-850 (2001)

The *SGS1* gene is a yeast homologue of the genes affected in Bloom's syndrome, Werner's syndrome, and Rothmund-Thomson's syndrome. We demonstrate that the C-terminal conserved region, as well as the helicase motifs, of Sgs1 are essential for complementation of MMS sensitivity and suppression of hyper-recombination in *sgs1* mutants. In contrast, the highly charged acidic regions, the HRDC domain, and the C-terminal 252 amino acids were dispensable for the complementation of these phenotypes. Surprisingly, the N-terminal 45 amino acids of Sgs1 were absolutely required for the suppression of the above phenotypes.

Keywords: Sgs1, RecQ, Top3

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Onodera, R*., Seki, M*., Ui, A*., Satoh, Y*., Miyajima, A., Onoda, F*. and Enomoto, T*. : **Functional and physical interaction between Sgs1 and Top3 and *Sgs1*-independent function of Top3 in DNA recombination repair.**

Genes Genet Syst., **77**, 11-21 (2002)

We found that several amino acids residues in the N-terminal region of Sgs1 between residues 4 and 33 were responsible for binding to Top3 and essential for complementing the MMS sensitivitye of *sgs1* cells. Although disruption of the *SGS1* suppressed the semi-lethality of the *top3* mutant, the *sgs1-top3* mutant grew more slowly and was more sensitive to MMS than the *sgs1* mutant, indicating that Top3 plays some role independently of Sgs1. The DNA topoisomerase activity of Top3 was required for the function to repair DNA damages induced by MMS, as shown by the fact that the Top3 gene carrying a mutation (Phe for Tyr) at amino acid residue essential for its activity (residue 356) failed to restore MMS sensitivity of *sgs1-top3* to the level of that of the *sgs1* mutant.

Keywords: Top3, Sgs1, slow-glowth

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West, M.*1, Blanchette, C.*2, Dressman, H.*2, Huang, E.*2, Ishida, S., Spang, R.*1, Zuzan, H.*1, Olson, JA. Jr*2, Marks, J.R.*2, Nevins, J.R.*2 : **Predicting the clinical status of human breast cancer by using gene expression profiles**

Proc. Natl. Acad. Sci. U.S.A., **98**, 11462-11467 (2001)

Prognostic and predictive factors are indispensable tools in the treatment of patients with neoplastic disease. Gene expression assays have the potential to supplement what were previously a few distinct features with many thousands of features. We have developed Bayesian regression models that provide predictive capability based on gene expression data derived from DNA microarray analysis of a series of primary breast cancer samples. These patterns have the capacity to discriminate breast tumors on the basis of estrogen receptor status and also on the categorized lymph node status. The practical value of such approaches relies on the ability not only to assess relative probabilities of clinical outcomes for future samples but also to provide an honest assessment of the uncertainties associated with such predictive classifications on the basis of the selection of gene subsets for each validation analysis.

Keywords: breast cancer, gene expression profiling, DNA

microarray

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Ishida, S., Huang, E.*2, Zuzan, H.*1, Spang, R.*1, Leone, G.*2, West, M.*1, Nevins, J.R.*2: **Role for E2F in control of both DNA replication and mitotic functions as revealed from DNA microarray analysis.**

Mol. Cell. Biol., **21**, 4684-4699 (2001)

We have used high-density DNA microarrays to provide an analysis of gene regulation during the mammalian cell cycle and the role of E2F in this process. Analysis of cell cycle samples identified seven distinct clusters of genes that exhibit unique patterns of expression. Genes tend to cluster within these groups based on common function and the time during the cell cycle that the activity is required. The analysis of genes induced by E2F proteins identified genes not previously described as regulated by E2F proteins; surprisingly, many of these encode proteins known to function during mitosis. A comparison of the E2F-induced genes with the patterns of cell growth-regulated gene expression revealed that virtually all of the E2F-induced genes are found in only G(1)/S and G(2). The activation of the G(2) genes suggests a broader role for E2F in the control of both DNA replication and mitotic activities.

Keywords: cell cycle regulated genes, E2F target genes, DNA microarray

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Sakimura, M.*, Hanzawa, S.*, Tsukada, A.*, Yamamoto, I.*, Saito, N.*, Usami, M., Ohno, Y., Shimada K.*: **Effect of estradiol and nonylphenol on mRNA levels of vitellogenin II in the liver of chicken embryos**

J. Poultry Sci., **38**, 250-257 (2001)

Nonylphenol is one of the endocrine disrupting agents with estrogenic activity in some vertebrates. The present study was conducted to assess estrogenic activity of *p*-nonylphenol (NP) in chicken embryos by determining mRNA levels of liver vitellogenin II (VTGII). Fertile chicken eggs were incubated using standard conditions. In the group 1, the eggs were treated with a single injection of either NP or estradiol (E2) on day 16 of incubation. In the group 2, the eggs were treated with double injection of either NP or E2 on days 13 and 16 of incubation. On day 18 of incubation the liver was collected and total RNA was extracted. VTGII mRNA levels were determined by reverse transcrition-polymerase chain reaction (RT-PCR) assay. No VTGII mRNA was detected in the control group, whereas distinct VTGII mRNA was revealed in the E2 treatment group where there was higher expression in the group 2 than in the group 1. Weak but distinct VTGII mRNA was detected in the NP treatment group. This study indicates that NP may have estrogenic activity in terms of liver VTGII mRNA assessed by RT-PCR assay in the chicken embryo.

Keywords: nonylphenol, estradiol, vitellogenin II

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Sakimura, M.*, Usami, M., Hanzawa, S.*, Tsukada, A.*, Saito, N.*, Ohno, Y., Shimada, K.*: **Suppressive effect of *p*-nonylphenol on male-specific mRNA expression in the embryonic gonad of chickens**

J. Poultry Sci., **39**, 91-99 (2002)

Effects of estradiol (E2) and *p*-nonylphenol (NP) on the mRNA expression of sex determination-related genes were examined in the embryonic gonad of chickens. Fertilized eggs were treated with either E2 (0.1 and 1.0 mg/egg) or NP (0.001, 0.01, 0.1 and 0.2 mg/egg) twice on days 13 and 16 of incubation. The mRNA expressions of anti-Müllerian hormone (AMH), SRY-related HMG box9 (SOX9), cytochrome P450 aromatase (P450arom) and steroidogenic factor 1 (SF-1) in the embryonic gonads were determined by the reverse transcription-polymerase chain reaction (RT-PCR) on day 18 of incubation. AMH, SOX9 and P450arom, but not SF-1, showed sexually dimorphic expression in the control; AMH and SOX9 were male-specific while P450arom was female-specific. E2 had no effects on these expressions in either sex. In contrast, NP reduced the expressions of AMH and SOX9 only in the males but had no effects on the expressions of P450arom and SF-1. These results suggest that NP has endocrine disrupting effects on the mRNA expression of sex determination-related genes in the gonads of chicken embryos.

Keywords: *p*-nonylphenol, AMH, SOX9,

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Ikezaki, S.*1, Nishikawa, A., Furukawa, F., Kudo, K.*1, Nakamura, H., Tamura, K.*1 and Mori, H.*2: **Chemopreventive effects of curcumin on glandular stomach carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine and sodium chloride in rats.**

Anticancer Res., **21**, 3407-3411 (2001)

The effects of curcumin on glandular stomach carcinogenesis were investigated in male Wistar rats. Rats were given N-methyl-N'-nitro-N-nitrosoguanisine (MNNG) in their drinking water at a dose of 100 ppm and simultaneously fed a diet supplemented with 5% NaCl for 8 weeks. They were then fed a diet containing either 0.2% or 0.05% curcumin or kept on a basal diet alone for 55 weeks. The mean number of atypical hyperplasias or adenocarcinomas of the glandular stomachs was significantly ($p<0.05$) reduced by the treatment of 0.05% curcumin. Thus, our results indicate that curcumin exerts chemopreventive effects, when given during the post-initiation phase of glandular stomach carcinogenesis in rats.

Keywords: curcumin, stomach carcinogenesis, rat

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Nakamura, H., Furukawa, F., Nishikawa, A., Miyauchi, M., Son, H-Y., Imazawa, T. and Hirose M.: **Oral toxicity of a tocotrienol preparation in rats.**

Food Chem. Toxic., **39**, 799-805 (2001)

A 13-week oral toxicity study on tocotrienol preparation was performed in F344 rats of both sexes at dose levels of 0, 0.19, 0.75 and 3% in diet. Because of pathological changes such as slight hepatocellular hypertrophy in male livers and hematological changes in females, the NOAEL was concluded to be 0.19% in the diet (120 mg/kg body weight/day for male rats and 130 mg/kg body weight/day for female rats). As a decrease in MCV, an increase in A/G, elevation of alkaline phosphatase and increase in adrenal weight were observed in all treated males, the NOEL could not be determined in this examination.

Keywords: tocotrienol, oral toxicity, rat

Morimura, K.*, Hori, T.*, Kaneko, M.*, Nishikawa, T.*, Nishikawa, A., Wanibuchi, H.*, Takada, N.*, Osugi, H.* and Fukushima, S.*: **Promotion of chemically induced rat esophageal tumorigenesis with post-initiation ethanol modification.**

Teratog. Carcinog. Mutagen., **21**, 295-301 (2001)

Post-initiation ethanol modification on esophageal carcinogenesis model was investigated in male rats receiving NMBA for the first 5 weeks and then were treated with 0%, 3.3%, and 10% ethanol in the drinking water for up to 20 weeks. There were no statistical differences in the incidence and multiplicity of esophageal tumors among the groups. However, the multiplicity of hyperplasias was statistically greater in the 10% ethanol group. BrdU-labelling indices of tumors and hyperplasias in NMBA-treated groups were essentially similar, although cycline D1 was overexpressed to a greater extent in tumors and also hyperplasias of the 10% ethanol group. The results indicated ethanol to exert weak promotion effects through cycline D1 overexpression on rat esophageal tumorigenesis initiated with NMBA.

Keywords: ethanol, esophageal tumorigenesis, rat

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Nishikawa, A., Suzuki, T., Masumura, K., Furukawa, F., Miyauchi, M., Nakamura, H,, Son, H-Y., Nohmi, T., Hayashi, M. and Hirose, M.: **Reporter gene transgenic mice as a tool for analyzing the molecular mechanisms underlying experimental carcinogenesis.**

J. Exp. Clin. Cancer Res., **20**, 111-115 (2001)

Recently, transgenic animal models with reporter genes have been developed as a tool for assessing *in vivo* mutagenicity as well as carcinogenicity. DMN significantly increased *lacI* mutant frequency (MF) in the liver, kidenys and lungs, but not in other non-target organs. The spectrum of mutant plaques induced by DMN was characterized by deletions as well as GC to AT base transitions. The remaining mice receiving DMN proved to have liver adenomas after 78 weeks. Meanwhile, MeIQx significantly increased *lacI* and *gpt* MFs in the liver and colon. The characteristic spectrum of mutant plaques induced by MeIQx was a GC to TA base transversion in both the *lacI* and *gpt* mutations. Our results thus suggest that these reporter gene transgenic animal models could offer a useful tool for analyzing molecular mechanisms underlying experimental carcinogenesis.

Keywords: reporter gene, transgenic mice, molecular mechanism

Ikeda, T., Nishikawa, A., Son, H-Y., Nakamura, H., Miyauchi, M., Imazawa, T., Kimura, S.* and Hirose, M.: **Synergistic effects of high-dose soybean intake with iodine deficiency, but not sulfadimethoxine or phenobarbital, on rat thyroid proliferation.**

Jpn. J. Cancer Res., **92**, 390-395 (2001)

The specificity and dose dependence of the synergistic effects of soybean intake with iodine deficiency on the induction of thyroid proliferation were investigated in

female F344 rats. In the first experiment, soybean feeding synergistically induced thyroid hyperplasias with iodine deficiency only at the 25% dose. In the second experiment, sulfadimethoxine (SDM) significantly ($p< 0.05$ or 0.01) increased the thyroid weights, but this increase rate was less prominent in the SDM + soybean group than in the SDM alone group. Phenobarbital (PB) was also associated with a similar tendency. Although the SDM or PB treatments reduced the serum T3 and T4 levels and consequently increased the serum TSH levels, the soybean feeding did not affect or rather attenuated these changes. Our results indicate that soybean feeding does not synergistically enhance the effects of SDM or PB on the rat thyroid.

Keywords: soybean, iodine deficiency, rat thyroid

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Furukawa, F., Nishikawa, A., Kasahara, K., Miyauchi, M., Nakamura, H., Son, H-Y., Uchida, K.* and Hirose, M.: **Involvement of lipid peroxidation in spontaneous pancreatitis in WBN/Kob rats.**

Pancreas, **22**, 427-430 (2001)

To cast light on the mechanisms underlying development of spontaneous pancreatitis lesions, tissues from WBN/Kob rats at various ages were investigated with special reference to the existence of the lipid peroxidation products 4-hydroxy-2-nonenal (HNE), 4-hydroxy-2-hexenal (HHE), and malondialdehyde (MDA). Histopathological changes were observed in exocrine pancreatic tissue from rats at 10-15 weeks of age. In animals aged 20 weeks, the lesions had progressed remarkably and deposits of hemosiderin were apparent with fibrosis. Immunohistochemical examination for lipid peroxidation product-modified proteins showed HNE and MDA to be negative in all pancreatic tissues, but HHE was positive in the areas involving atrophy of acinar cells and fibrosis in the islets. Our results thus suggest that lipid peroxidation during spontaneous pancreatitis in WBN/Kob rats may possibly be involved in the development of diabetes.

Keywords: pancreatitis, lipid peroxidation, WBN/Kob rat

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Son, H-Y., Nishikawa, A., Ikeda, T., Imazawa, T., Kimura, S.* and Hirose, M.: **Lack of effect of soy isoflavone on thyroid hyperplasia in rats receiving an iodine-deficient diet.**

Jpn. J. Cancer Res., **92** : 103-108 (2001)

In order to examine a possible contribution of soy isoflavone on thyroid tumorigenesis, female rats were given 0.2% soy isoflavone mixture (SI), 0.2% SI + iodine deficiency (ID), 0.04% SI, 0.04% SI + ID, 20% defatted soybean (DS) alone, 20% DS + ID, ID alone or basal diet alone for 5 weeks. Thyroid weight was not influenced by SI, but was increased by the ID and DS diets with a further significant increment in the DS + ID group ($p<0.01$). Compared to the control value, serum T4 was significantly ($p<0.01$) increased by 20% DS alone and decreased in all groups given the ID treatment ($p<0.001$). Serum TSH level was increased by ID, and further enhanced by DS ($p<0.01$) but not SI. Histopathologically, diffuse hypertrophy and/or hyperplasia of thyroid follicles were observed in the ID-treated groups, the severity being enhanced by DS but not SI. These results thus suggest that isoflavones may not be involved in the mechanisms under-lying the synergistic goitrogenic effect of soybean with ID.

Keywords: soy isoflavone, rat thyroid, iodine deficiency

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Akaike N*, Murakami N*, Katsurabayashi S*, Jin YH*, Imazawa T.: **Focal stimulation of single GABAergic presynaptic boutons on the rat hippocampal neuron**

Neurosci Res., **42**, 187-195 (2002)

Evoked inhibitory postsynaptic currents (eIPSCs) generated from a single GABAergic bouton were recorded and the functional properties were investigated. Native single boutons attached to mechanically dissociated rat hippocampal CA1 neurons, namely "synaptic bouton" preparation, were visualized with FM 1-43 dye and selectively stimulated by a glass pipette directed to a single bouton by focal stimulation. The GABAergic eIPSCs were elicited in like all-or-none fashion regarding both stimulus strength and pipette location, thus indicating that the eIPSCs result from the activation of a single bouton. As a result, this new experimental approach using both focal stimulation and a synaptic bouton preparation allows for a detailed study of the native synaptic machinery in nerve terminals measuring smaller than 1 micron in size in the CNS.

Keywords: Presynaptic terminal, GABA, Evoked IPSC

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Imazawa T, Nishikawa A, Shibutani M, Ogasawara H,

Furukawa F, Ikeda T, Suda K, Hirose M.: **Induction of pancreatic islet cell tumors in rats by repeated intravenous administration of 4-hydroxyaminoquinoline 1-oxide**
Toxicol. Pathol., **29**, 320-327 (2001)

The inducibility of pancreatic islet cell tumors by administration of 4-hydroxyaminoquinoline 1-oxide (4HAQO) was investigated in male SD rats. Rats were given 4HAQO intravenously at a weekly dose of 5 mg/kg 4 times (group 1) or a single dose of 10 mg/kg (group 2). Control rats received the vehicle alone (group 3). At fifty-six weeks, all surviving animals were killed and the pancreas was examined. The incidences islet cell tumors in groups 1, 2, and 3 were 52.3%, 19.2% and 0%, respectively. Islet cell carcinomas were induced only group 1, accounting for 26%. Islet cell tumors showed an increase in the number of insulin positive cells associated with cytological features indicative of enhanced insulin synthesis and secretion, and a decrease in the number of glucagon positive cells without ultrastructural signs of modified secretory activity. Thus our results indicate that repeated intravenous administration of 4HAQO to rats is useful for the induction of islet cell tumors at high incidence.

Keywords: Islet cell tumors, Immunohistochemistry, 4HAQO

Okazaki, K., Okazaki, S.*, Nishimura, S.*, Nakamura, H.*, Kitamura, Y.*, Hatayama, K.*, Nakamura, A.*, Tsuda, T.*, Katsumata, T.*, Nishikawa, A., Hirose, M.: **A repeated 28-day oral dose toxicity study of methoxychlor in rats, based on the 'enhanced OECD test guideline 407' for screening endocrine-disrupting chemicals**
Arch.Toxicol., **75**, 513-521(2001)

In association with the international validation project to establish an OECD Enhanced Test Guideline 407, we performed a 28-day repeated-dose toxicity study of methoxychlor. Seven-week-old Crj:CD(SD)IGS rats were given methoxychlor once daily by gavage at doses of 0 (control), 20, 100 or 500 mg/kg body weight per day. Effects of methoxychlor on endocrine-related organs were detected with regard to serum hormone, organ weights, histopathological examination in both sexes, estrus cycle in females and sperm examination in males. A no-observed-adverse-effect level (NOAEL) in the present study was estimated to be below 20 mg/kg per day. In particular, the adverse effects were effectively detected in organ weights of accessory sex organs and histopathological examination.

Keywords: Methoxychlor, Enhanced OECD test guideline 407, Endocrine disrupters

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Umemura, T., Kodama, Y., Hioki, K.*1, Inoue, T., Nomura, T.*1, Kurokawa, Y.*2: **Butylhydroxytoluene (BHT) increases susceptibility of transgenic rasH2 mice to lung carcinogenesis.**
J. Cancer Res. Clin. Oncol., **127**, 583-590 (2000)

rasH2 mice and wild littermates were pre-treated with carcinogens [urethane (UR), 4-nitro-quinoline 1-oxide (4NQO) or diethylnitrosamine (DEN)], and, one day later, given a 400 mg/kg dose of BHT. Six weeks after the initiation, carcinogenicity could be detected in male and female rasH2 mice that had received UR doses of ≧ 250 mg/kg and ≧ 125 mg/kg, respectively, prior to exposure to BHT, whereas only 500 mg/kg of UR was sufficient to induce tumors in female rasH2 mice given the carcinogen alone. The carcinogenicity of 15 mg/kg of 4NQO and 60 mg/kg of DEN could be detected within 9 weeks in male rasH2 mice given the carcinogen followed by BHT. These results suggest that the use of BHT in rasH2 mice might lead to the establishment of a rapid in vivo assay for lung carcinogens.

Keywords: rasH2 mice, lung carcinogen, BHT

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Shoda, T., Yasuhara, K., Moriyasu, M., Takahashi, T., Uneyama, C., Hirose, M., Mitsumori, K.: **Testicular toxicity of nitrofurazone causing germ cell apoptosis in rats**
Arch. Toxicol., **75**, 297-305 (2001)

Sequential observation of testes after a single gavage of NF to rats demonstrated that degeneration of pachytene spermatocytes in stages VII-VIII and vacuolation of Sertoli cells were first observed 12 h after treatment. By 24 h, degeneration of pachytene spermatocytes in stages VII-XII and diplotene spermatocytes were seen. On day 4, neither spermatocytes nor spermatids in stage VII were observed. Increase of serum testosterone and a decrease of progesterone at 6 h, and decreases of LH at 12 h and testosterone at 24 h were detected. PRL tended to decrease from 12 h and the decrease was significant at 48 h. The probability that NF damages germ cells by causing a hormonal imbalance is extremely low, since no pattern of hormonal imbalance that could be regarded as the

cause of the germ cell degeneration was observed until 12 h. The present experiments suggest that NF directly damages Sertoli cells and pachytene spermatocytes in stages VII-XII.

Keywords: nitrofurazone, testicular toxicity

Koujitani, T., Yasuhara, K., Tamura, T., Onodera, H., Takagi, H., Takizawa, T., Hirose, M., Hayashi, Y., Mitsumori, K.: **Lack of modifying effects of eugenol on development of lung proliferative lesions induced by urethane in transgenic mice carrying the human prototype c-Ha-ras gene**

J. Toxicol. Sci., **26**, 129-39 (2001)

To investigate the modifying effects of eugenol (EUG), a component of cigarette smoke, on lung carcinogenesis, rasH2 mice and their non-transgenic littermates (non-Tg mice) were given an urethane (UR) injection, followed by EUG administration for 26 weeks. Alveolar/bronchiolar adenomas and carcinomas were observed in all UR-treated rasH2 groups. In non-Tg mice, similar lesions were sporadically observed in UR-treated groups. However, there were no significant differences in the incidence and multiplicity of these lesions between the UR alone and UR+EUG groups in both rasH2 and non-Tg mice. These results suggest that the EUG treatment does not exert modifying effects on lung carcinogenesis induced by UR in rasH2 and non-Tg mice.

Keywords: eugenol, lung carcinogenesis, rasH2 mice

Koujitani, T., Yasuhara, K., Toyosawa, K., Shimada, A., Onodera, H., Takagi, H., Tamura, T., Hirose, M., Mitsumori, K.: **Immunohistochemical and ultrastructural studies of 2,6-dimethylaniline-induced nasal proliferative lesions in a rat two-stage nasal carcinogenesis model initiated with N-bis(2-hydroxypropyl) nitrosamine.**

Toxicol. Pathol., **29**, 300-307 (2001)

F344 rats received DMA in diet for 52 weeks after initiation with DHPN. Bowman's gland proliferation, glandular hyperplasias, dysplastic foci, adenomas, and carcinomas were observed. These nasal lesions mostly arose in the olfactory mucosa. They were positive for cytokeratin and/or collagen type IV. Ultrastructurally, intracytoplasmic dense secretory granules, identical to those in normal Bowman's glands, were observed in the lesions. Two morphological continua were evident, one from dysplastic foci to carcinomas and the other from Bowman's gland proliferation to hyperplasias and adenomas. These results suggest that dysplastic foci arise from Bowman's glands and progress to carcinomas, while proliferation of Bowman's glands result in glandular hyperplasias and adenomas.

Keywords: dimethylaniline, nasal tumor, histopathology

Tamura, T., Mitsumori, K., Onodera, H., Fujimoto, N., Yasuhara, K., Takegawa, K., Takagi, H., Hirose, M.: **Dose-threshold for thyroid tumor-promoting effects of orally administered kojic acid in rats after initiation with N-bis(2-hydroxypropyl) nitrosamine**

J. Toxicol. Sci., **26**, 85-94 (2001)

To evaluate the threshold dose of thyroid tumor-promoting effects of KA, rats received 0, 0.002, 0.008, 0.03, 0.125, 0.5 or 2%KA in diet for 20 weeks after DHPN initiation. The serum T4 levels were significantly decreased in the DHPN+0.125%KA or more. Thyroid weights were significantly increased in the DHPN+0.5 or 2%KA and in the 2%KA-alone group. Adenomas were observed in the DHPN+0.5 or 2%KA group, and carcinomas were observed in the DHPN+2%KA group. Only focal follicular cell hyperplasia was observed in 1/9 rats in the 2%KA-alone group. These results suggest that the no-observed-adverse effect for the thyroid tumor-promoting effect of KA is 0.03% (15.5 mg/kg/day) under the present experimental conditions, and that KA possesses weak tumorigenic activity in rats due to continuous serum TSH stimulation by a non-genotoxic mechanism.

Keywords: kojic acid, thyroid tumor, threshold

Tanakamaru, Z.[*1], Mori. I.[*1], Nishikawa, A., Furukawa, F., Takahashi, M. and Mori, H.[*2]: **Essential similarities between spontaneous and MeIQx-promoted aberrant crypt foci in the F344 rat colon.**

Cancer Lett., **172**, 143-149 (2001)

Aberrant crypt foci (ACFs) in the rat colon, of control or MeIQx-treated groups, were compared morphologically, immunohistochemically, and at the molecular biological level in order to elucidate their biological characteristics. Male 3-week-old F344 rats were fed a diet supplemented with or without MeIQx at doses of 100 ppm or less for 16 weeks. There were no morphological differences between MeIQx-promoted and spontaneous ACFs. There were also no differences in labeling for PCNA and *p53* protein between these ACFs. Dot blot hybridization revealed no c-K-ras mutations in codon 12 except in one ACF developing in a rat given 100 ppm MeIQx, in which a GGT

→GAT single base substitution was detected. Our results thus suggest that most ACFs found in rats given 100 ppm MeIQx are essentially identical to their spontaneous counterparts.

Keywords: aberrant crypt foci, rat colon, MeIQx

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Yasuhara, K., Koujitani, T., Takegawa, K., Nasu, M., Onodera, H., Takagi, H., Hirose, M., Mitsumori, K.: **Promoting effects of xylazine on development of thyroid tumors in rats initiated with N-bis(2-hydroxypropyl) nitrosamine and the mechanism of action**

Carcinogenesis, **22**, 613-618 (2001)

To cast light on whether xylazine hydrochloride (XZ), a veterinary medicine commonly used as a sedative agent for food-producing animals, has any promoting potential for thyroid carcinogenesis, XZ was administered to rats for 52 weeks after initiation with DHPN. The incidence and multiplicities of thyroid tumors in the DHPN+XZ group were significantly increased as compared with the DHPN alone case. Decreased serum T3 and T4 and increased TSH were observed in rats treated XZ for 1 week. Thyroid iodine uptake showed significant decrease in rats treated XZ for 2 weeks. These results indicate that XZ has tumor-promoting effects on thyroid follicular cells, and suggest an involvement of alterations in thyroid-related hormone levels due to inhibition of thyroid iodine uptake.

Keywords: xylazine, thyroid tumorigenesis, TSH

Takagi, H., Mitsumori, K., Onodera, H., Nasu, M., Tamura, T., Yasuhara, K., Takegawa, K., Hirose, M.: **Modifying effects of endocrine disrupting chemicals on N-bis (2-hydroxypropyl) nitrosamine and sulfadimethoxine-induced thyroid carcinogenesis in rats**

J. Toxicol. Pathol., **14**, 121-128 (2001)

To clarify whether 17βstradiol 3-benzoate (EB), methoxychlor (MXC), atrazine (ATR), or bisphenol A (BPA) have any modifying effects on relatively late stages of thyroid carcinogenesis, female castrated F344 rats received a single injection of N-bis(2-hydroxypropyl) nitrosamine and, starting one week later, were given drinking water containing sulfadimethoxine for 8 weeks were used. They were treated with EB, MXC ATR or BPA for 25-27 weeks. The results of the present study suggest that EB with strong estrogenic activity, but not MXC and BPA with only weak estrogenic activity or ATR, exerts promoting effects on thyroid carcinogenesis in rats.

Keywords: endocrine disrupter, thyroid carcinogenesis, rat

Ueda, M., Mitsumori, K., Onodera, H., Takagi, H., Yasuhara, K., Takizawa, T., Hirose, M.: **Lack of modifying effects of bisphenol A and roasted-ground soybean (kinako) on *N*-ethyl-*N*-nitrosourea-induced uterine carcinogenesis in heterozygous *p53* deficient CBA mice**

J. Toxicol. Pathol., **14**, 129-134 (2001)

To clarify the effects of endocine disrupting chemicals with weak estrogenic activity on development of uterine tumors, female *p53*(+/-) CBA mice received an injection of *N*-ethyl-*N*-nitrosourea (ENU) followed by the diet containing bisphenol A (BPA) or roasted-ground soybean (SB) for 26 weeks. Lower value in uterine weight was observed in BPA- or SB-treated groups compared to the ENU alone group, but no significant differences were observed in the incidence of uterine endometrial stromal sarcomas and their PCNA labeling indices among the groups. The results indicate that 1% BPA and 20% SB in diet have no modifying effects on uterine carcinogenesis in *p53*(+/-) CBA mice initiated with ENU.

Keywords: uterine carcinogenesis, bisphenol A, soybean

Takizawa, T., Mitsumori, K., Takagi, H., Onodera, H., Yasuhara, K., Tamura, T., Hirose, M.: **Modifying effects of flumequine on dimethylnitrosamine-induced hepato-carcinogenesis in heterozygous *p53* deficient CBA mice**

J. Toxicol. Pathol., **14**, 135-143 (2001)

Flumequine (FL), a quinolone-antibiotic used for veterinary treatment of infections was found to dlicit heptocellular tumors in a conventional 18-month carcinogenicity study in mice. In the present study, heterozygous *p53* deficient CBA [*p53* (+/-)] mice and their wild-type littermates [*p53* (+/+)] received an injection of dimethylnitrosamine (DMN) followed by the diet containing FL for 26 weeks were used. There were small numbers of hepatofelular adenomas (Ad) and carcinomas in the DMN+FL group of *p53* (+/-) mice, Ad in the FL alone group of *p53* (+/-) mice, and Ad in the DMN+FL group of *p53* (+/+) mice. Induction of hepatocelular tumors in these mice within a relatively short period suggests that mechanisms such as direct or indirect damage to DNA might be responsible for the hepatocarcinogenesis of FL.

Keywords: flumequine, hepatocarcinognesis, heterozygous *p53* deficient mice

Onodera, H., Mitsumori, K., Takagi, H., Yasuhara, K., Koujitani, T., Tamura, T., Imai, T., Hirose, M.: **Susceptibility of heterozygous p53 deficient CBA mice to induction of liver proliferative lesions by Phenobarbital after dimethylnitrosamine initiation**

J. Toxicol. Pathol., **14**, 273-278 (2001)

To investigate the susceptibility of heterozygous p53-deficient CBA [*p53* (+/-)] mice to promotion of liver proliferative lesions in a two stage hepatocarcinogenesis model, CBA [*p53* (+/-)] mice and their wild type littermates [*p53* (+/+)] received an injection of N-nitrosodimethylamine (DMN), and from one week later, each group was given drinking water containing Phenobarbital (PB) for 26 weeks. The incidences of eosinophilic foci and hepatocellular adenomas in *p53* (+/-) mice were much higher than those in *p53* (+/+) mice in the DMN+BP groups. The present results suggest that *p53* (+/-) CBA mice are very susceptible to promotion of the development of liver proliferative lesions by PB after DMN initiation.

Keywords: hepatocarcinogenesis, Phenobarbital, heterozygous *p53* deficient mice

Uneyama, C., Shibutani, M., Nakagawa, K., Masutomi, N., Hirose, M.: **Methacarn, a fixation tool for multipurpose gene expression analysis from paraffin-embedded tissue materials.**

Current Topics in Biochem. Res. **3**, 237-242 (2000)

We examined various fixatives for expression analysis of RNA and protein as well as genomic DNA analysis using paraffin-embedded tissues. Among fixatives, methacarn fixation showed best performance in terms of yield of molecules from paraffin-embedded tissue samples. Furthermore, isolated molecules retained high quality enough for quantitative analysis in Western blotting, RT-PCR and DNA-direct sequencing.

Keywords: Methacarn, Molecular aanalysis, Paraffin-embedded tissue

Niho, N., Shibutani, M., Tamura, T., Toyoda, K., Uneyama, C., Takahashi, N., Hirose, M.: **Subchronic toxicity study of gallic acid by oral administration in F344 rats.**

Food Chem. Toxicol. **39**, 1063-1670 (2001)

Subchronic toxicity of gallic acid (GA) was investigated in F344 rats by feeding diet containing 0.0, 0.2, 0.6, 1.7 and 5% GA for 13 weeks. Body weight gain in the 5% GA-treated animals of both sexes from week 1 was significantly lower than that of the untreated controls. Toxic effects following administration of 0.6% or more in males and 5% in females included reduction of hemoglobin concentration, hematocrit and red blood cell counts and increase in reticulocytes. Extramedullary hematopoiesis, hemosiderin deposition and congestion appeared in the spleens of 5% GA-treated animals, suggesting development of hemolytic anemia. Centrilobular liver cell hypertrophy, reflected in increase in liver weight, was observed in animals of both sexes from 1.7%. In the kidney, Berlin blue-negative brown pigment deposition in the proximal tubular epithelium was observed at 5% GA. 0.2% was determined to be a non-observed adverse effect level in rats. This level was translated into 119 and 128 mg/kg/day, respectively for male and female rats.

Keywords: Gallic acid, Subchronic toxicity study, hemolytic anemia

Masutomi, N., Toyoda, K., Shibutani, M., Niho, N., Uneyama, C., Takahashi, N., Hirose, M.: **Toxic effects of benzyl and allyl isothiocyanates and benzyl-isoform specific metabolites in the urinary bladder after a single intravesical application to rats.**

Toxicol. Pathol. **29**, 617-622 (2001)

To elucidate toxic effects of isothiocyanates (ITCs), benzyl isothiocyanate (BITC), allyl isothiocyanate (AITC), or BITC-metabolites conjugated either with GSH, Cys-Gly, Cys, or mercapturic acid were intravesically instilled into female F344 rats. Exposure to AITC and BITC at 2.8 mg/kg, and the same mol quantity of BITC-metabolites was for 2 hr. The animals were i.v. administered BrdU after 19 hr. and killed 1 hr. later. BITC caused more profound toxic damage than AITC. Among the BITC-metabolites, cytotoxicity was evident with intermediate glutathione or cysteinylglycine conjugates, while the mercapturic acid exerted little effects. BrdU labeling was essentially dependent on the degree of cytotoxic potential of each compound. The present results support that cytotoxic activity of orally administered ITCs is derived from free forms cleaved from conjugated metabolite(s) in urine.

Keywords: Isothiocyanates, Metabolites, Urinary bladder cytotoxicity

Shibutani, M., Mitsumori K., Satoh, S., Hiratsuka, H., Satoh, M., Sumiyoshi, M., Nishijima, M., Katsuki, Y., Suzuki, J., Nakagawa J., Akagi, T., Imazawa, T., Ando, M.: **Relationship between toxicity and cadmium accumulation in rats given low amounts of cadmium chloride or cadmium-polluted rice for 22 months.**
J. Toxicol. Sci. **26**, 337-358 (2001)

To clarify toxic effects of long-term oral administration of low dose cadmium (Cd) on the liver and kidney, six groups of female SD rats were fed diet containing Cd-polluted rice or CdCl2 at concentrations up to 40 ppm, and killed after 12, 18, and 22 months. There was no evidence of Cd-related hepato-renal toxicity. Dose-dependent accumulation of Cd was observed in the liver and kidneys at 18 months in animals treated with 40 ppm CdCl2. A dose-dependent increase in urinary Cd levels became evident with time. Induction of metallothionein (MT) was also observed in the liver and kidney with a high correlation to the corresponding Cd levels. The results demonstrated that renal toxicity is not induced by long-term oral administration of low amounts of Cd, although tissue accumulation does occur. Possible protective mechanisms may be operating.
Keywords: Cadmium, Low-dose long-term exposure, Renal toxicity

Hagiwara, A.*1, Takesada, Y*1., Tanaka, H.*1, Tamano, S.*1, Hirose, M., Ito, N.*2, Shirai, T.*2 : **Dose-dependent induction of glandular stomach preneoplastic and neoplastic lesions in male F344 rats treated with catechol chronically.**
Toxicol. Pathol. **29**, 180-186 (2001)

The dose-dependent of catechol glandular stomach carcinogenesis was investigated in male F344 rats. Groups of 30 male animals were fed catechol at dietary levels of 0 (control), 0.1, 0.2, 0.4 and 0.8 % for up to 104 weeks. Five rats of each group were killed at 34 weeks and the remaining animals were sacrificed at the termination, all undergoing histopathological examination. Submucosal hyperplasias and adenomas of the pyloric glands developed in the 0.4 and 0.8 % group. Incidences of adenocarcinoma development in the pylorus were 4 and 8 % in 0.4 % and 0.8 % groups, respectively, and 0 in the 0.1 % and 0.2 % groups, at the termination. Adenomas and submucosal hyperplasias were found in nearly all animals fed 0.2 % catechol or more, the incidences of those in 0.1 % group being 0 % and 56 %, respectively. These results demonstrated that dietary levels of 0.4 % and 0.8 % catechol long-term induce adenocarcinomas in the pyloric glands.

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Hirose, M., Hoshiya, T.*1, Mizoguchi, Y.*1, Nakamura, A.*2, Akagi, K.*1, Shirai, T.*1 : **Green tea catechins enhance tumor development in the colon without effects in the lung or thyroid after pretreatment with 1,2-dimethylhydrazine or 2,2'- dihydroxy- di- n-propylnitrosamine in amle F344 rats.**
Cancer Lett. **168**, 23-29 (2001)

Modifying effects of green tea catechins (GTCs) on the post-initiation stage of colon, lung and thyroid carcinogenesis were examined F344 male rats. Groups of 20 animals were given subcutaneous injections of 40 mg/kg body wt of 1,2-dimethylhydrazine twice a week for 2 weeks or oral administration of 0.1 % DHPN in the drinking water for 2 weeks for initiation. They then received diet containing 1 or 0.1 % green tea catechin or basal diet alone for 33 weeks. Although total incidence and multiplicity of colon tumors were not significantly different from controls, values for colon adenomas were decreased. Incidences and multiplicities of lung and thyroid lesions did not significantly vary among the DHPN-treated groups. These results indicate that GTCs do not inhibit, but rather may enhance colon carcinogenesis.

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Kimoto, N.*1, Hirose, M., Futakuchi, M.*1, Iwata, T.*2, Kasai *2, M., Shirai, T.*1 : **Site-specific modulating effects of conjugated fatty acids from safflower oil in a rat two-stage carcinogenesis model in female Sprague-Dawley rats.**
Cancer Lett. **168**, 15-21 (2001)

Modiyfying effects of dietary administration of conjugated fatty acids from safflower oil (CFA-S), rich in conjugated linoleic acids, on major organs were examined in the post-initiation stage of a two-stage carcinogensis model in female rats. Groups of 21 or 22 F344 female rats were treated sequentially with DHPN (i.g.), DMBA (i.g.), DMH (s.c.) and BBN (in drinking water) during the first 3 weeks for initiation, and then administered diet containing 1 or 0.1 % CFA-S for 33 weeks. The 1 and 0.1 % CFA-S treatment significantly decreased the incidence and multiplicity of mammary carcinomas, though a clear

dose response was not observed. In the urinary bladder, the incidence of papillary or nodular hyperplasia was significantly increased in the 1% CFA-S-treated group. The results indicate that low dose CFA-S may find application as a potent chemopreventor of mammary carcinogenesis.

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Ogawa, K.*, Hirose, M., Sugiura, S.*, Cui, L.*, Imaida, K.*, Ogiso, T*, Shirai, T.*: **Dose-dependent promotion by phenylethyl isothiocyanate, a known chemopreventer, of two-stage rat urinary bladder and liver carcinogenesis.**
Nutr. Cancer **40**, 134-139 (2001)

The effects of PEITC on urinary bladder and liver carcinogenesis were analyzed in a rat model. Diets containing 0.1%, 0.05%, or 0.01% PEITC were administered for 32 wks to male F344 rats with and without pretreatment with an injection of DEN (200 mg/kg, i.p.) and 0.05% BBN in the drinking water for 4 weeks for initiation. In the initiated groups, PEITC administration significantly increased the incidences of papillary or nodular hyperplasia, dysplasia and transitional cell carcinomas at higher doses of 0.01%, 0.01% and 0.05 %, respectively. In the liver, induction of GST-P foci was dose-dependently enhanced by PEITC administration, but the incidences of liver tumors were not different among the groups. We can conclude that >0.01% PEITC enhances rat urinary bladder carcinogenesis, while weakly promoting hepatocarcinogenesis. In addition, it is suggested that >0.05% PEITC has tumorigenic potential.

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Hamada, S.*1,*2, Yamasaki, K.*2, Nakanishi, S.*2, Omori, T., Serikawa, T.*2, Hayashi, M.: **Evaluation of the general suitability of the rat for the micronucleus assay: the effect of cyclophosphamide in 14 strains**
Mutat. Res., **495**, 127-134 (2001)

To evaluate the general suitability of the rat for the micronucleus assay, we conducted the assay in males of 14 different strains, 13 inbred (ACI, BN, BUF, COP, DRH, F344, IS, LEW, RCS, SHR, WAG, WKYO, WTC) and 1 outbred (SD), using cyclophosphamide as the test chemical. Cyclophosphamide at 0 (vehicle), 5, 10, or 20 mg/kg/day was administered orally twice, 24-h apart, to 5 rats per dosage group. Bone marrow and peripheral blood were collected 24 h after the second treatment. All 14 strains showed a positive response to cyclophosphamide, with slight differences in sensitivity. We concluded that the rat is suitable for the micronucleus assay regardless of strain.

Keywords: Micronucleus assay, Strain differences, Cyclophosphamide

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Nishikawa, T.*, Haresaku, M.*, Fukushima, A.*, Nakamura, T.*, Adachi, K.*, Masuda, M.*, Hayashi, M.: **Further evaluation of an in vivo micronucleus test on rat and mouse skin: results with five skin carcinogens**
Mutat. Res., **513**, 93-102 (2002)

In a previous paper, we presented a practical in vivo micronucleus (MN) test that used rat skin as the target organ. To evaluate the test, as well as to determine the reproducibility and applicability of the method to mice, we used it to test the effect of five skin carcinogens (*N*-ethyl-*N*'-nitro-*N*-nitrosoguanidine (ENNG), *N*-methyl-*N*'-nitro-*N*-nitroso-guanidine (MNNG), 4-nitroquinoline 1-oxide (4NQO), 7,12-dimethylbenz[*a*]anthracene (DMBA), and benzo[a]pyrene (B[*a*]P)) on rat and mouse skin. All five compounds significantly and dose-dependently increased the MN frequencies in the basal cells of the chemical-treated skin. These results indicated the reproducibility of the test results and also the applicability of the test to mice as well as rats.

Keywords: Skin micronucleus test, 2-methylbenz[*a*]anthracene, Benzo[*a*]pyrene

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Honma, M., Momose, M., Sakamoto, H., Sofuni, T., Hayashi, M.: **Spindle poisons induce allelic loss in mouse lymphoma cells through mitotic non-disjunction**
Mutat. Res., **493**, 101-114, 2001

Aneuploidy is an important contributor to reproductive failure and tumor development. Two spindle poisons, colchicine (COL) and vinblastine (VBL) are mutagenic in the mouse lymphoma assay (MLA), a gene mutation assay that targets the heterozygous thymidine kinase (tk) gene on chromosome 11 in mouse lymphoma L5178Y $tk^{+/-}$ 3.7.2c cells. To investigate the mechanisms of spindle poison mutagenesis, we analyzed the COL- and VBL-induced TK mutants at the molecular and cytogenetic level. Loss of heterozygosity (LOH) analysis employing a microsatellite region within the tk locus revealed that almost all mutants had lost the functional tk allele. To

determine the extent of the LOH, we further examined LOH mutants for heterozygosity at nine microsatellite loci spanning the entire chromosome 11. Our present study indicates that spindle poisons induce mutations through mitotic non-disjunction without structural DNA changes and supports a possible mechanism in which a recessive mutation mediated by aneuploidy may develop tumors.
Keywords: spindle poisons, aneuploidy, mouse lymphoma assay (MLA)

Morimoto, S.*, Kato, T.*, Honma, M, Hayashi, M., Hanaoka, F.*, Yatagai, F.*: **Detection of genetic alterations induced by low-dose X rays: analysis of loss of heterozygosity for TK mutation in human lymphoblastoid cells**

Radiat. Res., **157**, 533-538 (2002)

To elucidate the genetic influence of low-dose ionizing radiation at the chromosome level, we exposed human lymphoblastoid TK6-20C cells to 10 cGy of X rays. The TK mutation frequency was 5.7 +/- 1.3 x 10^{-6} at the background level and 6.9 +/- 2.8 x 10^{-6} after X irradiation. We applied multilocus analysis using 4 TK locus markers and 12 microsatellite loci spanning chromosome 17 for TK mutants exhibiting loss of heterozygosity (LOH). We observed radiation-specific patterns in the extent of hemizygous LOH in 14 TK mutants among the 92 mutants analyzed. Surprisingly, the radiation-specific LOH patterns were not observed among the 110 nonirradiated TK mutants in this study. They were identified previously in TK6 cells exposed to 2 Gy of X rays. We consider these hemizygous LOH mutants to be a result of end-joining repair of X-ray-induced DNA double-strand breaks.
Keywords: loss of heterozygosity (LOH), p53, mutation
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Noda, Y.*[1], Suzuki, T., Kohara, A., Hasegawa, A.*[2], Yotsuyanagi, T.*[1], Hayashi, M., Sofuni, T., Yamanaka, K.*[2], Okada, S.*[3] : **In vivo genotoxicity evaluation of dimethylarsinic acid in Muta^{TM}Mouse**

Mutat. Res., **513**, 205-212 (2002)

The present study was conducted to evaluate whether arsenite or its metabolite, dimethylarsinic acid (DMA), could initiate carcinogenesis via mutagenic DNA lesions in vivo. When DMA was intraperitoneally injected into Muta^{TM}Mice at a dose of 10.6 mg/kg per day for 5 consecutive days, it caused only a weak increase in the mutant frequency (MF) of the *lacZ* gene in the lung, which was at most 1.3-fold higher than in the untreated control animals. DMA did not appreciably raise the MF in the bladder or bone marrow. Further analysis of the *cII* gene in the lung, the organ in which DMA induced the DNA damage, revealed only a marginal increase in the MF. Following DMA administration, no change in the *cII* mutation spectra was observed. Administration of arsenic trioxide (arsenite) at a dose of 7.6 mg/kg per day did not result in any increase in the MF of the *lacZ* gene in the lung, kidney, bone marrow, or bladder. The periferal blood micronucleus assay gave marginally positive results with arsenite, but not with DMA. These results suggest that the mutagenicity of DMA and arsenite might be too low to be detected in the Muta^{TM}Mouse
Keywords: Muta^{TM}Mouse, arsenic, dimethylarsinic acid
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Kohara, A., Suzuki, T., Honma, M., Ohwada, T.*, Hayashi, M. : **Mutagenicity of aristolochic acid in the lambda/*lacZ* transgenic mouse (Muta^{TM}Mouse)**

Mutat. Res., **515**, 63-72 (2002)

Aristolochic acid (AA) is found in a plant that causes urothelial carcinomas in patients with Chinese herb nephropathy (CHN). To evaluate the in vivo mutagenicity of AA, we analysed the mutant frequency (MF) in the *lacZ* and *cII* gene of 10 organs of the lambda/*lacZ* transgenic mouse (Muta^{TM}Mouse) after intragastric treatment with AA (15mg/kg per week x 4). MFs in the target organs-forestomach, kidney, and bladder of AA-treated mice were significantly higher than those of control mice (forestomach 33- and 15-fold; kidney 10- and 9-fold; bladder 16- and 31-fold, for the *lacZ* and *cII*, respectively). The MFs in non-target organs, except the colon, showed only slight increases. Sequence analysis of *cII* mutants in target organs revealed that AA induced mainly A:T to T:A transversions whereas G:C to A:T transitions at CpG sites predominated among spontaneous mutations.
Keywords: *cII*, Aristolochic acid, Chinese herb nephropathy
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Kohara, A., Suzuki, T., Honma, M., Oomori, T., Ohwada, T.*, Hayashi, M.: **Dinitropyrenes induce gene mutations in multiple organs of the lambda/*lacZ* transgenic mouse (Muta^{TM}Mouse)**

Mutat. Res. **515**, 73-83 (2002)

Dinitropyrenes (DNPs), 1,3-, 1,6- and 1,8-dinitropy-

rene, are carcinogenic compounds found in diesel engine exhaust. A commercially available mixture of DNPs (1,3-, 1,6-, 1,8-, and unidentified isomer(s) was injected intragastrically at 200 and 400mg/kg once each week for 4 weeks. Seven days after the final treatment, liver, lung, colon, stomach, and bone marrow were collected for mutation analysis. Strongest increases in mutant frequencies (MFs) were observed in colon for both *lacZ* ($7.5x10^{-5}$ to $43.3x10^{-5}$) and *cII* ($2.7x10^{-5}$ to $22.5x10^{-5}$) genes. Three-four-fold increases were observed in stomach for both genes. A statistically significant increase in MFs was also evident in liver and lung for the *lacZ* gene, and in lung and bone marrow for the *cII* gene. The DNPs treatment increased the incidence of G:C to T:A transversion (2-43%) and decreased G:C to A:T transitions (70-22%). The present study showed a relevant use of the *cII* gene as an additional target for mutagenesis in the Muta^{TM}Mouse and revealed a mutagenic specificity of DNPs in vivo.

Keywords: *cII*, dinitropyrene, diesel exhaust

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Fujita, K.*, Nakayama, K.*, Yamazaki, Y.*, Tsuruma, K.*, Yamada, M., Nohmi, T., Kamataki, T.*: **Construction of *Salmonella typhimurium* YG7108 strains, each coexpressing a form of human cytochrome P450 with NADPH-cytochrome P450 reductase**

Environ. Mol. Mutagen., **38**, 329-338 (2001)

A series of *S. typhimurium* YG7108 strains, each coexpressing a form of human cytochrome P450 (CYP) together with human NADPH-cytochrome P450 reductase (OR), was established. The parental *S. typhimurium* YG7108, derived from TA1535, lacks two O^6-methylguanine-DNA methyltransferase genes and is highly sensitive to alkylating agents. The expression levels of CYP holo-protein and the OR in the genetically engineered *S. typhimurium* YG7108 cells ranged from 62 nmol/L culture for CYP3A4 and from 214 to 1029 units/L culture, respectively. Each form of expressed CYP efficiently catalyzed the oxidation of a representative substrate. The OR was sufficiently expressed to support the catalytic activity of CYP.

Keywords: heterologous expression, enzyme activity, kinetic parameter

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Gruz, P., Pisani, F.M.*[1], Shimizu, M., Yamada, M., Hayashi, I.*[2], Morikawa, K.*[2], Nohmi, T.: **Synthetic activity of Sso DNA polymerase Y1, an archaeal DinB-like DNA polymerase, is stimulated by processivity factors proliferating cell nuclear antigen and replication factor C**

J. Biol. Chem., **276**, 47394-47401 (2001)

The recent discovery of DNA polymerase Y family raises a question of whether the DNA polymerase activities are modified by accessory proteins such as proliferating cell nuclear antigen (PCNA). In fact, the activity of DNA pol IV (DinB) of *E. coli* is enhanced upon interaction with the beta subunit, the processivity factor of DNA pol III. In this report, the activity of *Sso* DNA pol Y1 encoded by the dbh gene of the archaeon *Sulfolobus solfataricus* is greatly enhanced by the presence of PCNA and replication factor C (RFC). *Sso* pol Y1 displayed a higher affinity for DNA compared with pol IV. The abilities of pol Y1 and pol IV to bypass DNA lesions and their sensitive sites to protease are also discussed.

Keywords: DNA polymerase, PCNA, bypass DNA lesion

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Abril, N.*, Luque-Romero, F.L.*, Yamada, M., Nohmi, T., Pueyo, C.*: **The effectiveness of the O^6-alkylguanine-DNA alkyltransferase encoded by the ogt_{ST} gene from *S. typhimurium* in protection against alkylating drugs, resistance to O^6-benzylguanine and sensitisation to dibromoalkane genotoxicity**

Mutat. Res., **497**, 111-21 (2001)

Here we demonstrate that the Ogt_{ST} from *Salmonella typhimurium* is a highly efficient O^6-alkylguanie-DNA alkyltransferase (AGT) in affording protection against antitumour chloroethylating drugs (1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)). In addition, Ogt_{ST} is refractory to O^6-benzylguanie (BG) inactivation and its expression provides only minor sensitization to genotoxicity by environmental dibromoalkanes (DBE). Our observations indicate that the Ogt_{ST} AGT might be, under some circumstances, of potential use to improve cancer chemotherapy.

Keywords: O^6-alkylguanine-DNA alkyltransferase, Dibromoethane, Ara^r-assay

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Kim, S.-R., Matsui, K., Yamada, M., Gruz, P., Nohmi, T.: **Roles of chromosomal and episomal *dinB* genes encoding DNA pol IV in targeted and untargeted mutagenesis in *Escherichia coli***

Mol. Genet. Genomics., **266**, 207-15 (2001)

This report presents that DNA pol IV is involved in -1 frameshift mutagenesis induced by 4-nitroquinoline N-oxide (4-NQO) and that the expression level of the chromosomal pol IV gene is 6-12 times higher than those for other SOS-inducible DNA polymerases in *E. coli*. Interestingly, the *dinB* gene is present not only on the chromosome but also on the F' plasmid in the *E. coli* CC108 strain. In this strain, 750 molecules of DNA pol IV are expressed form the F' *dinB* gene in the uninduced state and 250 molecules are expressed from the chromosomal gene. These cellular expression levels strongly affect -1 frameshifts induced by 4-NQO in runs of six guanine bases. The chromosomal *dinB* gene appeared to have little or no effect on the untargeted mutagenesis. These results suggest that DNA pol IV efficiently mediates targeted mutagenesis by 4-NQO, and that the expression levels substantially affect targeted and untargeted mutagenesis.

Keywords: *dinB*, 4-NQO, Targeted mutagenesis

Swiger, R.R.*, Cosentino, L.V.*, Maumura, K., Nohmi, T., Heddle, J.A.*: **Further characterization and validation of *gpt* delta transgenic mice of quantifying somatic mutations in vivo**

Environ. Mol. Mutagen., **37**, 297-303 (2001)

The utility of any mutation assay depends on its characteristics, which are best discovered using model mutagens. To this end, we report further on the characteristics of the lambda-based *gpt* delta transgenic assay first described by Nohmi et al. Our studies show that the *gpt* transgene responds similarly to other transgenic loci, specifically *lacZ* and *cII*, after treatment with acute doses of *N*-ethyl-*N*-nitrosourea (ENU). Because genetic neutrality is an important factor in the design of treatment protocols for mutagenicity testing, as well as for valid comparisons between different tissues and the results indicate that the *gpt* transgene, like *cII* and *lacZ*, is genetically neutral in vivo. The sensitivities of the loci are also equivalent, as evidenced by spontaneous mutant frequency data and dose-response curves after acute treatment with ENU. The results are interesting in light of transgenic target size and location and of host genetic background differences. Based on these studies, protocols developed for other transgenic assays should be suitable for the *gpt* delta. Additionally, a comparison of the *gpt* and an endogenous locus, Dlb-1, within the chronically treated animals shows differential accumulation of mutations at the loci during chronic exposure.

Keywords: *cII*, *lacZ*, preferential repair

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Tanabe, H., Müller, S.*[1], Neusser, M.*[1], von Hase, J.*[2], Calcagno, E.*[2], Cremer, M.*[1], Cremer, C.*[2], Cremer, T.*[1] : **Evolutionary conserved 3D positioning of orthologous chromosomes and chromosome segments in primate lymphoblastoid cell nuclei**

Annales de Genetique, **44**, s120 (2001)

Human chromosome territories 18 and 19 show a different position in the human lymphocyte cell nucleus. The gene-poor, late replicating chromosome 18 territory is preferentially located at the nuclear periphery, while the gene-rich and early replicating chromosome 19 territory is found in the nuclear center. Here we examined whether this topology is evolutionarily conserved among primates. In a comparative 3D-FISH analysis we used painting probes specific for human chromosome 18 and 19 or corresponding probes for orthologous chromosomes/chromosomal segments from seven primates-great apes (human, chimpanzee, gorilla and orangutan), lesser apes (white-handed gibbon) and New World monkeys (cotton-top tamarin, marmoset and squirrel monkey). In all species light optical serial sections were recorded by confocal microscopy from hybridized, 3D preserved nuclei and used for 3D reconstruction. For quantitative evaluation we scanned 30 to 40 S-phase nuclei per species. The nucleus was divided from its center to the periphery into 25 shells and the cumulative hybridization signal for the painted chromosome territories or orthologous segments in each shell was calculated and plotted versus the radius of the shell. The results showed that the distribution of the orthologous chromosome materials was nearly identical, irrespective of their evolutionary conservation or multiple translocations. We conclude that the more peripheral or central 3D positioning of gene poor and gene dense chromosome materials ortholog to human chromosome 18 and 19 chromosome territories is evolutionarily conserved among primates. Further studies will reveal whether other gene dense and gene poor chromosome segments follow the same pattern.

Keywords: chromosome territory, primates, 3D-FISH (three-dimensional fluorescence in situ hybridization)

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Masters, J.R.*1, Thomson, J.A.*2, Daly-Burns, B.*1, Reid, Y.A.*3, Dirks, W.G.*4, Packer, P.*5, Toji, L.H.*6, Ohno, T.*7, Tanabe, H., Arlett, C.F.*8, Kelland, L.R.*9, Harrison, M.*10, Virmani, A.*11, Ward, T.H.*12, Ayres, K.L.*13 Debenham, P.G.*1 : **Short tandem repeat profiling provides an international reference standard for human cell lines**

Proc. Natl. Acad. Sci. USA, **98**, 8012-8017 (2001)

Cross-contamination between cell lines is a longstanding and frequent cause of scientific misrepresentation. Estimates from national testing services indicate that up to 36% of cell lines are of a different origin or species to that claimed. To test a standard method of cell line authentication, 253 human cell lines from banks and research institutes worldwide were analyzed by short tandem repeat profiling. The short tandem repeat profile is a simple numerical code that is reproducible between laboratories, is inexpensive, and can provide an international reference standard for every cell line. If DNA profiling of cell lines is accepted and demanded internationally, scientific misrepresentation due to cross-contamination can be largely eliminated.

Keywords: STR (short tandem repeat) profiling, cross-contamination, human cell lines

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*12 Department of Drug Development, Paterson Institute for Cancer Research, United Kingdom
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Tanabe, H., Müller, S.*1, Neusser, M.*1, von Hase, J.*2, Calcagno, E.*2, Cremer, M.*1, Solovei, I.*1, Cremer, C.*2, Cremer, T.*1 : **Evolutionary conservation of chromosome territory arrangements in cell nuclei from higher primates**

Proc. Natl. Acad. Sci. USA, **99**, 4424-4429 (2002)

We demonstrate that the nuclear topological arrangement of chromosome territories (CTs) has been conserved during primate evolution over a period of about 30 million years. Recent evidence shows that the positioning of chromatin in human lymphocyte nuclei is correlated with gene density. For example, human chromosome 19 territories, which contain mainly gene-dense and early replicating chromatin, are located toward the nuclear center, whereas chromosome 18 territories, which consist mainly of gene-poor and later replicating chromatin, is located close to the nuclear border. In this study, we subjected seven different primate species to comparative analysis of the radial distribution pattern of human chromosome 18- and 19-homologous chromatin by three-dimensional fluorescence in situ hybridization. Our data demonstrate that gene-density-correlated radial chromatin arrangements were conserved during higher-primate genome evolution, irrespective of the major karyotypic rearrangements that occurred in different phylogenetic lineages. The evolutionarily conserved positioning of homologous chromosomes or chromosome segments in related species supports evidence for a functionally relevant higher-order chromatin arrangement that is correlated with gene-density.

Keywords: chromosome territories (CTs), evolutionary conservation, 3D-FISH (three-dimensional fluorescence in situ hybridization)

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Ishikawa, K.S.*, Masui, T., Ishikawa, K.*, Shiojiri, N.* : **Immunolocalization of hepatocyte growth factor and its receptor (c-Met) during mouse liver development**

Histochem. Cell Biol., **116**, 453-462 (2001)

Although hepatocyte growth factor (HGF) was discovered as a potent hepatotrophic factor responsible for liver regeneration and may involve some organ development in embryogenesis, it remains to be revealed what roles HGF plays in liver development. The present study was undertaken to determine which cells express HGF and its receptor c-Met and when c-Met is activated in mouse liver development by using immunoblotting and immunohistochemical techniques. HGF was detected in hepatocytes and non-parenchymal cells, including biliary epithelial cells, periportal connective tissue cells, megakaryocytes, endothelial cells, and sinusoidal cells, throughout liver development. Positive HGF immunostaining in hepatocytes increased during postnatal development, and reached the maximal level in the adult stage. c-Met protein was also expressed in hepatocytes throughout liver development, but maximal staining was obtained in 1- or 2-week-old livers. Phosphorylation of tyrosine residues in the c-Met beta chain also occurred in these stages. These results suggest that HGF signaling is implicated in hepatocyte growth during postnatal liver development, and its action could be in a paracrine mode; HGF produced by non-parenchymal cells such as sinusoidal cells acts on hepatocytes expressing c-Met receptors. Positive immunostaining in adult and postnatal hepatocytes may be derived from their blood clearance of HGF.

Keywords: HGF, c-Met, hepatocytes

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Iwashita, S.*1, Itoh, T.*2, Takeda, H.*2, Sugimoto, Y.*2, Takahashi, I.*3, Nobukuni, T.*4, Sezaki, M.*1, Masui, T., Hashimoto, K.*3 : **Gene organization of bovine BCNT that contains a portion corresponding to an endonuclease domain derived from an RTE-1 (Bov-B LINE), non- LTR retrotransposable element: duplication of an intra- molecular repeat unit downstream of the truncated RTE-1**

Gene, **268**, 59-66 (2001)

BCNT (a protein named after Bucentaur or craniofacial development protein 1) has a unique structure in Ruminantia. Bovine BCNT contains a region of the endonuclease domain derived from a truncated RTE-1 (previously called Bov-B LINE), a non-LTR retrotransposable repetitive element, and two repeat units (intramolecular repeat, IR) each with 40 amino acids in the C-terminal region. In contrast the human and mouse BCNT proteins contain one repeat unit and lack the RTE-1-derived portion. The 3' UTR of bovine bcnt cDNA also contains an approximately300-bp portion homologous to the 3'-part of RTE-1. We examined the bovine bcnt genomic DNA sequence to understand how the bovine bcnt gene has been organized. The sequence of 3' UTR homologous portion was found to more closely resemble the Art2 element than the bovine RTE-1. By PCR screening a bovine/hamster hybrid somatic cell panel, the bovine bcnt gene was mapped to chromosome 18, syntenic human chromosome 16q on which human BCNT is located. The bcnt genomic DNA sequence corresponding to the cDNA downstream of a RTE-1 derived portion reveals that each IR unit is flanked by both 5'-side and 3'-side introns and that 3'-UTR consists of one exon. The alignment of the above sequence with a bovine RTE-1 did not show any significant homology downstream of the endonuclease domain. On the other hand, the alignment of the intron sequences with each other revealed that the six sequential homologous segments ranging in size from 40 to 453 bp existed over a 1 kb long sequence between both the 5'- and 3'-side introns flanking each bovine IR unit. In addition, both the 174-bp of 5'-side intron and 80-bp of 3'-side intron neighboring each 120-bp IR exon are significantly homologous among the two bovine IRs, human IR and mouse IR. These results suggest that a truncated bovine RTE-1 was inserted into the intron upstream of an IR unit of an ancestor bcnt gene and that a duplication of a relatively long region that includes IR occurred in the bovine genome.

Keywords: BCNT, RTE-1(Bov-B LINE), exon intron structure

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Koizumi M, Yamamoto Y*1, Ito Y*1, Takano M*2, Enami T*2, Kamata E and Hasegawa R: **Comparative study of the toxicity of 4-nitrophenol and 2,4-dinitrophenol in newborn and young rats**

J. Toxicol. Sci., **26**, 299-311 (2001).

The toxicities of 4-nitrophenol and 2,4-dinitrophenol in newborn and young rats was examined and the susceptibility of newborn rats was analyzed in terms of presumed unequivocally toxic and no observed adverse effect levels (NOAELs). In the 18-day repeated dose

newborn rat study, 4-nitrophenol was orally given from day 4 to day 21 after birth but did not induce any toxicity up to 160 mg/kg in the main study, although it induced death in one of six males at 160 mg/kg, and three of six males and one of six females at 230 mg/kg in a prior dose-finding study. In the 28-day repeated dose oral toxicity study starting at 6 weeks of age, 4-nitrophenol caused the death of most males and females at 1,000 mg/kg but was not toxic at 400 mg/kg except for male rat specific renal toxicity. As unequivocally toxic levels were considered to be 230 mg/kg/day in newborn rats and 600 to 800 mg/kg/day in young rats, and NOAELs were 110 mg/kg/day in newborn rats and 400 mg/kg/day in young rats, the susceptibility of the newborn to 4-nitrophenol appears to be 2.5 to 4 times higher than that of young animals. In the newborn rat study of 2,4-dinitrophenol, animals died at 30 mg/kg in the dose-finding study and significant lowering of body and organ weights was observed at 20 mg/kg in the main study. In the 28-day young rat study, clear toxic signs followed by death occurred at 80 mg/kg but there was no definitive toxicity at 20 mg/kg. As unequivocally toxic levels and NOAELs were considered to be 30 and 10 mg/kg/day in newborn rats and 80 and 20 mg/kg/day in young rats, respectively, the toxicity of 2,4-dinitrophenol in newborns again seems to be 2 to 3 times stronger than in young rats. Abnormalities of external development and reflex ontogeny in the newborn were not observed with either chemical. Based on these results, it can be concluded that the toxic response in newborn rats is at most 4 times higher than that in young rats, at least in the cases of 4-nitrophenol and 2,4-dinitrophenol.

Keywords: Toxic response in the newborn rat, 4-Nitrophenol, 2,4-Dinitrophenol

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御船正樹*, 寺西俊幸*, 岩藤章正*, 秋澤宏行*, 小崎由香里*, 本橋範子**, 斎藤 寛*, 岡田敏史：**日本薬局方参照赤外吸収スペクトルと異常スペクトルについて**

医薬品研究, **33**, 259-266 (2002)

日本薬局方収載医薬品の確認試験に参照赤外吸収スペクトル法が多用されているが，その試料調製法の多くにKBr錠剤法が採用されている．塩酸塩などでKBr錠剤法を適用した場合，Br-/Cl-間でのイオン交換反応が起こり，薬物本来のスペクトルを与えないことがあることを具体的に指摘した．したがって，塩酸塩など塩の形をもつ薬物をIR法により確認する場合，ヌジョール法で得られるスペクトルと一致することを確認した後，錠剤法を適用すべきであることを提案した．

Keywords: Japanese Pharmacopoeia, IR Reference Spectra, ion-exchange reaction

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Miyazaki, T., Yomota C., Okada S.: **Ultrasonic depolymerizationof hyaluronic acid**

Polym. Degrad. Stab., **74**, 77-85 (2001)

Hyaluronic acid (HA) was depolymerized by ultrasonication. Changes in molecular weight and molecular weight distribution were observed by size exclusion chromatography with a low-angle laser light scattering photometer. We investigated the influence of sonication intensity, temperature, HA concentration, coexisting cations and ionic strength. Results demonstrated that, with an increase of intensity, initial depolymerization rate (k) increased and ultimate-depolymerized molecular weight (M_{lim}) converged to smaller size. The factors that change high-order structure of HA molecules had great influence upon the k, but not so much upon the M_{lim}. For example, continuous sonication with 55 W depolymerized the HA to almost the same M_{lim} (approximately $0.1x10^6$), with a few exceptions. Where exceptions occurred, they were in concentrated monovalent cation solutions; the M_{lim} increased up to about $0.3x10^6$. Consequently, by regulating the sonication conditions, HA with the desired lower molecular weight and a narrow distribution could be prepared from high molecular weight samples.

Keywords: hyaluronic acid, ultrasonication, molecular weight

Miyazaki, T., Yomota C., Okada S.: **Development and release characterization of hyaluronan-doxycycline gels based on metal coordination**

J. Control. Release, **76**, 337-347 (2001)

A simple mixing with hyaluronan (HA), doxycycline (DC) and divalent metal cation in an aqueous solution enabled a thermoreversible water-soluble gel to form. For the cross-linking, the two kinds of interactions were supposed. One was an electrostatic interaction between a positively charged group in DC and a negatively charged carboxyl function of HA. And the other was a chelation at the phenolic diketone moiety in DC. The hydrogel would

be formed holding water in the HA entanglement network when DCs on HA chains made coordinate bonds through metal chelation. By changing the mixing ratio, two types of gels with different characteristics in drug release could be prepared. One was a gel with zero-order release prepared by mixing the same amount of HA and DC in equivalent. And the other was a gel indicating Fickian diffusion-type release by mixing more DC than HA. Further, by controlling the absolute concentration of HA and DC, or the molecular weight of HA, some gels with desired release profiles could be prepared.

Keywords: hyaluronan, doxycycline, gel

Maekawa K., Tanimoto T., Okada S., Suzuki T.*1, Suzuki T.*1, Yabe-Nishimura C.*2: **Analysis of gene expression of the enzymes comprising polyol pathway in rat Schwann cells by competitive RT-PCR method using non-homologous DNA standards**

Brain Res. Protocols, **8**, 219-227 (2001)

Aldose reductase (AR) and sorbitol dehydrogenase (SDH) are the enzymes constituting the polyol pathway, an alternate route of glucose metabolism. A wealth of experimental data has indicated the involvement of the polyol pathway in the pathogenesis of diabetic complications. However, there has been surprisingly little research on the relative abundance of SDH to AR in the tissues affected in diabetes. We therefore developed a competitive RT-PCR system to simultaneously determine the mRNA levels of these two enzymes in small amounts of samples, and studied their expression in Schwann cells isolated from adult rat sciatic nerves. Although both AR and SDH mRNA were expressed in the Schwann cells, the levels of SDH cDNA were much lower than those of AR cDNA. The induction of AR mRNA expression in the Schwann cells under hyperosmotic conditions was similarly detected by Northern blot analysis and our competitive RT-PCR method. The RT-PCR system developed in this study may be a useful tool in ascertaining the relative contributions of AR and SDH to the metabolic derangements resulting from the acceleration of polyol pathway activity in the target organ of diabetic complications.

Keywords: Aldose reductase, Sorbitol dehydrogenase, Diabetic neuropathy, Hyperosmotic stress, Quantitative RT-PCR

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Maekawa K., Tanimoto T., Okada S.: **Gene expression of enzymes comprising polyol pathway in various rat tissues by competitive RT-PCR method**

Jpn. J. Pharmacol., **88**, 123-126 (2002)

The quantitative measurements of aldose reductase (AR) and sorbitol dehydrogenase (SDH) gene expression in various rat tissues were performed by competitive RT-PCR. AR mRNA was detectable in all tissues analyzed with pronounced differences in the amounts. SDH mRNA was most abundant in testes and liver, but was absent from lens. The estimation of the AR / SDH cDNA ratio showed that the relative abundance of SDH to AR differs among tissues. These results indicate that different tissues contain varying amounts of AR and SDH mRNA, that is, each tissue has its own polyol pathway activity.

Keyword: Aldose reductase, Sorbitol dehydrogenase, Diabetic complication

前川京子，小出達夫，斎藤博幸，原園 景，江馬 真，谷本 剛，岡田敏史：**エルカトニン製剤の含量評価**

医薬品研究，**32(7)**，465-471 (2001)

エルカトニン製剤（注射剤：9検体）の含量評価及び製造会社が有する自家エルカトニン標準品（5社：5検体）の品質評価を日局エルカトニン標準品を対照にしてHPLC法及び生物検定法で行った．すべての製剤が高度に純化された原料エルカトニンを用いて製造されていることがHPLC法で示された．製剤9検体中1検体の含量はHPLC法及び生物検定法の両法で規格値以下であり，両法の値はよく相関していた．他の製剤の定量値は規格に適合し，両法の値はよく相関した．このことから，HPLC法は生物検定法の簡便な代替法になることが示された．自家エルカトニン標準品の品質をHPLC法と生物検定法で検討したところ，生物検定法では血中カルシウム低下作用を示すにも関わらず，エルカトニン分子がHPLCで検出できないものが存在し，各企業が有する自家標準品には適切でないものがあり，標準品には日局エルカトニン標準品を使用すべきであると思われる．

Keyword: Elcatonin, Elcatonin Reference Standard, Quantitative evaluation

谷本 剛，八木澤守正*1，藤原 博*2：**日本抗生物質医薬品基準の日本薬局方への移行における問題点とその対応（その2）**

医薬品研究，**32(6)**，423-431 (2001)

日抗基の廃止に伴う一般試験法の取扱いを検討するために，一般試験法の日局と日抗基の間での相違点を明らかにし，日局に規定される抗生物質医薬品の品質試験の実施に支障が生じないように対策を講じることを目的に

検討を行った．日抗基一般試験法の種類及びそれらと日局一般試験法との関係を調査し，日抗基独自の一般試験法である①アセチル基定量法，②グラジュエントクロマトグラフ法，③結晶性試験法，④バイオオートグラフ法，⑤ヒスタミンの5種の試験法の日抗基廃止後の取扱いについて方策を示した．また，日局試験法と一部を異にする日抗基試験法には，注射剤の不溶性異物検査法，発熱性物質試験法，プラスチック製医薬品容器試験法，無菌試験法，乾燥減量試験法，水分試験法，pH測定法の7種の試験法があるが，これらについても日抗基廃止後の対応の仕方を提示した．

Keyword: antibiotics, The Minimum Requirements for Antibiotic Products of Japan, The Japanese Pharmacopoeia, general tests

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Kubo, E.*1, Maekawa, K., Tanimoto, T., Fujisawa, S.*2, Akagi, Y.*1 : **Biochemical and Morphological Changes during Development of Sugar Cataract in Otsuka Long-Evans Tokushima Fatty (OLETF) Rat**

Exp. Eye Res., **73**, 375-381 (2001)

The relationship between the polyol pathway and sugar cataracts has been studied extensively using streptozotocin-induced diabetic rats and galactose fed rats as animal models for insulin-dependent diabetes mellitus (IDDM). In these models, sugar cataracts progress quickly, leading to rapid lenticular polyol accumulation in the early stages of cataract formation. In 1992, a new animal model of non-insulin-dependent diabetes mellitus (NIDDM), the Otsuka Long-Evans Tokushima Fatty (OLETF) rat, was established. In the present study, we examined both biochemical and morphological changes in the lenses of the OLETF rats to determine whether these changes reflect those associated with diabetic cataract formation and to clarify their relationship with the polyol pathway. For the biochemical analysis, we measured the enzyme activity of aldose reductase (AR) and sorbitol dehydrogenase (SDH) and the sorbitol levels using 20, 40 and 60 week old OLETF or control Long-Evans Tokushima Otsuka (LETO) rats. Enzyme activities of AR and SDH, which were lower in 20 week old OLETF rats than in LETO rats, were increased in 60 week old OLETF rats. The lenticular sorbitol level of the OLETF rats was similar to the control level at 20 weeks of age, but it was markedly increased to 40 weeks of age, and slightly decreased at 60 weeks of age compared with rats at 40 weeks but not compared with controls. Slight lens fiber swelling was observed in the anterior and/or posterior subcapsular regions of 40 week old OLETF rats, accompanying elevated sorbitol level and slightly increased SDH activity in the lens. Swelling and liquefaction of lens fibers were observed in the subcapsular and supranuclear region of 60 week old OLETF rats, as well as decreased lenticular sorbitol, and markedly increased SDH activity compared with rats at 40 weeks. AR activity was also increased causing the elevation of sorbitol in lenses of OLETF rats during the early stages of cataract formation. Despite differences in the etiology of diabetes mellitus, the strain of rat and the rate of disease progression in the OLETF rat model compared with other diabetic models, the present results support the theory that the polyol pathway via AR is a factor in the development of sugar cataracts.

Keywords: Otsuka Long-Evans Tokushima Fatty rat, sugar cataract, sorbitol accumulation, polyol pathway, aldose reductase, sorbitol dehydrogenase

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Sakurai, F.*1, Nishioka, T.*1, Saito, H., Baba, T.*2, Okuda, A.*1, Matsumoto, O.*1, Taga, T.*1, Yamashita, F.*1, Takakura, Y.*1, and Hashida, M.*1 : **Interaction between DNA-Cationic Liposome Complexes and Erythrocytes is an Important Factor in Systemic Gene Transfer via the Intravenous Route in Mice: the Role of the Neutral Helper Lipid.**

Gene Ther. **8**, 677-686 (2001)

We investigated the effect of binding and interaction between erythrocytes, a major constituent of blood cells, and the cationic liposome complexes, in relation to the role of the helper lipid, on the in vivo gene delivery to the lung following intravenous injection. Although all three types of vectors bind to murine blood cells in vivo and in vitro, cholesterol-containing complexes with a higher in vivo transfection activity do not induce fusion between erythrocytes, whereas DOPE-containing complexes, a less efficient vector in vivo, induce fusion between the erythrocytes after a short incubation period. The differences in the physicochemical and structural properties of these complexes could explain the differences in interaction with erythrocytes and subsequent gene expression. These results indicate that the interaction with erythrocytes depends on the properties of the cationic lipid vectors and this is an important factor for intravenous gene delivery using

cationic lipid vectors.

Keywords: Cationic liposome, Erythrocytes, Gene transfer

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Komatsu, H., Saito, H., Okada, S., Tanaka, M.*, Egashira, M.*, and Handa, T.*: **Effects of the Acyl Chain Composition of Phosphatidylcholines on the Stability of Freeze-Dried Small Liposomes in the Presence of Maltose.**

Chem. Phys. Lipids **113**, 29-39 (2001)

The effects of the acyl chain composition of phosphatidylcholines (PCs) on the stability of small unilamellar vesicles during freeze-drying and rehydration in the presence of maltose were studied by monitoring the retention of a trapped marker, calcein, in the internal liposome compartment. In dipalmitoyl PC, beta-oleoyl-gamma-palmitoyl-PC and egg yolk PC liposomes, good or fair retentions (>50%) were observed in the presence of maltose, but maltose was ineffective in preserving retention in the dioleoyl PC (DOPC) liposomes (<10%). The extremely low retention in the DOPC liposome was ascribed to neither a formation of the inverted hexagonal phase of the liposomal membrane nor the fusion/aggregation of the liposomes in the drying-rehydration process. Differential scanning calorimetry measurements suggested that interactions of maltose with PC headgroups were essential to stabilizing the dry liposomes. These interactions were significant in the saturated or mixed chain liposomes but were markedly reduced in the DOPC liposomes.

Keywords: Freeze-dry, Saccharide, Differential Scanning Calorimetry

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Gorbenko, G.*1, Saito, H., Molotkovsky, J.*1, Tanaka, M.*2, Egashira, M.*2, Nakano, M.*2, and Handa, T.*2: **Resonance Energy Transfer Study of Peptide-Lipid Complexes.**

Biophys. Chem. **92**, 155-168 (2001)

Resonance energy transfer involving tryptophan as a donor and anthrylvinyl-labeled phosphatidylcholine (AV-PC), 3-methoxybenzanthrone (MBA) and 8-anilino-1-naphthalene sulfonic acid (ANS) as acceptors has been examined to obtain information on the structure of peptide-lipid systems consisting of 18A or Ac-18A-NH_2 peptides and large unilamellar phosphatidylcholine vesicles. The lower and upper limits for the tryptophan distance from the bilayer midplane have been assessed in terms of the models of energy transfer in two-dimensional systems, taking into account orientational effects. Evidence for the existence of preferential orientations of Ac-18A-NH_2 with respect to the lipid-water interface has been obtained.

Keywords: Resonance energy transfer, 18A peptide, Phosphatidylcholine vesicles

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Saito, H., Dhanasekaran, P.*1, Baldwin, F.*1, Weisgraber, K. H.*2, Lund-Katz, S.*1, and Phillips, M. C.*1: **Lipid Binding-Induced Conformational Change in Human Apolipoprotein E. Evidence for Two Lipid-Bound States on Spherical Particles**

J. Biol. Chem. **276**, 40949-40954 (2001)

To better understand apoE-lipid interactions on lipoprotein surfaces, we determined the thermodynamic parameters for binding of apoE4 and its 22-kDa and 10-kDa fragments to triolein-egg phosphatidylcholine emulsions using a centrifugation assay and titration calorimetry. Our data suggest that at maximal binding, the binding behavior of intact apoE4 is different from that of each fragment and that the N-terminal domain of intact apoE4 does not contact lipid. At a low surface concentration of protein, the binding enthalpy of intact apoE4 (69 kcal/mol) was approximately equal to the sum of the enthalpies for the 22-kDa and 10-kDa fragments, indicating that both the 22-kDa and 10-kDa fragments interact with lipids. In a saturated condition, however, the binding enthalpy of intact apoE4 (39 kcal/mol) was less exothermic and rather similar to that of each fragment, supporting the hypothesis that only the C- terminal domain of intact apoE4 binds to lipid. We conclude that the N- terminal four-helix bundle can adopt either open or closed conformations, depending upon the surface concentration of emulsion- bound apoE.

Keywords: Apolipoprotein E, Emulsion, Isothermal titration calorimetry

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Okamura, E.*1, Kimura, T.*1, Nakahara, M.*1, Tanaka, M.*2, Handa, T.*2, and Saito, H.: **^{13}C NMR Method for the Determination of Peptide and Protein Binding Sites in Lipid Bilayers and Emulsions**

J. Phys. Chem. **B 105**, 12616-12621 (2001)

The natural abundance ^{13}C NMR method was applied to directly determine the binding site of peptides and proteins in lipid bilayers and emulsions on the atomic level. Reliable NMR criteria for the location and depth of peptides and proteins in membranes were shown by the chemical shift and line width analyses, which reproduced not only the deep penetration of a transmembrane channel peptide gramicidin A but also the superficial binding of Ac-18A-NH_2, a synthetic model peptide of amphipathic helices of plasma apolipoprotein A-I (apoA-I). Membrane perturbation was most significant at EPC glycerol and ester carbonyl sites when apoA-I was bound to EPC small unilamellar vesicles. This indicates not deep but shallow penetration of apoA-I into the membrane interface whose polarity is intermediate between water and the hydrophobic core. Membrane structural modulation by apoA-I was, however, moderate at the bilayer headgroup and the alkyl chain region near the interface.

Keywords: ^{13}C NMR, apolipoprotein A-I, binding site

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Egashira, M.*1, Gorbenko, G.*2, Tanaka, M.*1, Saito, H., Molotkovsky, J.*2, Nakano, M.*1, and Handa, T.*1: **Cholesterol Modulates Interaction between an Amphipathic Class A Peptide, Ac-18A-NH_2, and Phosphatidylcholine Bilayers.**

Biochemistry **41**, 4165-4172 (2002)

Cholesterol (Chol) in phosphatidylcholine large unilamellar vesicles (PC LUV) modulated interaction of the bilayers with a class A amphipathic peptide, Ac-18A-NH_2: Chol increased the peptide binding capacity and reduced the affinity together with the peptide-induced leakage of calcein from LUV. The fluorescence spectral shift, quantum yield, anisotropy, and acrylamide-quenching of the peptide Trp indicated that in PC:Chol (3:2) LUV, Ac-18A-NH_2 was located in a more polar membrane environment with increased motional freedom and greater accessibility to the aqueous medium. Fluorescence energy transfer from the Trp indole ring to acceptors situated at different depths in the bilayers revealed that the amphipathic peptide penetrated the hydrophobic interior of PC bilayers, while the peptide was located at the polar zwitterionic surface in PC:Chol LUV. These findings imply that Chol in membranes affects the binding and motional freedom of exchangeable plasma apolipoproteins containing class A amphipathic sequences.

Keywords: Cholesterol, Amphipathic peptide, Bilayers

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小出達夫, 岩田美保, 前川京子, 斎藤博幸, 谷本 剛, 岡田敏史, 中根孝久, 川原信夫, 関田節子, 佐竹元吉*1, 横田洋一*2, 津野敏紀*2, 鈴木英世*2, 俣野 豊*3, 山本恵一*3：**国立医薬品食品衛生研究所プエラリン標準品の新規設定のための品質評価**

医薬品研究, **33(2)**, 118-123 (2002)

プエラリン標準品の新規設定のための品質評価試験を行った．試験成績は以下のとおりである．1) 元素分析：理論値と一致した．2) NMR：構造を支持した．3) 紫外吸収スペクトル：305.6, 249.4 nmに極大吸収が認められ，比吸光度$E^{1\%}_{1cm}$はそれぞれ243.5 (306nm), 732.4 (250nm)．4) 赤外吸収スペクトル：3364, 3236, 1634, 1515, 1060 cm^{-1}に特性吸収がみられた．5) 水分含量：4.36％．6) 融点：201.5℃ 7) 液体クロマトグラフ法による純度試験：複数個の類縁物質が検出され，類縁物質総量は約0.93％であった．

以上の試験成績から，本標準品原料は，国立医薬品食品衛生研究所プエラリン標準品に適した品質を有することを認めた．

Key words: Puerarin, NIHS Reference Standard, Quality evaluation

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Koide T., Nose M.*, Ogihara Y.*, Yabu Y.*, Ohta N.*: **Leishmanicidal effect of curcumin in vitro.**

Biol Pharm Bull, **25(1)**, 131-3(2002)

From a study to find anti-parasitic agents from natural resources, we found that curcumin showed the cytotoxicity against leishmania in vitro. The LD50 value of this activity was 37.6+/-3.5 microM.

Key words: Leishmania, curcumin

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Fuchino H., Koide T., Takahashi M., Sekita S., Satake M*.: **New sesquiterpene lactones from *Elephantopus mollis* and their leishmanicidal activities.**

Planta Med, **67**(7), 647-53(2001)

The leishmanicidal compounds isolated from whole plants of Elephantopus mollis H.B.K. were identified as follows. Three new sesquiterpenoid lactones, 2,5-epoxy-2beta-hydroxy-8alpha-(2-methylpropenoyloxy)-4(15),10(14),11(13)-germacratrien-12,6alpha-olide, (4betaH)-8alpha-(2-methylpropenoyloxy)-2-oxo-1(5),10(14), 11(13)-guaiatrien-12,6alpha-olide and (4betaH)-5alpha-hydroxy-8alpha-(2-methylpropenoyloxy)-1(10),11(13)-guaiadiene-12,6alpha-olide, were isolated from Peruvian and Brazilian collections together with four known sesquiterpenoids, molephantin, elephantopin, isoelephantopin and 2-deethoxy-2beta-methoxyphantomolin. They exhibited potent in vitro leishmanicidal activities against Leishmania major. The alpha-methylene-gamma-butyrolactone moiety was found to be essential to the potent leishmanicidal effect observed.

Key words: Leishmania, sesquiterpenes, *Elephantopus mollis*

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Nakamura, Y., Kaihara, A., Yoshii, K., Tsumura, Y., Ishimitsu, S., Tonogai, Y.: **Content and composition of isoflavonoids in mature or immature beans and bean sprouts consumed in Japan**

J. Health Sci., **47**, 394-406 (2001)

The content of 9 types of isoflavonoids (daidzein, glycitein, genistein, formononetin, biochanin A, coumestrol, daidzin, glycitin and genistin) in 34 domestic or imported raw beans including soybeans, 7 immature beans and 5 bean sprouts consumed in Japan were systematically analyzed. Each isoflavonoid was analyzed in total after acid hydrolysis to the aglycone, and intact individual isoflavonoids were also analyzed without hydrolysis. After the sample clean up, daidzein, glycitein, genistein, formononetin, biochanin A, daidzin, glycitin and genistin were determined by HPLC with a diode array detector and coumestrol was determined by spectrofluorimetry. The content and composition of isoflavonoids varied greatly between soybean sprouts, immature soybeans and mature beans of the same type but of different source. Isoflavonoid content was highest in mature soybeans. The composition of isoflavonoids differed in each growth stage of soybeans. In other beans, the largest content of isoflavonoids was found in mature chickpeas, but this value was less than 1/27 of the isoflavonoid content in mature soybeans. Thus, the contribution of beans other than soybeans to the daily intake of isoflavonoids in a Japanese diet is negligible.

Keywords: isoflavonoids, beans, high performance liquid chromatography

中村優美子，石光進，津村ゆかり，吉井公彦，開原亜樹子，外海泰秀：**HPLCによる農作物中フルスルファミド分析時におけるイオン交換系ミニカラムを用いた試料精製の工夫について**

食品衛生学雑誌，**42**, 398-403 (2001)

農産物中のフルスルファミド簡易分析法の改良を検討した．野菜試料をメタノールで抽出し，酢酸エチルで液－液分配を行った後，シリカゲルカラムクロマトグラフィーにより精製し，その溶出液をBond Elut^{R} SAX＋Bond Elut^{R} PSAミニカラムに負荷し，20％アセトン含有n-ヘキサン溶液10 mL及びアセトン5 mLで洗浄後，アセトン35 mLで溶出後，HPLCで定量した．本法ではカロチノイド色素及びあぶらな科野菜に特有の夾雑ピークを除去することができ，回収率の向上を図ることができた．

Keywords: flusulfamide, HPLC, Bond Elut^{R} SAX-PSA

Tsuji, S., Amakura, Y., Umino, Y., Nishi, M.*, Nakanishi, T.,* Tonogai, Y. : **Structural Determination of the Subsidiary Colors in Food Blue No. 1 (Brilliant Blue FCF) Aluminum Lake Detected by Paper Chromatography**

J. Food Hyg. Soc. Japan, **42**, 243-248 (2001)

One of eight Food Blue No. 1 aluminum lakes (B-1Als) used in the official inspection of coal-tar colors in fiscal year 1999 had a violet sub-spot during paper chromatography and was rejected. To clarify the orgin of the sub-spot, the violet subsidiary color (Sub-V) was isolated from the sample. On the basis of NMR and MS analyses and ion chromatography, the structure of the subsidiary color was elucidated to be 2-[[4-[*N*-ethyl-*N*-(3-sulfophenylmethyl)amino]phenyl][4-hydroxyphenyl]methylio]benzenesulfonic acid. The relative content of Sub-V to that of *m,m*-B-1 in the rejected sample was determined to be 39.5% by HPLC. The relative contents in other submitted samples of B-1Al were in the range of 1.1-3.6%.

Keywords: brilliant blue FCF aluminum lake, subsidiary color, NMR

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辻 澄子, 海野 有紀子, 天倉 吉章, 外海 泰秀：**食用赤色40号（アルラレッドAC）及び黄色5号（サンセットイエローFCF）のアルミニウムレーキ中有機性不純物のHPLC定量用試験液調製法の検討**

食衛誌, **42**, 379-384 (2001)

第7版食品添加物公定書に従った食用赤色40号アルミニウムレーキ（R-40Al）中の副成色素などの有機性不純物のHPLC定量では再現性などに問題があった．これは試験液に存在する高濃度のアルミニウムが原因と考えられた．そこで，R-40Alをアンモニアアルカリ性で煮沸し，水酸化アルミニウムのコロイド状沈殿を除去した上清を試験液とする試験液調製法を開発した．本法により，再現性のある定量結果が得られ，添加回収率も改善された．また，食用黄色5号アルミニウムレーキについても適用可能であった．

Keywords：allura red AC aluminum lake, sunset yellow FCF aluminum lake, subsidiary color

Amakura, Y., Umino, Y., Tsuji, S., Ito, H.*, Hatano, T.*, Yoshida, T.*, Tonogai, Y.: **Constituents and their antioxidative effects in eucalyptus leaf extract used as a natural food additive**

Food Chemistry, **77**, 47-56 (2002)

The components of a natural food additive, "eucalyptus leaf extract", were isolated and identified in order to determine their structures and contents. The structures of eight major compounds, namely the gallic and ellagic acids, and eucalyptone and macrocarpals A-E, isolated from them were elucidated by spectroscopic methods. The antioxidative activity of these isolated compounds were estimated by several assays, and it appears that the antioxidant activity is mostly due to the gallic and ellagic acids. On the other hand, in the determined eucalyptus product, the content of 1,8-cineole, a major component of the eucalyptus oil, was lower than the isolated compounds, and its activity as an antioxidant was negligible.

Keywords: eucalyptus leaf extract, natural antioxidant, food additive

*岡山大学

Ishimitsu, S., Kaihara, A., Yoshii, K., Tsumura, Y., Nakamura, Y., Tonogai, Y.: **Determination of Clethodim and Its Oxidation Metabolites in Crops by Liquid Chromatography with Confirmation by LC/MS**

J. AOAC Int., **84**, 1172-1178 (2001)

A method was developed for determination of the herbicide clethodim(C0) and its oxidation metabolites clethodim sulfoxide(C1) and clethodim sulfone(C2) in agricultural products. Upon extraction, both C0 and C1 were oxidized to C2 by m-chloroperoxybenzoic acid, and C2 was determined by liquid chromatography(LC). The C2 peak was confirmed by liquid chromatography/mass spectrometry(LC/MS) with electrospray ionization(ESI). Recoveries of C0 from radish, tomato, onion, sweet potato, kidney bean, carrot, cabbage, and lettuce ranged from 91 to 118% following fortification at 0.05-1.0 ppm. The detection limit of C2 in crops was 0.01 ppm(S/N>3). The fortified samples of onion, sweet potato, kidney bean, and carrot were confirmed by LC/MS(ESI), and the peak of C2 was detected.

Keywords: clethodim, clethodim sulfoxide, clethodim sulfone, crops, liquid chromatography

Akiko Kaihara, Kimihiko Yoshii, Yukari Tsumura, Susumu Ishimitsu, and Yasuhide Tonogai: **Multi-residue Analysis of 18 Pesticides in Fresh Fruits, Vegetables and Rice by Supercritical Fluid Extraction and Liquid Chromatography-Electrospray Ionization Mass Spectrometry**

J. Health Sci., **48**, 173-178, (2002)

A multi-residue screening method was developed for the determination of 18 pesticides in fresh fruits, vegetables and unpolished rice by supercritical fluid extraction (SFE), cleaned up with cartridge columns, and liquid chromatography-mass spectrometry with electrospray (LC/MS (ESI)). Comparing with our previous method, the number of fraction at purification was reduced to one fraction in order to reduce the preparation time, and detection limit and recovery value of almost pesticides were improved. The detection limits of the 18 pesticides were 0.04-148 ng/g in sample. Furthermore, in case of crops including many interfering peaks by UV detection, using a LC-MS (SIM) significantly improved the quantitative and qualitative analyses with less interfering peaks than UV detection.

Yoshii K, Kaihara A, Tsumura Y, Ishimitsu S, Tonogai Y.: **Simultaneous determination of residues of emamectin and its metabolites, and milbemectin, ivermectin, and abamectin in crops by liquid chromatography with fluorescence detection.**

J. AOAC Int., **84**, 910-917 (2001)

A liquid chromatographic (LC) method was developed for the determination of emamectin and its metabolites

(8,9-Z-isomer, N-demethylated, N-formylated, and N-methylformylated emamectin) in various crops. The analytes were extracted with acetone, cleaned up on cartridge columns (C18 and NH_2), derivatized with trifluoroacetic anhydride and 1-methylimidazole, and determined by LC with fluorescence detection. Because radish inhibited the formation of the fluorescent derivatives, an additional Bond Elut PRS cartridge was used in the cleanup of Japanese radish samples. During sample preparation, N-formylated emamectin partially degraded to emamectin B1b and emamectin B1a, and the 8,9-Z-isomer partially degraded to N-demethylated emamectin. Therefore, emamectin and its metabolites were determined as total emamectin, i.e., their sum was estimated as emamectin benzoate. Their recoveries from most crops were approximately 80-110% with the developed method. The detection limits for the analytes in vegetables were 0.1-0.3 parts per trillion (ppt). The results for these compounds were confirmed by LC/mass spectrometry (LC/MS; electrospray ionization mode). Because the fluorescent derivative of emamectin was undetectable by LC/MS, the results for the analyte were confirmed by using a sample solution without derivatization. Limits of detection by LC/MS were similar to the fluorescence detection limits, 0.1-0.3 ppt in vegetables. In addition to the emamectins, milbemectin, ivermectin, and abamectin were also determined by the developed method.

Tsumura, Y., Ishimitsu, S., Kaihara, A., Yoshii, K., Nakamura, Y., Tonogai, Y.: **Di (2-ethylhexyl) phthalate contamination of retail packed lunches caused by PVC gloves used in the preparation of foods**
Food Add. Contam., **18**, 569-579 (2001)

Plasticizers in retail packed lunch and set lunch in restaurants were determined by GC/MS. The phthalate esters were as follows: diethyl, dipropyl, dibutyl, dipentyl, dihexyl, butylbenzyl, dicyclohexyl, di(2-ethylhexyl), dioctyl, diisooctyl (mixture of isomers) and diisononyl (mixture). Di(2-ethylhexyl) adipate was also determined. Sixteen packed lunches and ten set lunches were analyzed, and in all samples the concentration of di(2-ethylhexyl) phthalate (DEHP) was highest, at 0.80 to 11.8 mg/kg in packed lunches and 0.012 to 0.30 mg/kg in set lunches. DEHP content in five packed lunches exceeded 1.85 mg, the EU TDI for a person of 50 kg body weight. Foodstuffs of the packed lunches at each step of preparation were taken from the factory and phthalates were determined. For example, uncooked chicken contained 0.08 mg/kg DEHP, 13.1 mg/kg after frying and 16.9 mg/kg after packing. Disposable PVC gloves used in preparation were apparently the source of high DEHP. The gloves used during cooking or packing process were sprayed by 68% (w/w) ethanol for the purpose of sterilization. PVC gloves from the factory contained 22 or 41 % by weight of DEHP. Boiled rice, croquette and boiled dry radish were handled with PVC gloves containing 30% w/w DEHP in the laboratory to confirm causality. DEHP migration was 0.05mg/kg in rice or 0.33 mg/kg in croquette, and 11.1 mg/kg in radish. The alcohol sprayed on gloves increased migration of DEHP to 2.03 mg/kg in rice and 2.45 mg/kg in croquette, 18.4 mg/kg in radish.
Keywords: phthalate, DEHA, packed lunch, disposable glove, GC/MS, PVC

Niino, T.[*1], Ishibashi, T.[*1], Itho, T.[*1], Sakai, S.[*1], Ishiwata, H., Yamada, T.[*2], Onodera, S.[*3]: **Monoester formation by hydrolysis of dialkyl phthalate migration from polyvinyl chloride products in human saliva**
J. Health Sci., **47**, 318-322 (2001)

The migration of dialkyl phthalate was tested in volunteers who chewed polyvinyl chloride (PVC) toys containing 100 mg/g di-n-butyl phthalate (DBP) and 185 mg/g di-2-ethylhexly phthalate (DEHP) (ball A), and 256 mg/g diisononyl phthalate (DINP) (ball B). The migration of dialkyl phthalate into simulated saliva was also examined in vitro by shaking toy pieces. Release of DBP, DEHP, and DINP in vivo from ball A was 11.7, 44.4, and 78.0 μg/10 cm^2/h, respectively, and in vitro was 339, 315, 535 μg/10 cm^2/h, respectively. The presence of mono-n-butyl phthaqlate (MBP) and mono-2-ethylhexyl phthalte (MEHP) in saliva collected during chewing ball A was confirmed by GC/MS-SIM. Saliva collected from volunteers was incubated with DBP and DEHP at 37℃ for 60 min. Monoesters of these diphthalates were detected in the collected salava.
Keywords: dialkyl phthalate, hydrolysis, human saliva

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Ishiwata, H., Nishijima, M.[*1], Fukasawa, Y.[*2]: **Estimation of preservative concentrations in foods and their daily intake based on official inspection results in Japan in fiscal year 1998**
J. Food Hyg. Soc. Japan, **42**, 404-412 (2001)

The mean concentration and daily intake of five preservatives were estimated based on the results of an analysis of 89,927 foods obtained in official inspedctions by Japanese local governments in fiscal year 1998. The mean concentrations of benzoic acid, dehydroacetic acid, p-hydroxybenzoic acid, propionic acid, and sorbic acid were 9.5%, 1.5%, 5.7%, 1.7%, and 23.9%, respectively, of the allowable limits. Daily intake per person was 6.23, 0.030, 1.02, 8.10, and 25.0 mg, respectively, and benzoic acid, p-hydroxybenzoic acid, and sorbic acid consumed were 2.5%, 0.2%, and 2.0% of their ADI, respectively. These results were similar to those obtained based on the results in fiscal years 1994 and 1996.

Keywords: preservative, concentration, daily intake

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Ishiwata, H., Abe, Y., Kubota, H., Kawasaki, Y., Takeda, Y., Maitani, T., Nishijima, M.*1, Fukasawa, Y.*2: **Estimation of concentrations of antifungal agents allowed as food additives in foods and their daily intake based on official inspection results in Japan in fiscal year 1998**

J. Food Hyg. Soc. Japan, **43**, 49-56 (2002)

The mean concentration and daily intake of four antifungal agents were estimated based on the results of an analysis of 7,005 foods obtained in official inspedctions by Japanese local governments in fiscal year 1998. The mean concentrations of diphenyl, imazalil, o-phenylphenol, and thiabendazole were 0.0004%, 14.0%, 3.5%, and 5.7%, respectively, of the allowable limits. Daily intake per person was 0.000326, 1.89, 11.5, and 23.3 μg, respectively, and these agents consumed were 0.000013%, 0.15%, and 0.12%, and 0.47% of their ADI, respectively. These results were similar to those obtained based on the results in fiscal years 1994 and 1996, except that the amount of diphenyl si much lower (1/100).

Keywords: antifungal agent, concentration, daily intake

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Ema, M., Miyawaki, E.: **Effects of monobutyl phthalate on reproductive function in pregnant and pseudopregnant rats**

Reprod. Toxicol., **15**, 261-267 (2001)

Rats were given monobutyl phthalate (MBuP) by gastric intubation at 250, 500, 750, or 1000 mg/kg on days 0-8 of pregnancy. The effects of MBuP on the uterine function, as a cause of early embryonic loss, were also determined in pseudopregnant rats, with an induced decidual cell response. The same doses of MBuP were given to pseudopregnant rats on days 0-8 of pseudopregnancy and the uterine weight on day 9 served as an index of the uterine decidualization. MBuP at 1000 mg/kg caused significant increases in the incidences of preimplantation loss in females successfully mated and of postimplantation loss in females having implantations. The uterine decidualization in pseudopregnant rats was significantly decreased at 1000 mg/kg. These findings suggest that early embryonic loss due to MBuP is mediated, at least in part, via the suppression of uterine decidualization, an impairment of uterine function.

Keywords: Monobutyl phthalate, early embryonic loss, decidual cell response

Ema, M., Miyawaki, E.: **Roles of progesterone on suppression of uterine decidualization and implantation failure induced by triphenyltin chloride in rats**

Cong. Anom., **42**, 106-111 (2001)

Although lower uterine weight was found in hormone-maintained ovariectomized rats given triphenyltin chloride (TPTCl) at 4.7 or 6.3 mg/kg on days 0-3 and induced decidual cell response on day 4, no statistical significance in the uterine weight was detected between the control group and the TPTCl-treated groups. The pregnancy rate and number of implantations in the groups given TPTCl at 4.7 or 6.3 mg/kg on days 0-3 of pregnancy and progesterone on days 0-8 of pregnancy were significantly higher than those in the groups given TPTCl alone. No significant differences in these parameters were found between the control group and the groups given TPTCl and progesterone. It can be concluded that the TPTCl-induced suppression of uterine decidualization is mediated, at least partially, via the ovarian hormones, and that progesterone protects against the TPTCl-induced implantation failure.

Keywords: Triphenyltin chloride, implantation failure, progesterone

Ema, M., Harazono, A.: **Toxic effects of butyltin trichloride during early pregnancy in rats**

Toxicol. Lett., **125**, 99-106 (2001)

Following successful mating, rats were given BTCl by gastric intubation at 56, 226, or 903 mg/kg on days 0-3 or

on days 4-7 of pregnancy. The pregnancy outcome was determined on day 20 of pregnancy. The maternal body weight gain and food consumption were significantly decreased at 903 mg/kg on days 0-3 or on days 4-7. The pregnancy rate in the BTCl-treated groups was comparable to the control value. The incidence of preimplantation loss was not significantly affected after administration of BTCl on days 0-3 or on days 4-7. In females having implantations, the numbers of corpora lutea, implantations, and live fetuses and the incidences of pre- and postimplantation loss in the groups given BTCl on days 0-3 were comparable to the controls. A significant decrease in weight of female fetuses was found at 903 mg/kg on days 0-3 or on days 4-7. It could be concluded that BTCl during early pregnancy is maternal and developmental toxic at 903 mg/kg.

Keywords: Butyltin trichloride, embryonic loss, reproductive and developmental toxicity

Ema, M., Fujii, S.*, Furukawa, M.*, Kiguchi, M.*, Ikka, T.*, Harazono, A.: **Rat two-generation reproductive toxicity study of bisphenol A**

Reprod. Toxicol., **15**, 505-523 (2001)

This study was conducted to determine the low-dose effects of bisphenol A (BPA) in a rat two-generation reproduction study. Male and female rats were given BPA at 0.2, 2, 20, or 200 μg/kg by gastric intubation throughout the study beginning at the onset of a 10- and 2-week premating period, in F0 males and females, respectively, and continuing through the mating, gestation, and lactation periods, for two generations. There were adult (F0, F1, F2) and postnatal day 22 (F1, F2) necropsies; the oldest F2 males and females being killed in postnatal weeks 7 and 14, respectively. The data indicate that oral BPA of between 0.2 and 200 μg/kg over 2 generations did not cause significant compound-related changes in reproductive or developmental parameters in rats.

Keywords: Bisphenol A, low-dose effects, development of reproductive system

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Ema, M., Miyawaki, E.: **Effects on development of the reproductive system in male offspring of rats given butyl benzyl phthalate during late pregnancy**

Reprod. Toxicol., **16**, 71-76 (2002)

Rats were given butyl benzyl phthalate (BBP) by gastric intubation at 250, 500, or 1000 mg/kg on days 15-17 of pregnancy. A significant decrease in maternal body weight gain and food consumption was found at 500 and 1000 mg/kg. A significant decrease in the number of live fetuses was found at 1000 mg/kg. The fetal weights were significantly lowered at 1000 mg/kg. A significantly increased incidence of fetuses with undescended testes was found at 500 and 1000 mg/kg. A significant decrease in the anogenital distance (AGD) and AGD/cube root of body weight ratio in male fetuses was detected at 500 and 1000 mg/kg. The AGD and AGD/cube root of body weight ratio of female fetuses in the BBP-treated groups were comparable to those in the control group. It was concluded that BBP on days 15-17 of pregnancy produced adverse effects on the development of the reproductive system in male offspring.

Keywords: Butyl benzyl phthalate, anogenital distance, undescended testes

Nakagawa, Y., Maeda, H., Murai, T.: **Evaluation of the in vitro pyrogen test system based on proinflammatory cytokine release from human monocytes: Comparison with a human whole blood culture test system and with the rabbit pyrogen test**

Clin. Diagn. Lab. Immun., **9**, 588-597 (2002)

The reliability of an in vitro pyrogen test system based on proinflammatory cytokine release from human monocytic cells was assessed by comparison with a test system using a human whole blood culture and also with the conventional rabbit pyrogen test. MM6-CA8 cells used as the pyrogen indicator cells, which were newly selected by subcloning of human monocytic Mono-Mac-6 cells, responded to various pyrogens including endotoxin, peptidoglycan (PG), Staphylococcus aureus Cowan I (SAC) and poly (I:C) with high sensitivity, and produced proinflammatory cytokines. The cytokine-producing responses of MM6-CA8 cells correlated significantly with the responses of cultured human whole blood. In terms of cytokine inducibility, the pyrogens were ranked in order endotoxin > PG > poly I:C > SAC in both culture systems which almost agreed with the rank of their pyrogenicity assessed by the rabbit pyrogen test. These results suggest that the in-vitro responsiveness of MM6-CA8 cells to various pyrogens is highly relevant for human pyrogenic reactions. Therefore, the in vitro test system is useful and reliable for detecting the presence of materials that are pyrogenic for humans.

Keywords: in vitro, pyrogen test, proinflammatory cytokine

Harazono, A., Ema, M.: **Effects of 4-tert-octylphenol on initiation and maintenance of pregnancy following oral administration during early pregnancy in rats**
Toxicol. Lett., **119**, 79-84 (2001)

4-tert-Octylphenol (OP) is an alkylphenol that is an intermediate in the production of alkylphenol ethoxylates. OP has been reported to be the most potent estrogenic alkylphenol in vitro. In the present study, the effects of OP on initiation and maintenance of pregnancy were investigated in rats. Inseminated female rats were orally given OP at 0, 15.6, 31.3, 62.5 and 125 mg/kg on day 0 through day 8 of pregnancy. Female rats were sacrificed on day 20 of pregnancy, and pregnancy outcome was determined. Decreases in body weight gain and food consumption on days 0-9 were found at 31.3 mg/kg and above, and at 15.6 mg/kg and above, respectively. The pregnancy rate was not adversely affected by OP administration during early pregnancy even at 125 mg/kg. The incidence of post-implantation loss per litter at 31.3 mg/kg and above was significantly higher than that in the control group. The body weights of live fetuses in the OP-treated groups were not significantly different from those in the control group. No increase in the incidence of fetuses with external malformations was found in any OP-treated group. We concluded that OP during early pregnancy caused post-implantation embryonic loss at doses that showed maternal toxicity.
Keywords: 4-*tert*-octylphenol, early pregnancy, rat

青柳光敏*, 姉帯正樹*, 林 隆章*, 柴田敏郎, 畠山好雄：**川芎の調製法と化学的品質評価（第2報）湯通しの条件が品質に及ぼす影響**
北海道立衛生研究所報告, **51**, 97-99 (2001)

生薬川芎は生根を湯で処理（湯どおし）した後乾燥して仕上げる．湯どおしの温度及び時間が希エタノールエキス，糖，エーテルエキス及びligstilide含量に及ぼす影響を検討した．その結果，希エタノールエキスと糖含量ならびに，エーテルエキスとligstilide含量間にいずれも高い正の相関が認められ，いずれも60℃以上の処理で著しく減少した．
Keywords：Cnidii Rhizoma, blanching condition
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Kraker, J. W.*[1], Franssen, M. C. R.*[2], Groot, A.*[1], Shibata, T., Bouwmeester, H. J.*[2]: **Germacrenes from fresh costus roots**
Phytochemistry, **58**, 481-487 (2001)

Four germacrenes were isolated from fresh costus roots (*Saussurea lappa*). Heating of (+)-germacrene A, germacra-1 (10), 4, 11(13)-trien-12-ol, germacra-1(10), 4, 11(13)-trien-12-al, and germacra-1 (10), 4, 11 (13)-trien-12-oic acid yields (-)-β-elemene, (-)-elema-1, 3, 11 (13)-trien-12-ol, (-)-elema-1, 3, 11 (13)-trien-12-al, and elema-1, 3, 11 (13)-trien-12-oic acid respectively. Acid induced cyclisation of the germacrenes yields selinene, costol, costal and costic acid respectively. It is highly probable that the elemenes reported in literature for costus root oil are artefacts.
Keywords：*Saussurea lappa*, sesquiterpene lactone biosynthesis, germacrenes
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Fuchino, H., Koide, T., Takahashi, M., Sekita, S. and Satake, M.: **New Sesquiterpene Lactones from *Elephantopus mollis* and Their Leishmanicidal Activities.**
Planta Medica, **67**, 647-653 (2001)

The leishmanicidal compounds isolated from whole plants of *Elephantopus mollis* H.B.K. were identified as follows. Three new sesquiterpenoid lactones, 2,5-epoxy-2β-hydroxy-8α-(2-methylpropenoyloxy)-4(15), 10(14), 11(13)-germacratrien-12,6α-olide, (4βH)-8α-(2-methylpropenoyloxy)-2-oxo-1(5), 10(14), 11(13)-guaiatrien-12,6α-olide and (4βH)-5α-hydroxy-8α-(2-methylpropenoyloxy)-1(10),11(13)-guaiadiene-12,6α-olide, were isolated from Peruvian and Brazilian collections together with four known sesquiterpenoids, molephantin, elephantopin, isoelephantopin and 2-deethoxy-2β-methoxyphantomolin. They exhibited potent *in vitro* leishmanicidal activities against *Leishmania major*. The α-methylene-γ-butyrolactone moiety was found to be essential to the potent leishmanicidal effect observed.
Keywords: Sesquiterpenes, *Elephantopus mollis*, leishmaniasis.

Li, S. Y.*, Fuchino, H., Kawahara, N., Sekita, S. and Satake, M.: **New Phenolic Constituents from *Smilax bracteata*.**
Journal of Natural Products, **65**, 262-266 (2002)

From the methanol extract of *Smilax bracteata* rhizomes, six new phenolic compounds, (2*S*,3*S*)-5-*O*-β-D-glucopyranosyloxy-6-methyl-3'-methoxy-3,7,4'-trihydroxyflavan, (2*S*, 3*S*)-5-*O*-β-D-glucopyrano-

syloxy-6-methyl-4'-methoxy-3,7,3'-trihydroxyflavan, 3β-(3', 5'-dihydroxyphenyl)-2α-(4''-hydroxyphenyl)-dihydrobenzofuran-5-carbaldehyde, (1-*p*-*O*-coumaroyl-6-*O*-feruroyl)-β-D-fructofuranosyl-α-D-glucopyranoside, (1-*p*-*O*-coumaroyl-3,6-di-*O*-feruroyl)-β-D-fructofuranosyl-α-D-glucopyranoside and (6-*O*-feruroyl)-β-D-fructofuranosyl-(6-*O*-acetyl)-α-D-glucopyranoside were isolated together with five known compounds. Their structures were established by spectral data interpretation.

Keywords : *Smilax bracteata*, stilben, flavonoid.

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Jung, D.W.*1, Sung, C.K.*1, Touno, K., Yoshimatsu, K., Shimomura, K.: **Cryopreservation of *Hyoscyamus niger* adventitious roots by vitrification**

Jounal of Plant Physiology, **158**, 801-805 (2001)

Auxin-independent adventitious root culture of *Hyoscyamus niger* was established, and the roots were successfully cryopreserved with a high regeneration rate of 93.3 % by vitrification method. The root tips cultured for 12 to 14 days in phytohormone-free Murashige and Skoog (MS) liquid medium were excised and precultured on Woody Plant (WP) solid medium supplemented with 0.3 M sucrose at 25 ℃ in the dark. After 1 day, they were treated with MS-based loading solution for 10 min followed by soaking in MS-based PVS2 for 10 min at 0 ℃. The root tips treated were immersed into liquid nitrogen (-196 ℃). For recovery, the root tips were thawed rapidly at 40 ℃ and washed with MS medium containing 1 M sucrose prior to plating onto WP solid medium. The regenerated roots were evaluated by their growth and tropane alkaloid production. The growth and alkaloid contents of the regenerated roots analyzed by HPLC were found to be almost the same as those of the non-treated roots.

Keywords: *Hyoscyamus niger*, adventitious root, cryopreservation

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Kojoma, M.*1, Iida, O., Makino, Y.*2, Sekita, S., and Satake, M.*3: **DNA fingerprinting of *Cannabis sativa* L. using Inter-Simple Sequence Repeat (ISSR) Amplification**

Planta Medica, **68**, 60-63 (2002)

Chemical analysis of cannabinoid, and Inter-Simple Sequence Repeat (ISSR) fingerprinting of DNA were used to identify different strains of *Cannabis sativa* L. for forensic purposes. Three strains were classified into two types, tetrahydrocannabinol (THC) chemo-type and cannabidiol (CBD) chemo-type, using high performance liquid chromatography (HPLC). The two strains of the CBD type were not distinguished by their HPLC chromatograph patterns. ISSR fingerprinting identified the definitive polymorphic DNA patterns between these strains. The use of ISSR fingerprinting enabled a clear differentiation between *cannabis* strains that could not be achieved using HPLC analysis.

Keywords: *Cannabis sativa*, DNA profiling, ISSR

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Kojoma, M.*1, Kurihara, K., Yamada, K., Sekita, S., Satake, M.*2 and Iida, O.: **Genetic identification of cinnamon (*Cinnamomum* spp.) based on the *trn*L-*trn*F chloroplast DNA**

Planta Medica, **68**, 94-96 (2002)

Genetic identification among cinnamon species was studied by analyzing nucleotide sequences of chloroplast DNA from four species (*Cinnamomum cassia*, *C. zeylanicum*, *C. burmannii* and *C. sieboldii*). The two regions studied were the intergenic spacer region between the *trnL* 3'exon and *trnF* exon (*trnL-trnF* IGS) and the *trnL* intron region. We found nucleotide variation at one site in the *trnL-trnF* IGS, and at three sites in the *trnL* intron. With the sequence data from analysis of these regions, the four *Cinnamomum* species used in this study were correctly identified. Furthermore, single-strand conformation polymorphism (SSCP) analysis of PCR products from the *trnL-trnF* IGS and the *trnL* intron resulted in different SSCP band patterns among *C. cassia*, *C. zeylanicum* and *C. burmannii*.

Keywords: *Cinnamomum* spp., chloroplast DNA, SSCP analysis

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高上馬希重*1, 飯田修, 関田節子, 佐竹元吉*2, 牧野由紀子*3：**大麻*Cannabis sativa* L. におけるISSR解析**

DNA多型, **9**, 77-81, 東洋書店 (2001)

DNAによる大麻鑑定手法の開発を目的として, ISSR-PCR (Inter Simple Sequence Repeats PCR) 解析を用いて, 異なった系統間の識別を試みた. 国内外の11系統の大麻を実験材料とした. 各プライマーにおいて実験に用いた系統間で異なるバンドパターンを示し, 得られた

データ解析からこれらの系統は数個のクラスターに分けられた．さらに麻薬性成分カンナビノイド類の成分分析からは識別の困難な系統間の比較を行った結果，ISSR-PCRによって明らかにこれらの系統を区別することが可能であった．以上のことからISSR-PCRフィンガープリント分析は大麻の系統間の違いを識別する有効な手法であることが認められた．また，PCR反応時のアニーリング温度を高く設定できることから実験結果の再現性も高く，大麻鑑定の初期スクリーニングに有効であると考えられた．

Keywords: *Cannabis sativa*, DNA, ISSR

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