Morihara, M., Aoyagi, N., Kaniwa, N., Kojima, S. and Ogata, H.*: Assessment of gastric acidity of Japanese subjects over the last 15 years

The gastric acidity of young to elderly Japanese subjects from 1989 to 1999 was assessed and compared with that obtained in 1984, using GA-Test capsules containing acid-dissolving granules of riboflavin. The percentage of achlorhydric subjects increased with the age as observed before, which, however, tended to decrease in all age categories year by year. The percentage of achlorhydric subjects of 50 years in 1995-1999 was about 40%, which was lower than that (60%) in 1984. However, such a chronological change was not observed when the percentage of achlorhydric subjects was determined according to the birth year, indicating that it is related with the birth year of subjects. The percentage of achlorhydric subjects is correlated with that of the infection of Helicobacter pylori. Considering the high percentage of achlorhydric elderly that still exists, bioavailability and bioequivalence studies should be performed by taking into consideration the effects of gastric acidity on the in vivo performance of drug products.

Keywords: gastric acidity, achlorhydric elderly, Helicobacter pylori

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Izutsu, K., Kojima, S.: Freeze-concentration separates proteins and polymer excipients into different amorphous phases

The miscibility of proteins and polymer excipients in frozen solutions and freeze-dried solids was studied as protein formulation models. Thermal profiles of frozen solutions and freeze-dried solids containing various proteins (lysozyme, ovalbumin, BSA), nonionic polymers (Ficoll, PVP), and salts were analyzed by DSC. The polymer miscibility in frozen solutions was determined from the glass transition temperature of maximally freeze-concentrated solute (Tg). All the protein and polymer combinations (5% w/w, each) were miscible in frozen solutions and presented single Tg's that rose with increases in the protein ratio. Various salts concentration-dependently lowered the single Tg's of the proteins and Ficoll combinations maintaining the mixed amorphous phase. In contrast, some salts induced the separation of the proteins and PVP combinations into protein-rich and PVP-rich phases among ice crystals. Freeze-concentration separates some combinations of proteins and nonionic polymers into different amorphous phases in a frozen solution.

Keywords: Freeze-drying, Phase separation, Formulation

吉田雅江，阿曽幸男，小嶋茂雄：タンパク質凍結乾燥製剤の安定性に及ぼす分子運動性の影響に関する検討
低温生物工学会誌, 46, 8-11 (2000)

種々の高分子添加剤を含有するタンパク質凍結乾燥製剤の分子運動性をパルス1H-NMRおよび高分解能13C-NMRを用いて測定し、製剤中のタンパク質の安定性との関連を検討した。湿度がプロトンのスピン-スピノ緩和から測定した分子運動性の限界温度（Tco）以上になると高分子の運動性が急激に高まり、それに連動してタンパク質の分子運動性も高まることが明らかになった。Tcoは添加剤の種類および水分含量に大きく依存することが分かった。さらに、湿度がTco以上に上昇すると製剤中のタンパク質分子の凝集が顕著になり、安定性が著しく低下することが明らかになった。

Keywords: protein stability, lyophilized formulation, molecular mobility

坂本知昭，花村環境，石橋雅雄，小嶋茂雄：日本薬局方の参照吸収スペクトルに関する研究 その2 一参照紫外可視吸収スペクトルに関する研究 (1)

第十四改正日本薬局方（JPI4）では、医薬品各条の紫外可視吸収スペクトルによる確認試験の参照スペクトルあるいは標準品を測定して得られた吸収スペクトルのパターンを、試料を測定して得られた吸収スペクトルのパターンとの比較により行う方法が全面的に導入される。著者らは、JPI4に取
A high-performance liquid chromatographic method is described for the simultaneous determination of the dapsone derivatives (2-aminodiphenyl) in plasma after derivatization with dapsone.

**Keywords:** Ultraviolet and visible absorption. The Japanese Pharmacopoeia. Reference spectrum

**References:**

1. **Pharmacology of Dapsone.**
2. **Therapeutic Drug Monitoring in Clinical Practice.**
3. **Dapsone: A Review of its Pharmacology and Therapeutic Uses in Dermatology.**

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**Received:** 31/7/18 (2000)

**Accepted:** 31/7/18 (2000)

**Correspondence:**

S. K. Tanaka, Y. Nakahara, Y. Hair Analysis for Pharmacologic Drugs. I. Effective Extraction and Determination of Dapsone in Rat and Human Hair.

**Reference:**


Keywords: transgenic animal, quality evaluation, clone animal

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* 星薬科大学薬学部

Kawakami, N.*, Kita, K.*, Hayakawa, T., Yamaguchi, T., and Fujimoto, S.*: Phorbol myristate acetate induces NADPH oxidase activity of cytochalasin B-primed neutrophils through the protein kinase C-independent pathway


We examined the effect of cytochalasin B (CB) or granulocyte colony stimulating factor (GCSF) on superoxide radical ($O_2^-$) production of neutrophils by phorbol myristate acetate (PMA)-stimulation. It was observed that $O_2^-$ generation of intact and GCSF-treated neutrophils by PMA-stimulation showed a lag during the early stage, and was largely inhibited by 1-(5-isoquinoline-sulfonyl)-3-methyl-piperazine (200 microM) or GF109203X (GF) (0.2 microM), but not by ethanol (1%) and wortmannin (100 nM). In contrast, $O_2^-$ generation of CB-pretreated neutrophils by PMA-stimulation did not show a lag, but was less than that of intact cells, and was only minimally depressed by the above inhibitors, but was markedly depressed by the simultaneous addition of GFX and ethanol or GFX and wortmannin. Although translocation of p47phox and p67phox to the membrane fraction by PMA-stimulation of intact and GCSF-treated neutrophils occurred in parallel with $O_2^-$ production, that of CB-treated neutrophils by PMA-stimulation was not always proportional to $O_2^-$ production. These findings suggest that pretreatment of neutrophils with CB dramatically alters the PMA response of the cells; that is, the protein kinase C-dependent pathway is largely depressed, and a phoshopholipase D-dependent one for NADPH oxidase activation appears in CB-treated cells.

Keywords: NADPH oxidase, protein kinase C, neutrophils

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High-performance liquid chromatography with electrospray ionization mass spectrometry (LC/MS) and liquid chromatography with tandem mass spectrometry (LC/MS/MS) were applied to the analysis of the site-specific carbohydrate heterogeneity in erythropoietin (EPO) used as a model of the sialylated glycoprotein. N-linked oligosaccharides were released from recombinant human EPO expressed in Chinese hamster ovary cells enzymatically and reduced with NaBH₄. Many different sialylated oligosaccharides of EPO were separated and characterized by LC/MS equipped with a graphitized carbon column (GCC). Glycosylation sites and the preliminary glycosylation pattern at each glycosylation site were determined by LC/MS of endoprotease Glu-C digested EPO. The detailed site-specific carbohydrate heterogeneity caused by the differences in the molecular weight, branch, linkage, and sequence were elucidated by GCC-LC/MS of the N-linked oligosaccharides released from the isolated gly-

Selective glycopeptide mapping of recombinant human erythropoietin (rEPO) used as a model glycoprotein was successfully carried out by online high-performance liquid chromatography/electrospray ionization mass spectrometry (ESI-LC/MS) using a Vydac C18 column eluted in acetonitrile-1M ammonium acetate, pH 6.8. rEPO expressed in a Chinese hamster ovary clone was exhaustively digested into four glycopeptides and nine peptides with endoprotease Glu-C. Both glycopeptides and peptides were eluted with trifluoroacetic acid as the eluent, whereas only glycopeptides were eluted selectively with ammonium acetate in the following order: N38, N24, O126, and N83. Furthermore, many glycoforms included in each glycopeptide were found to be separated by differences in the numbers of amino acids and N-acetyllactosaminyl repeats. Twenty, 16 and 22 different N-linked oligosaccharides were determined at Asn24, 38, and 83, respectively, and two different O-linked oligosaccharides were observed at Ser126. Our method is simple, rapid, and useful for determining the carbohydrate structures at each glycosylation site and for elucidating the site-specific carbohydrate heterogeneity.

Key words: glycopeptide mapping, ESI-LC/MS, erythropoietin


Crocus sativus lecin (CSL) is one of the truly mannose-specific plant lectins that has a unique binding specificity that sets it apart from others. We studied sugar-binding specificity of CSL in detail by a solution phase method (fluorescence polarization) and three solid phase methods (flow injection, surface plasmon resonance, and microtiter plate), using a number of different glycopeptides and oligosaccharides. CSL binds the branched mannotriose structure in the N-glycan core. Substitution of the terminal Man in the Manalph[1-3]Man branch with GlcNAc drastically decreases binding affinity much more than masking of the terminal Man in the Manalp[1-6]Man branch. Most interestingly, the beta-Man-linked GlcNAc in N-glycan core structure contributes greatly to the binding. The effect of this GlcNAc is so strong that it can substantially offset the negative effect of substitution on the nonreducing terminal Man residues. On the other hand, the GlcNAc that is usually attached to Asn in N-glycans and the l-Fuc linked at the 6-position of the GlcNAc are irrelevant to the binding. A bisecting GlcNAc neither contributes to nor interferes with the binding. This unique binding specificity of CSL offers many possibilities of its use in analytical and preparative applications.

Keywords: crocus sativus lecin, (Man),GlcNAc


We demonstrate the difference between the follistatin isoforms (FS-288 and FS-315), two activin-binding proteins, in the neutralizing activity for activin signalling. Transcriptional reporter assay using 3TP-Lux, an activin-responsive reporter construct, showed that the inhibitory effect of FS-288 on activin-induced transcriptional response is more potent than that of FS-315. The potency was not influenced by the presence of heparan sulfates, by which FS, in particular FS-288, associates with cell surfaces at a high affinity. Furthermore, FS-288 inhibited the binding of activin to its type II receptor more markedly than did FS-315, as evidenced by surface plasmon resonance and affinity cross-linking experiments. Moreover, the Kd of FS-288 and FS-315 for activin A was estimated to be 46.5+/−0.37 pM and 432+/−26 pM, respectively, by surface plasmon resonance experiments. These results indicate that the different potency between the two FS isoforms in the inhibition of activin activities depends on their affinity for activin A.

Keywords: Follistatin, FS-288, FS-315


Endogenous amine 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1BtTIQ) derivatives are synthesized, and their activity for dopaminergic systems are evaluated in vitro and in vivo by receptor binding assay and pharmacological tests. It is proposed that 1BtTIQ derivatives can act as endogenous dopaminergic antagonists.

Keywords: endogenous amine, dopamine receptor, locomotor activity

Kawai, H., Kotake, Y.*, and Ohta, S. *: Dopamine transporter and catechol-O-methyltransferase activities are required for the toxicity of 1-(3',4'-Dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline Chem. Res. Toxicol., 13, 1294-1301 (2000)
1-(3',4'-Dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline (3',4'-DHBSTIQ, 1) is an endogenous parkinsonism-inducing substance. It is taken up into dopaminergic neurons via the dopamine transporter, inhibits mitochondrial respiration and induces parkinsonism in mice. We synthesized four derivatives (aromatized, N-methylated, N-methyl-aromatized, and O-methylated, 2 - 5) and studied the cellular uptake and cytotoxicity of 1 - 5, as well as the metabolism of 1. All except the O-methyl derivative (5) were specifically taken up by the dopamine transporter, but 1 was taken up most efficiently. Relative to 1, oxidation reduced \( V_{\text{max}} \), N-methylation markedly increased \( K_m \), and O-methylation eliminated the uptake activity. Cytotoxicity of 1 - 5 was examined in a mesencephalic cell primary culture. Compound 1 reduced cell viability by nearly 80% at 100 \( \mu \)M, but the other compounds had little or no effect on cell viability. In vivo and in vitro studies revealed that 1 was O-methylated by soluble catechol-O-methyltransferase (COMT). Aromatization and N-methylation of 1 were not observed. We found that dopamine transporter inhibitors and a COMT inhibitor each blocked the cytotoxicity of 1, indicating that uptake and O-methylation are both necessary for neurotoxicity. Thus, we consider that 1 is taken up into dopaminergic neurons via the dopamine transporter and then converted by COMT to 5, which has cytotoxic and parkinsonism-inducing activities.

Keywords: metabolism, dopamine transporter, parkinsonism

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We previously showed that herbimycin A causes a decrease in glucocorticoid receptor (GR) level in primary cultured rat hepatocytes and suggested that the decrease was due to degradation of GR protein. In this paper, we examined the nature of the protease involved in the degradation by using a specific protease inhibitor. The proteasome-specific inhibitor lactacystin prevented the herbimycin-A-induced decrease in GR protein and binding capacity. Lactacystin also prevented the decrease in GR level during culture in the absence of herbimycin A. These results suggest that proteasome may be involved in herbimycin-A-dependent and independent decrease in GR protein.

Keywords: glucocorticoid receptor, proteasome, degradation

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Mizuguchi, H., Kay, M. A.*, and Hayakawa, T.: In vitro ligation-based cloning of foreign DNAs into the E3 as well as E1 deletion region for generation of recombinant adenoovirus vector

BioTechniques, 30, 1112-1116 (2001)

We develop a simple system that allows for the insertion of foreign genes into the E3 deletion region as well as into the E1 deletion region of adenoviral vector. By using this system, we constructed, as a model, a self-contained Ad vector carrying a tetracycline-regulatable gene expression system. The tet-responsive transcriptional activator gene was inserted into the E3 deletion region, while the luciferase gene with a tetracycline-regulatable promoter was inserted into the E1 deletion region. Tetracycline-regulatable luciferase expression was observed in the cells transduced with such a vector. This single vector system, combined with tetracycline-regulatable gene expression system, should greatly facilitate the application of the adenoovirus vector for gene therapy and gene transfer experiment.

Keywords: adenoovirus vector, gene therapy

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Mizuguchi, H., Koizumi, N., Hosono, T., Utoguchi, U.*, Watanabe, Y.*, Kay, M. A.*, and Hayakawa, T.: A simplified system for constructing recombinant adenooviral vectors containing heterologous peptides in the HI loop of their fiber knob

Gene Ther., 8, 730-735 (2001)

The use of recombinant adenoovirus (Ad) vectors containing genetically modified capsid proteins is an attractive strategy for achieving targeted gene transfer. The HI loop of the fiber knob is a promising candidate location for the incorporation of foreign ligands for achieving this goal. However, the method of constructing an Ad vector containing a foreign ligand in the HI loop of the fiber knob has proven difficult. In this study, we developed a simple system to construct fiber-modified vectors. To do this, a vector plasmid containing a complete E1/E3-deleted Ad type 5 genome and a unique Csp451 and/or Clal site between positions 32679 and 32680 of the Ad genome (residues threonine-546 and proline-547 of the fiber protein) was constructed. Oligonucleotides corresponding to the Arg-Gly-Asp (RGD) or Asn-Gly-Arg (NGR)-containing peptide motif (as a model) and containing a Csp451 and/or Clal recognition site, were ligated into the Csp451 and/or Clal digested plasmid. The simplicity of this method allows not only for easy construction of fiber-mutant Ad vectors, but also for screening of the peptides that target the vector to the desired cells and tissues.

Keywords: adenoovirus vector, gene therapy, targeting

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Recent studies have demonstrated the usefulness of dendritic cells (DCs) genetically modified by adenoovirus vectors (Ad) to immunotherapy, while sufficient gene transduction into DCs is required for high doses of Ad. The RT-PCR analysis revealed that the relative resistance of DCs to Ad-mediated gene transfer is due to the absence of Cxoxsakie-adenoovirus receptor expression, and that DCs expressed adequate alpha(v)-integrins. Therefore, we investigated whether fiber-mutant Ad containing the Arg-Gly-Asp (RGD) sequence in the fiber knob can efficiently transduce and express high levels of the LacZ gene into DCs. The gene delivery by fiber-mutant Ad was more efficient than that by conventional
Ad in both murine DC lines and normal human DCs (NHDC). Furthermore, NHDC transduced with fiber-mutant Ad and conventional Ad at 8000-vector particles/cell resulted in a 70-fold difference in beta-galactosidase activity. We propose that alpha(v)-integrin-targeted Ad is a very powerful tool with which to implement DC-based vaccination strategies.

Keywords: adenovirus vector, gene therapy, dendritic cells


The nuclear membrane is a tight barrier for cytoplasmic proteins, but nuclear proteins have the intrinsic ability to overcome this barrier by an active signal-mediated process. Specific cytoplasmic carrier proteins have the responsibility to escort these proteins into the nucleus through the nuclear pore. The nuclear membrane is also a tight barrier for exogenous DNA delivered by synthetic vehicles, while many of the karyophilic viruses have a mechanism to actively deliver their genome through the nuclear pore. Virus DNA and RNA cannot move into the nucleus by themselves and require the viral structural proteins for efficient nuclear transport. In this article, we review the recent progress in understanding the mechanism of the nuclear transport of proteins and the virus genome, and discuss the possibility of developing synthetic gene-delivery systems based on these outcomes.

Keywords: adenovirus vector, gene therapy, dendritic cells


In this article, we evaluated the feasibility of the restricted replication-competent adenoviruses for treatment of anaplastic thyroid carcinomas (ATCs), which are very aggressive and difficult to treat. Because ATCs very often harbor p53 mutations, we used wt-p53 as a regulatory factor to restrict virus replication and cytotoxic effect to p53-mutated cells. The recently reported "gene inactivation strategy" using p53-regulated Cre/IoxP system was employed; this system consists of two recombiant adenoviruses. One has an expression unit of the synthetic p53 - responsive promoter and the Cre recombinase gene (Axyp53RECre), and another contains two expression units; the first consists of E1A gene flanked by a pair of IoxP sites downstream of the constitutive CAG promoter and the second E1B19K gene under the control of the CMV promoter (AdCAlEiAL). In vitro data demonstrate that although infection of AdCAlEiAL alone led to E1A expression, viral replication and cytopsis in all the thyroid cells examined irrespective of their p53 status, the double infection did so in FRO cells (p53-null ATC) but not in FRO cells stably expressing wt-p53 and normal thyroid cells with wt-p53. These data indicate that our double infection method may have a potential for treatment of ATC and probably also other p53-defective cancer cells.

Keywords: adenovirus vector, gene therapy, p53


Here we report a simple and efficient method for nonviral gene transfer using liposomes which have envelope protein of vesicular stomatitis virus (VSV) on their surface (VSV-liposomes). We prepared VSV-liposome by fusing simple liposomes with VSV particles. To evaluate whether these particles can efficiently introduce their internal contents into the cytoplasm of mammalian cells, we examined the delivery of fragment A of diptheria toxin (DTA) by VSV-liposomes into the cytoplasm of FL cells. We found that VSV-liposomes encapsulating DTA were highly cytotoxic to the cells, while empty VSV-liposomes and plain liposomes encapsulating DTA were not, suggesting that VSV-liposomes delivered DTA into cytoplasm. Consistent with this, the cells cultured with plasmid DNA entrapped in VSV-liposomes and coding for firefly luciferase showed significant luciferase expression, whereas cells culture with plasmid DNA in plain liposomes and plasmid DNA-cationic liposomes complex did not. Thus, VSV-liposomes function as a simple and efficient nonviral vector for the delivery of DNA.

Keywords: vesicular stomatitis virus, gene therapy


We report here the antisense effect of phosphodiester oligodeoxynucleotide (DO-ODN) using fusogenic liposomes (FL) as its carrier. Using antisense (AS) DO-ODN 15-mer complementary to the c-myc proto-oncogene mRNA, including the translation initiation codon site, we analyzed the growth of HL-60 cells by [3H]-thymidine uptake. AS-ODNs encapsulated in FL inhibited the growth by about 70% that of the control HL-60 cells at 2.48 microM. In contrast, sense and scrambled DO-ODNs encapsulated in FL showed no effect of the growth of HL-60 cells at the same concentration. Even at 50 microM, free form DO-ODNs did not show any effect. These results suggest that FL is potentially a useful delivery vehicle for oligonucleotide-based therapeutics, and that DO-ODN may be a likely candidate for oligodeoxynucleotides when an efficient delivery system is used.

Keywords: vesicular stomatitis virus, gene therapy


We report here the antisense effect of phosphodiester oligodeoxynucleotide (DO-ODN) using fusogenic liposomes (FL) as its carrier. Using antisense (AS) DO-ODN 15-mer complementary to the c-myc proto-oncogene mRNA, including the translation initiation codon site, we analyzed the growth of HL-60 cells by [3H]-thymidine uptake. AS-ODNs encapsulated in FL inhibited the growth by about 70% that of the control HL-60 cells at 2.48 microM. In contrast, sense and scrambled DO-ODNs encapsulated in FL showed no effect of the growth of HL-60 cells at the same concentration. Even at 50 microM, free form DO-ODNs did not show any effect. These results suggest that FL is potentially a useful delivery vehicle for oligonucleotide-based therapeutics, and that DO-ODN may be a likely candidate for oligodeoxynucleotides when an efficient delivery system is used.

Keywords: vesicular stomatitis virus, gene therapy

Identification and characterization of cell lines with a defect in a post-adsorption stage of Sendai virus-mediated membrane fusion

J. Biol. Chem. 275, 17549-17555 (2000)

In the early stage of infection, Sendai virus delivers its genome into the cytoplasm by fusing the viral envelope with the cell membrane. Although the adsorption of virus particles to cell surface receptors has been characterized in detail, the ensuing complex process that leads to the fusion between the lipid bilayers remains mostly obscure. In the present study, we identified and characterized cell lines with a defect in the Sendai virus-mediated membrane fusion, using fusion-mediated delivery of fragment A of diphtheria toxin as an index. These cells, persistently infected with the temperature-sensitive variant Sendai virus, had primary viral receptors indistinguishable in number and affinity from those of parental susceptible cells. However, they proved to be thoroughly defective in the Sendai virus-mediated membrane fusion. We also found that viral RN protein expressed in the defective cells was responsible for the interference with membrane fusion. These results suggested the presence of a previously uncharacterized, HN-dependent intermediate stage in the Sendai virus-mediated membrane fusion.

Keywords: Sendai virus, fusion, HN protein

Kawahara N., Nozawa M., Flores, D., Bonilla P., Sekita, S., Motoyoshi Satake: Sesterterpenoid from Gentianella alborosea


Gentianella alborosea and G nitida (Gentianaceae), commonly known as "Hercampuri" or "Hircampure" are biennial medicinal plants growing in the Andes Region. The aqueous extracts of the whole plants have been used in traditional Peruvian folk medicine as a remedy for hepatitis, as a cholagogue and in treatment of; During our on going research on the plants above mentioned, we have isolated a novel sesterterpenoid, named alborosin, from the CHCl3 extract of G alborosea. The paper describes the structure elucidation of the above compound, occurring in the plant together with xanthones and phenolic compounds.

Keywords: Hercampuri, Gentianella alborosea, alborosin

Kuroyanagi, M., Kazumasa Sugiyama, Kanazawa M.,

Kawahara N.: Novel A-Seco-Rearranged Lanostane Triterpenoids from Abies sachiulensis


From the needles of Abies sachiulensis, novel rearranged lanostane type triterpenes, 1-4, were isolated along with a known triterpene (5). The structures of the new compounds, 1-4, were elucidated to be 3,4-seco-8(14→13)-fabo-17,13-friedo-9 β -lanosta-4(28),7,14(30),22Z,24-pentaen-26,23-olide-3-oxic acid, methyl 3,4-seco-8(14→13)-fabo-17,13-friedo-9 β -lanosta-4(28),7,14(30),22Z,24-pentaen-26,23-olide-3-oate, 3,4-seco-8(14 13)-fabo-17,13-friedo-9 β -lanosta-4(28),7,14,22Z,24-pentaene-26,23-olide-3-oxic acid and methyl 3,4-seco-8(14→13)-fabo-17,13-friedo-9 β -lanosta-4(28),7,14,22Z,24-pentaene-26,23-olide-3-oate, respectively, by means of spectral experiments, especially two dimensional NMR spectroscopy, such as 1H-detected multiple quantum coherence (HMQC), 1H-detected heteronuclear multiple bond connectivity (HMBC) and 1H-1H-correlation spectroscopy (COSY) experiments. These new compounds have novel structures containing A-seco, rearranged spiro structure and a γ -lactone conjugated with a diene. Some of these compounds showed potent antibacterial activity against gram positive bacteria.

Keywords: Abies sachiulensis, Pinaceae, rearranged lanostane

Yun Y. S., Sugimoto, N., Sekita S., Maitani T., Yamada T., Satake M.: Two New Alkaloids from Cigarette Smoke Condensate


The structural elucidation of minor constituents in mainstream smoke of cigarette was carried out. After mainstream smoke was collected on the glass filters by inhalation apparatus "Hamburg II", the condensate was extracted by MeOH. Seven known alkaloids and two new alkaloids named Cigatin A (1) and B (2) were isolated. Their structures were determined, on the basis of spectral data and chemical method.

Keywords: Cigarette, Cigatin A, Cigatin B

Tsuchiya, T.: A useful marker for evaluating tissue-engineered products: Gap-junctional communication for assessment of the tumor-promoting action and disruption of cell differentiation in tissue-engineered products


An in vitro system for evaluating the safety of tissue-engineered products is a convenient because of its rapidity and low cost. On the basis of recent studies, intercellular channels called gap-junctions are considered to play an important role on the tumor-promotion stage during the tumorigenesis induced by polycrylates. Further, we also demonstrate the significance of the intercellular communication during neuronal cell differentiation. From these results, we propose a survey of the function of the gap-junctonal communication as a probable useful marker for evaluating the safety of tissue-engineered products.

Keywords: tissue-engineered products, cell differentiation, tumor-promotion
角出泰造, 土屋利江: 代謝差異試験によるソフトコンタクト
レンズ用化学消毒剤の評価
生体材料, 19, 93-97 (2001)
For the evaluation of long-term safety of contact lens care solu-
tion used repeatedly for human eyes, inhibitory effects of chemical-
disinfectants on the gap-junctional intercellular communica-
tion (GJIC) were studied with the metabolic cooperation (MC)
assay. Three types of contact lens care solutions were investi-
gated using a colony formation test and MC assay, its ingredients,
poloxamine and polyhexamethylenebiguanide (PHMB), were in-
vestigated as well. Poloxamine showed an inhibitory effect on
the GJIC, whereas PHMB had no effect. The MC assay was con-
cluded to be suitable for evaluating the inhibitory activity of GJIC
at non-cytotoxic concentrations. To support this MC assay, fur-
ther studies are needed to evaluate the long-term safety of contact
lens care solutions.
Keywords: metabolic cooperation assay, colony formation test,
polyhexamethylenebiguanide

Kondo, S.*, Haishima, Y., Ishida, K.*, Ishikii, Y.*, Hisatsune,
K.*: The O-polyasaccharide of lipopolysaccharide isolated from
Vibrio fluvialis O19 is identical to that of Vibrio biserogroup
1875 Variant
Vibrio biserogroup 1875 Variant と同一の抗原性 (01 コ
レラ菌イタイプ因子 C, 1875 株共通抗原因子 D および E) を持
つ新規分離株 Vibrio fluvialis O19 (Kobe 株) の LPS O-抗
原多糖部のエピトープ構造解析を行った。その結果, Kobe
株の O-抗原特異性は N-3-hydroxypropionyl-D-persamine (4-
amino-4,6-dideoxy-D-mannopyranose) の a (1→2) 結合オホ
ポリマーにより構成されており, 1875 Variant 株 LPS O-抗
原特異性と同一の構造を持っていたことが明らかになった。
Keywords: LPS, Vibrio fluvialis, Vibrio biserogroup 1875
*城西大学

Haishima, Y., Hayashi, Y., Yamagi, T., Nakamura, A.: Elution
of bisphenol-A from hemodialyzers consisting of polycarbonate
and polysulfone resins
ポリカーボネートおよびポリスルフォン製血液透析カ
ラのビスフェノール A (BPA) の溶出状況を検討するため, 原
材料ベレット中のBPA含有量試験, ウシ血清を含めた各種溶
媒を使用したヘッドイングケースおよび血液透析器の溶
出試験を行った。BPAの溶出は全ての回収実験において認め
られたが, その溶出量は最大でも2000 ng/modal (ウシ血清
使用時) であり, 溶出したBPAは毎粘膜への影響は殆どな
いものと判断された。また, 血液透析器の実際の使用条件
でも最長17提示の回収条件と思われるウサ血清循環と同等のBPA
溶出を与える簡易型透析器を用いた検討試験を体験した結果, 17.2% エタ
ノールが使用できることが明らかになった。
Keywords: bisphenol-A, hemodialyzer, endocrine disruptor

Yamagi, T., Kitagawa, K.*, Aida, C.*, Fujiwara, H.*, Futaki,
S.*: Stabilization of a tyrosine O-sulfate residue by a cat-
tonic functional group: Formation of a conjugate acid-base
pair
硫酸化チロシン残基は, 基質間の相互作用に重要な役割
を果たすと考えられている。本研究では, 硫酸化チロシン残
基と塩基性官能基との相互作用を MS と IEP で調べた。硫酸
化チロシン残基の塩基性残基とオノパリアを形成している
場合, 気温及び液相中で脱硫酸反応を起こしにくくなるこ
とから, 脱硫酸の程度を塩基相互作用の強さを推定すること
ができる。まず, ベンゾイド中の塩基残基数が増すにつれ,
脱硫酸反応が起こりにくくなることが確かめられた。
アリニン残基は塩基残基よりも強い安定化能を示した。
また, 他のアミノ酸残基と硫酸化チロシン残基との分子間相互作用が MALDI-TOFMS スペクトルから確認された。以上,
結果は, 特にアリニン残基とオノパリアを形成するこ
とによる硫酸化チロシンの安定化が, 蛋白質のフォールディング
や基質間の相互作用を推進する力の一つとされている
ことを示唆するものであった。
Keywords: tyrosine O-sulfate, acid-base pair, protein interaction
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Yamagi, T., Haishima, Y., Nakamura, A., Osuna, H.*, Ikezawa,
Z.: Digestibility of allergens extracted from natural rubber
latex and vegetable foods
天然ゴムラテックスや植物性食品に由来する交差反応性
アレルゲンの数つかは, 植物の生体防御に関わる蛋白質であ
るゲンが既に明らかにされている。一方, 遺伝子組換え法
などを使ってこういった抵抗性蛋白質を人為的に作成させる
試みは多くなされており, 農作物中のアレルゲン量が増加す
る可能性が懸念される。本研究では, 発現させた蛋白質が食
物アレルゲンとなるかどうかを評価する手法の一つとして
用いられている消化性試験により, 天然ゴムラテックスや植
物性食品に含まれるアレルゲン蛋白質を実際に検出できるか
どうかを調べた。その結果, ラテックスアレルギーあるいは
口腔アレルギー症候群 (OAS) と診断された患者的 IgE 抗体
が認識する蛋白質抗原は, 必ずしも消化系に示さないこと
がわかった。この実験結果を基に, 消化試験は症候群を成
立させるような食物アレルゲンの検出法としては有効であ

Haishima, Y., Murai, T., Nakagawa, Y., Hirata, M.*, Yamagi,
T., Nakamura, A.: Chemical and biological evaluation of en-
dotoxin contamination on natural rubber latex products
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硫酸化チロシンが酸性溶液中で非常に不安定であること、この修飾残基を含むペプチドの化学合成は極めて困難であると考えられていた。そこで本研究は、硫酸化チロシン含有ペプチドの一般的な化学合成法を確立することを目的に行われた。まず、アミノ酸残基の酸性溶液中における脱硫酸化反応のマスコンを基に、硫酸化チロシン残基が分解しないような酸性保護基の条件を詳細に検討した。その結果、イオソーヌールの高いリフロール溶液中で低濃度という条件では、脱硫酸化反応の速度が極めて小さいことを見出した。そして、酸性に酸性なアミノチリル樹脂を用いる固相ペプチド合成法にこの酸脱保護基を組み合わせ、硫酸化ペプチドの一般的で容易な合成法を確立し、ビッググラストリン-IIやCCK-39の化学合成に応用了。

Keywords: tyrosine O-sulfate, peptide synthesis, deprotection

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HIV-1のTat蛋白を含む塩基性ペプチド（48-60）に、細胞膜を通過する性質を持つことが知られている。この研究では、多数のアミノ酸残基を持つ様々なペプチドがTat（48-60）と同様に細胞膜透過性を示すことを、バクテリアの発酵管酸を用いた研究により見出した。この研究で用いたペプチドの例として、各種ウイルスのRNA結合ドメインや、ライシンジッパー蛋白のDNA結合セグメントが挙げられる。アルギニンを含むペプチドを除き、これらはペプチドの1次構造を2次構造に共通性は認められなかった。細胞膜透過性は4℃においても認められなかった。さらに、アルギニンを8残基ほど含むペプチドが最も効率的に細胞膜を通過することが、(Arg)n (n=4 ~ 16)という合成ペプチドを用いた実験で明らかになった。これらの結果は、アルギニンを含むペプチドに、従来のエンコードデンドーナイソトープオフフェンスを示す共通の細胞膜透過機構がある可能性を示唆するものであった。

Keywords: internalization, protein delivery, translocation

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五十嵐良明, 鹿庭正昭, 中村晃: 椎子を用いる人工皮膚中の抗菌製品の部位別、nicebox-10-oxybis-10H-phenoxyarsineの分析


最近、抗酸化剤としての利用を期待されるペプチド（PVC）製のネジ用塗り地によってアレルギー性接触皮膚炎を起こした症例が報告されたことから、原因物質を特定するために人工皮膚中のOPAの定量を開始した。OPAは、メタノールで抽出し、酸化アルミウムカラムにかけ、ジェチルエステル: 95%ヘキサン95%酢酸で溶出された。溶出物をメタノールで溶解し、OD250nmに測定した。定量法は、ニトロフライアグリレート法で検討した。2個からそれぞれ52.7, 84, 99μg/gのOPAを検出された、皮膚炎を起こしたPVC製品からOPAは検出されなかった。

Keywords: antimicrobial, polyvinyl chloride, HPLC


We developed a method for the determination of PPD derivatives, such as N-(1-methylpentyl)-N-phenyl-p-phenylenediamine (MHPD), N-isopropyl-N-phenyl-p-phenylenediamine (IPPD) and N-1,3-dimethylbutyl-N-phenyl-p-phenylenediamine (DMBPPD). The PPD derivatives were extracted from rubber boots with acetone/chloroform (1:1). The extract was loaded then on to a silica-gel column. MHPD and DMBPPD were eluted in...
the diethyl ether:hexane (10:90) fraction. IPPD was detected in the diethyl ether:hexane (20:80) fraction. Each fraction was subjected to HPLC with an ODS column and a UV detector (detection wavelength 290 nm). The mobile phase was methanol:water (85:15). MHPPD was not found in any of the rubber boots, but DMBPPD and IPPD were detected.

Keywords: $\beta$-phenylenediamine, antioxidant, HPLC

Ikarashi, Y., Tsuchiya, T., Kaniwa, M., Nakamura, A.: Activation of osteoblast-like MC3T3-E1 cell responses by poly(lactide) 

This study examined the osteoblast-like MC3T3-E1 cell responses to poly($\varepsilon$-lactide) (PDLLA) and poly($\varepsilon$-lactide)/(PLLA) with different weight average molecular weight (Mw). The protein, DNA and hydroxyproline (HYP) content and alkaline phosphatase (ALP) activity for cells cultured on the PLLA (Mw: 270000 or 1370000) were almost similar to those on glass. The ALP activity of the cells cultured on low Mw PLLA (Mw: 20000) increased. The addition of low Mw PDLLA (Mw: 5000 or 10000), L-lactide or L-lactic acid into culture increased the protein, DNA and HYP content and ALP activity for cells. The release of L-lactic acid from PLLA and PDLLA into aqueous solution during incubation was little. These results suggested that increased osteoblast differentiation was induced by low Mw PDLLA and PLLA, and these may be used as an effective material in the field of orthopedic and drug delivery systems for the treatment of bone diseases.

Keywords: poly($\varepsilon$-lactide), poly($\varepsilon$-lactide), osteoblast

Takayama, M.*., Hayashi, Y.: Prediction of measurement precision based on FUMI theory for quantitative mass spectrometry with electron ionization 

The precision or relative standard deviation (RSD) of measurements in a sector type mass spectrometer equipped with an electron ionization system is examined experimentally and theoretically. The observed RSD is obtained by the usual replication and the theoretical RSD is predicted from the probabilistic properties of the baseline noise by the uncertainty theory called the FUMI theory (Function of Mutual Information). The peak corresponding to the limit of detection (RSD = 33%) is demonstrated.

Keywords: precision, mass spectrometry, FUMI theory

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Shintani, H.: Pretreatment and chromatographic analysis of phthalate esters and their biochemical behavior in blood products 
Chromatogr., 52, 721-726 (2000)

医学的に用いられているポリ塩化ビニールの可塑剤であるフタル酸エステルの内分泌障乱作用が問題になっている。本論文では血液バッグからのフタル酸ジェステル、フタル酸モノエステルならびにフタル酸が血液中の酵素によって生成すり、これらの成分をHPLC法で分離、分析した。またそれらの成分の選択的な固相抽出前処理法を開発した。

Keywords: phthalate esters, blood products, solid phase extraction

Shintani, H.: Determination of the endocrine disruptor bisphenol-A in the blood of uremia patients treated by dialysis 
Chromatogr., 53, 331-333 (2001)

透析患者に使用されている人工透析器にはポリサルフィン、ポリカーポネート、ポリステレンなどが使用されている。これらは内分泌障乱作用を有するとされている Bisphenol-A ならびにステレンオリゴマーなどを含んでいる。本論文では人工透析器からの Bisphenol-A、ステレンオリゴマーならびにステレンオリゴマーの放射線減菌ならびに高圧蒸気減菌での生成とその安全性について調査した。

Keywords: bisphenol-A, endocrine disruptor, artificial dialyzer

Shintani, H., Akers J.E.*.: On the cause of performance variation of biological indicator used for sterility assurance 

生物検査メーカーならびにメーカー間でロットが異なることに伴う抵抗値（D 値）の差の原因について調べた。

Keywords: biological indicator, D value, variation

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Sasski, K.*, Shintani, H., Itoh, J.*.: Effect of calcium in assay medium on D value of Bacillus stearothermophilus ATCC 7953 spores 

SCD 培地のロット間ならびにあるいはメーカー間の差により滅菌保証を達成するための D 値が異なることが報告されている。違いの要因については既に報告があるが、培地組成のどの成分が生物指標（BI）の D 値を起因しているのか明らかにされていない。著者らは SCD 液体培地（SCD）ならびに SCD 常温型培地（SCDA）の組成をおとす検討した。その結果 BI の差を生じさせるのは SCD 培地組成中のカルシウム（Ca）イオン量であることを見出した。SCDA での D 値は SCD 類似を有する高、それは SCD 培地中の Ca 量の差によることを明確した。

Keywords: sterility assurance, calcium ion, culture medium

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J. Food Production, 63, 1295-1298 (2000)

The inhibitory effect of methanol extract of Korean soybean paste on the mold growth and aflatoxin production of a toxigenic strain of Aspergillus parasiticus ATCC 15517 was studied using different concentrations of the extract in yeast-extract sucrose broth.

Keywords: soybean paste, aflatoxin, Aspergillus parasiticus

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安藤正典：インド・バンガラディシュにおける地下水と素汚染と健康影響
公衆衛生研究所、49(3), 266-274 (2000)
平成4年に水道水質基準の改正が行われたが、ビタミンは地下
水の利用や温泉水などの混入によって水質基準を超える
可能性の高い水深が存在していることが明らかに
なってきた。更に最近、US EPAではビタミンのリスクアセスメン
トにおける暴露評価の結果から高リスクがあることが明
らかに基準値の見直しを検討中である。著者は国際協力事業
の一つとして、インドやバンガラディシュの西ベンガル周辺
地域における地下水と素汚染に伴う健康影響の改善のため
と素の自然環境での挙動、健康影響及び暴露量を考察すると
と、ビタミンの水質基準の在り方に大きな課題を突きつけられること
に他ならない。本稿では、厚生労働省が新たに施行する
至った水質管理について水道事業者の観点からの課題点を
考察している。
Keywords: arsenic, India, contamination of ground water

安藤正典, 徳永裕司: 化粧品の将来に向けての産官学の共
同研究の展望
フレラランスジャーナル、29(1), 37-40 (2001)
化粧品の規制緩和が1991年4月を目指して推進されて
いる。化粧品の承認許可制度の大改査の根拠が、民間団体
の要望、化粧品の業界側からの要望、その安全性の
面から、化粧品の使用の承認的目的を明示するが、化粧品
の規制緩和以後は、製造、品質確保、安全性確立などに関
する企業自体の責任を前提に、消費者への化粧品の安全性と
efficiencyの情報の提供を通じてその重要性を重ねて
いる。本展で21世紀を展望した化粧品の在り方あるいは
化粧品に関する産官学の共同研究の在り方を展望する。
Keywords: cosmetics, efficacy, effectiveness

安藤正典, 五十嵐良明, 錦田栄一: ホルムアルデヒドの暴
露評価と健康影響
アレルギーの臨床、21(2), 113-118 (2001)
生活環境には数え切れないほどの化学物質や微生物その
他の生物が存在し、その発生原因も様々な多様な原因を示して
いる。これらの化学物質や微生物、食品、水などを介して経
口的に、また居住空間や環境污染の空気汚染の様式を通じて
経皮的に、更に化粧品等の事故の接触が発生している。特に、
ホルムアルデヒドはその代表的な化学物質で、日と夜などに対する刺激性と共
に、アレルギーとの関連性が指摘されている。本稿では、こ
うしたホルムアルデヒドによる発症原因や健康影響を検討
すると共に、アレルギーとの関連性について紹介した。
Keywords: formaldehyde, risk assessment, carcinogenic

安藤正典：水質管理の課題とその対応
資源環境対策、37(3), 242-246 (2001)
2000年度、生活環境審議会（厚生省の諮問機関）は、①
水道事業の一部を第三者に委託することを可能にすること。
②水道による専用水道の規制対象を居住人口（100人以上）
から、それと同等の給水の能力がある水道も加入させること。
③未規制の小規模受水槽（100m³以下）水道の管理の充実、④水
道事業における情報提供の制度の設立、などの水道法改
正案について答申した。これを受けて厚生労働省は水道法の
改正案を提出の予定である。
このような新たな施策を推進することは、石や美をもたらす
ことができるが、このことはぜひに来る水道との関係で
水質管理の在り方に大きな課題を突きつけられること
に他ならない。本稿では、厚生労働省が新たに施行する
至った水質管理について水道事業者の観点からの課題点を
考察している。
Keywords: control of drinking water quality, water sources, drinking
water treatment

安藤正典：室内化学物質の毒性と対策
建築雑誌、116(4), 75 (2001)
室内環境中存在する化粧品によって、シックハウス症
候群や化学物質過敏症などの症状が発生する可能性が高い
ことが社会的に大きな問題となっている。これらの疾病は、
慢性的な反応、長期又は多量・短時間暴露によって誘発され
その後、敏度の高め、発症すると考えられている。しかしなが
か、室内空気に関連する疾病であるシックハウス症候群は、
それと関連すると思われる揮発性有機化合物の特定あるいは
それらの毒性が動物実験によって証明されているわけではない
まず、暴露量と健康影響の相関関係性が示されて
いるわけではない。本稿では、室内化学物質の有害性の指標と
その有害性を回避する対策としてのガイドラインの設定の
基礎を紹介した。
Keywords: indoor air, guideline, sick house syndrome

熊谷一清*, 坊垣和明**, 池田耕一*, 坂 義宏**, 松村年
郎, 飯倉雄**, 吉津 晋**: 実大実験住宅における内装
材の室内化学物質濃度及び発生影響に関する研究
日本建築学会計画評価論文集,第542号, 77-83, (2001)
実大実験住宅において、各施工段階ごとの気体濃度を測定し
内装材の室内汚染に及ぼす影響を検討した。更に、使用する
材料の放散率、使用する建材・工法の放散量に及ぼす影
響、材料の組み合わせに伴い、外的条件（湿度）の影響
、放散量低減策の効果など、総合的に検討し、内装材の室内
汚染に及ぼす影響を明らかにした。
Keywords: voc, building material, full sized experiment
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德永裕司, 郎 焉, 内野 正, 安藤正典: ビスフェノー
ルAのIn Vitro被皮吸収に関する研究
日本化粧品技術者会誌, 34, 253-260 (2000)
化粧品に含まれると想定される内分泌を乱す化学物質
のビスフェノールA (BPA) の皮膚吸収の検討を行った。モルモット
の腹部の皮膚をFraunhofer酸素セルに装着した。
側に10mM ドデシル硫酸ナトリウム溶液、10mM 塩化ベンザルコニウム（BKC）溶液あるいは0.5% ポリオキシエチレン（10）オレイン酸エーテル（POE, 0E）溶液を加え、32℃ で 2 時間放置し
た。donor 側の界面活性剤を除き、0.05% BA 溶液を加え、32
℃で14～24時間後にreceptor 側に透過してくる BA を HPLC
法で測定した。また、同様に 0.05% BA を含む各種界面活
性剤を用いて 32℃で 14～24 時間後に receptor 側に透過して
くる BA を HPLC 法で測定した。HPLC 条件は、Unisil Q C18
(4.6 mm i.d. × 150mm) カラム及び水／アセトニトリル混合
液（3：2）を用い、蛍光検出器（励起波長：280nm、発光波長：
305nm）にて測定を行った。界面活性剤の BK 及び POE, 0E で
2 時間処理された無塵状態での BA の flux は 1.6 及び 1.2
倍と増加した。なお、BA を界面活性剤 BK あるいは POE, 0E と共
存させた場合、flux が 0.1 倍及び 0.2 倍と著しく低下した。

Keywords : bisphenol A, skin permeation, Franz type diffusion

 uid chromatography inductive coupled plasma mass spectrometry for speciation of arsenic compounds in urine

A high performance liquid chromatography inductive coupled plasma mass spectrometry(HPLC-ICP-MS) system for speciation of arsenic, arsenate, monomethyl arsenic acid, dimethylarsinic acid and arsenobetaine in a single run in urine samples has been
developed. Detection limits for the five arsenic species in urine
samples are between 0.01 and 0.04 μg/L. To validate the method,
SRM2670 containing both normal and elevated levels of arsenic
have been analyzed for arsenic species. The method has been ap
plied to determine the arsenic species in urine samples of two
groups of people from two arsenic-affected districts of two dis
tricts, out of the nine affected districts of Western Bengal, India.
From their urine speciation, the nature of exposure of individuals to
arsenic compound could be predicted. It is concluded that, even though
these groups are using safe water, they cannot avoid, from time to
time, arsenic contamination as any water sources of the surrounding
areas are arsenic contaminated.

Keywords : arsenic species in urine samples, HPLC-ICP-MS

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内野 正, 徳永裕司, 安藤正典 : 高速液体クロマトグラフ
による有機水, 乳液, クリーム中の塩酸プロカイン及び塩
酸ジプシンの定量
薬誌誌, 34, 261-266 (2000)

塩酸ジプロカイン及び塩酸ジプシンは局所麻酔剤として
広く用いられているが、昭和61年の厚生省通知（昭和61年
3月12日薬第2号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塩酸ジプロカイン及び塩
酸ジブシ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。化粧水や乳液
及びクリーム中の塩酸プロカイン又は塩酸ジプシンを水
又はメタノールで抽出し、ODS カラム（Shiseido CAPCELL
PACK C8, 4.6 × 250mm）、移動相として50mL リン酸塩緩衝液
(pH 5)／アセトニトリル混液（37:3）又は（65:35），検出器と
して紫外吸収光度計（測定波長 296nm 又は 228nm）を用い、
高速液体クロマトグラフィーにて分析した。この方法を用いる
ることにより、化粧水や乳液及びクリーム中の塩酸プロカイン
又は塩酸ジブシ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 : procaine hydrochloride, dibucaine hydrochloride,
HPLC

Hanioka, N., Tanaka-Kagawa, T., Chung, Y.S., Nishimura, T.,
Jinno, H., Ando M.: Changes in hepatic cytochrome P450 en
zymes by biodegradation products of 4-tert-octylphenol
polyethoxylates in rats.


The effects of some biodegradation products of 4-tert-octyl
phenolpolyethoxylates (OPEO), namely 4-tert-octylphenol
polyethoxylate (OPEO), 4-tert-octylphenol diethoxylate (OPEO)
and 4-tert-octylphenol monooxyborate (OPEO1EC), on hepatic cytochrome P450 enzymes in rats were studied. Among the cytochrome P450-dependent
monoxygenase activities, testosterone 2a-hydroxylase activity
was significantly decreased by OP1OPE0EC and OPEO1EC.
CYP3A2-dependent monoxygenase, testosterone 6b-hydroxylase
activity was also decreased by 50% by OP. Furthermore, immunoblotting showed that OP significantly decreased by 49
and 43%, the CYP2C11/6 and 3A2/1 protein level, respectively.
By contrast, CYP1A1/2, CYP1A2, CYP1B1, CYP2A and 4A1/2/3-
dependent monoxygenase activities were not affected by any
OPEO biodegradation product. These results suggest that OPEO
biodegradation products change the enzyme-specific cytochrome
P450 isoform(s) in rat liver (OP<OP1OPE0EC), and that these
changes may relate to the toxicity of OPEO and its biodegrada
tion products.

Keywords: Cytochrome P450; 4-tert-Octylphenol

Hanioka, N., Tatarazako, N.* Jinno, H., Arizono, K.*, Ando,
M.: Determination of cytochrome P450 1A activities in mam
malian liver microsomes by high-performance liquid chroma
tography with fluorescence detection.


A sensitive method for the determination of CYP1A activities
such as ethoxyresorufin O-deethylation (EROD) and methoxy
resorufin O-demethylation (MROD) in liver microsomes from hu
man, monkey, rat and mouse by HPLC with fluorescence detec
tion is reported. The newly developed method was found to be
more sensitive than previous methods. The detection limit for
resorufin was 0.80 pmol/assay. Intra-day and inter-day prece
isions (RSD) were less than 6% for both enzyme activities. With
this improved sensitivity, the kinetics of EROD and MROD activities
in mammalian liver microsomes could be determined more pre
cisely. EROD activities in human and monkey liver microsomes,
and MROD activities from all animal species exhibited a monophasic
kinetic pattern, whereas the pattern of EROD activities in
rat and mouse liver microsomes was biphasic. Therefore, this
method is applicable to in vivo and in vitro studies on the interaction
of xenobiotic chemicals with CYP1A isoforms in mammals.

Keywords: Cytochrome P450; EROD; MROD

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A simple and sensitive method for the determination of UDP-glucuronosyltransferase UGT1A6 activity using 4-methyl umbellifone (4-MU) and 4-nitrophenol (4-NP) as substrates in human and rat liver microsomes by HPLC with UV detection was reported. The method was validated for the determination of 4-methylumbelliferyl b-D-glucuronic acid (4-MUG) and 4-nitrophenyl b-D-glucuronic acid (4-NPG). The method was found to be more sensitive than previous methods using a spectrophotometer, a spectrofluorimeter and HPLC. The detection limit for 4-MUG and 4-NPG was 14 and 23 nM, respectively. The intra- and inter-day reproducibility (RSD) of UGT1A6 enzyme assay in liver microsomes was less than 6%. With this improved sensitivity, the kinetics of UGT activities toward 4-MU and 4-NP in human and rat liver microsomes could be determined more precisely. Therefore, this method is applicable to in vivo and in vitro studies on the interaction of xenobiotic chemicals with UGT1A6 isofroms in mammals using small amounts of biological samples.

Keywords: UDP-Glucuronosyltransferase; 4-MU; 4-NP


4種有機スズ化合物の人体暴露量を推定することを目的に、2004年度の1日摂取量はTBTCが平均1.73 (範囲0.87-4.70) μg/人、DBTCが平均0.45 (範囲0.17-0.9) μg/人、TPTCが平均0.09 (範囲0.01-0.25) μg/人、PPTCが6 μg/人であった。TBTC及びTPTCはほとんど10群 (魚介類) 由来であり、本群の1日摂取量の経年変化を7年間でわが国での1999年度において比較した。1999年度にTBTCは1991年度の1/2に低下し、TPTCは1/4 (約21% ) に低下した。TBTCの1日摂取量は我が国のTBTCの一定値の2.3%に相当した。TPTCの平均1日摂取量は、MPFRのADIの0.3%相当であった。

Key words: organotin compounds, tributyltin, triphenyltin, total diet study, daily intake

佐々木久美子, 辻晃 隆*, 中村宗知*, 六沢 晃*, 桑原 正, 石部 基, 塚田 隆, 鈴木 実, 三浦 謙, 佐藤 正, 食品衛生法告示エスペロカルプ等5農薬試験法の評価


Keywords: method-performance study, notified analytical method, esprocarb

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Keywords: method-performance study, notified analytical method, bromine

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佐々木久美子, 辻晃 隆*, 中村宗知*, 六沢 晃*, 桑原 正, 石部 基, 塚田 隆, 鈴木 実, 三浦 謙, 佐藤 正, 食品衛生法告示ダミノジド試験法の評価

食品衛生学雑誌, 41, 228-232 (2000)

ダミノジド (D2) 告示法検査法の評価のために共同実験を実施した。6群でD2を添加した玄米等5物を分析したとき、平均回収率は92.2-94.4%であった。併行精度及び室内再現精度の相対標準偏差はそれぞれ4.4-5.5%, 7.4-11.8%であった。5農薬のD2試験液のクロマトグラムには妨害ビックは検出されなかった。検出限界値は5C-NPDの状態に依存し、0.1 μg/kg以下であった。

Keywords: method-performance study, notified analytical method, daminozide

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根本 了, 佐々木久美子, 衛藤修一*, 斎藤 慎*, 酒井 洋*, 高橋哲夫*, 外海泰秀, 永山敏廣*, 嶋田二郎*, 前川 吉明*, 豊田正武: GC/MS (SIM) による農作物中の 110 薬剤の 一斉分析


農作物からアセトニトリル抽出後、GPC及びカートリッジカラムによるクリーンアップを行い、GC/MS (SIM) で 110 薬剤の 一斉分析法を作成した。65 種類の農薬に 4 他又は 5 分析機関で添加回収試験を行った結果、60種類の回収率データのうち 70%以上の良好な結果を得たデータが全種類以上の検出位で 77 種種類である。各農薬の検出限界値は 0.004~0.08 μg/g 以下であった。

Keywords: agricultural product, pesticide residue, multiresidue analysis

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根本 了, 高附 巧, 佐々木久美子, 豊田正武: 市販魚中の ニフレールの分析


6 店舗で購入した市販魚の ニフレール (NP) 汚染を調査した結果、35検体中31検体から 9~800ng/g の NP が検出された (検出限界 8ng/g)。購入した店舗の各魚の NP 汚染度が高かったことが報告されたことから、包装容器の一つ一つパックによって影響されることが判明した。耐化学性ポリスチレン製トレイからの NP が溶出されたが、それらの污染物質は NP に相当する包装材料、特にラミネートフィルムから移行の可能性が強く示唆された。

Keywords: fish, nonylphenol, wrapping film

今中雅章*, 佐々木久美子, 根本 了, 植田英一*, 村上和子*, 宫田大典*, 外海泰秀: GC/MS による各種食品中の ピスフェノール-A の分析

食品衛生学雑誌, 42, 71-78 (2001)

各種食品中のピスフェノール-A をアセトンで抽出、クリーンアップ後、ヘプタフロリコ酸で誘導体化し、GC/MS (SIM) で定量する方法の分析法を開発、加熱食品、鮮魚食品など 95 検体の実験調査を行った。その結果、散合、生鮮魚介類、肉類、コンピュータ非当からそれぞれ痕跡量~60ng/g、痕跡量~6ng/g 及び痕跡量のピスフェノール-A が検出された。一方、乳製品、野菜、果物、精白米からは検出されなかった。

Keywords: bisphenol A, GC/MS, fresh food

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高附 巧, 根本 了, 堤 智昭, 佐々木久美子, 豊田正武: HPLC による農作物中の Acibenzolar-S-methyl 及び分解物の分析

食品衛生学雑誌, 41, 381-386 (2000)

HPLC による農作物中の アシベンゾラール-S-メチル (BTH) 及び分解物の benzol[1,2,3]dihydroxyl-7-carboxylic acid (BTC) の分析法を確立した。試料をリン酸緩衝液 (pH 8.0) とアセトンで抽出後、BTH は塩基性溶液 (pH 7.5~8.5) からエーテルへキサン (1:1) で再抽出し、BTC はリン酸緩衝溶液 (pH 3.0) から同溶液で再抽出した。BTC は脱塩後、フラジリスで精製した。BTC は、直接フラジリスで精製し、UV 検出器付 HPLC 及び LC/MS で測定を行った。

米及及びトウモロコシに BTH 又は BTC を 0.1 μg/g 添加したときの回収率は、平均 76.8~86.7%、標準偏差は 0.3~2.7%であった。検出限界は UV 検出器付 HPLC で 0.003 μg/g (BTH) 及び 0.008 μg/g (BTC) であった。

Keywords: pesticide residue, HPLC, acibenzolar - S-methyl

豊田正武, 堤 智昭, 柳 俊彦*, 河野雅一*, 高野博*, 堤 誠*, 場 糠*, 島田隆雄*, 末野や大規オキシ類測定における振とう抽出法と還流抽出法の比較

食品衛生学雑誌, 41, 316-320 (2000)

野菜試料における、アセトンヘキサン振とう抽出法とトルエン溶液抽出法のデイオキシン類抽出効率の比較を行った。抽出試験を 3 回行った結果、ほうれん草では、振とう抽出で FCDs, PCBs 及び Co-PCBs が平均 0.48, 0.80 及び 7.70pg/g 検出され、還流抽出では同様の順に 0.43, 0.72 及び 7.39pg/g 検出された。また、してきたんマでは、振とう抽出で FCDs, PCBs 及び Co-PCBs が平均 0.67, 0.50 及び 2.6pg/g 検出され、還流抽出では同様の順に 0.81, 0.64 及び 2.6pg/g 検出された。両抽出法の間で、抽出率には有意差はなく、両市の野菜中デイオキシン類の抽出効率は同様であることが判明した。

Keywords: shaking extraction, reflux extraction, dioxin

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食品衛生学雑誌, 42, 18-23 (2001)

1996年から1998年に、トルックダイエット試料のアルミウム濃度を測定しアルミウムの一日常取量を推定した。10カ所の機関でトルックダイエット試料の調製及びアルミウム濃度の測定を行った。アルミウムの一日常取量は平均 3.6mg であり、範囲は 2.8mg から 8.4mg であった。分析結果の正当性は、認証標準試料の分析により保証された。

Keywords: aluminum, daily intake, total diet sample

*新潟県保健環境科学研究所
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Kondo, K., Kurihara, M., Fukuhara, K.: Mechanism of antioxidative effect of catechins


The antioxidative mechanisms of EC, EGC, ECG and EGCG were studied using LC/MS, spectrophotometry, chemiluminescence and electrochemical analyses, and semiempirical MO calculations. These results showed the antioxidative effect of catechins and their mechanisms were complicated. EGC has a lower oxidation potential (159 mV) than EC (307 mV) and, therefore, can scavenge peroxyl radicals more quickly (kinh/kp = 232) than EC (kinh/kp = 41). This indicates that the pyrogallol structure in the B ring play an important role in the rapid scavenging ability. EGC having the gallate group at the C-3 position show much more rapid scavenging ability (kinh/kp = 628) than EC and EGC, suggesting that as the number of phenolic OHs in catechins increases, the more rapidly they can scavenge peroxyl radicals. However, EGC and EGCG with the pyrogallol structure have a negative aspect. They may generate superoxide and the antioxidative effect of them does not last for a long time. Catechins have both aspects as antioxidants and prooxidants as do other flavonoids. Catechin with the lowest oxidation potential does not exert the strongest antioxidative effect. Also, the effect may change based on the experimental conditions such as the solvent system or radical species being used as an initiator.

Keywords: catechin, oxidation potential, bond dissociation enthalpy


Two anthocyanins were isolated from the highly pigmented callus derived from the storage root of purple sweet potato (Ipomoea batatas L.) cultivar 'Ayamurasaki'. One was identified as cyanidin 3-O-sophoroside-5-O-glucoside, and the other as cyanidin 3-O-(2-O-(6-O-(E)-p-coumaryl)-β-D-glucopyranosyl)-β-D-glucopyranoside-5-β-D-glucopyranoside, by chemical and spectroscopic analysis.

Keywords: anthocyanin, Ipomoea batatas, natural food colorant

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J. Health, Sci., 46, 89-93 (2001)

The relationship between the cardiac glycoside contents in Corchorus olitorius seeds and the seed color was examined. The seed color was assigned a shade (color value) (L value in UCS system). The dark grayish green seeds showing lower L value, contained more cardglycosides than dark grayish yellow seeds showing higher L value. When the total cardiac glycoside contents were plotted against the L values, a positive correlation (r = 0.913) was observed. Also, there was a higher content ratio of strophanthidin glycosides (erysimoside and olitoside) in the seeds showing lower L value, while there was a lower content ratio of digitoxigenin glycosides (coroside and glucovatromonoside) in the seeds showing lower L value.

Keywords: Corchorus olitorius, cardiac glycoside, color value

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Seven lines of genetically modified (GM) maize were authorized in Japan as foods and feeds imported from the USA. We improved a multiplex PCR method described in the previous re-
port in order to distinguish the five lines of GM maize. Genomic DNA was extracted from GM maize by a silica spin column kit, which could reduce experimental time and improve safety in the laboratory and potentially in the environment. We sequenced recombinant DNA (r-DNA) introduced to GM maize, and re-designed new primer pairs to increase the specificity of PCR to distinguish five lines of GM maize by multiplex PCR. A primer pair for the maize intrinsic zein gene (Ze1) was also designed to confirm the presence of amplifiable maize DNA. The lengths of PCR products using these six primer pairs were different. The Ze1 and the r-DNAs from the five lines of GM maize were qualitatively detected in one tube. The specific PCR bands were distinguishable from each other on the basis of the expected length. The r-DNA could be detected from the maize sample containing 0.5% of each of the five lines of GM maize. The sensitivity would be acceptable to secure the verification of non-GMO materials and to monitor the reliance of the labeling system.

Keywords: genetically modified maize, recombinant DNA, PCR

*National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries

Miyahara, M., Saito, A.*1, Ito, H.*1, Toyoda, M.: Capability of identification of gamma-irradiated bovine liver by new high sensitivity assay


DNA in food will get damage by gamma radiation. The high sensitive Comet assay was studied using fluorescence microscopy. Beef liver was irradiated at range of 1 Gy to 8 kGy. Single cells were obtained from the irradiated liver, and were analyzed by agarose-gel electrophoresis. The pH of the buffer for electrophoresis was pH13, which is generally utilized for sensitive detection of DNA damage. The pattern formed by DNA was visualized by staining ethidium bromide. The resulted comets were evaluated by the scale we developed and influence scores were calculated based on the Tice method. It is possible to detect irradiation of beef liver at 10 Gy. In the other hand, DNA damage will be caused not only by irradiation, but also by the other treatments. Therefore influence of freezing, preservation, irradiating temperature, atmosphere of irradiation, cooking, and homogenizing device were also examined. This new comet assay will be a useful detection procedure of irradiated foods.

Keywords: comet assay, irradiated food detection, DNA damage, beef liver

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Miyahara, M., Ito, H.*1, Saito, A.*1, Nagasawa, T.*1, Kariya, M.*1, Toyoda, M., Saito, Y.: Detection of meats by HPLC determination for o-tyrosine using novel LASER fluorometric detection with automatic pre-column reaction

J. Health Science., 46, 304-309 (2000)

An o-Tyrosine method for detection of irradiation of foods was studied by HPLC using a novel LASAR fluorometric detection system with pre-column reaction. Sample was prepared and purified by eliminating fat and sugars using a mixture of acetone and chloroform, and then the purified protein was hydrolyzed using hydrochloric acid at 110°C for 24 hours in a vacuum. The sample was reacted with NDB-F reagent by an automatic pipetting system and was introduced into the HPLC system. Irradiated chicken, pork, beef, and tuna were examined by irradiating at 0.1, 1.5, 10 kGy. Irradiation of chicken and pork irradiated at or over 10 kGy was successfully detected, but that of beef and tuna were more difficult to detect. After 3 months storage at 20°C, the irradiation was still detectable in chicken irradiated at 10 kGy. Thus this detection procedure can be used to detect irradiation in some chilled meats irradiated at 10 kGy. Non-irradiated o-tyrosine formation and reduction of o-tyrosine by hydroxylation are also discussed.

Keywords: o-tyrosine method, irradiated food detection, NBD-F, HPLC, pre-column derivatization

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後藤典子*1, 田辺寛子*1, 宮原 誠: 照射鶏肉の炭化水素法およびESR法による検知


Chicken meat with bone was irradiated by gamma ray at a-19~10°C, and both amount of hydrocarbons formed from fatty acids and intensity of ESR signals in bone fragments were measured. Very good correlation was found between the amount of hydrocarbons and the intensity of ESR signals. The amount of hydrocarbons (Cn-2:1) had 2 carbon atoms less than the original fatty acids and an additional double bond, was almost constant irrespective of the irradiation temperature raised. As the ratio between corresponding fatty acids, the ratio between hydrocarbons (Cn-2:1) is a suitable index in the detection of the irradiation. On the contrary, the ratio of hydrocarbons from same fatty acid, (Cn-2:1)/(Cn-1:0), varied according to the kind of fatty acid and temperature used at the irradiation.

It was found that under irradiation temperature of -19~10°C, intensity of ESR signals of bone is not affected by the irradiation temperature.
Keywords: irradiated chicken, hydrocarbon method, ESR method, identification

DNA in food will sustain damage by gamma radiation. The detection capability of the high sensitivity comet assay was studied using fluorescence-microscopy. Meats were irradiated at a range of 1 Gy to 2kGy. Single cells were obtained from the irradiated meats, then analyzed by agar-gel electrophoresis. The pH of the buffer for electrophoresis was pH13, which is generally utilized for sensitive detection of DNA damage. The pattern formed by DNA was visualized by staining with ethidium bromide. The resulting comets were evaluated with a scale we developed, and influence scores were calculated based on the Tice method. Pork, beef, and chicken that were irradiated at or above 0.5kGy were identified by the method.
Keywords: comet assay, irradiated meats, identification

Nagaoka M.H. and Maitani T.: Binding patterns of co-existing aluminium and iron to human serum transferrin studied by HPLC/high resolution ICP-MS
Serum transferrin (Tf) is an Fe-binding glycoprotein. Al in the blood is bound to Tf. In the present study, the chemical forms of co-existing Al and Fe bound to human serum Tf were studied by on-line combined HPLC/HR-ICP-MS. Samples were subjected to HPLC equipped with an anion-exchange column. The levels of 27Al, 56Fe and 32S, which are interfered by polyatomic ions such as 13C14NO3+, 13C15N+ and 14N15NH+, "AlO4" and "CaO4", respectively, in the case of quadrupole ICP-MS, were monitored simultaneously by HR-ICP-MS at resolution m/Δm = 3000. Al added to apo-Tf as Al-citrate was preferentially bound to the N-lobe site almost selectively. Al in serum from a healthy person without any in vitro Al spike was present both as Al3-Tf and Al3Fe2-Tf. The chemical states were reproduced in apo-Tf solution supplemented with Fe (Fe/Tf = 0.6) and Al (Al/Tf = 1) successively. The cleanup procedures of column were devised, and with the procedures, the detection limit for 27Al was lowered to 0.1 μg/l (3σ) at the middle resolution.
Keywords: HPLC/HR-ICP-MS, Al, transferrin

Nagaoka M.H., Maitani T.: Effects of sialic acid residues of transferrin on the binding with aluminum and iron studied by HPLC/high-resolution ICP-MS.
Carbohydrate-deficient transferrins (TfS) (CDTs) with fewer sialic acids increased in several diseases. In this study, the affinity of metals (Al and Fe) to TfS was compared between native- and asiato-Tf by on-line HPLC/HR-ICP-MS, to clarify whether the presence of sialic acids influences the metal binding. Fe added as Fe-citrate in the presence of bicarbonate preferred the N-lobe site and the binding affinity was similar between native- and asiato-TfS. Al-citrate added also preferred the N-lobe site, while the binding affinity was higher to asiato-Tf than to native-Tf. In Al-oxalate addition, the affinity to the N-lobe site of both TfS increased further. In the absence of bicarbonate, Al-oxalate showed a preference for the C-lobe site in native-Tf and comparable affinity to both lobes in asiato-Tf. In asiato-Tf, Al-Tf was the largest peak. Thus, the lack of sialic acid in glycans and the presence of oxalate enhanced the binding affinity of Al to Tf. Therefore, it was suggested that the binding affinity of Al in patients with CDTs may be enhanced.
Keywords: carbohydrate-deficient Tfs, HR-ICP-MS, Al

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: GPC/ICP-AES 法によるカラギナン分子量測定方法

日本食品化学学会誌，8, 33-43 (2001)

カラギナンは分子中に多数の硫酸を有する天然増粘安定剤である。その平均分子量を求めるために、GPC カラムを装着した HPLC 法と硫酸取量の ICP 発光分光法を直結した、GPC/ICP-AES 法を応用した。注入した試料を高分子由来の硫酸量の回収率は、97-98%と良好であった。測定した平均分子量は、GPC/RI 法による分子量と同程度であった。その原因として、カラギナン分子量による硫酸含有率の違いと、ICP-AES 側の要因が考えられた。

Keywords: carrageenan, GPC/ICP-AES, average molecular weight

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HPCE with a UV detector was applied for the analyses of enzymatically glucosylated flavonoids, which are used as natural food additives in Japan. Four items, which have flavonol or flavanone as aglycon, were analyzed. Each of these items is a mixture of glycosides with various lengths of maltooligosaccharide chain. On capillary zone electrophoresis with untreated fused-silica capillary at alkaline pH, glycosides with longer sugar chain migrated more rapidly. Flavonol glycosides with 1-13 glucose units were distinguished with the borate buffer (pH 10.0). Flavanone glycosides needed higher pHs for good separation than flavanol glycosides.

Keywords: capillary zone electrophoresis, flavonoid, malto-oligosaccharide

Akiyama, T., Yamada, M., Yamada, T. and Maitani, T.: Naringin glycosides α-glucosylated on ring B found in the natural food additive, enzymatically modified naringin


Enzymatically modified naringin is a natural food additive, which is prepared with cyclodextrin glucanotransferase. Its constituents were structurally analyzed. Four constituents were isolated from the glucoamylase-treated sample. An NMR analysis revealed that two of them were novel compounds having 4′-O-α-glycosyl moieties on ring B of the naringenin aglycone. Both the aglycone and the glucose moiety in naringin are shown to be simultaneously glucosylated.

Keywords: enzymatically modified naringin, cyclodextrin glucanotransferase, nuclear magnetic resonance


Enzymatically hydrolyzed licorice extract (EHLE) is also used as a sweetener in Japan. Three oleane-type monoglycosides along with glycyrrhizin (1) and 3-O-[β-D-glucuronopyranosyl-(1→2)]-β-D-glucuronopyranosyl]-18β-liquiritic acid (2) were isolated from EHLE. The structures of the three compounds have been determined to be 3-O-β-D-glucuronopyranosyl-24-hydroxy-18β-glycyrrhetinic acid (3), 3-O-β-D-glucuronopyranosyl-18β-glycyrrhetinic acid (4) and 3-O-β-D-glucuronopyranosyl-18β-liquiritic acid (5) based on MS and NMR. 4 was the monoglycosylated derivative of glycyrrhizin (1). Compounds 3 and 5 are the monoglycosylated derivatives of the minor constituents in licorice extract. They were first isolated from EHLE, and compound 5 was a new compound.

Keywords: glycyrrhizin, sweetener, enzymatically hydrolyzed licorice


Enzymatically modified licorice extract (EMLE) is a natural sweetener, which is prepared with cyclodextrin glucanotransferase. The structures of six major constituents isolated from EMLE were determined, and their sweetness was studied. The isolated compounds were glycyrrhizin (1), 3-O-[β-D-glucuronopyranosyl-(1→2)]-β-D-glucuronopyranosyl]-liquiritic acid (2), and their derivatives glucosylated at the C-4 position of the terminal glucuronopyranose with additional one (3 and 4, respectively) and two (5 and 6, respectively) glucose moieties. Compounds 3-6 were new compounds isolated for the first time. Compound 2 was sweeter than compound 1. Interestingly, compound 3, which was a monoglucosylated derivative of compound 1, was sweeter than compound 4. Compounds 5 and 6, which have additional two glucose moieties, showed only slight sweetness.

Keywords: Glycyrrhiza, sweetener, enzymatically modified licorice

石川憲志*1，久保木俊**1，佐藤孝志，米谷民雄，布村伊*1: 4 倍体シシト果実の形態とカプサイシンノイド含量 日本食品化学学会誌，7, 74-77 (2000)
トウガラシ属における染色体倍数による倍数体を利用した品種改良の可能性を検討するため、シシトウ（Capsicum annuum L. cv. 'Shishitoh'）の4倍体を抽出し、当該の果実について種子数、果実の大きさ、カプサイシンイソド及びその前駆体含有量を調べた。その結果、4倍体の果実は2倍体の約4倍の種子数を持ち、果実の幅は同じであるが、長さは6割に減少していた。カプサイシンイソド及びその前駆体含有量は2倍体と同様であり、カプサイシンイソドの生成には促進される麻抑制されているステップはないことが明らかとなった。

Keywords: Shishitoh, tetraploid, capsicainoid

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阿部有希子，武田由比子，石綿 慶，山田 隆：甘味料アセスルファムカルムの規格試験体の検討及び純度と含有量
食品衛生学雑誌，41，274-279（2000）
アセスルファムカルムの規格作成のための試験方法について検討し、試験方法を適した。試験方法はJECA及びFCCの方法を大きく変わる必要があった。4.6エタオの試験方法について、性状、極大波長、沈殿反応、フッ化物、紫外線吸収不純物、乾燥減量、及び含量について測定したところ、JECA及びFCCの規格に適合した。

Keywords: aceulfame potassium, specification, purity test

杉田たき子，平山クニ*1，新野竜太*2，石橋 亨*1，山田 隆：ポリ塩化ビニル製玩具中のフタル酸エステル含有量
食品衛生学雑誌，42，48-55（2001）
1998年10月に入手した玩具68検体の材質試験を実施したところ、全ての玩具からフタル酸無水物（PAG）が検出された。検出されたPAGはフタル酸エチレノール（DEH），フタル酸ジエチル（DEHP），フタル酸ジジェチル（DEJH），フタル酸ジジェチルの4種類で、その他にアジビン酸ジエチレノールが検出された。DEHPは48検体から検出され、含有量は1.5〜5800mg/g、平均308mg/g、含有率が最も多かったのはおしゃれ玩具であった。DEHPは20検体から検出され、含有量は2.0〜3800mg/g、平均78mg/g、国産品では15検体、60%から検出された。

Keywords: polyvinyl chloride toy, diisononyl phthalate, di(2-ethylhexyl) phthalate

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武村由比子，川崎洋子，石綿 慶：スクラロースの食品添加物成分規格のための定量法に関する検討
日本食品化学会誌，7，56-59（2000）
平成11年7月30日、厚生省令第75号で食品添加物として指定され、告示第167号により、規格が定められた。告示に先立ち、純度試験など成分規格設定に関する検討を行った。含有量に関する定量法は米国食品添加物規格（FCC）および国際規格（CFAS）では標準品を用いたHPLC法であるが、標準品の入手が困難で、また、わが国の指定の食品添加物の含有規格の定量法は標準品不要の方法が一般的である。そこで、スクラロース分子中の塩基を水酸化ナトリウム溶液で逆流し、塩酸鉄で測定する簡便な方法を考察した。試料1gを精密に量り、100mlとした試験溶液10mlに水酸化ナトリウム溶液（1→10）10mlを加え、30分間反応後、指示電極に鉛、参照電極に鉛塩化鉄電極を装着した自動滴定装置を用い、0.1mol/1硝酸鉄溶液で滴定した。n=8の変動係数は0.4%であった。

Keywords: sucralose, sweetener, potentiometric titration

武村由比子，阿部有希子，石綿 慶，山田 隆：HPLCによる粉末スプール中のポリソルベートの分析法
食品衛生学雑誌，42，91-95（2001）
HPLCによる粉末スプール中のポリソルベートの定量法について検討した。試料中の粉末を、ヘキサンで除去後、アセトニトリルでポリソルベートを抽出した。抽出溶媒をポリエチルテトラメチルカルタール（500ppm）に溶解し、酸で繰り返し粉末スプールを60℃に加熱し、生成した生成物を、GPCカラムを使用したHPLCで測定した。粉末スプール中の添加回収率は75%以上であり、定量限界は0.04mg/gであった。市販の粉末スプール16種につき本法で分析したところ、いずれも除外であった。

Keywords: polysorbate, powdered soap, cobalt biocyanate

河村英子，前原英枝，飯塚昭広，山田隆：食品用プラスチック製品及び玩具中のニトリフェノール
食品衛生学雑誌，41，212-218（2000）
プラスチック製品・容器包装及び乳児用玩具中のニトリフェノール(NP)残存量をGC/MS-SIMにより測定したところ、ポリ塩化ビニリデン(PVC)製ラッフルフィルムと手袋において、全検体で10〜2,600μg/gと高濃度の残存が認められた。ポリステレン(PS)製使い捨てカップでも高濃度であった。その他、ポリカーボネート、ポリプロピレン、ABS樹脂、ステレンタクシン製品からも検出されたが、ポリエチレン、AS樹脂、ポリ塩化ビニリデン製品では検出されなかった。NPが残存していたPSカップ、PVCラッフルフィルム及び手袋について測定を行ったところ、nへプタンにより極めて高い溶解が認められた。一方、NPを含有する試料から酸化防止剤のトリス(ニトリフェノール)フェノスフェイトが検出され、これが分解してNPが生成したものと推察された。

Keywords: nonylphenol, polyvinyl chloride, tris(nonylphenyl) phosphate

河村英子，前原英枝，鈴木 隆，山田 隆：ガスクロマトグラフィー／原子発光検出器(GC/AED)による食品用器具・容器包装及び玩具中の有機スズ化合物の分析
食品衛生学雑誌，41，246-253（2000）
ガスクロマトグラフィー／原子発光検出器を用いた9種類の有機スズ化合物の分析法を検討した。選択性が高く、検出限界は標準液で1μgと高感度であった。食品用器具・容器包装及び玩具を分析したところ、ポリ塩化ビニリデン(PVC)製容器では全検体から安定剤のジオキシフェルスズ(DOT)が検出された例はなかった。
それ、残存量はほぼ数百万μg/gであった。さらにその不純物や分解物のモノ及びジオクチンズ(MOT及びTOT)、グリチルスズ(DBT)など検出され、ジチルスズの残存量もみられた。また、PVC製張袋からDOT、MOT、TOT、シクロオクタン、クロムとシラコンの影響を調べた結果、モノ及びジオクチンズが検出され、PVC製構成物のDOT及びDBTは比較的小さくみなかったが、BD及びRBDからは極めて容易に溶出した。

Keywords: organotin compounds, gas chromatography/atomic emission detection method (GC/AED), polyvinyl chloride

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河村薫子、左山佳代、山田 隆：食品用ポリエチレン、ポリプロピレン及びポリスチレン製品へのガム線照射の影響—添加剤及びその他の化合物
食品照射、33, 7-14 (2000)

市販ポリエチレン及びポリプロピレン製品に10～50kGyのガム線を照射したところ、残存していた添加剤のうち、酸化防止剤はいずれもすみやかに減少し、減少の程度はIrgafos 168の減少が最も顕著であり、Yoshinok 2246RやYoshinok SRはやや照射抵抗性があった。溶剤では、脂肪酸アミド類が減少されたが、脂肪族炭化水素には変化はみられなかった。また、可溶型のDBPは比較的安定であった。酸化防止剤の分解物のうち、1,3-di tert-butylbenzene及び2,6-di tert-butyl-1,4-benzoquinoneは照射分解物と考えられたが、2,4-di tert-butylphenolは非溶剤試料をも検出され、製品の製造時に生成するものと考えられた。ポリスチレン製品では、添加剤は含まれず、残存していた不純物のスチレンダイマー及びトリマーについては、ガム線照射による含有物の変化は認められなかった。

Keywords: gamma irradiation, food contact plastic, additives

河村薫子、左山佳代、山田 隆：食品用ポリエチレン、ポリプロピレン及びポリスチレン製品へのガム線照射の影響—揮発性物質

市販ポリエチレン及びポリプロピレン製品に10～50kGyのガム線を照射した。全てのポリエチレン及びポリプロピレンにおいて、照射により酸、アルデヒド、ケトン、アルコール等の揮発性物質が生成し、特に酢酸及びアセトンの生成量が多く、また、ポリプロピレンでは、分枝化した化合物など生成物の種類が多く、しかも生成量も高く、ポリエチレンよりも照射分解を受けやすいことが示された。一方、ポリスチレンは、非照射時に残存していた残留モノマーであるスチレン及びエチレンの含有量が照射により減少し、照射分解物と思われる揮発性物質の生成もわずかであり、ポリエチレンやポリプロピレンよりも照射耐性があることが示された。

Keywords: gamma irradiation, food contact plastic, volatiles

河村薫子、前原正枝、和久井千世子、山田 隆：ポリ塩化ビニル製張袋中の可溶剤及びノルフェノールの溶出
食中毒学生物雑誌、41, 330-334 (2000)

ポリ塩化ビニル(PVC)製張袋に残存するフタル酸ジ(2-エチルヘキシル) (DEHP)、フタル酸ジイソソノール(DINP)、ジフタル酸ジエチルヘキシル (DEHA)及び4-ノルフェノール (NP)は、水、20%エタノール及び4%酢酸で0.005〜0.416 μg/cm²の溶出であったが、n-ヘプタン(25℃60分間)ではDEHPが1,400〜2,500 μg/cm²、DINPが120 μg/cm²、DEHAが137〜841 μg/cm²、NPが2.72〜36.4 μg/cm²と極めて高い溶出を示した。ナフェノールの溶出量は、60℃30分間で薄手張袋ではn-ヘプタンの1/2〜1/4、や厚手張袋では1/4〜1/10に相当し、試験温度が高く、時間が長いほど溶出量は多くなったが、低温又は短時間でもかなりの溶出が見られた。以上より、PVC製張袋を食塩性食品に使用すると、残存するDEHP、DINP、DEHAが大量に食品へ移行することが示唆された。

Keywords: polyvinyl chloride glove, migration test, di(2-ethylhexyl) phthalate

河村薫子、井之上浩一,*中澤裕之,*山田 隆、米谷美雄：飲料食品からのブクフェノールA移行原因の解明と改良の評価
食衛衛生学雑誌、42, 13-17 (2001)

ブクフェノールA(BPA)含有量が高かったコーヒー及び紅茶飲料の相関検査を検討したところ、いずれもエポキシ樹脂コーティングに由来したが、サティシム条件でBPA濃度が極めて高く、試料がやや高め且つ全体の残存量が高すぎて原因物質が探れない状態であった。試料60℃952%のエタノール、n-ヘプタンではBPAは溶出しなかったが、水120℃30分間では35〜124mg/mL溶出した。担当分のBPA溶出量は材質中の残存量とほぼ一致し、缶内飲料のBPA含有量と近い値であった。以上よりBPAの移行は、コーティング中のBPA残存量と飲料の加熱条件に依存することが示された。一方、改良例ではコーティング中のBPA量が大幅に減少し、溶出量は1/10以下に低減された。

Keywords: bisphenol A, can for drink, epoxy resin

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Superoxide radical anion (O_2^-), generated by KO_2/crown ether, is effective for hydroxylation of nitratednaphthalenes. When mono- and di-nitratednaphthalenes are treated with KO_2/crown ether, hydroxylation results at the electron-deficient site caused by the electron withdrawing effect of the substituted nitro group. Kinetic experiments suggest that the hydroxylation proceeds by two different mechanisms dependent on the first one-electron reduction potential of nitratednaphthalenes.

Key words: superoxide; nitroarene; hydroxylation

Kurihara, M., Tanaka, M.¹, Oba, M.¹, Suehime, H.¹, Miyata, N.: Computational study on conformation of oligopeptides prepared from α,α-disubstituted amino acids Peptides 2000, 427-428 (2001)

Recently the conformation of peptides consisting of non-proteinogenic amino acids (β-amino acid, α, α-disubstituted amino acid, etc.) has received considerable attention. α, α-Disubstituted amino acids have two alkyl substituents at the α-position of normal α-amino acids and are conformationally restricted. To predict the conformation of these peptides presents an interesting challenge. We report the conformational analysis of oligopeptides prepared from α, α-disubstituted amino acids
(isovaline, diethylglycine, butyloxethylglycine) by computational study. Conformational energy computations on oligopeptides of α, α-disubstituted amino acids were performed using molecular mechanics. Conformational search calculations were carried out by the Monte Carlo method of MacroModel® (ver. 6.5, Schrodinger, Inc.). When AMBER® was used as the force field, the global minimum energy conformations were found to be 31p-helix. These results are in agreement with their conformational properties in the solid state determined by X-ray crystallographic analysis. In the case of the MMFF force field the global minimum energy conformations of diethylglycine and butyloxethylglycine peptides are planar structures, which are in agreement with their conformations in solution.

Key words: α, α-disubstituted amino acid, oligopeptide, molecular mechanics calculation


Visible light irradiation of poly(vinylpyrrolidone) (PVP)-solutebilized C60 in water in the presence of NADH and molecular oxygen results in the formation of superoxide anion (O2•−) which was detected with use of 5-diethoxypyrophosphoryl-5-methyl-1-pyrroline-N-oxide (DEPMPO) as a O2•−-trapping agent. Formation of O2•− having a characteristic g// value of 2.18 was also evidenced by the direct observation with use of a low-temperature ESR technique at 77 K. Photoinduced O2•− formation was also observed for the N-methyl-2-pyridone (NMP) solution of C60 and 1-benzyl-1,4-dihydronicotinamide (BNAH) in the presence of O2, whereas C60 radical anion (C60•−) was formed in the absence of O2 under otherwise the same experimental conditions. These results suggest that C60•− formed in the photoinduced electron-transfer reduction of C60 by BNAH acts as an electron donor to molecular oxygen to give O2•− in NMP.

Key words: Fullerene, superoxide, photosensitization


To investigate the chemical conformations and functions of the -Phe-Phe-Val- or -Phe-Phe- sequences contained in the Alzheimer's disease related β-amyloid peptide, a series of mini parallel double-stranded peptides conjugated with two peptide residues to one spacer were designed and prepared. The structure of the compounds was elucidated by circular dichroism (CD) spectrum and NMR two dimensional (2D) nuclear overhauser enhancement and exchange spectroscopy (NOESY) measurements. The structure of 1,2-ethano-bis(L-Phe-L-Phe-L-Leu), 1,12-dodecan-bis(L-Phe-L-Phe-L-Leu), 1,12-dodecan-bis(L-Phe-L-Phe-L-Val), and 1,12-dodecan-bis(D-Phe-D-Phe-D-Leu) conjugated with L-Leu and L-Val residues show a β-turn-like structure. The dihedral angles (θ = 75°, ω = 90°, Φ = -87°, Ψ = 180°) obtained from experimental coupling constant (J) data, etc. support that 1,12-dodecan-bis(L-Phe-L-Phe) adopts β-turn mimic the structure. The 1,12-dodecan-bis(L-Leu-L-Leu-L-Phe), 1,12-dodecan-bis(L-Leu-L-Phe-L-Leu), and 1,12-dodecan-bis(L-Phe-L-Val-L-Leu), etc. adopt most probably a random structure by CD studies. It was found by titration spectrum that an inclusion complex of 1:1 ratio (association constant; K=1.0x10^4 M^-1) is formed between 1,12-dodecan-bis(L-Phe-L-Phe-L-Leu) and azobenzene (guest,
Moreover, the stability of the complexes was increased in order of 1,12-dodecane-bis(L-Phe-L-Phe-L-Leu) > azobenzene > 1,12-dodecano-bis (L-Phe-L-Phe-L-Val) > azobenzene > 1,12-dodecano-bis (L-Phe-L-Val-L-Leu) > azobenzene. The data show that X-Phe-L-Phe-L-space (S)-L-Phe-L-Phe-L-X (X=amino acids; S=1,2-ethano- and 1,12-dodecano-) plays an important role as a binding site of the artificial receptor. The hydrophobic interaction of the four Phes in the two strands is a very interesting view in the physiological action of proteins as well as the conformation of the backbone of X-L-Phe-L-Phe-space (S)-L-Phe-L-Phe-X.

Key words: double-stranded peptide, β-turn mimetics, β-amiloid peptide

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All eight possible A-ring diastereomers of 2-methyl-1, 25-dihydroxyvitamin D3 (2) and 2-methyl-20-epi-1, 25-dihydroxyvitamin D3 (3) were convergently synthesized. The A-ring enyne synthons 19 were synthesized starting with methyl (S)-(+)- or (R)-(−)-3-hydroxy-2-methylpropionate (8). This was converted to the alcohol 14 as a 1:1 epimeric mixture in several steps. After having been separated by column chromatography, each isomer led to the requisite A-ring enyne synthons 19 again as 1:1 mixtures at C-1. Coupling of the resulting A-ring enynes 20a-h with the CD-ring portions 5a,b in the presence of a Pd catalyst afforded the 2-methyl analogues 2a-h and 3a-h in good yield. In this way, all possible A-ring diastereomers were synthesized. The synthesized analogues were biologically evaluated both in vitro and in vivo. The potency was highly dependent on the stereochemistry of each isomer. In particular, the alpha alpha-beta-isomer 2g exhibited 4-fold higher potency than 1 alpha,25-dihydroxyvitamin D3 (1) both in bovine thymus VDR binding and in elevation of rat serum calcium concentration and was twice as potent as the parent compound in HL-60 cell differentiation. Furthermore, its 20-epimer, that is, 20-epi-alpha alpha beta 3g, exhibited exceptionally high activities: 12-fold higher in VDR binding affinity, 7-fold higher in calcium mobilization, and 590-fold higher in HL-60 cell differentiation, as compared to 1 alpha,25-dihydroxyvitamin D3 (1). Accordingly, the double modification of 2-methyl substitution and 20-epimerization resulted in unique activity profiles. Conformational analysis of the A-ring by 1H NMR and an X-ray crystallographic analysis of the alpha alpha beta-isomer 2g are also described.

Key words: vitamin D3, conformational analysis, MacroModel

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A convenient and potentially valuable synthetic approach to the novel 2αlpha-functionalized 1α,25-dihydroxyvitamin D3 [1α,25(OH)2D3] derivatives (1a-c), which are the C2-epimer of ED-71 and its analogues, has been developed. The C2alpha-modified ring A precursors (1,7-enynes 16, n = 0, 1, and 2) were constructed stereoselectively starting from D-glucose in high yield. In the synthesized 2αlpha-(omega-hydroxyalkoxy)-1α,25(OH)2D3 derivatives, 1a and 1b showed a greater binding affinity to vitamin D receptor (VDR), up to 1.8 times that of the native hormone.

Key words: vitamin D3, vitamin D receptor, MacroModel

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Tanaka, M.*, Oba, M.*, Imawaka, N.*, Tanaka, Y.*, Kurihara, M., Suemune, H.*: Conformational study of heteropentapeptides containing an α -ethylated α , α -disubstituted amino acids: (S)-Butylethylglycine (=2-amino-2-ethylhexanoic acid) within dimethylglycine (=2-aminoisobutyric acid) residues


Heteropentapeptides containing the α -ethylated α , α -disubstituted amino acid (S)-butylethylglycine and four dimethylglycine residues, i.e., CF3CO-[{(S)-Beg}-(Alb)2]-OEt (4) and CF3CO-(Alb)2-[(S)-Beg]-(Alb)2-2OEt (7), were synthesized by conventional solution methods. In the solid state, the preferred conformation of 4 was shown to be both a right-handed (P) and a left-handed (M) 310-helical structure, and that of 7 was a right-handed (P) 310-helical structure. IR, CD, and 1H-NMR spectra revealed that the dominant conformation of both 4 and 7 in solution was the 310-helical structure. These conformations were also supported by molecular-mechanics calculations.

Key words: α , α -disubstituted amino acid, oligopeptide, molecular mechanics calculation

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Peptides 2000, 441-442 (2001)

α , α -Disubstituted amino acids and their peptides have attracted considerable attention since these amino acids and peptides show unique biological activities and very stable secondary structures. The conformation of the peptides prepared from achiral α , α -disubstituted amino acids such as 2-aminoobutyric acid: Alb, dialkylglycine has been studied extensively because the achiral α , α -disubstituted amino acids could be easily prepared. The property of Alb is known to be a 310-helical structure, and that of dialkylglycine such as diethylglycine and dipropylglycine is proved to be a fully planar C2-conformation. Recent develop-
ment of asymmetric reaction enables the peptide chemists to synthesize several chiral $\alpha$, $\alpha$-disubstituted amino acids, and the properties of $\alpha$-methylated $\alpha$, $\alpha$-disubstituted amino acids are known to be $3_{\alpha}$-helical structure.

We studied the conformation of peptides prepared from a chiral $\alpha$-ethylated $\alpha$, $\alpha$-disubstituted amino acid; (S)-butylethylglycine (=2-amino-2-ethylhexanoic acid: Beg). The homopeptides containing (S)-Beg (up to hexapeptide) were prepared by using solution-phase methods employing an ethyl ester as the C-terminal protection and a trifluoracetyl group as the N-terminal protection. The heteropeptidetides containing (S)-Beg as a guest molecule in the Aib sequence were also prepared in the similar manner. The conformations of these peptides in the solid state were studied using X-ray crystallographic analysis, and those in solution were studied using IR, $^1$H NMR, and CD spectra. The dominant conformation of homopeptides containing (S)-Beg was a fully planar C5-structure, and that of heteropeptides containing (S)-Beg and Aib was a $3_{\alpha}$-helical structure.

Key words: $\alpha$, $\alpha$-disubstituted amino acid, oligopeptide, molecular mechanics calculation


Nitropyrenes are carcinogenic pollutants. Adduct formation following nitro-reduction is considered to be a major cause of nitropyrene-mediated DNA damage. We investigated the role of 1-nitropyrene, a metabolite of 1-nitropyrene, in causing oxidative DNA damage, using $^{31}$P-$^5$-end-labeled DNA. 1-Nitropyrene was found to facilitate Cu(II)-mediated DNA damage in the presence of NADH. Catalase and a Cu(I)-specific chelator attenuated DNA damage, indicating the involvement of $H_2O_2$ and Cu(I). Typical $OH$ scavenger did not have a significant effect. These results suggest that the main reactive species is probably a DNA-copper-hydroperoxocomplex. We also measured 8-oxo-7,8-dihydro-2′-deoxygenosine formation by 1-nitropyrene in the presence of Cu(II) and NADH, using an electrochemical detector coupled to a high-pressure liquid chromatograph. We conclude that oxidative DNA damage, in addition to DNA adduct formation, may play an important role in the carcinogenesis of nitropyrenes.

Keywords: Nitropyrene, Nitrosopyrene, DNA damage, Copper, Hydrogen peroxide

Hachisuka, A., Nakajima, O., Yamazaki, T., Sawada, J.: Developmental expression of opioid-binding cell adhesion molecule (OBCAM) in rat brain


OBCAM, a neuron-specific protein, has been presumed to play a role as a cell adhesion/recognition molecule, but its function has not been fully elucidated. We investigated the developmental expression of OBCAM in rat brain by using a monoclonal anti-OBCAM peptide antibody (OBC3). OBCAM was clearly detectable on embryonic day 16 (E16) as assessed by immunoblotting. The expression level increased by the second postnatal week and was maintained at a constant level until week 17. During the early developmental period OBCAM was found to be expressed on postmitotic neurons and to be strongly expressed in the fiber tracts containing expanding axons, in contrast to the adult brain, in which OBCAM is principally expressed in the gray matter. These findings suggest that the function of OBCAM involves axonal outgrowth.

Keywords: OBCAM, development, immunohistochemistry


Communication between nerves and mast cells is a prototypic demonstration of neuro-immune interaction. Recently, we used an in vitro co-culture approach comprising cultured murine superior cervical ganglia (SCG) and rat basophilic leukaemia (RBL) cells to study this interaction. In the present work, we studied the communication from mast cells to neurites. We observed that binding of anti-IgE receptor antibodies to mast cells increases calcium ion concentration [Ca$^{2+}$] in SCG neurites. This indicates that mast cell-nerve communication is bi-directional. Confocal fluorescence microscopic images indicated that [Ca$^{2+}$] in neurites increased after an increase of [Ca$^{2+}$] in mast cells. The lag-time of neurite activation was several times longer than that of mast cell activation.

Keywords: nerve-immune interaction, co-culture, rat basophilic leukaemia cells

Aketani S.*, Teshima R., Umezawa Y.*, Sawada J.: Correlation between cytosolic calcium concentration and degranulation in RBL-2H3 cells in the presence of various concentrations of antigen-specific IgEs.


We studied the dependence of $\beta$-hexosaminidase release from RBL-2H3 cells on the antigen-specific IgE concentrations. The cells were sensitized with DNP-specific IgE (0.5-5000 ng/ml) or OVA-specific IgE (5-50 ng/ml) and stimulated with DNP$\_x$-HSA (10$^2$-100 ng/ml) or OVA (10-ng/ml-1$\mu$g/ml). It was found that the $\beta$-hexosaminidase release increased in a dose-dependent manner with the concentration of the IgEs added to the mast-cell suspension. The percentage of $\beta$-hexosaminidase release from the cells was well correlated with [Ca$^{2+}$], increase, and the correlation coefficient was 0.88 for DNP-specific IgE and 0.99 for OVA-specific IgE. Therefore, the [Ca$^{2+}$] monitoring system is a sensitive marker of degranulation from RBL-2H3 cells and can be used to measure even low amounts of antigen-specific IgE.

Keywords: degranulation, calcium signal, IgE


*The University of Tokyo

Mast-cell-deficient W/W(v) mice were sensitized by oral administration of 0.1 and 1.0 mg ovalbumin (OVA) by gavage every day for 9 weeks, and active systemic anaphylaxis (ASA) was induced by intraperitoneal injection of OVA. The production of OVA-specific IgE and IgG1 by oral immunization of the W/W(v) mice was high, and the production of IL-4 by splenocytes re-stimulated with OVA in vitro was increased. In contrast, production of OVA-specific IgG2a and IgG2b was low, and production of IFNgamma by splenocytes after re-stimulation with OVA in vitro was rather decreased. These findings suggest that Th2-dominant helper T-cell activation had occurred. The plasma platelet-activating factor (PAF) levels of mice sensitized with 0.1 and 1.0 mg OVA by gavage increased significantly. W/W(v) mice seem to be a good model for studying induction of food allergy.

**Keywords:** Mast cell-deficient mice, PAF, food allergy


A simple screening method is presented for the measurement of antigen-specific IgEs in sera in which mast cells are used. This method is based on the intracellular calcium signal in mast cells induced by cross-linking the surface high-affinity Fe receptors (Fe ε RIs) with IgEs and multivalent antigens. Two kinds of rodent mast cells, RBL-2H3 cells and mouse BMMCcs, were used. Two antigen-specific IgEs (DNP-specific IgE, OVA-specific IgE) was used. It was found that free 

**Keywords:** calcium signal, RBL-2H3 cells, BMMC


The effects of two Ca²⁺-ATPase inhibitors, thapsigargin (TG) and cyclopiazonic acid (CPA), and three hydroquinone-antioxidants, DTBHQ, DTAHQ, MTBHQ on the production of various cytokines and degranulation and LTC4 release from RBL-2H3 cells and BMMC (bone marrow-derived mast cells) were investigated. TG, CPA, DTBHQ and DTAHQ, all of which induce intracellular free Ca²⁺ concentration ([Ca²⁺]i) increase, induced degranulation and TNF-α release in the presence of TPA in a dose-dependent manner. In contrast, MTBHQ, which does not induce increase in [Ca²⁺], did not induce the release of histamine and TNF-α. LTC4 production and IL-4 and MCP-1 release were increased by CPA and DTBHQ in a dose-dependent manner without TPA. There were two types of mediator release process in RBL-2H3 cells; 1.([Ca²⁺]i) increase itself is sufficient, 2.([Ca²⁺]i) increase and PKC work synergistically.

**Keywords:** Ca²⁺-ATPase inhibitors, TNF-α, mast cells


Subchronic animal feeding studies to examine the effect of glyphosate-tolerant soybeans, which contain the bacterial 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, on the immune system were conducted with BN rats and B10A mice. The studies were designed to compare the feeding value of a line of genetically modified glyphosate-tolerant soybeans (GM soybeans) to that of closely-related and one-parent same cultivar (non-GM soybeans). Heat-treated soybean meal was incorporated into the diets of the rats and mice at a concentration of 30%. The study duration was 15 weeks. Growth, food intake and weights of the liver and the spleen were compared between animals fed the non-GM and GM lines. Growth, feeding value, and the histopathology of immune-related organs showed no significant differences between animals fed GM and non-GM lines. No immunotoxic activity was found in GM-soybean-fed rats or mice.

**Keywords:** GM soybeans; CP4-EPSPS; immune system


We found that U73122, an inhibitor of phospholipase C (PLC), suppressed both oposoned zymosan (OZ)-induced dephosphorylation and translocation of cofilin in macrophage-like U937 cells. OZ triggered an increase in inositol 1,4,5-trisphosphate (IP3), and U73122 inhibited it. U73343, which was employed as an inactive analogue, had no such inhibitory activities as did U73122. Furthermore, herbimycin A, an inhibitor of src-type tyrosine kinase, also inhibited OZ-triggered IP3 formation. These results suggest that the activity and localization of cofilin are regulated by PLC at the downstream of src-family tyrosine kinase.

**Keywords:** cofilin, U73122, phospholipase C

**共立薬科大学**


We investigated chemotaxis of neutrophil-like HL-60 cells induced by NO, as well as the influence of NO on cofilin, an actin-binding phosphoprotein. Two NO donors were shown to cause chemotaxis, and a NO-specific scavenger inhibited the chemotaxis. Inhibitors of soluble guanylate cyclase inhibited this NO action. We also found that NO caused translocation of cofilin to the cell periphery, though dephosphorylation of cofilin was not detected. These results demonstrated that NO has chemotactic activity for neutrophils and caused the translocation of cofilin to
the plasma, membrane regions without its phosphorylation.

Keywords: nitric oxide, chemotaxis, coflin

*Tohoku University*


The lipid A from *Flavobacterium meningosepticum* exhibited generally moderate activity compared to *Salmonella abortus equi* LPS used as a control in all the assay systems tested. The moderate activity of the lipid A may be explained by the unique fatty acid composition and the lack of a phosphate group in position 4'. Noticeably, the lipid A apparently induced TNF-a release from peritoneal macrophages in LPS-unresponsive C3H/HeJ, and the activation was suppressed by the LPS-specific antagonist. Taken together with the previous results concerning *Porphyromonas gingivalis* lipid A, which has high structural similarities to that of *F. meningosepticum*, and the induction of TNF-a release in macrophages from C3H/HeJ mice, the lipid A having novel fatty acids may possibly play an role for the activation of C3H/HeJ macrophages.

Key words: lipid A, C3H/HeJ mice, Flavobacterium meningosepticum

Ken-ichi Tanamoto, Takatoshi Iida, Yuji Haishima and Satoko Azumi: Endotoxic property of lipid A from *Comamonas testosteroni*. Microbiology, 147, 1087-1094 (2001)

The lipid A from *Comamonas testosteroni* was characterized by its relatively short-chain length (C10) of the 3-hydroxy fatty acid components directly bound to the glucoseamin disaccharide backbone by either amide or ester linkage. The lipid A exhibited endotoxic activity in all of the assay systems tested to the same extent with those of *Salmonella* lipid A or *E. coli* lipopolysaccharide used as a control. The strong endotoxic activity of the lipid A indicates that the composition of 3-hydroxydecanoic acid is not responsible for the low endotoxicity of lipid A. Furthermore both the defect of second acylation of 3-hydroxy fatty acid attached to the position 3', and the substitution of a hydroxyl group to the 3'-hydroxy fatty acid attached at position 2 do not affect the manifestation of endotoxic activity or species specificity.

Key words: LPS, TNF-α, NO


Rescue of contractile parameters and myocyte hypertrophy in calasequstrin overexpressing myocardium by phospholamban ablation


To test the hypothesis that inhibition of phospholamban activity may rescue myocardial abnormalities due to defects in sarcoplasmic reticulum, calasequstrin overexpressing mice were crossed with phospholamban-knockout mice. Phospholamban ablation in calasequstrin overexpressing mice led to reversal of the depressed cardiac contractile parameters, restoration in the ability of ICa to trigger SR Ca2+ release, and normalization of ventricular myocyte size. These results indicate that attenuation of phospholamban function may prevent or overcome functional and remodeling defects in hypertrophied hearts.

Keywords: phospholamban, transgenic mice, cardiac hypertrophy

*University of Cincinnati, USA*

*Case Western Reserve University, USA*
We investigated the role of NF-κB in lipopolysaccharide (LPS)-
induced desensitization of TNF-α gene expression in P388D1
cells. Gel-shift assays revealed that nuclear localization of p65/
p50, c-rel/p50 and p65/c-rel, and p65 homodimers was reduced in
LPS-tolerant cells, whereas that of p50 homodimers was only
slightly decreased. Western analysis showed that the phosphory-
lation of Ser32 on IκB α and its degradation did not occur in
LPS-tolerant cells. These results suggest that desensitization of
TNF-α gene expression in LPS-tolerant cells is closely associ-
ated with down-regulation of transactivating NF-κB and may in-
volve a defect in the LPS-induced IκB α kinase pathway.

Keywords: NF-κB, signal transduction, Toll-like receptor 4

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Yukiko Hara-Kudo*, Michiko Miyahara, and Susumu Kumagai

*1: Loss of O157 O Antigenicity of Verotoxin-Producing Es cherichia coli O157:H7 Surviving under Starving Conditions

Verotoxin (VT)-producing Escherichia coli O157:H7 was cul-
turable on agar media after being left in water for 21 months.
However, there were a number of colonies which had lost O157
O antigenicity. These colonies produced VTs, which are patho-
genic to humans. These observations suggest that the immuno-
logic methods based on O157 O antigenicity are unable to detect
and isolate VT-producing E. coli in foods and other environments
if the organism has been under starvation conditions for a long
period.

Keywords: Escherichia coli O157:H7; O-antigenicity; verotoxin

*1: National Institute of Health

Michiko Miyahara, Makoto Miyahara: Effects of Gamma-
Identification of E-beam Irradiation on Survival of Anaerobic and Aerobic Bacte-
ria

On line; 12th International Meeting on Radiation Processing
Extended Synopsis

Extended approvals of irradiated foods are requested, due to
increase of food poisoning cases in the world. There are some
discussions on effects on survival of bacteria by both irradiations
(gamma rays and electron beam). Some anaerobic and aerobic bacteria irradiated will be tested on survival effects on agar in
packaged atmosphere. We may propose preferable irradiation for
reduction of some bacteria population.

Keywords: gamma irradiation; electron beam irradiation; bacte-
ria

Yukiko Hara-Kudo*, Susumu Kumagai*, Takashi Masuda*,
Koukichi Goto*, Kayoko Ohitsuka*, Hiroyuki Masaki*,
Hiroyuki Tanaka*, Kenji Tanno*, Michiko Miyahara, and
Hirotaka Konuma: Detection of Salmonella enteritidis in shell
and liquid eggs using enrichment and plating

Int. J Food Microbiol., 64, (2001), 395-399

Detection methods using various enrichment and plating me-
dia and immunoconcentration for Salmonella enteritidis in shell
and liquid eggs were evaluated. For liquid egg samples naturally
contaminated with S. enteritidis, pre-enrichment in 225 ml of buff-
ered peptone water with cysteine followed by selective enrich-
ment in 10 ml of tetraionate broth was the superior, resulting in
the detection of S. enteritidis in all samples on six of the seven
types of selective agar substrate investigated. This enrichment
procedure also enabled detection of S. enteritidis in most of arti-
ficially inoculated shell egg and pasteurized liquid egg samples.

Keywords: Salmonella enteritidis; shell Egg; Detection

*1: National Institute of Health

工藤由起子*, 小西良子*, 春日文子*, 伊藤嘉典*, 岩部
正昭*, 齋藤典子*, 小沼博隆, 熊谷 進*: 腸管出血性
大腸菌O157:7によって実験的に汚染した野菜種子に関す
る研究

日食微誌, 17, 201-205(2000)

野菜種子が食中毒菌に汚染され、生産した野菜が食中毒
を引き起こすことが知られている。腸管出血性大腸菌O
157:7（E. coli O157:H7）による実験的汚染種子の冷蔵保存
中の菌の消長を調べた結果、カワレ大根種子 10 g 当たり
約10^2 cfu の污染濃度の場合は2週間に以上生存し、約10^2 cfu
の場合を20週目以上生存した。小松菜種子において本菌は
カワレ大根よりも速く死滅した。また、E. coli O157:H7で
実験的に汚染させたカワレ大根種子を栽培し、得られた可
食部におけるE. coli O157:H7の分布を電子顕微鏡下で観察
し、気孔周辺に多くの本菌が存在することが判明した。

Keywords: Escherichia coli O157:H7, radish sprout, seed,

*1: National Institute of Health

Nakagawa, H*1, Hara-Kudo, Y*1, Kojima,T, Ikeda, M*1,
Kodaka, H*1, Konuma, and Kumagai, S*1: Detection of freeze-
injured Escherichia coli O157:H7 from foods by resuscitation
prior to selective enrichment


We tried to detect Escherichia coli O157:H7 in food samples
artificially contaminated with freeze-injured Escherichia coli
O157:H7 using an enrichment method with modified EC broth
supplemented with novobiocin. When the samples were cul-
tured for enrichment immediately after inoculation of freeze-injured
cells, E. coli O157 was not detected in 13 out of 18 samples.
Ground beef or radish sprouts inoculated with 6 colony forming
units of E. coli O26 were homogenized in 225 ml of various broths.
After static incubation at 37°C or 42°C for 6h or 18h, we isolated
the inoculated bacterium by plating onto Rainbow Agar O157 with
novobiocin. In combination with the immunomagnetic separation
method, E. coli O26 was isolated from all samples by using enrich-
ment in tryptone soy broth at 37°C for 6h and in modified E. coli
broth with novobiocin(mEC+n) at 42°C for 18h ground beef and
radish sprouts, respectively. Enrichment E. coli O157 from both
ground beef and radish sprouts.

Keywords: Escherichia coli O26, Escherichia coli O157:H7,
ground beef

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後藤公吉†*, 佐野拓*, 瀬野浩文*, 奉口真一*, 佐田
高志*, 城の定三*, 小沼博隆, 常口邦邦**: 食肉のサーモ
モラ専門インタ


わが国の対輸入出食肉等事情により, サルモネラのモラ専門インタを行なうことが義務づけられ
た。このサルモネラの検査法は, 米国農務省の食品安全検
査局(FSIS)より食衛検査97%以上, 薬局検査56%以上の方法
ことが規定されている。今回, サルモネラの検査と
して, わが国で通常行われている食品衛生検査指針に示されて
ている食衛法とFSISの方法(FSIS法)について検討し
た。その結果, コロニー(28)生産サルモネラの検
査食衛法はFSIS法と同等の成績であったが, H2S非生産
サーモラネラでは食衛法はFSIS法に比べ明らかに低い検出率
であった。

Keywords: HACCP, H2S, Salmonella

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*鹿児島県衛生検査所
*静岡県環境衛生科学研究所
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*岩手大学農学部

Sakai, A., Teshima, R.: 2,5-Di-tert-butyl-1,4-dihydroquinone en-

hances cell transformation accompanied by an increase in in-

tracellular free calcium ion concentration


Two hydroquinones with similar structures, 2,5-dit-tert-butyl-

1,4-dihydroquinone (DTBHQ) and 2-tert-butyl-1,4-dihydroquinone

(MTBBQ), are used as antioxidants in the environment. DTBHQ

and MTBBQ were examined for their ability to induce cell trans-

formation using BALB/3T3 cells. DTBHQ at concentrations of

2.5-15 μM enhanced cell transformation initiated by a subthresh-

old dose of 3-methylcholanthrene (MCA) in a two-stage cell trans-

formation agent. Because DTBHQ is known to act as a calcium

ion mobilizing agent in other cells, we examined the effects of

DTBHQ on intracellular free calcium ion concentration (Ca2+) in

BALB/3T3 cells. DTBHQ elevated [Ca2+], with a dose depend-

ency similar to that of its enhancing effect on the MCA-initiated cell

transformation. DTBHQ neither enhanced cell transfor-

mation nor induced increase of [Ca2+]. Aberrant calcium signaling

produced by DTBHQ might contribute to the enhancement of

MCA-initiated transformation in BALB/3T3 cells.

Keywords: 2,5-di-tert-butyl-1,4-dihydroquinone, cell transforma-

tion, calcium ion

Sakai, A.: p-Nonylphenol acts as a promoter in the BALB/3T3
cell transformation

Mutation Res., 493, 161-166 (2001)

p-Nonylphenol (NP) has attracted attention as an estrogenic con-
taminant, and the environmental pollution by NP has been found to
be extensive. NP is classified as a phenoic antioxidant. Some
phenolic antioxidants are known to induce and/or enhance car-
cinogenesis. We examined the effects of NP on the two-stage trans-
formation of BALB/3T3 cells, a model of two-stage carcinogenesis.
The treatment by NP in the promotion phase markedly en-
hanced the transformation of the cells pretreated with a subthresh-
old dose of a carcinogen, 3-methylcholanthrene (MCA), but not
that of non-pretreated cells. The promoting activity of NP was
approximately one hundredth of that of 12-O-tetradecanoyl-
phorbol-13-acetate (TPA). The treatment by NP in the initiation
phase did not induce cell transformation with and without post-
treatment by TPA. These results indicate that NP acts as a pure
promoter of cell transformation. The enhancement by NP of MCA-
initiated transformation was suggested not to be mediated by es-
trogen receptors in BALB/3T3 cells because 17 β-estradiol did
not promote cell transformation in our experiments, and it has
been reported that BALB/3T3 cells do not express estrogen re-
ceptors at a detectable level.

Keywords: Nonylphenol; cell transformation; tumor promotion

村松芳多子*, 太田利子*, 村原兼記*, 朴隆吉*, 徐頜
*, 奉木明子, 成田紀子, 高島浩之: 真菌定量試験におけ
る適宜期間


真菌定量試験の基本的条件として, 培養期間の問題があ
る。国内にある真菌試験の規格をみてもかなり条件が異
なっている。そこで, 今後の基準設定をするための基礎実験として, 多
種真菌を用いて, 適宜期間と定量値をあわせて検討した。

Keywords: optimal growth period, quantitative test, CFU

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*3お茶の水女子大学
*4Yonsei University, Korea

相原兼記*, 田中昌明*, 高島浩之: 居住環境壁面にみる真
菌の垂直分布


居住環境にみる真菌の生態を研究しており, 微生物と真菌
の関係を検討した。真菌の分布をみると最も影響するであ
ら低碳酸の基盤から研究する必要がある。そこで, 同一居
住環境での壁面にみる真菌垂直分布を検討した。環境条件と
して, 温湿度を測定したところ, 温湿度は少ないが, 温度
は下方ほど高い傾向にあった。そこで真菌分布を見るとこ
る, 下方ほどCFUが有意に高かった。さらに真菌変化にも特
徴がみられた。

Keywords: fungal CFU and distribution, vertical point of dwell-

ing, micro meteorology

*お茶の水女子大学

小菅秀吉*, 後藤義隆*, 木村敏弘*, 安方了*, 高島浩之:
ウマ飼育環境における数料からの真菌包
Emericella nidulansの検出とその意義

真菌誌, 41: 251-256 (2000)

真菌性喉頭炎の起因真菌であるEmericella nidulansは
ウマ特有の感染症であり、いままで本菌の起因性、疫学を調査してきた。ここでは、衛生学的観点からE.nidulansの環境での生息性を数値で検討し、さらに分布する因子を研究した。さらに本菌の分布意義について考察した。

Keywords: Emericella nidulans, horse bedding, guttural pouch mycosis

*官崎大学

日本中央競馬会

高橋淳子*, 田谷正昭*, 田中重則*, 中瀬崇*, 山口英世*, 高島浩介：環境水と浄水にみる真菌とその化学的背景

株野研. 23: 18-23(2000)

水環境での真菌は、環境汚染のひとつとパラメーターとなりうる。同時に水質の程度を知るうえでも重要である。ところが、こうした水環境での真菌に関する規格基準は必要であるにもかかわらず整文化されていない。そこで環境水として河川、湖沼、浄水、工業用水にみる真菌と水質の分析をおこない、今後の規格基準の基礎資料とする。

Keywords: water, fungi, chemical analysis

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Toru Anzai*, K.Takatori,J.Kosuge*, M.Akai* and T. Higuchi

: Distribution of Emericella nidulans in the environment of Thorobred stables


In order to understand the distribution of Emericella nidulans, the main causative agent of guttural pouch mycosis, in the environment of stable, fungal isolation was carried out from samples collected from a racehorse training center and breeding farms. E. nidulans was mainly isolated from bedding straw at the training center stables and from straw at the breeding farms. The main habitat of E. nidulans in the stables included straw and hay for bedding or feeds and that these materials were often contaminated with fungi before use.

Keywords: Emericella nidulans, guttural pouch mycosis, horse environment

*Japan Racing Association

*Miyazaki University

*Hidaka Agriculture Mutual Aid Association

Aihara, Maki*, Tanaka T.* and K. Takatori: 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 highly isolated from them. A high frequency of contamination was seen to involve Cladosporium, of which C. sphaerospermum and C. cladosporioides were detected frequently at rates of 63.6 and 14.6%, respectively.

Keywords: Cladosporium, fungal contamination, dwelling environments

*Ochanomizu University


To investigate oleic acid biodegradation, 7 Aspergillus niger strains were tested with 3 different types of Czapek broth 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 Czapek broth with both 14C-labeled and non-labeled oleic acid, 2 strains of A. niger oxidized more than 58% of the supplied substrate within 72 hours. 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, Aspergillus niger, biodegradation

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Personalized medicine is an idealized medical practice aiming to give the right drugs to the right patients at the right times. It has been widely admitted that many projects for finding Single nucleotide polymorphisms (SNPs) are the basis for such practice. New informatics is also needed in order to utilize these data and knowledge effectively. Such an information environment, i.e., data and knowledge bases and computational tools, would be called the infrastructure for personalized medicine. The infrastructure would also be useful in pharmaceutical research for finding leads and analyzing detailed mechanisms of drug actions. As the only national institution for pharmaceutical research in Japan, we have started to implement some components of the infrastructure and put their prototypes on the Web. Further research initiatives are being discussed in the Chem-Bio Informatics Society as of its Grand Challenge Projects.

Keywords: Informatics, Genome, Infrastructure

山本 部：化学物質のデータベース構築と研究の交流

公衆衛生, 64 (6), 405-409 (2000)

1990年代半ばから、サン事件、重油流出事故、和歌山県温泉のカーテン球事件など化学物質が係わる重大事故、事件が相次ぎ、「健康危機管理」や「情報ネットワーク」の重要性がクローズアップされてきたことから、化学物質情報部で作成している化学物質情報のデータベース等の紹介と共に、分野・機関横断的な情報ネットワークおよび情報の共同利用を目指したシステムの構築について考察した。

山本 部：化学物質情報へのアクセス--その３：化学物質情報の情報収集一実践編


化学物質の安全性に関わる情報が収載されている各種データベースや資料等の情報を調査し、情報収集の際に情報の信頼性、検索の簡便性、コストなどの点から有用と思われる情報源、媒体等を考察した。
中野達也、高島一*、長崎賢二*：タンパク質の第一原理電子状態計算－生体化学反応の理路的解析を目指して－シミュレーション、19, 271-281 (2000)

Information of electronic state of biomolecules such as protein is indispensable to understanding of property of biomolecules and mechanism of biochemical reactions. Molecular orbital calculation is applied to simulate its electronic states. However molecular orbital calculation for large scale system as biosystem is very difficult to execute even on supercomputer because the amount of calculation is proportionally increased with the fourth power of system size. In order to realize cost low and post execution of ab initio MO, we are developing a special purpose computer for molecular orbital calculation: MOEngine to increase calculation power. A novel method: the fragment MO method was also developed to decrease the amount of computation in MO calculation. We report that the calculation of electronic state of biomolecule will be inexpensively and personally executable using MOEngine and the fragment MO method showing some examples.

Keywords: Fragment MO method, MOEngine, biomolecules


Recently we have proposed the fragment molecular orbital method for calculating large molecules such as proteins. The method, with some modifications for a practical convenience, was applied to the model peptides of (Gly)n and (Ala)n (n=5-20), [Met]enkephalin (YGGFM), and the synthetic designed peptide ALPHA-1 (Acetyl-ELLKLEELKG). The calculated total energies were well compared with those from the conventional ab initio MO method; the errors were within about 2 kcal/mol. It indicates that the fragment MO method is sufficiently accurate and useful to study electronic properties of large molecules.

Keywords: Fragment MO method, ab initio MO method, polypeptides

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*4 Osaka Prefecture University

T. Nakano, T. Kaminuma, M. Uebayasi*2, Y. Nakata*2: 3D Structure Based Atomic Charge Calculation for Molecular Mechanics and Molecular Dynamics Simulations

CBII, 1, 35-40 (2001)

We propose a new charge equilibration approach that depends upon molecular 3D structure. Nishimoto - Mataga equation is used to express the shielding effect. With the present approach, it is not necessary to iterate simultaneous equations for evaluating charge equilibration, although that is required in the EQ method. Atomic charge calculations were carried out for several organic molecules. Calculated charge distributions are in good agreement with experimental values.

Keywords: atomic charge, QEq, Nishimoto - Mataga equation

*5 National Institute of Bioscience and Human-Technology
*6 Gunma University

Sekizawa J., Suter, G.*1, Vermeire T.*2 and Munns W.*4: An Example of Integrated Approach for Health and Environmental Risk Assessment: Case of Organotin Compounds


Because environmental decision making based solely on simple compilation of toxicological data on either wild life or humans in isolation can not give effective answers about the nature and levels of risk, an integrated approach for risk assessment of adverse effects of chemicals is required. Integration of available information on health and environmental effects, from in vitro to the level of humans, across various species, across different endpoints, and combination with integrated exposure data, permits enhanced estimation of the potential risks posed by various agents. Mechanistic and quantitative consideration are the keys in this process. The value and utility of the integrated approach is shown using the example of organotin compounds.

Keywords: integration, organotin compounds, risk assessment

*1 US Environmental Protection Agency
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関澤 純：化学物質のリスクにおける不確実性の評価

日本リスク研究学会誌, 12 (2), 4-9 (2000)

リスクと不確実性とは本来不可分の関係にあると解

されていない。本論文は筆者が日本リスク研究学会の春季シンポジウム（2000年6月）で講演した内容を基に書かれた。リスク評価の枠組みから解きおき、リスク評価における不確実性の要因としてメカニズムの未解明による「真の不確実性」と、分布や変動のために一定の値をとれない「不確定で幅があることによる不確実性」の区別について解説した。より具体的にリスク評価における不確実性と問題点について、推測の必要性と情報をにおける不確実性の原因、感受性の多様性や環境条件における分布と変動、データ取得の困難による不確実性、パラメータの不確実性とサンプリングや測定法の問題点と誤差、シナリオやモデルにおける不確実性について論じた。さらに「浸透流体化物質のリスク評価における不確実性分析」についての筆者の研究を踏まえて、健康リスク評価における不確実性の検討の問題を、動物データから人への外挿における不確実性（データに基づく不確実性係数の選択）と、健康へのリスクと環境へのリスクの統合的評価の観点から論じた。

Keywords: uncertainty and its components, chemical risk, endocrine disruptor, International Programme on Chemical Safety, uncertainty factor

関澤 純：環境リスクの評価


「環境と測定技術」誌環境リスクの評価をシリーズとしておりあげるという企画の導入として、化学物質による人の健康と環境中生物へのリスクの評価において、筆者が国際協力により積み重ねてきた成果の一部を紹介しつつ、現在発展しつつある環境リスク評価の基礎的な枠組みとその背景、および今後の課題について概説した。1995年による評価とその仕組みを基本に、リスク評価の目的を明らかにした.
データの組織化と解析手法の体系的研究。健康リスク評価の手順およびリスク管理との関係を論じ、リスク管理評価についてUS EPAの環境リスク評価のスケールと欧州連合で化学物質の健康と環境への影響の初期評価に用いられているEUSESの概念を紹介した。さらに、情報が不十分な中での評価と不確定性の解消、CICAD計画の紹介、今後の課題として確率論的と不确定性解析、統合的リスク評価、透明なプロセスと批判検討、枠組みと判定の基準という手法の確立、リスクの比較による優先順位付けと社会の判断、その基礎としてリスク評価とリスク対応のリンクおよび、そのサイクリックな関係による両者の改善について提案した。

キーワード: データの組織化と解析手法の体系的研究、統合的リスク評価、不確実性の解析

関澤 純：環境管理におけるリスク・コミュニケーションにおける実例

環境問題におけるリスク・コミュニケーションの都市内外の事例から、環境ホルモン・ダイオキシン問題、O.17フタロの主要被害者の対応、消費者・政治・業界の関係が同席したワークショップでの対応、日本化学会における環境機構と連携委員会の考え方における国内における化学物質リスクコミュニケーションの現状分析および今後のあり方の検討、米国における「地域居住者の知る権利法」などを分析し、リスクコミュニケーションにおけるマネジメント、専門家、住民、NGOの役割について論じた。今後のあり方については、独自に行行ったアンケートの結果、ＩＰＳＲとCICAD（国際標準評価文書計画）に関与する物質安全に関する住民の役割を対象としたATSDRの健康影響評価、環境影響マネジメント、地域公衆衛生の指針の作成と情報の提供、米国大統領府・議会諮問委員会の「リスク管理の新たな枠組み」も参考にして、リスクコミュニケーションが21世紀社会における共存の必須の基盤であることを示した。

キーワード：科学への関与と信頼性、評価プロセス・判断基準と決定過程の明示、リスクの予測と未然防止

関澤 純：化学物質管理とリスクコミュニケーションのあり方

高圧ガス、37 (10), 12-17 (2000)

化学物質は危険なもの、やっかいなものというイメージが広くある。しかしそのイメージは、一方では当たりでおり、正しくリスクを予測して管理しないと、もとれないことになる。したがってリスクを予測して、よりリスクを少なくする手段を講じるより、正しい手法を模索するために、関係者がそれぞれの立場から理解し、意見を交換しあって適切に運営してゆくことが求められている。

すなわち、考えられるリスクを分析し、リスクの予防と管理のための方策を検討することが、まずなければならえない。この段階でも、できうる限り関係者がいっぱいして可能な問題点を探り、また新たな方策を検討する必要がある。それぞれの自由性を尊重し、その考慮した考え方が検討され、新たな一歩を踏み出して適切な方策を模索するために、関係者がそれぞれの立場から理解し、意見を交換しあって適切に運営してゆくことが求められている。

キーワード: 知的推進と価値判断、化学物質への不安、リスクコミュニケーションの発展段階

関澤 純：リスクコミュニケーションと情報公開

環境技術、29 (10), 52-57 (2000)

情報公開とリスクコミュニケーションとは違うこと、なぜリスク・コミュニケーションについて研究されるようになったのか「地域住民の知る権利法」制定の背景や、環境ホルモン・ダイオキシン問題と市民の不安の分析を進めると同時に、リスクと有害性、コミュニケーションと情報公開・説明責任・情報公開・情報提供・情報交換の違いについて解説した。さらに科学的なリスク評価、価値判断とリスク対応の意思決定の関係、公表のリスク受容性に影響を及ぼす要因と、違いの尊重、違いの理由や情報の相互理解の重要性、透明性と批判的検討の関係では仮定の立て方や予測の道筋の明示と、批判的検討を許すプロセスについて記した。危機管理とリスクコミュニケーションの違いに立って、日常的な対応や緊急時の即応、リスクコミュニケーションの枠組みの構築の重要性、社会の各グループの役割について論じた。

キーワード：リスク・コミュニケーションの専門能力、立場の違いの尊重と自主的な判断、相互間の意見交換

関田清司、桜井隆志、斎藤 実、小川幸男、上野克典、金子豊男、松本裕子、川崎 靖、黒川雄二、田上 達: F344ラットによるフクロウリ抽出物90日間反復投与と毒性試験

食品衛生学雑誌、42, 96-101 (2001)

食品添加物、フクロウリ抽出物（FE）の0.0, 0.5, 1, 5及び5.0％添加飼料を雄ラットの80日間摂取させる毒性試験を行った。結果、雄の1.5％群以上で摂取量の増加が、また、雄5.0％群で血清脂質（総コレステロール、中性脂肪）の減少傾向が認められた。しかし、いずれの変化も毒性変化とは考えられなかった。以上のことから、F344ラット飼料摂取、平均FE摂取量をmg/kg/day、雄で3,362mg、雌で3,594mgは毒性変化が認められない用量であると結論した。

キーワード: fukuronori (gloiopeptis furacata) extract, 90-day toxicity study, funoran


The Notch signaling pathway plays an important role in establishing metameric pattern during somiteogenesis. In mice, the lack of either of Mesp2 or Presenilin-1 (PS1) results in contrasting phenotypes, caudalized vs. rostralized vertebrae. We adopted a genetic approach to analyze the molecular mechanism underlying the establishment of the rostro-caudal polarity in somites. By focusing on the fact that expression of a Notch ligand, DI11 is critically important for prefiguring somite identity, we found that Mesp2 plays an important role to initiate establishment of rostro-caudal polarity by controlling two Notch signaling pathways. Mesp2- and PS1-dependent activation of Notch signaling pathways might differentially regulate DI11 expression resulting in the establishment of the rostro-caudal polarity of somites.

Keywords: Somitogenesis, Notch signaling, Molecular genetics

*Chiba University School of Medicine

The transcription factors, MesP1 and MesP2, sharing an almost identical bHLH motif, have an overlapping expression pattern during gastrulation and somitogenesis. To understand the cooperative functions of MesP1 and MesP2, either a deletion or sequential gene targeting strategy was employed to inactivate both genes. The double-knockout (dKO) embryos died around 9.5 days postcoitum (dpc) without developing any posterior structures such as heart, somites or gut. The major defect in this double-knockout embryo was the apparent lack of any mesodermal layer between the endoderm and ectoderm. In the chimeric embryos, dKO cells were scarcely observed in the anterior-cephalic and heart mesoderm, however, they did contribute to the formation of the somites, notochord and gut. These results strongly indicate that the defect in the cranial-cardiac mesoderm is cell-autonomous, whereas the defect in the paraxial mesoderm is an non-cell-autonomous secondary consequence.

Key words: MesP1, MesP2, heart morphogenesis


We have characterized zebrafish mesp-a and mesp-b genes that are segmentally expressed in the somite primordia. Observation in fused somites (fss) embryos suggests that these genes are downstream targets of fss at the segmentation stage. Ectopic expression of Mesp-b in embryos causes a loss of the posterior identity within the somite primordium, leading to a segmentation defect. These observations suggest that mesp genes are involved in anteroposterior specification within the presumptive somites.

Key words: Somitogenesis, Notch signalling, Zebrafish

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Objective: The aim of this study was to clarify previously reported controversial data and hypotheses concerning the effect of benzene on the cell cycle of hemopoietic stem cells. Materials & Methods: In this study, the bromodeoxy-uridine UV (BUVU) suicide assay was performed in normal C57BL/6 and p53 knockout (KO) C57BL/6 mice during and after expo-sureto 300 ppm of benzene for 2 weeks. Conclusions: Our present study revealed the mechanism of action of benzene hematotoxicity. Benzene suppresses the cell cycle by p53-mediated over-expression of p21, a cyclin-dependent kinase inhibitor, resulting not simply in suppression of hemopoiesis but rather in a dynamic change of hemopoiesis during and after benzene exposure. Thus, the controversies raised by previously reported data are resolved by our present findings of hemopoietic stem cell kinetics.

Keywords: Cell cycle, CFU-GM, Benzene

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We report a case of a 50-year-old male with ulcerative colitis who developed well-differentiated adenocarcinoma in the ileal J-pouch, which had been functioning for 18 years. The extension of the carcinoma in the pouch suggested that it had recently appeared in the pouch. Monitoring by endoscopic examination and biopsy or pouch excision seems to be an appropriate action if a pouch is out of the fecal stream.

Keywords: Ulcerative colitis, Ileal J-pouch, Adenocarcinoma

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OBJECTIVE: To assess the production of prostaglandin E(2), an important chemical mediator in diarrhea induced by laxative administration, a prostaglandin E-main urinary metabolite (1alpa-hydroxy-5,11-diketotetranor-prosta-1,16-diolic acid, PGE-MUM) was measured in healthy volunteers and compared with the values of patients with ulcerative colitis. METHODS: PGE-MUM was determined by a simplified immunoadass of bicyclic PGE-MUM. CONCLUSION: Laxative administration induces production of prostaglandin E(2) as one of the chemical media tors, although its production grade is relatively low as compared with ulcerative colitis in the active phase.

Keywords: Laxatives, ProstaglandinE, Ulcerative colitis

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根本哲夫*, 森山美樹*, 坂田健子*, 山本夏子*, 江石信信 *, 菅野 純: 家族性大腸腺腫に合併した甲状腺頭腺腫の細胞像
日本臨床細胞学会雑誌, 39, (5) (2000)

背景: 家族性大腸腺腫（Familial adenomatous polyposis 以下FAP）はAPO遺伝子のgermaine mutationに起因する遺伝性疾患であり、若年女性の甲状腺頭腺腫をしばし
は合併することが知られている。近年、甲状腺癌癌細胞に細胞の筋状配列や有形細胞増殖などの組織形態的特徴を有するcitriform-murular variant (CMV)が報告され、FAPに伴う甲状腺癌癌細胞は、多くがその遺伝子11にかかわるものと考えられる。われわれは家族性大腸腺癌に合併した2例を経験し、穿刺細胞診を検討した。症例1: CMV 2a、症例2: CMV 2b。家族性大腸癌11の遺伝子異常の有無を検査しているが、多くの遺伝子異常が存在する例を経験している。}

**Keywords:** Thyroid papillary carcinoma, Familial adenomatous polyposis, Aspiration cytology

*東京医科歯科大学

横河哲郎*, 岩間毅*, 安成和夫*, 金田篤*, 松崎 淳*, 菅野 杏: 長期の観察中に回腸癌が発生した家族性を示す若年性ポリポーシスの1例

胃と腸, 35, (3) (2000)

要旨 患者は49歳、女性、长期間にわたって追跡を行っていた、11の11家族の発症者は、腹部、嘔吐を主訴とし、腸重篤の診断を医師が施行した。回腸癌の発症を疑うと、家族にこれらの例を示したが、異常は認めなかった。11の遺伝子異常がSMAD4の異常であることが多く、再発を防ぐためには神経異常の有無を検査することが重要であると考えられた。

**Keywords:** 若年性ポリポーシス、回腸癌、遺伝子診断

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保冷保存を含む腸管状態の保存方法についてカリバックの影響をもって検討したところ、MEM保存法及びKrebsリンガルで保存した保存する保存法はリン酸緩衝液中で保存した保存するシール維持が大きく違った。同様の結果はノルアドレナリンによる弛緩反応においても認められた。

**Keywords:** preservation, smooth muscle, contractility

*国立医科大学

*日本製薬工業協会


Lamb等の雄性生殖器薬物を検査するために必要ならば

遊離での反復投与毒性試験の期間と観察法について明らかにするために、28日の方法のもと、雄性生殖器薬物へ影響するものとは知られている。この薬物投与に伴う battalionについて、その毒性発現を調査した。2週間および4週間の反復投与毒性試験で比較した。その結果、適切な大きさの発現を行い、精子のステージを目と詳細な調査学的観察を行うことにより、2週間で検出できることが明らかになった。

**Keywords:** validation, male fertility test, repeated dose toxicity study

*山之内製薬

*昭和大学

*東京農工大学

*化合物安全性研究所

*ラビトン

Takahiko, B. *, Touchi, A. *, Yamaguchi, Y. *, Ito, K. *, Yamazoe, Y. *, Sugiyama, Y. * and Ohno, Y.: Can in vitro metabolism and enzyme inhibition be explained by unbound drug concentration?

*Drug metabolism Reviews, 32 (suppl.), p. 222 (2000)

薬物濃度論的解析においては、適切な反復投与毒性試験の観察の薬物を受けた、阻害性を有する事前提にしている。すなわち、反復投与試験の適用の有無にかかわらず、適切な毒性試験を基礎として求めたKm値やKi値は変わらないはずである。しかしながら、薬物によってはアプロピルや肝薬溶性分画の反復投与試験の添加によりそれらの値が変化することを示した。

**Keywords:** pharmacokinetics, protein binding, free form

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The TNF-a release was maximally elicited by 1 mM ATP and also induced by a P2x and selective agonist, 2-3'-O-(4-benzoylbenzoyl)-adenosine 5'-triphosphate (BzATP), suggesting the involvement of P2X ATP-induced TNF-α release was Ca2+-dependent and a sustained Ca2+ influx in response to ATP was correlated with the extent of TNF-α release. TNF-α release induced by ATP was inhibited by PD98059, an inhibitor for MEK1 which activates extracellular signal-regulated protein kinase (ERK), and SB203580, an inhibitor for p38 MAP kinase. However, both ERK and p38 were rapidly activated by ATP even in the absence of extracellular Ca2+. These results indicate that extracellular ATP triggers TNF-α release in rat microglia via P2 receptor, likely P2X3, by a mechanism, which is dependent on both the sustained Ca2+ influx and ERK/p38 cascade regulated independently from Ca2+ influx.

**Keywords:** ATP, microglia, TNF-α

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Extracellular ATP and ADP induced membrane ruffling and markedly enhanced chemokinesis in Boyden chamber assay. Further analyses using the Dunn chemotaxis chamber assay, which allows direct observation of cell movement, revealed that both ATP and ADP induced chemotaxis of microrna. The elimination of extracellular calcium or treatment with pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid, suramin, or adenosine-3'-phosphate-5'-phosphosulfate did not inhibit ATP- or ADP-induced membrane ruffling, whereas AR-C69931MX or pertussis toxin treatments clearly did so. As an intracellular signaling molecule underlying these phenomena, the small G-protein Rac was activated by ATP and ADP stimulation, and its activation was also inhibited by pretreatment with pertussis toxin. These results strongly suggest that membrane ruffling and chemotaxis of microrna induced by ATP or ADP are mediated by G (i/o)-coupled P2Y receptors.

Keywords: ATP, microrna, chemotaxis


Here, we found a novel nociceptive response induced by ATP, mechanical allodynia. Injection of α, β-methylene ATP (β meATP), an agonist to P2X receptor, into plantar surface in rats produced the mechanical allodynia along with previously described nocicepive behavior and thermal hyperalgesia. This allodynic response was blocked by pretreatment with the P2 receptor antagonist pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate. Interestingly, only the mechanical allodynia evoked by α β meATP selectively remained in neonatal capsaicin-treated adult rats that had selectively lost the capsaicin-sensitive neurons. Taken together with our previous finding that the α β meATP-activated slow desensitizing current in DRG neurons is mediated by heteromic P2X3 (P2X; and P2X3) receptors, it is hypothesized that activation of heteromic P2X3 receptors in peripheral terminals of capsaicin-insensitive primary afferent fibers leads to the induction of mechanical allodynia.

Keywords: P2X receptors, Mechanical allodynia, Capsaicin sensitivity


Electrical signals in rat hippocampal slices were optically monitored using a voltage-sensitive dye to determine whether extracellular ATP exhibits direct effects through its own receptors or indirect effects after its hydrolysis to adenosine. The dentate gyrus was stimulated and electrical signals in the CA1 and the CA3 region were analyzed. ATP, ADP and AMP inhibited the excitation in the CA1 region. The inhibition by ATP was antagonized by adenosine receptor antagonists and α, β-methylene ADP, an inhibitor of 5'-nucleotidases. The results suggest that extracellular ATP inhibits neuronal electrical signals in hippocampal slices after its metabolism to adenosine.

Keywords: ATP, Hippocampus, Optical recording


A simple colorimetric assay for phenotyping the major human thermostable phenol sulfotransferase (SULT1A1) using platelet cytosol


A thermostable phenol sulfotransferase, SULT1A1, has been implicated in numerous detoxification and bioactivation pathways. A simple endpoint colorimetric assay is described that can be used for rapid phenotyping of SULT1A1 activity in human populations. The reaction concomitantly results in generation of p-nitrophenol that can be quantified colorimetrically at 405 nm (ε = 18,200 M(-1)) to give an indirect measure of sulfotransferase activity.

Keywords: phenol sulfotransferase assay, human, genetic polymorphism


The in vitro metabolic activation of o-phenylenediamine has been evaluated as yielding a toxic metabolite, 2,5-dihydroxybiphenyl (phenylhydroquinone), by p-hydroxylation in liver microsomes of rat and human. The involvement of rat CYP2C11, CYP2E1 and human CYP1A2 in the p-hydroxylation of o-phenylenediamine is suggested.

Keywords: o-phenylenediamine, phenylhydroquinone, cytochrome P450

Nowell, S.*', Ambrosone, C. B.*, Ozawa, S., MacLeod, S.L.*', Mrackova, G.*', Williams, S.*', Plasco, J.*', Kadlubar, F.F.*', Lang, N.P.*': Relationship of phenol sulfo transferase activity (SULT1A1) genotype to sulfo transferase phenotype in platelet cytosol


To date, one genetic polymorphism (Arg213His) has been identi-
E2F3 product suggests a mechanism for determining specificity of repression by Rb proteins


The tumor suppressor function of Rb is intimately related to its ability to interact with E2F and repress the transcription of E2F target genes. Here we describe a novel E2F product that specifically interacts with Rb in quiescent cells. This novel E2F, which we term E2F3b, is encoded by a unique mRNA transcribed from an intronic promoter within the E2F3 locus. The E2F3b RNA differs from the previously characterized E2F3 RNA, which we now term E2F3a, by the utilization of a unique coding exon. In contrast to the E2F3a product that is tightly regulated by cell growth, the E2F3b product is expressed equivalently in quiescent and proliferating cells. But, unlike the E2F4 and E2F5 proteins, which are also expressed in quiescent cells and form complexes with the p130 protein, the E2F3b protein associates with Rb and represents the predominant E2F-Rb complex in quiescent cells. Thus, the previously described specificity of Rb function as a transcriptional repressor in quiescent cells coincides with the association of Rb with this novel E2F product.

Keywords: cell cycle, E2F, Rb

Onoda, F.*, Seki, M.*, Miyajima, A., Enomoto, T.*: Elevation of sister chromatid exchange in Saccharomyces cerevisiae sgs1 disruptants and the relevance of the disruptants as a system to evaluate mutations in Bloom's syndrome gene


The SGS1 of Saccharomyces cerevisiae is a homologue of the Bloom's syndrome and Werner's syndrome genes. The sgs1 disruptants show hyperrecombination, higher sensitivity to methyl methanesulfonate and hydroxyurea, and poor sporulation. In this study, we found that sister chromatid exchange was increased in sgs1 disruptants. We made mutated SGS1 genes coding a protein proved to lack DNA helicase activity (sgs1-hd), having equivalent missense mutations found in Bloom's syndrome patients (sgs1-BSI, sgs1-BS2). None of the mutated genes could suppress the higher sensitivity to methyl methanesulfonate and hydroxyurea and the increased frequency of interchromosomal recombination and sister chromatid exchange of sgs1 disruptants. On the other hand, all of the mutant genes were able to complement the poor sporulation phenotype of sgs1 disruptants, although the values were not as high as that of wild-type SGS1.

Keywords: SGS1, sister chromatid exchange, Bloom's syndrome

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The SGS1 gene of Saccharomyces cerevisiae is a homologue for the Bloom's syndrome and Werner's syndrome genes. The disruption of the SGS1 gene resulted in very poor sporulation, and the majority of the cells were arrested at the mononucleated stage. The recombination frequency measured by a return-to-growth
assay was reduced considerably in sgs1 disruptants. However, double-strand break formation, which is a key event in the initiation of meiotic DNA recombination, occurred. The spores produced by sgs1 disruptants showed relatively high viability. The sgs1 spo13 double disruptants sporulated poorly, like the sgs1 disruptants, but spore viability was reduced much more than with either sgs1 or spo13 single disruptants. The poor sporulation of sgs1 disruptants was complemented with a mutated SGS1 gene encoding a protein lacking DNA helicase activity; however, the mutated gene could suppress neither the sensitivity of sgs1 disruptants to methyl methanesulfonate and hydroxyurea nor the mitotic hyperrecombination phenotype of sgs1 disruptants.

Keywords: SGS1, helicase, meiotic function
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Miyajima, A., Seki, M.*, Onoda, F.*, Uji, A.*, Satoh, Y., Ohno, Y., Enomoto, T.*: Different domains of Sgs1 are required for mitotic and meiotic functions

The SGS1 of Saccharomyces cerevisiae is a homologue for human Bloom's syndrome, Werner's syndrome, and Rothmund-Thomson's syndrome causative genes. Disruptants of SGS1 show high sensitivity to methyl methanesulfonate (MMS) and hydroxyurea, and hyper recombination phenotypes including interchromosomal homologous recombination in mitotic growth. In addition, sgs1 disruptants show poor sporulation and a reduced level of meiotic recombination as assayed by return-to-growth. We examined domains of Sgs1 required for mitotic and meiotic functions of Sgs1 by transiently mutated SGS1 into sgs1 disruptants. The N-terminal 1-401 amino acid region was required for complementation of MMS sensitivity and suppression of hyper heteroallelic recombination of sgs1 disruptants in mitotic growth and for complementation of poor sporulation and of reduced meiotic recombination. Although DNA helicase activity of Sgs1 was not required for Sgs1 to complement the meiotic functions, a deletion of helicase motifs III-IV (842-1046 amino acid) abolished the complementing activity of Sgs1, indicating that a structurally intact helicase domain is necessary for Sgs1 to fulfill its meiotic functions.

Keywords: SGS1, DNA repair, DNA recombination
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Onoda, F.*, Seki, M.*, Miyajima, A., Enomoto, T.*: Involvement of SGS1 in DNA damage-induced heteroallelic recombination that requires RAD52 in Saccharomyces cerevisiae

The SGS1 gene of Saccharomyces cerevisiae is homologous to the genes that are mutated in Bloom's syndrome and Werner's syndrome in humans. Disruption of SGS1 results in high sensitivity to methyl methanesulfonate (MMS), poor sporulation, and a hyper-recombination phenotype including recombination between heteroalleles. In this study, we found that SGS1 forms part of the RAD52 epistasis group when cells are exposed to MMS. Exposure to DNA-damaging agents causes a striking, Rad52-depen-
dent, increase in heteroallelic recombination in wild-type cells, but not in sgs1 disruptants. However, in the absence of DNA damage, the frequency of heteroallelic recombination in sgs1 disruptants was several-fold higher than in wild-type cells, as described previously. These results imply a function for Sgs1: it acts to suppress spontaneous heteroallelic recombination, and to promote DNA damage-induced heteroallelic recombination.

Keywords: SGS1, RAD52, heteroallelic recombination
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The developmental toxicity of indium was examined in both rats and mice using comparable experimental protocols. In rats, indium caused fetal weight decrease and fetal gross malformations. In mice, however, indium did not cause fetal gross malformations although it caused fetal weight decrease and fetal death. It was concluded from these results that rats and mice were susceptible to the embryotoxicity of indium at similar developmental stages in the early organogenetic period, but mice were less susceptible to the teratogenicity of indium than rats in terms of gross malformation.

Keywords: indium trichloride, malformation, embryotoxicity
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In order to cast light on the significance of lipid peroxidation products for carcinogenesis, the lacI mutant frequency (MF), micronuclear induction and cell proliferation were analyzed in lacI transgenic mice treated with trans-4- hydroxy-2-nonenal (HNE), a typical example. Male mice were ip injected with HNE at doses of 0, 5 or 50 mg/kg bw and 48 h thereafter, peripheral blood was collected for analyzing micronucleus induction. After 14 days, the mice were sacrificed to allow tissue sampling for examination of lacI MF and cell proliferative activity. Sixty percent of the mice given 50 mg/kg HNE died within 5 days after the treatment, but no other mortalities were observed. Histopathologically, marked pulmonary hemorrhage was found in the 50 mg/kg HNE group mice that survived until day 14. Immunohistochemically, HNE-modified proteins were detected in their alveolar macrophages. The HNE treatment did not increase lacI MF in the liver, kidney and lung and no significant increase in micronuclear induction or cell proliferation in major organs was found in either treatment. Moreover, no tumors developed in the 5 mg/kg HNE-treated mice which survived until week 78. Our results thus indicate that HNE lacks in vivo genotoxicity in lacI transgenic mice even when lethal doses are applied.

Keywords: Trans-4-hydroxy-2- nonenal, lacI mutation, Micro-

The effects of long-term concurrent administration of powdered fish meal (FM) and sodium nitrite (SN) were examined in F344 rats. Rats in groups 1-3 and 7-9 were respectively fed diets supplemented with 64%, 32% and 8% (basal diet) FM, and simultaneously given 0.12% SN in their drinking water. Groups 4-6 and 10-12 were respectively given 64%, 32% and 8% FM and tap water. At the 104th week, all surviving animals were killed and examined histopathologically. Treatment with FM dose-dependently increased the incidences and multiplicities of atypical tubules, adenomas and renal cell carcinomas in SN-treated males. Females were less susceptible than males for renal tumor induction. In males given the 64% FM diet alone, the incidence and multiplicity of atypical tubules were also significantly increased as compared with the 8% FM alone case. Nephropathy was apparent in FM-treated groups in a clear dose-dependent manner, irrespective of the SN treatment, and was more prominent in males than in females. Dimethylnitrosamine was found in the stomach contents after 4-week treatment with 64% FM plus 0.12% SN, at a level twice that in the 8% FM plus 0.12% SN group. The results clearly indicate that concurrent administration of FM and SN induces renal epithelial tumors. Further studies are required to elucidate how nephropathy and nitrosamines produced in stomach contents may contribute to the observed renal tumor induction.

Keywords: Powdered fish meal, sodium nitrite, renal cancer


A time-course study of ultrastructural changes and immunoelectron microscopic localization of neurocalcin was performed on motor endplates of the lumbar muscles of female rats given a single administration of 2,5-di(tert-butyl)-1,4-hydroquinone (DTBHQ) at a dose of 120 mg/kg. At the ultrastructural level, neurotoxicity characterized by a decrease or loss of synaptic vesicles and mitochondria was observed after 24 h and at the 1 week time point. After 6 weeks, newly formed reinnervated endplates were observed. Immunoelectron microscopically, the synaptic vesicle membranes were heavily labeled for neurocalcin in the control rats, but not at 24 h after DTBHQ treatment. The results strongly suggest that DTBHQ targets the motor endplates in the rat lumbar muscles, causing depletion of neurocalcin in the synaptic vesicles followed by their loss.

Keywords: DTBHQ, rat, motor endplate


In order to establish a quantitative method for analysis of gene expressions in small areas of tissue after paraffin-embedding, preliminary experiments with RT-PCR were performed using methacarn-fixed rat tissues. A sufficient amount of total RNA for quantitative RT-PCR of many genes could be extracted from a deparaffinized liver section by a simple, single step extraction method. Low concentration of contaminating genomic DNA and resolution of ribosomal RNAs proved the purity and integrity of extracted RNA samples, allowing PCR-amplification of long mRNA sequence and mRNA species expressing low-copy numbers. Thus, methacarn-fixed paraffin-embedded tissue has benefits for analysis of RNAs in the cells of histologically defined areas.

Keywords: Methacarn, Paraffin-embedded tissue, Gene expression analysis


The carcinogenicity of 5-fluouracil (5-FU) was investigated in F344 rats of both sexes. 5-FU was administered to groups of 50 male and 50 female rats ad lib. for 104 weeks, added to drinking water at concentrations of 0, 62, 125 ppm. Body weight gains were slightly depressed in the 125 ppm group of both sexes. While not statistically significant in females, final survival rates at week 111 in the 125 ppm group of both sexes were higher than those in the control group, suggesting an ability of 5-FU to prolong the life span. Histopathologically, there was no significant induction of any neoplastic or non-neoplastic lesions, indicating a lack of carcinogenicity of 5-FU under the present experimental conditions using rats.

Keywords: 5-Fluorouracil, Carcinogenicity, Rat


The sensitivity of heterozygous p53-deficient CBA mice [p53 (+/−)] to N-ethyl-N-nitrosourea (ENU) was investigated. p53 (+/−) and wild-type littermates [p53(+/+)] were given an i.p. injection of ENU and were maintained for 26 weeks. The incidence of uterine tumors and lung adenomas in p53 (+/−) mice was significantly greater than that in p53 (+/+)/ mice. Malignant lymphomas were only induced in p53 (+/−)/ mice. Gene analysis revealed GGC→ GTG point mutations in codon 135 of exon 5 of the p53 allele in the uterine endometrial stromal sarcomas. Our results suggest that female p53(+−)/ CBA mice are very susceptible to uterine carcinogenesis, providing a useful model for ENU-induced uterine tumors.

Keywords: p53-deficient mouse, MNU, uterine tumor


Olitpraz is known to inhibit tumorigenesis induced by variety of carcinogens in several animal model systems. In the present experiment, the modifying effects of dietary olitpraz given during BOP initiation of carcinogenesis, were investigated in Syrian hamsters. Groups 1-3 were thrice given subcutaneous injections of BOP at 1 week intervals and fed diets supplemented with 400 or 200 ppm of olitpraz or basal diet alone, starting 1 week prior and finishing 1 week after the carcinogen exposure. The incidences and multiplicity of adenocarcinomas of the pancreas were higher in groups 1 and 2 than in group 3 although without statistical significance. The incidence of pancreatic duct dysplasias was significantly increased in group 2 but not in group 1 as compared with group 3. While the incidences of alveolar adenomas and carcinomas were significantly decreased by the high dose, the multiplicities of hepatocellular adenomas, cholangiocellular carcinomas and gall bladder adenomas were elevated in the BOP/olitpraz groups. The results of the present study suggest that olitpraz exerts organ-dependent modifying effects on BOP-induced carcinogenesis in hamsters when given in the initiation stage.

Keywords: olitpraz on N-nitrosobis (2-oxopropyl)amine, hamster


The carcinogenicity of gardenia blue colour was examined in Fischer 344 (F344) rats.

Groups of 50 males and 50 females were given the material at dietary doses of 0 (control), 2.5 or 5% for 104 weeks and then sacrificed. The doses were selected on the basis of results from a 13-week subchronic toxicity study. A slight increase in relative organ weights of the left lung was observed in male rats of the 5% group. However, no significant differences between the control and treated groups were noted with regard to clinical signs, mortality and haematological findings. A variety of tumours developed in all groups, including the controls, but all were histologically similar to those known to occur spontaneously in F344 rats, and no statistically significant increase in the incidence of any type of neoplastic lesion was found for either sex in the treated groups. Thus, it was concluded that, under the present experimental conditions, gardenia blue colour is not carcinogenic in F344 rats.

Keywords: Carcinogenicity, gardenia blue colour, F344 rat

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Chung, F-L.*, Nath, R.G.*, Ocando, J.*, Nishikawa, A. and Zhang, L.*: Deoxyguanosine adducts of 1,4-hydroxy-2-nonenal are endogenous DNA lesions in rodents and humans: detection and potential sources


1,4-Hydroxy-2-nonenal (HNE) is a free radical-mediated oxidation product of polyunsaturated fatty acids. As an electrophile, HNE readily binds to proteins and yields diastereomeric cyclic 1,3-propano adducts with deoxyguanosine (dG). Here, we report the detection and identification of the HNE-derived cyclic 1,4-propano-dG adducts as endogenous DNA lesions in tissues of untreated rats and humans using a highly sensitive 32P-postlabeling method in conjunction with high-performance liquid chromatography. These adducts were first verified by their comigration with the synthetic UV standards of HNE-dG adducts. Subsequently, their identities were unequivocally established by two independent reactions. An approximately 37-fold increase in the levels of HNE-dG adducts was observed in the liver DNA of F344 rats after treatment with CCl4, suggesting that tissue lipid peroxidation is a likely source of their formation. Our studies in vitro further indicate that α,ω polyunsaturated fatty acids are likely a unique class of fatty acids involved in HNE-dG adduct formation.

Keywords: 1,4-Hydroxy-2-nonenal, adduct, endogenous DNA lesion

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β-naphthoflavone (BNF) is a strong inducer of cytochrome P450 (CYP) 1A but not CYP 2B. To determine its liver tumor promotion potential, a F344 rat model initiated with diethylnitrosamine (DEN) and subjected to two-thirds partial hepatectomy was used. The incidence of hepatocellular adenomas and glutathione S-transferase placental form (GST-P)-positive altered hepatocellular foci were elevated in the DEN+BNF group as compared to the DEN alone group at week 28. In conclusion, BNF promoted hepatocarcinogenesis initiated by DEN. The data of proliferating cell nuclear antigen labeling indices supported this conclusion.

Keywords: β-naphthoflavone, hepatocarcinogenesis, rat

Shinoda, K., Mitsumori, K., Uneyama, C. and Uehara, M.: Induction and inhibition of testicular germ cell apoptosis by fluororacetate in rats


Fluororacetate (FA), an inhibitor of acanitase, has been suggested to be a possible determinant of the form of cell death, apoptosis or necrosis. To investigate FA-induced testicular toxicity, adult SD rats were given a single dose of FA and sequentially analyzed. Germ cell degeneration was first found in round spermatids and in spermatogonia. Degenerating spermatogonia exhibited characteristic features of apoptosis by both electron microscopy and in situ terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL), whereas spermatids did not. After that, the degenerating/TUNEL-labeled spermatogonia were drastically decreased compared to those from the control. It was concluded
that FA induces either apoptosis or necrosis of male germ cells in the early stage after dosing and subsequently inhibits spontaneous apoptosis.

Keywords: fluoroacetate, testicular toxicity, apoptosis


To investigate risk of 2,6-dimethylaniline (DMA), a metabolite of xylazine, which intake may increase by ingestion of edible tissues from its treated domestic animals, the following studies were performed. Male F344 rats fed a diet containing 3000 or 300 ppm of DMA for 4 weeks. Bowman’s gland atrophy and irregular arrangement of olfactory epithelial cells were observed at 3000 ppm. The plasma concentration of DMA was 0.20 to 0.36 μg/ml at the 3000 ppm, but under the detection limit at 300 ppm. So, it was suggested that the probability of nasal carcinogenic effects of DNA on consumers via ingestion of edible tissues from food-producing animals treated with xylazine is extremely low.

Keywords: 2,6-dimethylaniline, rat, plasma concentration

Carcinogenesis., 21, 707-713 (2000)
The effects of soybean and/or iodine-deficient diet feeding were investigated in female F344 rats. Rats were fed basal gluten (Group1), iodine-deficient gluten (Group 2), 20% defatted soybean (Group 3) or iodine-deficient defatted soybean (Group 4) diets. At week 10, relative thyroid weights were significantly higher in Groups 2 and 4 than in Group 1 and pituitary weights were significantly higher in Groups 3 and 4 than in Group 1. T4 was significantly lower in Groups 2 and 4 than in Group 1. Serum TSH was significantly higher in Groups 3 and 4 than in Group 1. Histologically, marked diffuse follicular hyperplasia of the thyroid was evident in Group 4 rats. Ultrastructurally, severe disorganization and disarrangement of mitochondria were apparent in thyroid follicular cells of Group 4. In the anterior pituitary, dilated rSER and increased secretory granules were remarkable in this group. Our results thus strongly suggest that dietary defatted soybean synergistically stimulates the growth of rat thyroid with iodine deficiency.

Keywords: soybean, iodine deficiency, thyroid

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The influence of 1-acetoxcyavicol acetate (ACA) during the initiation stage was investigated in the N-nitrosobis (2-oxopropyl) amine (BOP)-initiated hamster tumorigenesis model. Ninety male 5-week-old hamsters were divided into three groups, each consisting of 30 animals, and s.c. injected with 20 mg / kg of BOP twice with a one-week interval. Groups 1 through 3 were fed diet supplemented with ACA at concentrations of 500, 100 and 0 ppm, respectively, for 3 weeks starting one week before the first carcinogen application. At the termination of experimental week 54, the total incidence and multiplicity of cholangiocellular adenomas and carcinomas in group 1 were significantly decreased as compared to the group 3 values. Our results thus indicate that ACA exerts an inhibitory effect on BOP-induced cholangiocarcinogenesis in hamsters. Taken together with previous findings of inhibited colon, oral and skin carcinogenesis in rats and mice, they suggest that ACA is a candidate chemopreventive agent with a wide spectrum of activity.

Keywords: 1-acetoxycavicol acetate, N-nitrosobis (2-oxopropyl) amine, cholangiocarcinogenesis
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The modifying effects of concurrent administration of fish meal (FM) and sodium nitrite (SN) on the development of renal tumors after initiation with N-ethyl-N-hydroxyethylnitrosamine (EHEN) were investigated. A total of 120 male 6-week-old Wistar rats were divided into six groups. Groups 1-3 were given 1000 ppm EHEN in their drinking water for 3 weeks as an initiation treatment for renal cancer induction and thereafter fed respective diets containing 64, 32, and 8% FM, and simultaneously given 0.12% SN in the drinking water for 33 weeks. Groups 4-6 were similarly treated without the prior application of EHEN. At the end of the 37th experimental week, all surviving animals were autopsied and examined histopathologically for the existence of renal proliferative lesions. The incidences of dysplastic lesions, adenomas or adenocarcinomas of the kidney were not significantly different among groups 1-3. No renal proliferative lesions were found in groups 4-6. Chronic nephropathy was slightly but significantly enhanced in the 64 and 32% FM-treated groups as compared with group 3. Our results suggest that concurrent administration of FM and SN does not affect the post-initiation phase of EHEN-induced renal carcinogenesis in the rat.

Keywords: Fish meal, sodium nitrite, renal carcinogenesis

Genetic changes in urethane-induced lung tumors induced in
transgenic mice carrying a human prototype c-Ha-ras gene (rasH2 mice) were investigated. rasH2 mice and non-transgenic littermates (non-Tg) were i.p. injected urethane once or three times at 2-day intervals. The multiplicities of lung proliferative lesions including hyperplasias, adenomas and carcinomas in treated rasH2 mice were significantly higher than those in treated non-Tg mice. The variation of the lesions induced by different doses of urethane was not the cause of the variation of the mutation spectrum, and mutations of both transgene and mouse c-K-ras genes were not principal genetic events in urethane-induced lung proliferative lesions in rasH2 mice.

Keywords: urethane, lung tumor, rasH2 mouse


Phenolphthalein has carcinogenic activity, causing malignant lymphomas in B6C3F1 mice at a dietary dose of 3000 ppm in a 2-year carcinogenicity study and in heterozygous p53-deficient female mice at the same dose in a 6-month study. To examine whether phenolphthalein carcinogenic potential can be detected in male and female transgenic (Tg) mice carrying the human c-Ha-ras gene (rasH2) and their wild-type littermates (non-Tg), a diet containing 3000, 6000 or 12000 ppm was given for 6 months. Unequivocal induction of neoplastic lesions was not apparent, suggesting that rasH2 mice are resistant to the induction of malignant lymphomas by the treatment of phenolphthalein.

Keywords: phenolphthalein, carcinogenicity, rasH2 mouse


The modifying effects of dietary L-methionine in the post-initiation phase of pancreatic carcinogenesis were investigated in hamsters treated with N-nitrosobis(2-oxopropyl)amine (BOP). Groups consisting of 20 and 30 animals, respectively, were given BOP subcutaneously, once a week five times at a dose of 10 mg/kg body wt. and then continuously fed diet supplemented with 2% (group 1) or 0% (group 2) methionine (weeks 5-32). The incidence of pancreatic ductal adenocarcinomas was significantly lower in group 1 than in group 2. Multiplicity of adenocarcinomas was also significantly lowered. Similarly, total numbers of combined adenocarcinomas and dysplastic lesions were significantly decreased in group 1 as compared with group 2. Methionine enhanced atrophic change of pancreatic acinar cells in hamsters given BOP, indicating that the inhibitory effects on the post-initiation stage of BOP-induced pancreatic carcinogenesis in hamsters could be generally linked to suppression of growth.

Keywords: methionine, pancreatic carcinogenesis, hamster *Keimyung University


Proliferative lesions in rat thyroid were induced by N-bis (2-hydroxypropyl) nitrosamine (DHPN) initiation followed by thiourac (TU) treatment. Simultaneous administration with a high level of vitamin A (VA) enhanced their induction. Iodine uptake and iodination of tyrosine residue in thyroglobulin, of the thyroid, were decreased by DHPN/TU treatment compared to the DHPN alone. Data from the DHPN/TU+VA and DHPN/TU animals were comparable. Therefore, the possibility that modification of hormone synthesis contributes to the enhancing effect of simultaneous treatment with VA on thyroidal tumor induction by TU is considered to be very minimal.

Keywords: thyroid tumor, rat, vitamin A


Chemopreventive effects of arctii, a lignan isolated from Arctium lappa (burdock) seeds, on the initiation or post initiation period of PhIP induced mammary carcinogenesis in female rats and on MelQx-associated hepatocarcinogenesis in male rats were examined. In experiment 1, female 5D rats were given intragastric doses of 100 mg/kg body wt of PhIP once a week for 8 weeks as initiation. Groups of 20 rats each were treated with 0.2 or 0.02% arctii during or after PhIP initiation. Control rats were fed 0.2 or 0.02% arctii, or basal diet alone during the experimental period. Animals were killed at the end of week 48. Although the incidence of mammary carcinomas did not significantly differ among the PhIP-treated groups, multiplicity was significantly decreased in rats given 0.2 or 0.02% arctii after PhIP initiation as compared with the PhIP alone controls. The average number of colon aberrant crypt foci was also significantly decreased in these two groups. Pancreas acidophilic foci were induced in PhIP treated animals with slight decrease in the multiplicity with arctii during the initiation phase. For liver carcinogenesis, groups of 15 male F344 rats were given a single intraperitoneal injection of DEN and starting 2 weeks later, they were administered 0.03% MeQx in the diet, MelQx together with 0.5% arctii, 0.1% arctii or basal diet for 6 weeks. They were subjected to two-three partial hepatectomy 3 weeks after DEN initiation and killed at the end of week 8 for glutathione GST-P immunohistochemistry. The numbers and areas of preneoplastic GST-P positive foci were elevated by the treatment with MelQx, and further increased by the simultaneous treatment with arctii. These results indicate that arctii has a protective effect on PhIP-induced carcinogenesis particularly in the mammary gland in the promotion period. On the other hand, it may have a weak co-carcinogenic influence on MelQx-induced hepatocarcinogenesis. In addition, the results suggested
that PhIP is a weak pancreatic carcinogen in female SD rats, target- ing acinar cells.

Keywords: Chemoprevention, arctiin, PhIP

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Maronpot, R.R., Mitsumori, K., Mann, P., Takaoka, M., Yamamoto, S., Usui, T., Okamiya, H., Nishikawa, S., Nomura, T.: Interlaboratory comparison of the CB6F1-Tg rasH2 rapid carcinogenicity testing model
Toxicology., 146, 149-159 (2000)

We have undertaken an interlaboratory comparison of the performance of the CB6F1-Tg rasH2 transgenic mouse in cancer bio-assays concurrently conducted in the United States and Japan. Chemicals selected for study included known human carcinogens (cyclosporin A) and known rodent carcinogens (p-cresidine and vinyl carbamate) tested at carcinogenic doses, and non-carcino- gens (p-anisidine and resorcinol) tested at appropriate high doses. All studies showed similar results between the two laboratories conducting each study. Although only five chemicals were success- fully tested in this interlaboratory comparison, there was good concordance in outcome for the strong carcinogens and for the non-carcinogens.

Keywords: rasH2 mouse, carcinogenicity test, validation

Iwata, H., Hosoi, M., Miyajima, R., Mikami, S., Yamakawa, S., Enomoto, M., Imazawa, T. and Mitsumori, K.: Morphogenesis of craniohyrgeal derivatives in the neurohypophysis of Fisher 344 rats: abnormally developed epithelial tissues including parotid glands derived from the stomatodeum

Morphogenesis of craniohyrgeal derivatives of the neurohypophysis found in 14 Fischer 344 rats was studied. They were composed of aberrant epithelial structures consisting of serous acinar and tubular and fusiform cell structures. Cells forming these structures were positive for cytokeratin, and basal cells of the aci- nar or tubular structures and some of the fusiform cells were posi- tive for alpha-smooth muscle actin, and they were regarded to be myoepithelial cells. These findings indicate that the craniohyr- gopal derivatives are a developmental aberration derived from the stomatodeum, which is known to be the origin of both nasal and oral epithelial tissues, including the parotid glands.

Keywords: craniohyrgeal derivatives, rat, diagnosis


Nasal lesions in male F344 rats administered 2,6-dimethylaniline (DMA) after initiation with N-bis (2-hydroxypropyl) nitrosamine (DHPN) were examined. Severe atrophy of Bowman's glands and epithelial disarrangement were apparent from week 4, followed by dilatation/proliferation of Bowman's glands, degeneration of epithelial cells, and undifferentiated epithelial cell proliferation. Focal glandular hyperplasias, dysplastic foci, and adenomas were observed from week 26, and carcinomas at 52 week. Carcinoma cells had ultrastructural characteristics identical to those in normal Bowman's glands. It was concluded that Bowman's glands are the target of DMA, giving rise to nasal carcinomas after DHPN-initiation.

Keywords: nasal carcinogenesis, rat, DHPN


To elucidate the molecular mechanism of NGF-induced down-regulation of the EGFr, we have cloned da 2.7-kilobase promoter sequence of the rat EGFr from a rat P1 library. Six transcriptional start sites were identified by 5'-rapid amplification of cDNA ends and primer extension. Reporter gene assay was performed with 1.1 kilobases of the 5'-flanking sequence, and this sequence exhibited functional promoter activity in transient transfection experiments with PC12, C6, and CV-1 cells. Treatment of PC12 cells with NGF inhibited promoter activity, and TCC repeat sequence appeared to be at least partially responsible for the down-regulation. Supportive evidence for the relevance of this sequence was obtained from EMSA and by transfection of TCC mutation constructs.

Keywords: NGF, PC12, Transcriptional down-regulation of EGFr


Interrelationships among induction of cytochrome P-450 (CYP) 1A1/2, decrease in connexin 32 (Cx32), and liver tumor-promot- ing activity by beta- naphthoflavone (BNF) in the promotion stage were examined in a 2-stage liver carcinogenesis model. Male Fisher 344 rats initiated with diethy- l nitrosamine (DEN) were fed a diet containing 2%, 1%, or 0% BNF for 6 weeks and subjected to a two-thirds partial hepatectomy at week 3. At week 8, all rats were sacrificed and examined. BNF induced adenoma- like hepatic foci and glutathione S-transferase-P foci, unlike pheno- nobarbitol, does not induce CYP 2B1/2 isozymes, and there seems to be no direct relationship between CYP 1A1/2 induction and Cx32 reduction in BNF hepatocarcinogenesis.

Keywords: beta-naphthoflavone, rat, hepatocarcinogenesis


A carcinogenicity study, in which F344 rats were given potas- sium iodide (KI) in the drinking water at 10, 100 or 1000 ppm for
104 weeks, and a two-stage carcinogenicity study of application
at 1000 ppm for 83 weeks following an injection of N-bis (2-
hydroxypropyl) nitrosamine (DHPN), were conducted. In the
former, squamous cell carcinoma was induced in the salivary
glands at 1000 ppm, but no tumors in the thyroid. In the two-stage
carcinogenicity study, the incidence of thyroid tumors derived from
the follicular epithelium was increased in the DHPN+KI as com-
pared with the DHPN alone group. The results suggest that ex-
cess KI has a thyroid tumor-promoting effect, but KI per se does
not induce thyroid tumors in rats. In salivary gland, KI was sug-
gested to have carcinogenic potential via an epigenetic mecha-
nism, only active at a high dose.

Keywords: potassium iodide, salivary gland, thyroid tumor

Mori, I., Hayashi, S., Nonoyama, T., Yasuhara, K., Mitsumori,
and Masegi, T.: Point mutations of the c-H-ras gene in spont-
aneous pulmonary tumors of transgenic mice carrying the hu-
man c-H-ras gene


Spontaneous proliferative pulmonary lesions were found in 10
(6 males and 4 females) of 244 (122 of each sex) transgenic
(Tg) mice carrying the human prototype c-H-ras gene (rasH2).
The mutation patterns of the human c-H-ras codon 61 and endogenous
mouse c-K-ras codons 12, 13, and 61 in these lesions were ana-
yzed. Immunohisto-chemical detection for p53 protein, hsp70 or
mdm2 gene protein was also performed. Obtained findings sug-
gested that, at least, a point mutation of the human c-H-ras tran-
gene may be an important step in progression of spontaneous lung
tumors, whereas p53 abnormality may not play an important role
of the pulmonary carcinogenesis in rasH2 Tg mice.

Keywords: rasH2 mouse, lung tumor, ras mutation

Toyoda, K., Shibutani, M., Tamura, T., Kojitani, T., Uneyama,
C., Hirose, M.: Repeated dose (28 days) oral toxicity study of
flutamide in rats, based on the draft protocol for the 'Enhanced
OECD Test Guideline 407' for screening for endocrine-disrupting
chemicals


To establish a test protocol for the 'Enhanced OECD Test Guide-
line 407', we performed a preliminary 28-day, repeated-dose toxi-
city study of flutamide at doses of 0 (control), 0.25, 1 and 4 mg/
kg b.w./day in rats of both sexes. Male rats receiving 1 and 4 mg/
kg showed lobular atrophy of the mammary gland and a decrease
in epididymal weight. At 4 mg/kg, increased levels of serum tes-
tosterone and estradiol and decrease of the weight of the access-
ory sex glands. In females, a slight prolongation of the estrous
cycle was also observed at 4 mg/kg. Thus, among the parameters
tested in the present experimental system, the weight of endo-
crine-linked organs and their histopathological assessment, se-
rum hormone levels, and estrous cycle stage allowed the detec-
tion of endocrine-related effects of flutamide

Keywords: Enhanced OECD Test Guideline 407, Flutamide, En-
docrine disrupters

Takegawa, K., Mitsumori, K., Yasuhara, K., Moriyasu, M.,
Sakamori, M., Onodera, H., Hirose, M. and Nomura, T.: A
mechanistic study of ovarian carcinogenesis induced by nitro-
furazone using rasH2 mice


Tumorigenic mechanisms of nitrofurazone (NF) in mouse ova-
rries were evaluated in a short-term model using transgenic mice
 carrying the human c-Ha-ras gene (rasH2). Both rasH2 mice and
their wild littermates (non-Tg) fed a diet containing 500 to 1,000
ppm NF for 7 weeks demonstrated ovarian atrophy with decreased
labeling indices (LI) for PCNA in granulosa cells. Increased
atretic follicles and decreased LI in granulosa cells were recog-
nized in rasH2 mice at 250 or 500 ppm for 26 weeks, but no tu-
mors. Ovarian atrophy was observed with increased serum luteni-
zing hormone levels at 1,000 ppm for 11 days. In conclusion,
oviduct tumor induction by NF was associated with continuous
stimulation of gonadotropins via a negative-feedback phenom-
eon secondary to ovarian atrophy.

Keywords: nitrofurazone, rasH2 mouse, ovarian tumor

Furukawa, F., Nishikawa, A., Nakamura, H., Miyauchi, M., Son,
H.Y. and Hirose, M.: Effects of octreotide, a somatostatin ana-
ologue, on initiation of pancreatic carcinogenesis in hamsters
with N-nitrosobis (2-oxypropyl) amine


The modifying effects of octreotide acetate, a somatostatin
(SMS) analogue shown to inhibit secretion of digestive enzymes, bi-
carbonate and pancreatic juice, on the initiation phase of pan-
creatic carcinogenesis were investigated in hamsters simulta-
neously treated with BOP. Groups 1-3, each consisting of 20 ani-
mals, were given BOP subcutaneously once a week three times at
a dose of 10 mg/kg body weight during administration of octreotide
acetate for 28 days via osmotic pumps implanted subcutaneously
at doses of 6 μg/day (group 1), 3 μg/day (group 2) or 0 μg/day
(saline) (group 3). At the termination of experimental week 40, the
incidences and multiplicities of pancreatic ductal adenocarcin-
omas and dysplastic lesions did not significantly differ among
groups 1-3. Subcutaneous administration of octreotide acetate
resulted in obviously increased plasma octreotide levels. Our
results Thus suggest that this SMS analogue may not modulate
the initiation of BOP-induced pancreatic carcinogenesis, regard-
less of its pharmacological action.

Keywords: Octreotide acetate, pancreatic carcinogenesis, ham-
ster

Son, H.Y., Nishikawa, A., Ikeda, T., Nakamura, H., Miyauchi,
M., Imazawa, T., Furukawa, F. and Hirose, M.: Lack of modi-
fying effects of environmental estrogenic compounds on the
development of thyroid proliferative lesions in male rats pre-
treated with N-bis (2-hydroxypropyl) nitrosamine (DHPN)


The modifying effects of various environmental estrogenic com-
ounds on thyroid carcinogenesis were investigated in a rodent
two-stage carcinogenesis model. The compounds examined were
a soy isoflavone mixture (SI) and genistein (GEN) as phytoestrogens, nonylphenol (NP) as a xenoestrogen, 3-chloro-
4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) as a thyroid
carcinogen and sulfadimethoxine (SDM) as a known thyroid tu-
mor promoter. Five-week-old male F344 rats were given a single subcutaneous injection of DHPN or the vehicle alone. Starting one week thereafter, GEN, SI, NP, MX or SDM was administered for 12 weeks. Thyroid weights were significantly increased only in the SDM treatment groups, especially with DHPN pretreatment. Kidney weights were slightly increased in the NP or MX treatment groups, albeit without statistical significance. There were no organ weight changes or histopathological lesions in the major organs including the thyroid in the GEN, SI, NP, and MX treatment groups regardless of DHPN pretreatment. Our results thus indicate that the weakly estrogenic compounds GEN, SI and NP and the environmental rat thyroid carcinogen MX do not exert any modifying effects on thyroid carcinogenesis in rats under the present experimental conditions.

Keywords: Estrogenic compounds, thyroid carcinogenesis, genistein


The effects of environmental estrogenic compounds, soy isoflavone mixture (SI), genistein (GEN), and nonylphenol (NP), and the possible goitrogen 3-chloro-4-((dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), on thyroid carcinogenesis were investigated in ovariectomized (OVX) female rats. Five-week-old OVX F344 rats were given a single subcutaneous injection of DHPN. Starting 1 week later, GEN, SI, NP, MX, sulfadimethoxine (SDM), a known thyroid tumor-promoter, or beta-estradiol 3-benzoate (EB), a synthetic estrogen were administered for 12 weeks. SDM and EB were included as positive controls. Renal tubule lesions, uterine squamous metaplasia, vaginal keratinization and telangiectasia of pancreatic islets were also observed with EB. There were no organ weight changes or histopathological lesions in the major organs, including the thyroid, in the GEN, SI, MX or NP treatment groups. Our results thus indicated a lack of modifying effects on thyroid carcinogenesis in female OVX rats, in agreement with our previous finding in males.

Keywords: Thyroid carcinogenesis, ovariectomy, genistein


The chemopreventive effects of protease-inhibitor (PCA) were investigated during the post-initiation stage of the BOP-initiated hamster pancreatic tumorigenesis model. Animals in groups 1-3 were given two s.c. injections of 20 mg/kg body weight of BOP with a one week interval as an initiation treatment. After the BOP injection, hamsters in groups 1 and 2 were respectively fed diet supplemented with 1000 or 500 ppm of PCA for 49 weeks. The animals in group 3 were treated with BOP alone. The animals in groups 4-6, each consisting of 10 hamsters, were given 1000 or 500 ppm PCA, or basal diet alone without prior BOP injection. At the termination of experimental week 52, the incidences and multiplicities of neoplastic lesions in the pancreas were comparable among the BOP-treated groups. However, the incidence of pancreatic tumors larger than 3 cm was significantly lower in the PCA-treated high dose groups than in the control group. Moreover, the incidence of advanced pancreatic cancers which had directly invaded adjacent tissues such as the diaphragm, spleen and stomach was reduced by the PCA treatments, being significantly lower in group 2 than in group 3. Our results thus indicated that PCA can inhibit the late post-initiation or progression phase of BOP-induced pancreatic carcinogenesis in hamsters.

Keywords: protease-inhibitor, pancreatic carcinogenesis, BOP


To investigate the modifying effects of cinamaldehyde (CNMA) on the development of lung proliferative lesions, male transgenic mice carrying the human prototype c-Ha-ras gene (rasH2) were given a single injection of urethane (UR) or saline, followed by a diet containing 0.5% CNMA or basal diet for 26 weeks. Lung tumors were induced in the treated groups except for CNMA-alone group without significant intergroup differences in their incidences and multiplicities. There were no intergroup differences between UR-alone and UR+CNMA groups in the PCNA labeling indices and the areas of lung tumors. It was concluded that the CNMA treatment does not influence the development of lung proliferative lesions induced by urethane in rasH2 mice.

Keywords: cinamaldehyde, rasH2 mouse, lung tumor


Adenophora triphylla (AT), an oriental medicinal plant, was extracted using water and several organic solvents and each fraction was assayed for its antiproliferative effects on human Jurkat T cells with 3-(4,5-dimethylthiazolyl)-2, 5-diphenyltetrazolium bromide (MTT). The influence on induction of apoptosis and G1 arrest was also examined. The ethyl acetate fraction showed the most pronounced inhibitory effects on proliferation of Jurkat T cells. Apoptosis was induced in line with up-regulation of FasL, tyrosine phosphorylation and c-fos mRNA levels. Arrest in G1 of the cell cycle was observed in A2780 cells with a wild type p53 gene but not HT-29 cells with a mutant p53 gene. Modifying effects of AT on cell turnover and glutathione(GSH) levels in vivo were also investigated in the stomach of rats given 150 mg/kg of MNNG by gavage and then fed a diet supplemented with 5% or 1% pulverized AT and 0.5% or 0.2% ethylacetate-extracted AT for 42 hours. The 5% AT and both of the ethylacetate fractions caused significant reduction in PCNA-labeling in the glandular
stomach epithelium as compared with the value for the MNNG alone group. In addition, the treatments significantly increased the gastric GSH levels. These results suggest that AT could be a chemopreventive agent against gastric cancer.

Keywords: *Adenophora triphylla*, apoptosis, FasL

*Keimyung University*


The modifying effects of dietary 4-phenylbutyl isothiocyanate (PBIC), given during the initiation stage of carcinogenesis, were investigated in hamsters treated with BOP. Animals in groups 1-3, each consisting of 30 hamsters, were given BOP by two s.c. injections, 1 week apart, at a dose of 20 mg/kg body weight, plus 0, 10 or 100 micromol/animal of PBIC in corn oil by gavage 2 h prior to each carcinogen treatment. Ten animals in group 4 served as a vehicle control, and animals in groups 5 and 6, each consisting of ten hamsters, were given 10 and 100 μ of PBIC alone in corn oil. Sacrifice was 52 weeks after the first BOP injection. The PBIC treatments significantly inhibited the development of pancreatic ductal dysplasias and adenocarcinomas. Also, lung tumors (adenomas and adenocarcinomas) were significantly reduced in a dose-dependent manner. In contrast, both hepatocellular and cholangiocellular tumors tended to be or were significantly increased by PBIC. These results, taken together with our previous findings, indicate that the natural isothiocyanate, phenethyl isothiocyanate (PETIC), has a more potent chemopreventive action against BOP-induced tumorigenesis than synthetic isothiocyanates with longer alkyl chains, such as 3-phenylpropyl isothiocyanate (PPTIC) and PBIC. Thus, their lipophilicity does not necessarily reflect the chemopreventive potential because the strength of lipophilicity is PETIC<PPTIC<PBIC.

Keywords: 4-phenylbutyl isothiocyanate, BOP, hamster

*Keimyung University*


The effects of a long-acting synthetic ACTH on 4-hydroxyaminoquinoline 1-oxide (4HAQQ)-induced adrenocortical lesions were investigated in female rats. A total of 140 rats were divided into 4 equal groups, given a single s.c. injection of 7 mg/kg 4HAQQ or vehicle, followed by repeated s.c. administration of the synthetic ACTH or no further treatment. Subgroups of 10 rats in each group were sequentially sacrificed at weeks 20, 30, and 40. Adenomas and adenomatous nodules developed in the adrenal cortex of animals receiving 4HAQQ and the chronic ACTH stimulation. From week 20, middle zone, cortical cystic degeneration, which mimics the age-associated degenerative change named adrenal peliosis, was frequently observed in the adrenal glands of animals treated with 4HAQQ alone. Its development was inhibited by ACTH. These results indicate that long-term stimulation of ACTH promotes the development of adrenocortical tumors but suppresses the occurrence of adrenal peliosis in rats treated with 4HAQQ.

Keywords: Synthetic ACTH, 4HAQQ, adrenal cortex, peliosis


Gastric tumorigenic sensitivity to N-methyl-N-nitrosourea (MNU) was examined in heterozygous p53 knockout (p53(+/-)) CBA mice and their wild-type littermates (p53(+/-)) or p53(+/+) CBA mice were given MNU in their drinking water at concentration of 50, 10, 5 or 0ppm for 26 weeks. The incidences of hyperplasias in the glandular stomach observed in p53(+/+) CBA mice treated with 50ppm and 10ppm MNU were significantly increased, as compared with the control group. No tumors were induced in the stomach of any treated groups. The present study suggests that p53(+/-) CBA mice have low susceptibility to MNU-induced gastric carcinogenesis.

Keywords: p53 knockout CBA mouse, MNU, gastric tumor


It is unknown whether endocrine-disrupting chemicals (EDCs) with estrogenic activities have any modifying effects on uterine carcinogenesis. To investigate the effects of ethinylestradiol (EE) and methoxychlor (MXC) on development of N-ethyl-N-nitrosourea (ENU)-induced uterine tumors, female p53-deficient CBA mice [p53 (+/+) mice] and their wild-type littermates [p53 (+/+) mice] received an injection of ENU, followed by a diet containing EE or MXC for 26 weeks. The present study suggests that 2.5 ppm EE, but not MXC, exerts tumor-promoting effects on stromal and epithelial proliferative lesions of the uteri in p53 (+/-) mice initiated with ENU.

Keywords: p53-deficient mouse, uterine tumor, ethinylestradiol


Six groups of female SD rats were fed a diet containing low amounts of CdCl2 or Cd-polluted rice at concentrations up to 40 ppm, and were killed after 12, 18, and 22 months. Animals dem-
onstrated spontaneous chronic nephropathy and fluctuation in the tubular PCNA LI, but these findings were not correlated with renal Cd levels at 22 months. PCNA LI on the other hand, appeared to be linked to the severity of chronic nephropathy. The results demonstrated that treatment with 40 ppm or less for 22 months did not influence tubular regeneration as a component of nonspecific chronic nephropathy, suggesting that long-term oral administration of low levels of Cd does not injure renal tubules in female rats.

Keywords: Cd-induced nephrotoxicity, PCNA-LI, chronic nephropathy

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Hirose, M., Yamaguchi, T.†, Lin, C.‡, Kimoto, N.§, Futakuchi, M.¶, Kono, T.¶, Nishibe, S.** and Shirai, T.**: Effects of arctiin on PhIP-induced mammary, colon and pancreatic carcinogenesis in female Sprague-Dawley rats and MeIQx-induced hepatocarcinogenesis in male F344 rats


Chemopreventive effects of arctiin, a lignan isolated from Arctium lappa (burdock) seeds, on the initiation or post initiation period of PhIP induced mammary carcinogenesis in female rats and on MeIQx-associated hepatocarcinogenesis in male rats were examined. In experiment 1, female SD rats were given intragastric doses of 100 mg/kg body wt of PhIP once a week for 8 weeks as initiation. Groups of 20 rats each were treated with 0.2 or 0.02% arctiin during or after PhIP initiation. Animals were killed at the end of week 48. Although the incidence of mammary carcinomas did not significantly differ among the PhIP-treated groups, multiplicity was significantly decreased in rats given 0.2 or 0.02% arctiin after PhIP initiation as compared with the PhIP alone control. The average number of colon aberrant crypt foci was also significantly decreased in these two groups. Pancreas acinaric foci were induced in PhIP treated animals with slight decrease in the multiplicity with arctiin during the initiation phase. For liver carcinogenesis, groups of 15 male F344 rats were given a single intraperitoneal injection of DEN and starting 2 weeks later, they were administered 0.03% MelIQx in the diet, MeIQx together with 0.5% arctiin, 0.1% arctiin or basal diet for 6 weeks. They were subjected to two-third partial hepatectomy 3 weeks after DEN initiation and killed at the end of week 8 for GST-P immunohistochemistry. The numbers and areas of preneoplastic GST-P positive foci were elevated by the treatment with MeIQx, and further increased by the simultaneous treatment with arctiin. These results indicate that arctiin has a protective effect on PhIP-induced carcinogenesis particularly in the mammary gland in the promotion period. On the other hand, it may have a weak co-carcinogenic influence on MelIQx-induced hepatocarcinogenesis. In addition, the results suggested that PhIP is a weak pancreatic carcinogen in female SD rats, targeting acinar cells.

Keywords: Actiin, PhIP, MeIQx

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Boesenbergia pandurata (Zingiberaceae), Languas galanga (Zingiberaceae) and Citrus hystrix (Rutaceae) are edible plants that are commonly used as flavors or condiments in various Thai food dishes. They are known to exert strong anti-promoting activity in a test of tumor promoter-induced Epstein-Barr virus (EBV) activation. In the present study their effects on hepatocarcinogenesis were investigated in a medium-term bioassay using F344 male rats. C. hystrix significantly enhanced 2-amino-3,8-dimethylimidazo (4, 5-f) quinoxaline-associated preneoplastic liver cell focus development while B. pandurata and L. galanga had borderline effects. The results suggest that C. hystrix as well as B. pandurata and L. galanga may contain agents augmenting the hepatocarcinogenicity of 2-amino-3,8-dimethylimidazo(4,5-f) quinoxaline.

Keywords: Thai edible plants, 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline, medium-term bioassay

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Kawabe, M.†, Lin, C.‡, Kimoto, N.§, Sano, M.¶, Hirose, M. and Shirai, T.**: Modifying effects of propolis on MeIQx promotion of rat hepatocarcinogenesis and in a female rat two-stage carcino genesis model after multiple carcigen initiation


The modifying effects of the dietary administration of water-and ethanol-extracted propolis produced in Brazil (WB and EB, respectively) on MeIQx promotion of rat hepatocarcinogenesis were investigated in a medium-term liver bioassay system with use of male Fischer 344 rats. The number and area of GST-P-positive foci in rats given 0.5% WB were significantly increased compared with the group given MeIQx alone. Furthermore, the numbers of GST-P-positive foci were higher in rats given 0.1% WB or EB than in those given the basal diet alone. The modifying effects of propolis on other organs were also examined in female Fischer 344 rats given multiple carcinogens for initiation. Rats received water- and ethanol-extracted propolis produced in Brazil and Uruguay (WB, EB, WU, and EU, respectively) in the diet after exposure to three different carcinogens. The incidence of total mammary tumors was significantly lower in rats given EU than in the control group. These results indicate that a water extract of propolis exerts a cocarcinogenic effect on MeIQx hepatocarcinogenesis while promoting the effect at low dose in a two-stage hepatocarcinogenesis model. Moreover, they suggest that ethanol-extracted propolis may be an inhibitor of mammary gland carcinogenesis.

Keywords: Propolis, rat hepatocarcinogenesis, two-stage carcino genesis
Hamada, S.†, Sutoh, S.†, Morita, T.†, Wakata, A.†, Asanami, S.†, Hosoya, S.†, Ozawa, S.†, Kondo, K.†, Nakajima, M.†, Shimada, H.†, Osawa, K.†, Kondo, Y.†, Asano, N.†, Sato, S.†, Tamura, H.†, Yajima, N.†, Marshall, R.†, Moore, C.†, Blakey, D.H.†, Schechtman, L.M.†, Weaver, J.L.†, Torous, D.K.†, Proudlock, R.†, Ito, S.†, Namiki, C.†, and Hayashi, M.: Evaluation of the rodent micronucleus assay by a 28-day-treatment protocol: Summary of the 13th collaborative study by CSGMT/JEMS・MMS


To examine whether micronucleus tests (MNT) can be incorporated into general toxicology assays, we performed MNT applying the treatment protocols typically used in such assays. Our results indicate that the integration of the MNT into a 28-day toxicological assay is feasible. To serve this purpose, blood samples collected 4 days after the beginning of treatment and blood and bone marrow samples collected at autopsy should be examined. We propose that rats can provide biologically important and relevant information regarding potential chemical mutagens.

Keywords: UVB, epidermis, deletion

‡‡ See article.

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Mutant cells generated in vivo can be eliminated when mutant gene products are presented as altered MHC/peptide complexes and recognized by T cells. Diminished expression of MHC/peptide complexes enables mutant cells to escape recognition by T-cells. In the present study, we tested the hypothesis that mutant lymphocytes lacking expression of MHC class I molecules are eliminated by autologous NK cells.

Keywords: MHC, mutation, NK cells


Chromosomal double strand breaks (DSBs) occurring in mammalian cells, which are responsible for initiating genomic instability, are usually repaired by end-rejoining or homologous recombination. We demonstrated here that wild-type p53 protein contributes to the maintenance of genomic integrity through recombinational DNA repair. Human lymphoblastoid cells deficient in p53 were defective in the ability to carry out recombinational DNA repair of spontaneously arising DSBs. Broken chromosomes by DSBs in p53-deficient cells triggered the breakage-fusion-bridge cycle, and were occasionally stabilized by end-rejoining or nonhomologous recombination to other chromosomes. Our results support a model in which p53 protein regulates recombinational DNA repair, providing a mechanism for the maintenance of genomic integrity in mammalian cells.

Key words: p53, DNA double strand break (DSB), recombination repair

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Mutation Res., 491, 211-220 (2001)

The o-aminoozotoluene (AAAT) is carcinogenic mainly in the liver, and also in lung following long term administration. In the present report, we reveal the molecular nature of mutations induced by AAAT in the lambda eII gene (the eII gene, a phenotypically selectable marker in the lambda transgenes, has 294 bp, which makes it easier to sequence than the original target, the 3kb lacZ gene). The eII mutant frequency in liver and colon was five and nine times higher, respectively, in AAAT-treated mice than in control mice. Sequence analysis revealed that AAT-induced G:C to A:T transversions, whereas spontaneous mutations consisted primarily of G:C to A:T transitions at CpG sites.

Keywords: mutation spectra, Muta™Mouse, o-aminoozotoluene


Mutation Res. 456, 73-81 (2000)

Quinoline is carcinogenic to the liver in rodents, but it is not clear whether it acts by a genotoxic mechanism. In the present report, we reveal the molecular nature of the mutations induced by quinoline in the lambda eII gene, which is also a phenotypically selectable marker in the lambda transgene. The liver eII mutant frequency was nine times higher in quinoline-treated mice than in control mice. Sequence analysis revealed that quinoline induced primarily G:C to C:G transversions (25 of 34). Thus, we have confirmed that quinoline is genotoxic in its target organ, and the G:C to C:G transversion is the molecular signature of quinoline-induced mutations.

Keywords: mutation spectra, Muta™Mouse, quinoline


Mutation Res. 468, 19-25 (2000)

2-Amino-3,8-dimethylimidazo[4,5-f]quinoline (MelQx), a heterocyclic amine found in cooked meat, is a strong mutagen and was proven to be a hepatocarcinogen in rodents. We used the lacI transgenic (Big Blue®) mouse to investigate MelQx genotoxicity in vivo. Intragastric treatment with MelQx (100 mg/kg) did not increase mutant frequency (MF) in liver or colon. No
apparent increase in PCNA-positive foci was observed in any of tissues analyzed 14 days after the treatment. Administration of MelQx (300 ppm) in diet for 12 weeks, however, caused MF increases in liver and colon in male and female mice, with greater increases in the females. An increase was also obvious after 4 weeks, but only in females. These results demonstrated that in the transgenic mouse mutation assay, long-term feeding of MelQx was more effective than single gastric exposures and that sex differences in susceptibility can also be observed.

Keywords: organ specificity, Big Blue®, lacI


In order to get a cumulative marker of UV exposure, we have established a sensitive allele-specific polymerase chain reaction (AS-PCR) assay capable of detecting one CC to TT mutation in Mt DNA among 107 wild-type genes using a mismatch allele-specific primer. With this assay, we found no mutation-positive samples from internal non-exposed tissue (colon, and blood) (0/50). In contrast, 17 out of 111 skin samples were positive. In normal skin tissue, the prevalence of positive samples was higher in those from exposed sites (13/51) than in those from less-exposed sites (1/26) (p<0.05), however, a quantitative correlation was not found. We conclude that the UV-associated CC to TT mutation in Mt DNA can be detected in normal skin, but further studies are required to develop this as a quantitative marker for UV exposure.

Keyword: allele-specific PCR, UV, mitochondria

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Tumor development in the skin could be a multi-step process where various genetic alterations. We demonstrate that UVB irradiation efficiently induces deletions in the epidermis using a novel transgenic mouse gpt delta. In this mouse model, deletions in lambda DNA integrated in the chromosome are preferentially selected as Spi' (sensitive to P2 interfence) phages, which can then be subjected to molecular analysis. The mice were exposed to UVB at single doses. Four weeks later, lambda phage was rescued from the genomic DNA of the epidermis. The mutant frequencies of Spi having large deletions in the epidermis increased more than 15-fold at a UVB dose of 0.5 KJ/m2 over the control. Molecular sizes of most of the large deletions were greater than 1,000 base pairs. These results suggest that UVB irradiation induces deletions in the murine epidermis, and most of the deletions are generated through end-joining of DNA double strand breaks.

Keywords: UVB, epidermis, deletion


Mutations induced by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), were characterized using gpt delta transgenic mice. This system has two selection methods to efficiently detect the point mutations or deletions. The mice were fed with a diet containing 400 ppm PhIP for 13 weeks and gpt and Spi mutations were analyzed from the colon. Concerning the types of gpt mutations from PhIP-treated mice, G:C to T:A transversions and single base pair deletions at G:C base pairs predominated. Concerning Spi' mutants from PhIP-treated mice, G:C base pair deletions in monotonic G or C run sequences and base run sequences predominated. These results suggest that PhIP induces point mutations, rather than larger deletions in vivo and that run sequences may play an important role in PhIP-induced G:C base pair deletions.

Keywords: PhIP, gpt delta, mutation spectrum

*国立がんセンター研究所


The mutagen-activating capacity of human CYP2E1 for N-Alkylnitrosamines was compared with that of CYP2A6 using Salmonella typhimurium YG7108 2E1/OR and YG7108 2A6/OR strains. Eight N-Alkylnitrosamines, including N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosodiisopropylamine, N-nitrosodibutylamine, N-nitrosomethylphenylamine, N-nitrosopyrrolidine, N-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanal were examined. We conclude that human CYP2E1 is mainly responsible for the metabolic activation of N-nitrosamines with a relatively short alkyl chain(s), whereas CYP2A6 was predominantly responsible for the metabolic activation of N-Alkylnitrosamines possessing a relatively bulky alkyl chain(s).

Keywords: N-Alkyl nitrosamines, human CYP2E1, human CYP2A6

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We developed a new Salmonella tester strain highly sensitive to promutagenic N-nitrosamines by introducing a plasmid carrying human cytochrome P450 2A6(CYP2A6) and NADPH-cytochrome
P450 reductase (OR) cDNA into the ada-and ogr-deficient strain YG7108. The expressed CYP2A6 efficiently catalyzed coumarin 7-hydroxylation. N-nitrosomethylphenylamine (NMPHA) and some other chemicals were mutagenic in the new strain into the absence of any exogenous activation system. We believe that this is the first demonstration that CYP2A6 is responsible for the metabolic activation of NMPHA. The established tester strain may be useful to predict human activation of N-nitrosamine promutagens. Keywords: YG7108, mutation assay, heterologous expression

Wagner, J.*, Nohmi, T.: *Escherichia coli* DNA Polymerase IV Mutator Activity: Genetic Requirements and Mutational Specificity
The dinB gene of *Escherichia coli* encodes a novel DNA polymerase, DNA Pol IV, which is able to dramatically increase the untargeted mutagenesis. At the amino acid level, DNA Pol IV shares sequence homologies with *E. coli* UmuC (DNA Pol V), Rev1p and Rad30p (DNA polymerase α) of *Saccharomyces cerevisiae* and human Rad30A (XPV) proteins, all of which are involved in translesion DNA synthesis. Here we report that the DNA pol IV mutator activity clearly promotes single nucleotide substitutions as well as one base deletions in the ratio of about 1:2. The base changes were drastically biased for substitutions toward G:C base pairs and about 70 % of them occurred in 5'-GX-3' sequences where X represents the base (T, A or C) that is mutated to G. These results are discussed in the view of the recently described biochemical characteristics of DNA Pol IV. Keywords: dinB, DNA polymerase IV, untargeted mutagenesis

*EMBO Report*, 1, 484-488 (2000)
The recent discovery of a new family of ubiquitous DNA polymerases involved in translesion synthesis has shed new light onto the biochemical basis of mutagenesis. Among these polymerases, the dinB gene product (Pol IV) is involved in mutagenesis in *Escherichia coli*. We show here that the activity of native Pol IV is drastically modified upon interaction with the s subunit, the processivity factor of DNA Pol III. In the absence of the s subunit Pol IV is strictly distributive and no stable complex between Pol IV and DNA could be detected. In contrast, the s clamp allows Pol IV to form a stable initiation complex (t1/2 approximately 2.3 min), which leads to a dramatic increase in the processivity of Pol IV reaching an average of 300-400 nucleotides. In vivo, the s processivity subunit may target DNA Pol IV to its substrate, generating synthesis tracks much longer than previously thought. Keywords: dinB, DNA polymerase IV, processivity

Adenine paired with 8-hydroxyguanine (ohG), a major component of oxidative DNA damage, is excised by MYH base excision repair protein in human cells. We compared the repair activity of type 1 mitochondrial and type 2 nuclear MYH proteins on A:ohG and A:G mismatches. In a reaction buffer with a low salt (0-50 mM) concentration, adenine DNA glycosylase activity of type 2 protein was detected on both A:ohG and A:G substrates. However, in a reaction buffer with a 150 mM salt concentration, similar to physiological conditions, the glycosylase activity on A:G, but not on A:ohG, was extremely reduced and the binding activity of type 2 protein for A: G, but not for A: ohG, was proportionally reduced. The glycosylase activity on A: ohG and the ability to suppress spontaneous mutagenesis were greater for type 2 than type 1 enzyme. These results indicate that human MYH protein specifically catalyzes the glycosylase reaction on A:ohG under physiological salt concentrations. Keywords: oxidative DNA damage, 8-hydroxyguanine, base excision repair

The human monochromosome hybrid cell panel in the Japanese Collection of Research Bioresources (JCRB) consists of 23 mouse cell lines, each containing a different human chromosome. In order to determine the state of the human chromosomes and to supply the information to investigators, we characterized the cells by fluorescence in situ hybridization (FISH) with corresponding human chromosome-specific painting probes. Here we report the frequency of intact human chromosomes maintained in each hybrid and the retained subregions of corresponding human chromosomes with relative frequencies estimated by fluorescent intensity. This characterization will provide valuable information to investigators using the panel. Keywords: FISH (fluorescence in situ hybridization), human monochromosome hybrid cell panel, JCRB (Japanese Collection of Research Bioresources)

*食品薬品安全性研究所 黒野研究室
*国立感染症研究所
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髙田和子，増井 等，田辺幸之，原沢 亮*，水沢 博：培養細胞系でのマイコプラスマのPCR検出法
マイコプラスマの汚染を迅速・正確に検出することを目的に、新製テクノロジーを用いた2段階のPCR法（テクノロジーファン法）を利用して検出系を開発した。2段階のPCRは検出感度を高めると同時に、1段階目のPCRの特異性を確認できる利点がある。陽性コントロールとしてMycoplasma orale DNAを用い、テープブレイドDNA濃度3〜10μg/μl（1反応あたり2-6コピー）が検出限界であることが明らかに

216 国 立 衛 研 報 第119号 (2001)
なった。更に、PCR 法で増幅された DNA を回収し、塩基配列を決定した結果、細胞に感染しているマイコプラズマ菌種を確実に同定することができた。この一連の方法の開発により、培養細胞に感染しているマイコプラズマの検出感度、迅速性、また、依頼性を高め、細胞バンクの品質管理を向上させることができとなった。

Keywords: Mycoplasma, nested PCR, DNA sequencing

*東京大学医学部附属動物実験施設


Oncogene 20, 1143-1151 (2001)

Bloom syndrome (BS) is a recessive human genetic disorder characterized by short stature, immunodeficiency and an elevated risk of malignancy. BS cells have mutator phenotypes such as hyper-recombination, chromosome instability and an increased frequency of sister chromatid exchange (SCE). We generated BLM-/- mutants of the chicken B-cell line DT40. They are hypersensitive to genotoxic agents such as etoposide, bleomycin and 4-nitroquinoline-1-oxide and irradiation with the short wave length of UV (UVC) light. UVC irradiation to BLM-/- cells during G1 to early S phase caused chromatid instability, leading to eventual cell death. These results suggest that BLM is involved in surveillance of base abnormalities in genomic DNA that may be encountered by replication forks in early S phase. Such surveillance would maintain genomic stability in vertebrate cells, resulting in the prevention of cellular tumorigenesis.

Keywords: RecQ-type DNA helicase, Bloom syndrome, DT40

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増井 徹，祖父尼俊雄，石井美智子*，今田由紀夫*，安井英明*，高田宏子，林 真，水沢 博：厚生省細胞バンクにおけるヒト組織・細胞取扱い規則に関する倫理問題への取り組み


医学・生物学研究の中でヒトの組織・細胞を利用した研究が重要な位置を占めるようになってきた。そして、社会意
識の変化に対応するために、ここにおいてヒト試料を利用する際の倫理問題に関する複数ガイドラインが並在する現
状である。研究現場では、いくつかのガイドラインに沿う形で研究を行わなければならないことになり、倫理審査が困
難な状況となっても予想されている。そこで、この分野の社会認知の得るためにも、研究体制が整えられようとしてい
る今こそ、基本的事柄に立ち直ることが重要であると考えている。我々は、厚生科学審議会先端医療技術評価部の1998年12月の考案と日本細胞培養学会の倫理問題検討委員
会報告を踏まえて、ヒト試料の公的研究資源化のプロセスに
について検討を試みた。本稿では厚生省細胞バンク（JCRB細胞バンク）の取り組みをケーススタディとして報告す
る。

Keywords: human material, guideline, public research resource

*東京都立大学法学部
*関東中央病院

長谷川隆一，小泉聡子，広瀬明彦，前川昭彦*：アルキルフェノールのエストロゲン様作用と生殖器系への影響

日本食品化学学会誌，7, 1-9 (2000)

内分沁剤と乱化学物質の一つとして注目されているアルキルフェノール類として、nonylphenol および tert-
octylphenolについて、文献情報を収集・整理するとともに、評価を行った。両物質とともに、in vitro および in vivo で
エストロゲン受容体を介したエストロゲン様作用が認められる。後記投与によって雄の生殖器、乳腺、雄の生殖器
系への強い影響は認められていないが、nonylphenol が出生直後の雄に生殖腺内投与することにより、顕著な精子並び
副生殖器重量の低下、卵巣肥大及び受精率の低下が引き起こされている。生体内動態は速いが、半減期は数時間以内と短
い。ラットの発育中の個体投与試験で、nonylphenol の生殖器
に関する無毒性量は15mg/kg/dayである。この値を人の最大推定1日摂取量と比較したところ、安全性は30倍以上
と推定され、人への危険性は現在のところ低いと考えられ

Keywords: nonylphenol, octylphenol, estrogenic activity

佐佐木研究所

小泉聡子，江馬 龍，広瀬明彦，長谷川隆一：フタル酸エストロゲン様作用の生殖器および発生系に対する毒性影響についての最近の研究：主としてdi(2-ethylhexyl) phthalateおよび
Di-n-butyl phthalateについて


フタル酸エストロゲンのうち、主にdi(2-ethylhexyl) phthalateおよびdi-n-butyl phthalateについて、げっ歯類の生殖
器および発生に対する毒性影響およびその推定されるメカ
ニズムについて、最近の文献を中心に整理した。血圧において
は、生殖機能と精子形成機能が関与している。特に、性周明の延長や
排卵阻害が引き起こす。生殖毒性としては、雌雄両性に対
しても不妊を、器官形成期の投与で口蓋裂、顕椎、胸椎、肋
骨の崎形および腎孟拡張等の催形形を引き起こす。妊娠後期および
出生期の胸腹部への投与では、妊娠代謝性機能の低下を。
したがって、胎児雄性の影響は、アンドロゲン受容体を介さ
ない抗アンドロゲン作用と考えられている。

Keywords: di(2-ethylhexyl) phthalate, di-n-butyl phthalate, reproductive
devvelopmental toxicity

小泉聡子，江馬 龍，広瀬明彦，黒川真二，長谷川隆一：フタル酸エステルの生殖・発生毒性性と、繁殖毒性の種類
差、種差およびDEHP の1日耐容摂取量

日本食品化学学会誌，8, 1-10 (2001)

フタル酸エステルの雌生殖器に対する影響、雌生殖器に対する影響、生殖毒性および発生毒性について、構造活性相関に注目して、毒性発現の種類と程度（無毒性発現投与量）を
比較、整理した。また、フタル酸エステルの繁殖毒性に関する種差および種差の発現機構に関する情報を解析し、考察
した。最後に、平成12年に設定されたDEHPのTDI薬物の根
拠となって情報を受け、解説するとともに、その適正、情報
の欠落等について考察した。

Keywords: NOAELs of phthalate esters, Species and age differ-
ences of testicular toxicity, TDI of DEHP

Miyazaki, T., Yamada, C. and Okada, S.: Hyaluronate depolymerization following thermal decomposition of oxytetracycline
Depolymerization of hyaluronate (HA) by oxytetracycline (OTC) was investigated. On mixing with OTC and incubating at 37°C, HA was gradually depolymerized. OTC is known as a photosensitizer, however, HA depolymerization required no irradiation. As time passed, OTC solution incubated at 37°C got colored reddish brown, even in the dark. With reversed-phase HPLC separation, several peaks derived from decomposed OTC appeared. One of the peaks had an absorbance in the visible range. A quantitative correlation between the discoloration and the HA depolymerization rate was obtained. On the other hand, when samples were incubated below 25°C change of color was slight, and practically no HA depolymerization was observed after up to 4 hours. Oxygen depletion by nitrogen saturation or addition of mannitol also prevented the depolymerization. Under anaerobic conditions, the color of the solution did not change, whereas it turned red under aerobic conditions in the presence of mannitol. The mannitol did not inhibit the OTC decomposition, but it preserved HA from damage. On the basis of the known decomposition of OTC and the results of HPLC separation, anhydrooxytetracycline can be proposed as the derivative causing HA depolymerization.

Keywords: hyaluronic acid, hydroxyethyl acrylate, gel

Enantiomer ratio of hyoscymamine from Scoporia extract was determined by chiral HPLC-CD analysis. It was found that circular dicroism(CD) detection allowed the analysis of the sample without any special pretreatment whereas UV detection required ammonia-ether extraction. To obtain a shorter analysis time for the determination, reversed-phase HPLC-CD analysis was applied by using a g-factor calibration curve (EE% vs. CD/UV). The analysis time was shortened from 35 to 18 min. EE% values obtained were consistent with those by chiral HPLC analysis.

Keywords: Hyoscymamine, CD detector, Chiral
ing surface acyl chain fluidity, and fluorescence lifetime of N-(5-dimethylaminonaphthalene-1-sulfonyl)diaplatimoyl-phosphatidylyl-
ethanamine in H2O and D2O buffer, assessing the degree of
hydration in the head group region. The results revealed that in-
corporation of egg SM into triolein-egg phosphatidylcholine emu-
sions markedly increased acyl chain order and decreased head
group hydration of the surface monolayers. In contrast, chole-
sterol was shown to increase head group hydration despite a strong
increase in acyl chain order. The close correlation between the
apparent Km values of LPL and the degree of head group hydra-
ation indicated that LPL interacts with the head group region rather
than with the hydrophobic interior of the surface monolayers.
However, apparent Vmax did not show a simple correlation with
any surface structure, and the finding in which SM had no effect
on apparent Vmax of medium-chain triglyceride emulsions sug-
gested that the hydrophobic interaction between acyl chains of
SM and triglyceride at the emulsion surface is important for
determining the apparent Vmax. These results showed conclusively
that SM inhibits LPL activity mainly by changing the emulsion
surface structure and not by a specific interaction between SM
and LPL.

Keywords: sphingomyelin, lipoprotein lipase, cholesterol

Saito, H., Okuhara, K.*, Tsuchimoto, N.*, Vertut-Doi, A.*, 
Matsumoto, C.*, Tanimoto, T., Okada, S. and Handa, T.*

Modulation of Apolipoprotein E-Mediated Plasma Clearance
and Cell Uptake of Emulsion Particles by Cholesteryl Ester
Lipids, 36, 27-33 (2001)

Cholesteryl ester, along with triglyceride (TG), is the major core
component of plasma lipoproteins. We investigated the effect of
core composition on the physical state and metabolic behavior of
lipid emulsions, as model particles of lipoproteins. Fluorescence
studies using 1,6-diphenylhexatriene analogs showed that although
cholesteryl olate (CO) significantly decreased core mobility, the
surface rigidity of phosphatidylcholine (PC) monolayers was in-
dependent of core composition. When intravenously injected into
rats, the increased amount of core CO tended to retard TG emu-
sion removal from plasma, and the initial clearance rate was cor-
related with the amount of apolipoprotein E (apoE) bound from
plasma. In addition, PC liposomes with a similar emulsion particle
size showed negligible binding of apoE and were cleared at a slower
rate compared to all emulsions. Furthermore, the effect of
CO on the binding behavior of apoE to the emulsion surface
and the emulsion uptake by hepatocytes was assessed in vitro.
Replacing core TG with CO was found to decrease the apoE binding
capacity to emulsions markedly without changing the binding
affinity and thereby to reduce the cell uptake of emulsion par-
ticles by HepG2 cells. These results indicate that the physical state
of core lipids, which can be modulated by CO content, plays a
role in emulsion metabolism through the alteration in apoE bind-
ing.

Keywords: apolipoprotein E, cell uptake, cholesteryl ester

Saito, H., Tanaka, M.*, Okamura, E.*, Kimura, T.*, Nakahara,
M.* and Handa, T.*

Interaction of Phosphatidylcholine Surface
Monolayers with Triglyceride Cores and Enhanced Apo-
A-I Binding in Lipid Emulsions
Langmuir, 17, 2528-2532 (2001)
The binding maximum of apoA-I (N) in triolein (TO)-egg yolk
phosphatidylcholine (PC) emulsions was 10-fold larger than that
in PC large unilamellar vesicles (LUV) of similar size (100 nm)
with no significant difference in the affinity. Replacement of the
long-chain triglyceride, TO, by medium-chain triglycerides or
cholesteryl olate in emulsion cores significantly decreased the N
value. The 13C NMR chemical shifts of the PC carbonyl carbon
at the surface layers indicated that PC polar headgroups are more
separated and exposed to water molecules in emulsions than in
vesicles. The N values were satisfactorily correlated with the
chemical shift, that is, the degree of separation between the car-
bonyl groups at the surface. Although apoA-I binding to the PC
monolayers of emulsions brings about bending of the surface lay-
ers and creates local defects in the hydrocarbon regions in a simi-
lar manner as PC LUV, the surface-core interaction seems to fill
the defects with the core neutral lipids, compensates for the bend-
ing stress, and eventually increases the N value. Dependence of
the core effect upon the acyl chain length of triglycerides implied
important roles of the acyl chains in the surface-core interaction
between PC and triglycerides.

Keywords: apolipoprotein A-I, NMR, emulsions

Mackawa, K., Tanimoto, T., Okada, S., Suzuki, T.*, Suzuki,
T.*, Yabe-Nishimura, C.*, Expression of Aldose Reductase
and Sorbitol Dehydrogenase Genes in Schwann Cells Isolated
from Rat: Effects of High Glucose and Osmotic Stress
To investigate the polyol pathway activity in Schwann cells, we
determined the mRNA levels of Aldose reductase (AR) and sorbi-
tol dehydrogenase (SDH) in cultured cells under hyperglycemic
or hyporesmotic conditions using competitive RT-PCR technique.
The expressions of AR and SDH mRNAs in Schwann cells were
unaltered by high (30 mM) glucose content in the medium. On
the other hand, osmotic stress elicited significant increases in AR
mRNA without any effect on SDH mRNA expression. These find-
ings suggest that in contrast to the induction of AR expression by
osmotic stress, high glucose per se does not up-regulate expres-
sion of the enzymes constituting the polyol pathway in Schwann
cells. The RT-PCR system developed in this study may be a use-
ful tool in ascertaining the relative contributions of AR and SDH
to the metabolic derangements leading to diabetic complications.

Keywords: polyol pathway, diabetic neuropathy; competitive RT-
PCR

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小出達夫，岩田真恵，前川京子，斎藤博幸，谷本 剛，岡 田敏史：国立薬品食品衛生研究所スウェルテアマル
標準品の新規設定のための品質評価
医薬品研究，32 (3)，118-123 (2001)
スウェルチアマリン標準品の新規設定のための品質評価試験を行った。試験結果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：236.2nmに極大吸収が認められ、比吸光度はそれぞれ257.2（236nm）と348（317nm）である。4）赤外吸収スペクトル：3346, 1697, 1619, 1282, 1068, 1013cm⁻¹に特性吸収がみられた。5）水分含量：3.42%。6）液クロマトグラフ法による純度試験：複数個の不純物が検出され、不純物総量は約0.3%であった。7）ガスクロマトグラフ法による残留溶媒試験：プラノールが約3.96%検出された。以上の試験結果から、本標準品原料は、国立薬品食品衛生研究所スウェルチアマリン標準品に産した品質を有することを認めた。

Keywords : Swertiamarin, NIHs Reference Standard, Quality evaluation

斎藤博幸, 岩井美保, 小出達夫, 前川京子, 谷本 剛, 岡田敏史・国立薬品食品衛生研究所ニコチン酸トフェロール標準品の新規設定のための品質評価

薬品研究, 31 (11), 818-823 (2000)

ニコチン酸トフェロール標準品の新規設定のための品質評価試験を行った。試験結果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：264nmに極大吸収が認められ、比吸光度は83.5であった。4）赤外吸収スペクトル：1742, 1590, 1462, 1214, 1099cm⁻¹に特性吸収がみられた。5）水分含量：0.03%。6）融点：43.9℃。7）液体クロマトグラフ法による純度試験：4個の不純物が検出され、不純物総量は約0.91%であった。以上の試験結果から、本標準品原料は、国立薬品食品衛生研究所ニコチン酸トフェロール標準品に適した品質を有することを認めた。

Keywords : Tocopherol nicotinate, NIHs Reference Standard, Quality evaluation

斎藤博幸, 岩井美保, 前川京子, 谷本 剛, 岡田敏史, 鎌倉浩之, 川原信夫, 中根孝久, 関田節子, 佐竹元吉, 横田洋一*, 森野英紀*, 鈴木英彦*, 岩崎 冲*, 松浦敬一*：国立薬品食品衛生研究所バイカルニン標準品の新規設定のための品質評価


パイカン標準品の新規設定のための品質評価試験を行った。試験結果は以下のとおりである。1）元素分析：理論値と一致した。2）紫外吸収スペクトル：277.2nmと317.0nmに極大吸収が認められ、比吸光度はそれぞれ609（277nm）と384（317nm）である。3）赤外吸収スペクトル：3385, 1728, 1662, 1611, 1575cm⁻¹に特性吸収がみられた。4）水分含量：3.07%。5）融点：210.4℃。6）液体クロマトグラフ法による純度試験：1個の不純物が検出され、不純物総量は約0.9%であった。以上の試験結果から、本標準品原料は、国立薬品食品衛生研究所バイカールニン標準品に適した品質を有することを認めた。

Keywords : Baicalin, NIHs Reference Standard, Quality evaluation

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谷本 剛, 八木澤正*, 藤原 博*：日本抗生物質薬品

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1）—医薬品各条—

薬品研究, 31 (9), 674-680 (2000)

日局13（第二追補を含む）に収載されている抗生物質薬品医薬品108品目についての日局基準規格の設定を行い、その他の日局薬品各条における規格内容との差異を比較検討する。各条の判定基準等に対する日局と薬局に関する考え方の相違点について検討した。更に、これらの検討結果に基づいて、薬品の抗生物質薬品を日局に規定する際に日局薬品各条との整合を図るために必要な措置や留意点などについて考察した。

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Tsumura, Y., Ishimitsu, S., Saito, I.*., Sakai, H.*., Kobayashi, Y.*, Tonogai, Y.: Eleven phthalate esters and di(2-ethylhexyl) adipate in one-week duplicate diet samples obtained from hospitals and their estimated daily intake

Food Add. Contam., 18, 449 - 460 (2001)

Plasticizers in one-week total diet samples were determined for the purpose to estimate daily intake. The phthalate esters were as follows: diethyl, dipropy, dibutyl, dipentyl, dihexyl, butylbenzyl, dicyclohexyl, di(2-ethylhexyl), dioctyl, disoocetyl (mixture of isomers) and diisononyl (mixture). Di(2-ethylhexyl) adipate was also determined. For analysis, homogenized meal samples was extracted with acetonitrile, lipids were removed by extraction into n-hexane and the acetonitrile layer was cleaned using Florisil® and Bondesiv PSA® dual layer column. Phthalates were determined by GC/MS (SIM). Phthalate recovery from fortified meal mixture by this methods was 62.5 to 140.8%. Quality assurance as assessed by three laboratories indicated coefficient of variance in the levels of detected phthalates in same lot samples as below 10%. Detection limits were 0.1 to 23 ng/g for each Phthalate. One-week diet samples provided at three hospitals in three remote prefectures of Japan were analyzed for individual meal. In all 63 samples, DEHP was the highest among all phthalates, 10 to 4400 ng/g. Daily intake of phthalates estimated from all samples was 519 ng DEHP/day, 86 µg DEHA/day, 65 µg DNBP/day, and 4.7 µg BBP/day. Calculated DEHP in two-day samples out of 21 days exceeded EU TDI for a person of 50 kg body weight (1850 µg per day). Disposable PVC gloves used during preparation of meals were suspected as the source of high DEHP content. One-day intake of the other phthalates and DEHA was below 7% of TDI in all cases. High concentration of DEHP (5990 ng/g) was found in baby food used in quality assurance. The source of contamination was PVC-tube in production and effectively reduced by replacing the tube to stainless steel one.

Keywords : phthalate, DEHP, DEHA, total diet sample, hospital food, GC/MS.

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津村ゆかり, 石光連, 中村俊美子, 吉井公彦, 関原薬樹子, 外海泰夫：調理用PVC製手袋使用規制下における市販弁当中のフタル酸エステル類及びアジビン酸ジ（2-エチルヘキシル）濃度
Liquid chromatographic determination of emamectin, milbemectin, ivermectin and abamectin in crops and confirmation by liquid chromatography-mass spectrometry.


Emamectin, milbemectin, ivermectin and abamectin are similar macrocyclic lactone chemicals used as an acaricides or parasiticides. We developed a simultaneous analytical method for determining the residual amounts of these compounds and emamectin metabolites in crops. A sample extracted with acetone was cleaned up with Bond Elut Car and NH2. The sample was then fluorescence-derivatized with trifluoroacetic anhydride and 1-methylimidazole in acetonitrile. The analyte was measured by HPLC with fluorescence detection using an octadecylsil column with 3 microm particle size and gradient elution. In most crops, their recoveries by the developed method were ca. 80-110%. The detection limits of the analytes in vegetables were 0.1-0.3 ppt. Using the developed method, we surveyed the residues of these compounds in 20 commercial crops in Osaka, Japan. The result of the surveillance was that emamectin benzoate of 0.2-6.7 ppb was detected in nine cases and milbemectin of 16.7-279.3 ppb was detected in four cases. The detected samples were confirmed by LC-electrospray ionization (ESI) MS. The limit of detection by LC-ESI-MS was similar to the fluorescence detection level of 0.1-0.3 ppt in vegetables except for milbemectin.

Keywords: emamectin, LC/MS, determination, derivatization


Residual malathion in wheat was estimated at a lower value when analysis was performed by extraction with acetone after addition of water to swell the wheat, according to the Japanese Bulletin Method. The supernatant of the wheat homogenate showed degradation not only of malathion but also of phenthoate. Malathion and phenthoate were not degraded by the boiled supernatant of the wheat homogenate. It was presumed for this reason that glutathione reductase (GR; EC 1.6.4.2) in the wheat degraded malathion. The following results were obtained: (1) GR originating in wheat could degrade malathion and phenthoate. (2) The degradation of malathion by the GR was inhibited by excessive GSSG. (3) There was a high correlation between GR activity and malathion degradation activity of the supernatant of wheat homogenates. It is likely that GR acted on the specific structure of malathion and phenthoate, the S=S bond, and the branched structure bonding with the sulfur atom. Following the above, extraction with acetone after addition of water (the Japanese Bulletin Method) should be replaced by extraction with pure organic solvent and without addition of water for swelling.

Keywords: wheat, malathion, degradation, phenthoate, enzyme, glutathione reductase
Nakamura, Y., Tsuji, S., Tonogai, Y.: Determination of the levels of isoflavonoids in soybeans and soy-derived foods and estimation of isoflavonoids in the Japanese daily intake

*JAOAC Int.*, 83, 635-650 (2000)

The levels of 6 kinds of isoflavonoids found in 11 domestic and imported soybeans, and 12 kinds of soybean-based processed foods in Japan were systematically analyzed, and the Japanese daily intake of isoflavonoids from those foods was estimated. The total isoflavonoids (daidzein, glycine, and genistein) were analyzed with acid hydrolysis and the intact isoflavonoids (daidzein, glycine, genistein, daidzin, glicitin, and genistin) were analyzed without hydrolysis. This was followed by cleanup with ODS cartridge column and determined by LC with a diode array detector (DAD). The highest content of isoflavonoids was found in kinako (a roasted soybean powder) and the lowest was found in soy sauce. The contents and composition of the isoflavonoids in the 11 soybeans varied by species and country of origin. The level of isoflavonoids found in the processed foods varied by manufacturing method or ingredients. The percentage of aglycon tended to be higher in miso (fermented soybean paste) and soy sauce, which are heated and fermented during the manufacturing process. Japanese daily intake of isoflavonoids from soybeans and soybean-based processed foods was estimated as 27.80 mg per day (daidzein 12.02 mg, glycine 2.30 mg and genistein 13.48 mg).

Keywords: Isoflavonoids, Soybeans

Nakamura, Y., Ishimitsu, S., Tonogai, Y.: Effects of quercetin and rutin on serum and hepatic lipid concentrations, fecal steroid excretion and serum antioxidant properties

*J. Health Sci.*, 46, 229-240 (2000)

Effects of quercetin and rutin on serum and hepatic lipid concentrations, fecal steroid excretion and their antioxidant properties were investigated in rats by oral administration. No toxic symptom was observed even at the dose of 1.0 g/kg of quercetin or rutin. Serum and hepatic lipid concentrations and fecal steroid excretion was not influenced remarkably, but serum thiobarbituric acid reactive substances (TBARS) decreased dose-dependently with the administration of quercetin or rutin. The decrease of serum TBARS was significantly correlated with the increase of serum free flavonoids (p<0.05-0.001). Serum flavonoid concentrations, especially free quercetin, were higher in rutin-administered rats than in quercetin-administered rats at doses of 1.0 g/kg for 10 days (p<0.05-0.001). When 1.0 g/kg of quercetin or rutin was administered in a single dose, they remained in the blood as aglycone or their conjugates of quercetin and isorhamnetin, even three days after administration. Recovered flavonoids were only 0.13% and 0.89% in urine for 3 days and 0.03% and 0.13% in serum on day 3 by administration of quercetin and rutin, respectively. Thus, some part of the administered quercetin or rutin was metabolized and showed antioxidant property, but had no remarkable influence on serum or hepatic lipid concentrations or fecal steroid excretion in rats.

Keywords: Quercetin, Rutin
Polyphenol administration. We found that alterations in the compositions of fecal neutral steroids and bile acids were independent of the tannic acid or Polyphenol dose: the ratio of coprostanol to cholesterol decreased significantly in rats given 0.05-0.2 g/kg of Polyphenol or 0.5 g/kg of tannic acid; and the ratio of cholic-acid-derived bile acids to chenodeoxycholic-acid-derived bile acids decreased significantly after administration of 0.05, 0.2 and 0.5 g/kg of Polyphenol or 0.1 and 0.5 g/kg of tannic acid. Primary bile acid excretion significantly only in rats given a dose of 0.1 g/kg of Polyphenol. This is the first report that documents the changes occurring in fecal steroid excretion induced by oral administration of green tea polyphenol or tannic acid.

Keywords: Green tea polyphenol; Tannic acid, Fecal steroid excretion

Amakura., Y., Okada, M., Tsuji, S., Tonogai, Y.: Influence of jam processing on the radical scavenging activity and phenolic contents in berries


Selected six phenolic (aglycons; caffeic and ellagic acids, kaempferol, quercetin, myricetin and morin) contents and their changes in nine berries influenced by jam processing have been evaluated using optimized HPLC with diode array detection. The samples, fresh and after jam processing from the berries, were analyzed, and the total amounts of selected phenolics as aglycons were identified and determined by acid hydrolysis of them. Their contents in fresh and jam samples did not indicate appreciable changes; therefore the influence of jam processing on selected phenolics in berries was suggested to be small and mostly present in berries as several conjugated forms glycosylated, esterified, etc., in the samples. The total phenolic contents of each sample also were determined by the Folin-Ciocalteu method. Three samples, namely fresh, jam and acid hydrolysate of the berry, had similar contents. On the other hand, the scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured, and acid hydrolysates produced many aglycons showed stronger activity than that of the fresh and jam processed samples as a whole. Keywords: berry, phenolics, jam.

Amakura., Y., Okada, M., Tsuji, S., Tonogai, Y.: Determination of phenolic acids in fruit juices by isocratic column liquid chromatography


A simple and rapid analytical method of five phenolic acids, gallic, chlorogenic, caffeic, ellagic and ferulic acid, which are naturally occurring bioactives, were determined in fruit juices by isocratic LC using photodiode array UV detection. The sample was pretreated by solid-phase extraction (a combination of Sep-Pak Plus C18 and Bond Elut PSA).

Keywords: phenolic acids, fruit juices, solid-phase extraction

Amakura., Y., Okada, M., Tsuji, S., Tonogai, Y.: High-performance liquid chromatographic determination with photodiode array detection of ellagic acid in fresh and processed fruits


A high-performance liquid chromatographic (HPLC) procedure based on an isocratic elution with photodiode array detection has been developed for a simple and rapid determination of ellagic acid (EA) in fresh and processed fruits. The homogenized sample was refluxed with methanol, and the extract was refined using a solid phase cartridge before HPLC. We analyzed EA in 40 kinds of fresh fruits and 11 kinds of processed fruits by the developed method. EA was found in several berries, fujioja, pineapple and pomegranate. This is the first occurrence of the detection of EA in bayberry, fujioja and pineapple.

Keywords: HPLC, food, ellagic acid

Murai, T., Nakagawa, Y., Maeda, H., Terada, K.: Altered regulation of cell cycle machinery involved in interleukin-1-induced G1 and G2 phase growth arrest of A375S2 human melanoma cells

J. Biol. Chem., 276, 6797-6806 (2001)

Interleukin-1 (IL-1) inhibits the growth of A375S2 human melanoma cells by arresting them at G1 and G2 phases of the cell cycle. The arrests are preceded by a rapid decrease in kinase activities of cyclin E-Cdk2 and cyclin B1-Cdc2, which are critical for G1-S and G2-M progression, respectively. IL-1 quickly enhances the protein expression of the CDK inhibitor p21Cip1. The induced p21 binds preferentially to cyclin E-Cdk2, and the increase in p21 binding parallels the decrease in cyclin E-Cdk2 activity. Thus, p21 is likely to be responsible for the inhibition of cyclin E-Cdk2 activity and G1 arrest. Coinciding with the decrease in cyclin B1-Cdc2 activity, there is an increase in tyrosine phosphorylation of Cdc2, suggesting that an increase in the inactive Tyr-15-phosphorylated form of Cdc2 is involved in the decrease in cyclin B1-Cdc2 activity and G2 arrest. Furthermore, we found that IL-1 causes rapid dephosphorylation of p107, but not of pRb or p130, while the total protein levels of p130 are increased. Thus, IL-1 may exert its growth-arresting effects via p107 and p130 pathways rather than through pRb.

Keywords: IL-1, cell growth arrest, cell cycle machinery

Ema, M., Harazono, A.: Adverse effects of dibutylin dichlo-
Ride on initiation and maintenance of rat pregnancy

The present study was conducted to evaluate the adverse effects of dibutyltin dichloride (DBTCI) on initiation and maintenance of pregnancy after maternal exposure during early pregnancy in rats. Following successful mating, female rats were given DBTCI by gastric intubation on days 0 to 3 or on days 4 to 7 of pregnancy at 0, 3.8, 7.6, or 15.2 mg/kg. Food-restricted pregnant rats were given an amount of feed equal to the feed intake of female rats treated with DBTCI at 15.2 mg/kg on days 0 to 3 or on days 4 to 7 of pregnancy. Female rats were sacrificed on day 20 of pregnancy and pregnancy outcome was determined. After administration of DBTCI on days 0 to 3, the rate of nonpregnant females and the incidence of preimplantation embryonic loss in the 7.6 mg/kg group were significantly higher than those in the control group, and those in the 15.2 mg/kg group were significantly higher than those in the control and pair-fed groups. In females with implantations, the numbers of implantations and live fetuses and the incidence of postimplantation embryonic loss in the groups given DBTCI on days 0 to 3 were not significantly different from those in the control group. The incidence of postimplantation embryonic loss in the groups given DBTCI on days 4 to 7 at 7.6 and 15.2 mg/kg was significantly higher than that in the control and pair-fed groups. It can be concluded that DBTCI adversely affects initiation and maintenance of pregnancy when administered during early pregnancy and that the manifestations of the adverse effects of DBTCI vary with the gestational stage at the time of maternal exposure.

Keywords: Dibutyltin dichloride, pregnancy failure, early embryonic loss

Ema, M., Miyawaki, E.: Adverse effects on development of reproductive system in male offspring of rats given monobutyl phthalate, a metabolite of dibutyl phthalate, during late pregnancy

The objective of this study was to determine the adverse effects of monobutyl phthalate (MButP), a major metabolite of dibutyl phthalate (DBP), on development of reproductive system in offspring following maternal administration during late pregnancy, and to assess the role of MButP in the antiandrogenic effects of DBP. Pregnant rats were given MBuP by gastric intubation at 250, 500, or 750 mg/kg on days 15 to 17 of pregnancy. Maternal body weight gain and food consumption during the administration period were significantly decreased at 500 mg/kg and higher and at 750 mg/kg, respectively. A significant increase in the incidence of postimplantation embryonic loss was found at 500 mg/kg and higher. The body weights of male and female fetuses were significantly lower at 750 mg/kg. A significant increase in the incidence of fetuses with undescended testes was found at 250 mg/kg and higher. A significant increase in the anogenital distance (AGD) of male fetuses was found at 250 mg/kg and higher. The AGD/body weight ratio and AGD/cube root of body weight ratio in male fetuses was also significantly reduced at 250 mg/kg and higher. The AGD, AGD/body weight ratio and AGD/cube root of body weight ratio in female fetuses in the MBuP-treated groups were comparable to those in the control group. The data of the present study indicate that MBuP on days 15 to 17 of pregnancy produced adverse effects on the development of reproductive system in male offspring and suggest that MBuP may be responsible for the induction of the antiandrogenic effects of DBP.

Keywords: Monobutyl phthalate, anogenital distance, undescended testes

Ema, M.: Reproductive and developmental toxicity of triphenyltin chloride in rats

Reproductive and developmental toxicity of triphenyltin chloride (TPTCI) was evaluated in rats. Although no significant increase in the incidence of fetuses with malformations was observed following administration of TPTCI during organogenesis, a significant increase in the incidence of postimplantation embryonic loss was found. TPTCI during early pregnancy, especially on days 0-3 of pregnancy, caused implantation failure, i.e., preimplantation embryonic loss. The effects of TPTCI on the uterine function, as a cause of implantation failure, were determined using pseudopregnant rats. TPTCI was given on days 0-3 of pseudopregnancy and the decidual cell response was induced on day 4 of pseudopregnancy. Rats were sacrificed on day 9 of pseudopregnancy and the uterine weight served as an index of the uterine decidualization. A significantly lower weight of the uterus, which indicates the suppression of uterine decidualization, was found at the doses which induced implantation failure. These doses of TPTCI also caused a significant decrease in the progesterone levels, which indicates reduced ovarian function. These findings suggest that TPTCI exerts adverse effects on uterine decidualization correlated with the reduction in serum progesterone levels and these effects are responsible, at least in part, for the implantation failure induced by TPTCI.

Keywords: Triphenyltin, diphenyltin, Embryonic loss, decidualization

Ema, M., Harazono, A.: Developmental and reproductive toxicity of tributyltin and its metabolite, dibutyltin, in rats

Developmental and reproductive toxicity of tributyltin chloride (TBTCl) and dibutyltin dichloride (DBTCl) was evaluated in rats. Tributyltin chloride (TBTCl) was teratogenic when administered on day 8 and days 11-14 of pregnancy, and the most pronounced effect was seen after administration on day 13 of pregnancy. Cleft palate was predominantly observed. DBTCl was teratogenic when administered on days 7-8 of pregnancy, and day 8 of pregnancy was the most susceptible to the teratogenicity of DBTCl. Cleft jaw, ankyloglossia, omphalocoele, anomaly of the tail, and deformity of the vertebral column and ribs were frequently observed. TBTCl and DBTCl on days 0-3 of pregnancy caused implantation failure, preimplantation embryonic loss. TBTCl and DBTCl on days 4-7 of pregnancy affected the viability of the implanted embryos. The effects of TBTCl on uterine function, as a cause of early embryonic loss, were determined using pseudopregnant rats. TBTCl was given on days 0-3 or days 4-7 of pseudopregnancy and the decidual cell response was induced on day 4. The uterine
weight on day 9 served an index of the uterine decidualization. A significantly lower weight of the uterus, which indicates suppression of uterine decidualization, was found at the doses that induced early embryonic loss. These doses of TBTCI also caused a significant decrease in the serum progesterone levels. The findings suggest that TBTCI exerts adverse effects on uterine decidualization correlated with a reduction in serum progesterone levels, and that these effects are responsible, at least in part, for the early embryonic loss induced by TBTCI.

Keywords: Tributyltin, dibutyltin, reproductive and developmental toxicity

Harazono, A., Ema, M.: Effects of 4-tert-octylphenol on initiation and maintenance of pregnancy following oral administration during early pregnancy in rats


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


In our previous studies, tributyltin chloride (TBTCI) at doses of 16.3 mg/kg and above caused implantation failure (preimplantation embryonic loss) and postimplantation embryonic loss in rats following administration on gestational day (GD) 0 through GD 3 and GD 4 through GD 7, respectively. This study was designed to assess the effects of TBTCI on uterine function, as a cause of early embryonic loss in pseudopregnant rats. TBTCI was given orally to pseudopregnant rats at doses of 4.1, 8.1, 16.3 and 32.5 mg/kg on pseudopregnant day (PPD) 0 to PPD 3 or 8.1, 16.3, 32.5 and 65.1 mg/kg on PPD 4 to PPD 7. The decidual cell response was induced by bilateral scratch trauma on PPD 4. The uterine weight on PPD 9 served as an index of uterine decidualization. Uterine weight and serum progesterone levels on PPD 9 were significantly decreased after administration of TBTCI at doses of 16.3 mg/kg and above on PPD 0 to PPD 3 or PPD 4 to PPD 7. Administration of TBTCI at doses of 8.1 mg/kg and above on PPD 0 to 3 also significantly decreased serum progesterone levels on PPD 4. TBTCI had no effect on ovarian weight and number of corpora lutea. It can be concluded that TBTCI suppresses the uterine decidual cell response and decreases progesterone levels, and these effects are responsible for early embryonic loss due to TBTCI exposure.

Keywords: tributyltin chloride, decidual cell response, pseudopregnancy

草野源次郎*, 芝野真喜雄*, 鈴木直樹*, 渡辺 齋*, 尾崎和男*, 柴田敏郎, 昌山好雄, 前島 蔵**: 甘草屋数のウラルカンゾウ復活


山梨県塩山市にある高野家（別称、甘草屋数）では、江戸幕府の命により1720年頃からカンゾウの栽培が行われていたが、近年管理がゆきとどく絶滅寸前の所であった。今川、現地で栽培を復活させると同時に栽培試験場の畑を植栽して増殖させ、また、品種栽培により増殖も進み、完全復活に成功した。この栽培は、形態や成分の比較によりウラルカンゾウであることを確認した。

Keywords: Glycyrrhiza uralensis, Kanzo Yashiki

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Touno, K., Harada, K.*, Yoshimatsu, K., Yazaki, K.** and Shimomura, K.: Histological observation of red pigment formed on shoot stem of Lithospermum erythrorhizon

Plant Biotechnology, 17, 1, 127-130 (2000)

Shikonin production on the stem of shoot cultures of Lithospermum erythrorhizon was controlled by use of various culture media and light irradiation. Microscopic analysis of the shoot cultures revealed that the red pigment formation was only observed on the stem surface and the stem hairs of shoots cultured in the dark. Cross section of the shoot stem which formed red pigment in the dark was morphologically similar to that of shoots cultured under illumination. Red pigment accumulation was strictly localized in the outer surface of epidermal cells. The localization of these pigments was similarly observed in root tissues generated from the cultured shoots as well as field-grown roots. Northern blot analysis indicated that LED1-2 gene, which is one of the candidates for the regulatory element of shikonin biosynthesis and specifically expressed in the root system of the intact plants, was also expressed in the stem of shoot cultures when producing shikonin.

Keywords: Lithospermum erythrorhizon, shoot cultures, pigment localization

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Nakanishi, F.*, Sasaki, K.** and Shimomura, K.: Kinetics of littorine content in various developing stages of regenerates of Atropa belladonna

Plant Cell Reports, 19 (10), 1021-1026 (2000)
Aseptically propagated regenerates were cultivated in hydroponic apparatus, phytotron or field, and their growth and litorine contents were investigated. No litorine was detected in aseptic regenerates cultured on solidified MS medium, nor in leaves through any condition tested. In roots, it was common features to all conditions used here that litorine increased dramatically after transplantation from culture tubes, and was a major alkaloid up to week 4. After then, the litorine contents varied in different cultivation conditions. The roots cultivated in the field showed marked thickening and rapid disappearance of litorine: those cultivated in hydroponic apparatus were fine and maintained litorine at high level for long. In a plant cultivated for 16 weeks in a pot, the litorine content in roots decreased with increasing their diameter.

Keywords: *Aropa belladonna*, regenerates, litorine
* Kyoto University

Touno, K., Harada, K.*, Yoshimatsu, K., Yazaki, K.* and Shimomura, K.: *Shikonin derivative formation on the stem of cultured shoots in Lithospermum erythrorhizon*


Shoot cultures of *Lithospermum erythrorhizon*, which are capable of producing red pigments, have been established. The red pigments were formed on the stem of *L. erythrorhizon* shoots cultured both on solid and in liquid media without phytohormone at 25°C in the dark. TLC and HPLC analyses revealed that the red pigments observed on the shoot cultured on those media were shikonin derivatives. The effects of various basal media and phytohormones (IAA, IBA and kinetin) on the growth and the formation of shikonin derivatives were investigated. When the shoots were cultured on Murashige and Skoog solid medium, the addition of kinetin remarkably enhanced shikonin derivative accumulation in the shoots. However, these effects of kinetin were not observed in the liquid culture when cultured in Gamborg B5 medium. The maximum content of shikonin derivatives (2.3% as dry weight, ca. 1.5 mg / flask) was observed in the shoots cultivated in phytohormone-free B5 liquid medium for 5 weeks.

Keywords: *Lithospermum erythrorhizon*, shikonin derivatives, shoot culture

* Kyushu University


*Plant Physiology*, 125, 1831-1841 (2001)

*Lithospermum erythrorhizon* produces red naphthoquinone pigments that are shikonin derivatives. They are accumulated exclusively in the roots of this plant. The biosynthesis of shikonin is strongly inhibited by light, even though other environmental conditions are optimized. Thus, *L. erythrorhizon* dark-inducible genes (LeDIIs) were isolated to investigate the regulatory mechanism of shikonin biosynthesis. LeDI-2, showing the strict dark-specific expression, was further characterized by use of cell suspension cultures and hairy root cultures as model systems. Its mRNA accumulation showed a similar pattern with that of shikonin. In the intact plants LeDI-2 expression was observed solely in the root, and the longitudinal distribution of its mRNA was also in accordance to that of shikonin. LeDI-2 encoded a very hydrophobic polypeptide of 114 amino acids that shared significant similarities with some root-specific polypeptides such as ZRP3 (maize) and RecC3 (rice). Reduction of LeDI-2 expression by its antisense DNA in hairy roots of *L. erythrorhizon* decreased the shikonin accumulation, whereas other biosynthetic enzymes, e.g. p-hydroxybenzoic acid:geranyltransferase, which catalyzed a critical biosynthetic step, showed similar activity as the wild-type clone. This is the first report of the gene that is involved in production of secondary metabolites without affecting biosynthetic enzyme activities.

Keywords: *Lithospermum erythrorhizon*, shikonin derivatives, LeDI-2

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We analyzed the nucleotide sequences of the non-coding region of chloroplast DNA: the intergenic spacer between trnL(UAA) 3'exon and trnF(GAA). Two kinds of sequence, "type-1" and "type-2", were detected in 33 populations of *Cannabis sativa*. The length of the "type-1" fragment was 354bp. In contrast, the "type-2" fragment from 3 population was 353 bp long, with only one base deletion compared to "type-1". The fragment length from *Humulus lupulus* was 353bp with a 1bp deletion, and ten 1-bp substitutions compared to the sequences from *C. sativa* "type-1". Furthermore, we could clearly identify differences between *C. sativa* and *H. lupulus* using single-strand conformation polymorphism of PCR products (PCR-SSCP) analysis.

Keywords: *Cannabis sativa* L., chloroplast DNA; intergenic spacer; intraspecific variation; PCR-SSCP

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