[*J. Chem. Soc., Perkin Trans. 1*, 2043-2045 (2002)] [Lab. of Pharm. Chemistry]

The Chalcogeno-Baylis-Hillman Reaction of Ketones and α-Dicarbonyl Compounds.

Tadashi KATAOKA * Hironori KINOSHITA, Sayaka KINOSHITA and Tatsunori IWAMURA

We conducted the reaction of 1-[(2-methylsulfanyl)phenyl]prop-2-en-1-one with various carbonyl compounds which are not subject to the traditional Baylis-Hillman reaction and showed the first example of ketones undergoing the chalcogeno-Baylis-Hillman reaction under mild conditions and of enolizable α-dicarbonyl compounds such as diacetyl and ethyl pyruvate giving Morita-Baylis-Hillman adducts.

[*Tetrahedron Lett.*, **43**, 7039-7041 (2002)] [Lab. of Pharm. Chemistry]

Tandem Michael-aldol Reaction via 6-*Endo*-dig Cyclization of Ynone-chalcogenides: Synthesis of 2-Unsubstituted 3-(Hydroxyalkyl)chalcogenochromen-4-ones.

Tadashi KATAOKA,* Hironori KINOSHITA, Sayaka KINOSHITA and Tatsunori IWAMURA

The tandem Michael-aldol reaction of 1-[(2-methylsulfanyl)phenyl]propyn-1-one (**1**) or the seleno congener **3** with aldehydes in the presence of $BF_3{\cdot}OEt_2$ gave 3-(hydroxyalkyl)thiochromen-4-ones **2** or selenochromen-4-ones **4** via the 6-*endo*-dig cyclization and aldol reaction. No 5-*exo*-dig cyclization product was obtained. Selenochromen-4-ones **4** were obtained in higher yields than the thiochromen-4-ones **2.** This is the useful method for the synthesis of 2-unsubstituted 3-(hydroxyalkyl)chalcogeno- chromen-4-ones.

[*J. Am. Chem. Soc.*,**124**, 1866-1867 (2002)] [Lab. of Pharm. Chemistry]

A Highly Enantioselective Route to Either Enantiomer of Both α- and β-Amino Acid Derivatives.

Armando CÓRDOVA, Shin-ichi WATANABE, * Fujie TANAKA, Wolfgang NOTZ, and Carlos F. BARBAS, III

This report describes the unprecedented use of unmodified aldehydes as donors in a catalytic asymmetric Mannich-type reaction. The proline-catalyzed reaction of *N*-PMP-protected α-imino ethyl glyoxylate with unmodified aliphatic aldehydes provided a general and very mild entry to either enantiomer of β-amino and α-amino acids and derivatives in high yield and stereoselectivity. In addition, the corresponding chiral β-amino aldehyde adducts can be readily converted to the corresponding amino acid derivatives. Most significantly, this approach provides facile access to substituted β-lactams.

[*Org. Lett.*, **4**, 4519-4522 (2002)] [Lab. of Pharm. Chemistry]

One-Pot Asymmetric Synthesis of β-Cyanohydroxymethyl α-Amino Acid Derivatives: Formation of Three Contiguous Stereogenic Centers.

Shin-ichi WATANABE, * Armando CÓRDOVA, Fujie TANAKA, and Carlos F. BARBAS, III

One-pot asymmetric Mannich-hydrocyanation reactions are described. Reaction of unmodified aldehydes with *N*-PMP-protected α-imino ethyl glyoxylate in the presence of catalytic amounts of L-proline followed by the addition of Et_2AlCN provided highly enantiomerically pure β-cyanohydroxymethyl α-amino acid derivatives possessing three contiguous stereogenic centers as single diastereomers (93-99% ee). Control of reaction temperature during the cyanation step directed whether cyclization of the products to lactones occurred.

[*Synlett.*, 1149-1151 (2002)] [Lab. of Medicinal Chemistry]

Pd/C-H_2-catalyzed Deuterium Exchange Reaction of the Benzylic Site in D_2O.

Hironao Sajiki, Kazuyuki Hattori, Fumiyo Aoki, Kanoko Yasunaga and Kosaku Hirota*

Pd/C is found to catalyse efficient and chemoselective exchange of deuterium derived from D_2O with hydrogens on a benzylic carbon in the presence of a catalytic amount of hydrogen at room temperature. Less than 2 mol% of palladium metal in the presence of a catalytic amount of hydrogen can carry out efficient H-D exchange at room temperature under ordinary pressure. The reaction is general for a variety of substrates. The simplicity of this method makes it an attractive new tool for the post-synthetic deuterium labeling to a wide variety of disciplines.

[*Tetrahedron Lett.*, 635-655 (2002)] [Lab. of Medicinal Chemistry]

Regioselective BH_3-hydride Reduction of Inosine Derivatives.

Kosaku Hirota,* Hironao Sajiki, Ryuji Hattori, Yasunari Monguchi, Genzoh Tanabe and Osamu Muraoka

We have developed a mild and efficient method for the regioselective conversion of inosines to 2,3-dihydro-inosines that proceeds at room temperature. The regioselective BH_3-THF reduction was successfully applied to various inosine derivatives and related compounds. The reaction with 1-substituted inosine derivatives smoothly proceeded to afford the corresponding 2,3-dihydro products in good to high yields. The BH_3-THF reduction of 2-methylinosine hardly proceeded while a 1,2-disubstituted inosine, 2',3'-isopropylidene-1,2-dimethylinosine, which possesses a substituent at both N1 and C2 positions, was reduced . The reaction is general for the hypoxanthine nucleus, and the products are of interest as transition state analogs of IMP dehydrogenase. Studies to further elucidate the scope of this method and biological activities are currently under way.

[*Tetrahedron Lett.*, 7247-7250 (2002)] [Lab. of Medicinal Chemistry]

Mild and General Procedure for Pd/C-catalyzed Hydrodechlorination of Aromatic Chloride.

Hironao Sajiki, Akira Kume, Kazuyuki Hattoiri and Kosaku Hirota*

A mild and efficient one-pot hydrodechlorination using a Pd/C-Et_3N system proceeds at room temperature, which is general for the dechlorination of a variety of aromatic chlorides. During the course of our current study on the chemoselective hydrogenation using the Pd/C catalyst, we found that the catalytic activity of Pd/C toward the hydrodechlorination of only aromatic chlorides was remarkably and selectively activated by the addition of Et_3N, contrary to our expectation. Although it is suggested that dechlorination of aromatic chlorides cannot readily be achieved, both the conversion yield and the reaction rate of the dechlorination of 4-chlorobiphenyl (1) could be brought to outstanding levels by running the hydrodechlorination using commercial 10% Pd/C (3% of the weight of 1) and 1.2 equivalents of Et_3N in MeOH at room temperature and under hydrogen pressure. The reaction was completed smoothly within 1 h to afford the corresponding biphenyl **2** in 100% conversion yield (GC/Mass) and no products other than **2** were detected by GC/Mass although the dechlorination was incomplete even after 3 days when the reaction was carried out without Et_3N. The reaction is general for a variety of aromatic chlorides. The simplicity and reliability of this method makes it an attractive new tool for organic and environmental chemists.

[*Tetrahedron Lett.*, 7251-7254 (2002)] [Lab. of Medicinal Chemistry]

Complete and Truly Catalytic Degradation Method of PCBs Using Pd/C-Et_3N System under Ambient Pressure and Temperature.

Hironao Sajiki, Akira Kume, Kazuyuki Hattoiri, Hisamitsu Nagase and Kosaku Hirota*

Since PCBs are persistent toxic pollutants and do not degrade easily, the development of safe and perfect methodology for destruction of such remains of great importance. We have found that the catalyst activity of Pd/C toward the hydrodechlorination of PCBs was outstandingly activated by the addition of triethylamine. PCBs could be thoroughly dechlorinated under ambient temperature and pressure, and no product other than biphenyl has been detected by GC/MS. All reagents and solvents used are reusable, and the reaction mixture only contains biphenyl and triethylammonium chloride (triethylamine is regenerated by neutralization using NaOH). Nevertheless, this study clearly sets the framework for a practical and truly catalytic degradation method of PCBs and, by implication, of structurally related chlorinated aromatic environmental pollutants, such as chlorinated *p*-dioxins, dibenzofurans and benzenes and DDTs. The results are easily applicable to industrial degradation processes of PCBs for future protection of the environment from additional dispersion of PCBs.

[*J. Med. Chem.*, 5419-5422 (2002)] [Lab. of Medicinal Chemistry]

Discovery of 8-Hydroxyadenines as a Novel Type of Interferon Inducer.

Kosaku Hirota,* Kazunori Kazaoka, Itaru Niimoto, Hiroshi Kumihara, Hironao Sajiki, Yoshiaki Isobe, Haruo Takaku, Masanori Tobe, Haruhisa Ogita, Tetsuhiro Ogino, Shinji Ichii, Ayumu Kurimoto and Hajime Kawakami

9-Benzyl-8-hydroxyadenine (**1**) was found to possess interferon-inducing activity *in vitro* as a lead compound. Although replacement of the 9-benzyl group of **1** did not improve the activity, the introduction of a substituent such as alkyl, alkylthio, alkylamino, and alkoxy groups into the 2-position of the adenine ring resulted in a remarkable increase in the activity. The 2-alkylthio, 2-butylamino, and 2-butoxy analogues indicated the highest activities by oral administration to mice.

[*J. Med. Chem.*, 3465-3474 (2002)] [Lab. of Medicinal Chemistry]

The Effect of Phosphodiester Linking Group on Albumin Binding, Blood Half-Life, and Relaxivity of Intravasscular Diethylenetriaminepentaacetato Aquo Gadolinium (III) MRI Contrast Agents.

Thomas J. McMurry, David J. Parmelee, Hironao Sajiki,* Daniel M. Scott, Hillori, S. Ouellet, Richard C. Walovitch, Zoltan Tyeklar, Stephane Dumas, Paul Bernard, Samuel Nadler, Katarina Midelfort, Matthew Greenfield, Jeffrey Troughton, Randall B. Lauffer

The human serum albumin binding, blood half-life, and relativity of amphiphilic gadolinium complexes containing a phosphodiester moiety were compared with those of complexes which are lacking the negatively charged, hydrophilic linker. A modest increase in albumin binding was observed for the compounds containing the phosphodiester linker. Moreover, a dramatic shift from predominantly biliary excretion to mostly renal excretion was observed following addition of the phosphodiester moiety. Concomitantly, an extended blood half-life was observed for the phosphodiester compounds, presumably because glomerular filtration is a relatively enhancement observed in albumin solution, the gadolinium chelate phosphodiester compound **5a** is now being investigated in blood vessel enhancement studies.

[*Chem. Pharm. Bull.*, **50**, 615—622 (2002)] [Lab. of Pharm.Synthetic Chemistry]

Diastereoselective Imino—Aldol Condensation of Chiral 3-(*p*-Tolylsulfinyl)-2-furaldimine and Ester Enolates.

Yoshitsugu ARAI, * Shinya YONEDA, Tsutomu MASUDA and Yukio MASAKI

(S_S)-3-(*p*-Tolylsulfinyl)-2-furaldimine was synthesized, and condensation of the chiral furaldimine with lithium ester enolates has been examined. The product distribution of the reaction is dependent upon reaction conditions and on the kind of the substituent placed on the esters. Disubstituted ester enolates resulted in the exclusive formation of (4*R*)-β-lactam, while unsubstituted, *tert*-butyl ester enolate preferentially gave (3*R*)-β-amino ester. With the monosubstituted ester enolates, the condensation afforded (4*R*)-β-lactams and/or (3*R*)-β-amino esters as major products. This method has been applied to an efficient route to chiral furyl β-lactams.

[*Synlett.*, 522-524 (2002)] [Lab. of Pharm. Synthetic Chemistry]

Oxidative Cleavage of the Double Bonds of Styrenes with a Combination of Mesoporous Silicas FSM-16 and I_2 under Photoirradiation.

Akichika ITOH,* Tomohiro KODAMA, Yukio MASAKI and Shinji INAGAKI

A mesoporous silica FSM-16 was found to be a recyclable promoter for the oxidative cleavage of double bonds, which are conjugated with an aromatic nucleus, to afford the corresponding carboxylic acid in the presence of catalytic iodine under photoirradiation conditions.

[*Chem. Pharm. Bull.*, **50**, 1434-1438 (2002)] [Lab. of Pharm. Physical Chemistry]

Synthesis of Water-Soluble Polymeric Prodrugs Possessing 4-Methylcatechol Derivatives by Mechanochemical Solid-State Copolymerization and Nature of Drug Release.

Shin-ichi KONDO,* Yasushi SASAI, Masayuki KUZUYA, and Shoei FURUKAWA

In this study we synthesized the water-soluble polymeric prodrugs possessing a 4-methylcatechol (4MC) derivative as a side chain by mechanochemical solid-state copolymerization. 1-Benzoyl-4-methylcatechol (Bz4MC) was selected as a compound of 4MC, and its methacryloyl derivative (**1**) was synthesized. 6-*O*-Methacryloyl-D-galactose (**2**) was also prepared as a water-soluble monomer. The mechanochemical copolymerization of **1** and **2** proceeded to completion, and the polymeric prodrug produced possessed a narrow molecular weight distribution. Three kinds of polymeric prodrugs, whose compositions were different from one another, were hydrolyzed *in vitro*. The rate constants of hydrolysis decreased with increasing the mole fraction of **1** in polymeric prodrug. It was suggested that the rate constant of hydrolysis could be controlled by the composition.

[*Drug Delivery System*, **17**, 127-133 (2002)] [Lab. of Pharm. Physical Chemistry]

A New Drug Delivery System Using Plasma-Irradiated Pharmaceutical Aids-X Controlled-Release of Theophylline from Plasma-Irradiated Double-Compressed Tablet Composed of Poly(Styrene-Maleic Anhydride) or Poly(Styrene-Maleic Acid) as a Single Wall Material.

Shin-ichi KONDO, Kousuke ITO, Yasushi SASAI, and Masayuki KUZUYA*

As a part of the development of the DDS device using plasma-irradiated polymer, the utility of poly(styrene-maleic anhydride) (SMAn) and poly(styrene-maleic acid) (SMA) was investigated. It was found that SMAn and SMA were of intramolecular bifunctionality, cross-linkable phenyl group and degradable maleic anhydride or maleic acid group as an effect of plasma irradiation. Using these plasma characteristics, controlled-release tablets were designed by argon plasma irradiation on the outer layer double-compressed tablet prepared from theophylline as a core material and SMAn or SMA as a single wall material. The dissolution profiles of theophylline from plasma-irradiated double-compressed tablet were sufficiently modified in comparison with one of non-plasma-irradiated tablet, depending on the set of chosen plasma operational conditions.

[*Thin Solid Films*, **407**, 144-150 (2002)] [Lab. of Pharm. Physical Chemistry]

Mechanically-Amplified Plasma Processing for Drug Engineering.

Masayuki KUZUYA*, Yasushi SASAI, Motoaki MOURI and Shin-ichi KONDO

We report here special features of mechanochemical reaction of plasma-irradiated polyethylene (PE), low density PE (LDPE) and high density PE (HDPE). A dangling bond site (DBS) of three component radicals formed on PE surface by argon plasma-irradiation disappears rapidly by mechanical vibration with a Teflon twin-shell blender, causing successively solid state radical recombination reaction. When mechanical vibration of plasma-irradiated PE is similarly conducted together with a powdered drug, the sustained-release powders are obtained due to trapping of drugs into the powder matrix formed by mechanochemical solid state recombination of plasma-induced PE surface radicals.

[*J. Photopolym. Sci. Technol.*, **15**, 331-334 (2002)] [Lab. of Pharm. Physical Chemistry]

Preparation of Floating Drug Delivery System by Plasma Techniques

Masayuki KUZUYA,* Tomoya NAKAGAWA, Shin-ichi KONDO, Yasushi SASAI and Yoshimitsu MAKITA

The double-compressed tablet containing theophylline as the core drug with the outer layer composed of poly(methyl vinyl-ether-co-maleic acid) (PMVEMAC) and hydroxypropylmethyl cellulose phthalate (HPMCP) expanded by argon plasma-irradiation so that it floated on the dissolution test solution (pH 1.2) surface for more than 13 hours. Furthermore, the profile of drug release showed the sustained release pattern. It is considered that the expansion of tablet and the sustained drug release result mainly from decarboxylation and/or dehydration of PMVEMAC inside of tablet by plasma heat flux and the plasma-induced cross-link reaction of HPMCP on the surface of tablet, respectively. These results provide the basis for the preparation of Floating Drug Delivery System by plasma-irradiation.

[*Chem. Lett.*, **2002**, 652-653 (2002)] [Lab. of Pharm. Anal. Chemistry]

Amperometric Detection of Endocrine Disruptive Alkylphenolic Compounds Based upon the Redox Recyclization on an Alkyl Chain-Coated Electrode.

Bunji UNO,* Yoshiaki KATO

A sensitive detection method based upon continuous regeneration of the oxidized form of the ferrocenoyl phenols by reaction with ferrocyanide is proposed for HPLC analyses of endocrine disruptive alkylphenolic compds. The detection limits were 5.4×10^{-11} and 9.4×10^{-11} mol dm^{-3} of 4-*tert*-butylphenol and 4-nonylphenol, respectively. The detection limit of the method is 100–1000 times higher than that of the published HPLC methods, and is on the same level as that of the GC-MS methods. The proposed method is promising in that it provides conventional HPLC trace analysis of endocrine disruptive alkylphenolic compounds and serves for the construction of their electrochemical sensor.

[*Anal. Sci.*, **18**, 685-687 (2002)] [Lab. of Pharm. Anal. Chemistry]

Simultaneous Determination of Endocrine Disruptive Alkylphenolic Compounds as Dansyl Derivatives.

Bunji UNO,* Yoahiaki KATO, Shigeru MIWA

A simple and highly sensitive assay method for simultaneous detrmination.of endocrine disruptive alkylphenolic compounds as dansyl derivatives for fluorescent detection has been developped. The detection limit of the fluorescent detection involving the dansylation is several times higher than that of the direct fluorescent detection (4.5×10^{-8} mol/L). We believe that the proposed method is a simple and highly sensitive one for simultaneous determination of a trace amount of alkylphenolic compounds in environmental and biological samples, coupled with the sample preparation techniques published.

[*Nippon Kagaku Kaishi.*, **2002**, 289-300 (2002)] [Lab. of Pharm. Anal. Chemistry]

Intermolecular Charge-Transfer Interaction and Molecular Complex Formation of the Electrogenerated Organic Dianions.

Noriko OKUMURA, Bunji UNO*

Recent developments in the research on charge-transfer (CT) complex formation based on molecular recognition of organic π-dianions are described. It has been found that the hydrogen bonding of the quinine dianions is contributed from the strong n–σ charge transfer interaction and the TCNE and chloranil dianions form the π–π charge-transfer complexes characterized by the geometrical and chromatic proterties. These results are important for extended discussion on the function of biological quinones as a charge separator and the development of highly designed, redox-mediated recognition systems involving the electrogenerated π-dianions.

[*Talanta*, **57**, 481-490 (2002)] [Lab. of Pharm. Anal. Chemistry]

Anthraquinone-2-sulfonyl Chloride: A New Versatile Derivatization Reagent–Synthesis Mechanism and Application for Analysis of Amines.

Fang FENG, Bunji UNO,* Masashi GOTO, Zhengxi ZHANG, Dengkui AN

A new sulfonating agent, anthraquinone-2-sulfonyl chloride, was synthesized. The mechanism about the synthetic reaction was 1st elucidated in aid of mass spectrometry. Several primary and secondary amines were selected to evaluate the new reagent and their standard derivatives were prepered via a facile pathway. Analitical derivatization carried on through a 1-step procedure at room temperature within 3 min. The new reagent reacts quantitatively with amines to form stable sulfonamides, which are readily amenable to analysis with normal-phase and reversed-phase HPLC. Compared with standard derivatives, excellent response linearity is demonstrated over the concentration range 0.4-400 μM at 320 nm for normal-phase HPLC and 4 nM to 4 μM at 256 nm for reversed-phase HPLC. Detection limits are 0.8 nmol and 8 pmol, respectively.

[*Sepu*, **20**, 486-492 (2002)] [Lab. of Pharm. Anal. Chemistry]

Evaluation of Anthraquinone-2-sulfonyl Chloride for Determination of Phenol in Water by Liquid Chromatography Using Pre-Column Phase-Transfer Catalysed Derivatization.

Fang FENG, Bunji UNO,* Masashi GOTO, Zheng-xing ZHANG, Deng-kui AN

A new reagent, anthraquinone-2-sulfonyl chloride (ASC), has been used for the derivatization of phenols. Several componds with different polarity were selected to evaluate the new reagent and derivatives of these phenols prepared via a facile pathway. The optimal conditions for analytical derivatization and mechanism of the derivatization reaction are discussed. The derivatization procedure involves an ion-pair extraction of the deprotonated phenols with a tetrabutylammonium counter ion to an organic phase. At the interface of two phases, the derivatization reaction occurs quantitatively at room temperture within 3 min. The derivatives are stable and readily amenable to analysis with normal-phase and reversed-phase HPLC. Excellent Linearity response was demonstrated over the concentration range of 0.2–200 μmol/L.

[*J.Chromatogr.A*, **979**, 91-96 (2002)] [Lab. of Pharm. Anal. Chemistry]

Analysis of Active Chemical Species Generated by Electrolysis Using Non-aqueous Capillary Electrophoresis; Detection of the Anion Radical and the Divalent Anion of Tetacyanoquinodimethane.

Yukihiro ESAKA*, Noriko OKUMURA, Bunji UNO, Masashi GOTO

We have investigated detection of the anion radical and the divalent anion of tetracyanoquinodimethane (TCNQ) by acetonitrile-CE under an anaerobic condition. With electrolysis at a potential of 0.0 V (vs. Ag/AgCl), an acetonitrile solution of TCNQ turned green being characteristic of the TCNQ anion radical ($TCNQ^-$). Only one peak of the anionic compound was observed in CE of the electrolysis solution and it should be that of $TCNQ^-$. Then, the electrolysis potential was shifted to −0.8 V expected to be sufficient potential for further reduction of $TCNQ^-$, and the solution turned almost colorless. In CE analysis of the latter solution, another anionic component possessing a larger electrophoretic mobility than that of $TCNQ^-$ was detected, and it was decomposed immediately under aerobic conditions. This product was strongly suggested to be the divalent anion of TCNQ, and the present method would contribute notably to detection of the unstable species.

[*Pharm Res.*, **19**, 1439-1445 (2002)] [Lab. of Pharm. Engineering]

Improved inhalation behavior of steroid KSR-592 in vitro with Jethaler by polymorphic transformation to needle-like crystals (beta-form).

Kazuhiko IKEGAMI, Yoshiaki KAWASHIMA*, Hirofumi TAKEUCHI, Hiromitsu YAMAMOTO, Nobuyuki ISSHIKI, Den-ichi MOMOSE, Kiyohisa OUCHI

The aim of the present study was to improve the dry powder inhalation behavior of steroid KSR-592 with lactose by altering the crystal shape and the particle size of the drug for use in a newly designed inhalation device, Jethaler. The shape of the crystals was changed by polymorphic transformation of original crystal (alpha-form) to beta-form by agitating alpha-form crystals in hexane containing 5% ethanol. Needle-like crystals (beta-form) were obtained by the polymorphic transformation, the kinetics of which was described by the Avrami equation. The beta-form crystals loaded on lactose particles were easily separated and crushed into fine particles in the airstream produced in the Jethaler, which increased dramatically the respirable fraction deposited in the twin impinger and the fine particle fraction of the cascade impactor compared with their values for the original crystals.

[*Chem Pharm Bull.*, **50**, 1430-1433 (2002)] [Lab. of Pharm. Enginnering]

Determination of optimum processing temperature for transformation of glyceryl monostearate.

Toshio YAJIMA, Shigeo ITAI, Hirofumi TAKEUCHI, Yoshiaki KAWASHIMA*

The purpose of this study was to clarify the mechanism of transformation from alpha-form to beta-form via beta'-form of glyceryl monostearate (GM) and to determine the optimum conditions of heat-treatment for physically stabilizing GM in a pharmaceutical formulation. From a strong correlation observed between the beta-form content in the mixture of alpha-form and beta-form and the enthalpy change, beta-form content was expressed as a function of the enthalpy change. An inflection point existed in the time course of transformation of alpha-form to beta-form. Based on this aspect, the transformation rate equations were derived as consecutive reaction. Experimental data coincided well with the theoretical curve. In conclusion, GM was transformed in the onsecutive reaction, and 50 degrees C was the optimum heat-treatment temperature for transforming GM from the alpha-form to the stable beta-form.

[*Pharm Res.*, **19**, 403-410 (2002)] [Lab. of Pharm. Engineering]

Prolonged anti-inflammatory action of DL-lactide/glycolide copolymer nanospheres containing betamethasone sodium phosphate for an intra-articular delivery system in antigen-induced arthritic rabbit.

Eijiro HORISAWA, Tsuyoshi HIROTA, Satoko KAWAZOE, Jun YAMADA, Hiromitsu YAMAMOTO, Hirofumi TAKEUCHI, Yoshiaki KAWASHIMA *

The objective of the present study was to develop prolonged anti-inflammatory action of DL-lactide/glycolide copolymer (PLGA) nanosphere incorporating a water-soluble corticosteroid (betamethasone sodium phosphate; BSP). Another aim was to demonstrate the biocompatibility and biologic efficacy of these BSP-loaded nanospheres directly administered into ovalbumin-induced chronic synovitis in the rabbit. BSP-loaded nanospheres were prepared by an emulsion solvent diffusion method in oil. Direct intra-articular injection of a PLGA nanosphere system with a water-soluble steroid provided a prolonged pharmacological efficacy in the joints of arthritic rabbits. The local anesthetic in the knee-joints was evaluated to be safe and without biologic damage.

[*Pharm Res.*, **19**, 132-139 (2002)] [Lab. of Pharm. Engineering]

Size-dependency of DL-lactide/glycolide copolymer particulates for intra-articular delivery system on phagocytosis in rat synovium.

Eijiro HORISAWA, Katsuaki KUBOTA, Izumi TUBOI, Keiichi SATO, Hiromitsu YAMAMOTO, Hirofumi TAKEUCHI, Yoshiaki KAWASHIMA*

The present study evaluated the size-dependency of DL lactide/glycolide copolymer (PLGA) particulates for an intra articular delivery system on phagocytosis in the rat synovium after administering directly into the joint cavity. We also investigated the biocompatibility of PLGA particulate systems administered directly into the joint cavity of the rat. Fluoresceinamine bound PLGA (FA-PLGA) nanospheres and microspheres were prepared by the modified emulsion solven diffusion method. PLGA nanospheres should be more suitable for delivery to inflamed synovial tissue than microspheres due to their ability to penetrate the synovium. PLGA particulate systems with biocompatibility in the joint can provide local-therapy action in joint disease in a different manner depending on the size of the system.

[*Int J Pharm.*, **247**, 69-77 (2002)] [Lab. of Pharm. Engineering]

Moisture induced polymorphic transition of mannitol and its morphological transformation.

Tomohiro YOSHINARI, RT FORBES, Peter YORK, Yoshiaki KAWASHIMA*

The effects of moisture on the polymorphic transition of crystalline mannitol were investigated. Mannitol has three polymorphic forms, and was classified as alpha, beta, and delta form. The water uptake of delta form crystalline was greater than that of the beta form when each crystalline form was stored at 97%RH. The different powder X-ray diffraction patterns obtained before and after humidification confirmed that a moisture induced polymorphic transition from the delta to beta form had occurred. Morphological changes were also observed with an increase in the specific surface area of the delta sample from 0.4 to 2.3 m(2)/g being found on exposure to humidity. Thus it was suggested that the observed higher hygroscopicity of the newly formed beta form arose from the gradual increase in the surface area with the polymorphic transition from the delta to beta form.

[*Yakuzaigaku.*,**62**, 189-202 (2002)] [Lab. of Pharm. Engineering]

Size-Dependency of Copoly (DL-Lactic/Glycolic Acid) Carrier Particles In Vitro Cytotoxicity, Phagoeytosis and the Research of Local Therapeutic Action.

Eijiro HORISAWA, Katsuhide IWASAKI, Kohshi HIJIKURO, Hiroshi TAKEI, Hiromitsu YAMAMOTO, Hirofumi TAKEUCHI, Yoshiaki KAWASHIMA*

Safety and efficacy information is necessary for drug delivery systems that use particulate systems prepared with copoly (DL-lactic/glycolic acid) (PLGA). To investigate the size-effect of PLGA particulate systems, the present study first evaluated the in vitro cytotoxicity, and then assessed the prolonged efficiency of lidocaine-loaded PLGA nanospheres. The fluorescence microscopy method was used to identify the phagocytosed PLGA partic-ulate systems by cultured L929 cells. These in vitro cytotoxicity tests showed that PLGA microspheres were safer than PLGA nanospheres. To confirm the pharmacological effect of lido-caine-loaded PLGA particulate systems, localized pain-responses were assessed using the subcutaneously administered guinea pig model. Lidocaine-loaded nanospheres were found to provide remarkably prolonged anesthetic effects compared with lidocaine solution.

[*Int J Pharm.*, **241**, 203-211 (2002)] [Lab. of Pharm. Engineering]

The role of the kneading paddle and the effects of screw revolution speed and water content on the preparation of solid dispersions using a twin-screw extruder.

Kouichi NAKAMICHI, Tomio NAKANO, Hiroyuki YASUURA, Shogo IZUMI, Yoshiaki KAWASHIMA*

The twin-screw hot-melt extrusion process is useful for preparing solid dispersions which can improve the dissolution and absorption of drugs. The kneading paddle elements of the screws play an important role in changing the crystallinity and dissolution properties of a solid dispersion of kneaded nifedipine-hydroxypropylmethylcellulose phthalate. Slow revolution of the screws and the addition of a suitable amount of water to the mixture increased the rate of drug dissolution, although no super-saturation occurred. As the kneading paddle elements can retain the mixture in the machine for a longer period under intense shear, desired solid dispersions can be prepared routinely irrespective of the operating conditions. Moreover, a capillary rheometer can be useful to predetermine the amount of water added and the temperature for the preparation of solid dispersions using a twin-screw extruder.

[*Chem.Pharm. Bull.*, **50**, 147-152 (2002)] [Lab. of Pharm. Engineering]

Method of Evaluation of the Bitterness of Clarithromycin Dry Syrup.

Toshio YAJIMA, Yumiko FuKUSHIMA, Shigeru ITAI, Yoshiaki KAWASHIMA*

The degree of bitterness of clarithromycin (CAM) dry syrup was evaluated using several methods. Using the inversion method, shaking method, and paddle method, a reasonable correlation between the bitter taste and the amount dissolved was not observed. The release rate of CAM in test solution was then measured to evaluate bitterness. The release rate of CAM in the release test using the mini-column correlated well with the results of a sensory test for the bitterness of CAM dry syrup. The dissolution rate constant, defined as the percentage of CAM dissolved from the unit void surface multiplied by the void volume, was inversely proportional to the linear velocity of the test solution. The optimum linear velocity and void volume were 0.048-0.021 cm/min and 0.27-0.12 cm^3 respectively. The threshold of bitterness of CAM dry syrup was defined as the concentration in the sensory test. This threshold was found to be 135 pg/ml using the mini-column.

[*Powder Technol.*, **126**, 266-274 (2002)] [Lab. of Pharm. Engineering]

Primary crystal growth during spherical agglomeration in liquid: designing an ideal dry powder inhalation system.

Kazuhiko IKEGAMI, Yoshiaki KAWASHIMA*, Hirofumi TAKEUCHI, Hiromitsu YAMAMOTO, Nobuyuki ISSHIKI, Den-ichi MOMOSE, Kiyohisa OUCHI

Spherical agglomerates of steroid KSR-592, consisting of fine primary drug crystals suitable for dry powder inhalation (DPI), were prepared. It was found that the particle size of primary crystals increased until the dispersing medium was saturated with the bridging liquid introduced to the system, whereas the spherical agglomeration of primary crystals continued even after the saturation. The growth rates of primary crystals and agglomerates increased with an increase in the temperature and/or a reduction in the agitation speed. The primary crystals were mechanically stronger than their agglomerates so that the agglomerates were disintegrated easily into the primary crystals, which retained their original size, under the shear force generated on being mixed with carrier particles for DPI.

[*Cancer Lett.*, **179**, 121-132 (2002)] [Lab. of Pharmaceutics]

Indipendent Variation in Susceptibilities of Six Different Mouse Strains to Induction of Pepsinogen-altered Pyloric Glands and Gastric Tumor Intestinalization by N-methyl-N-nitrosourea.

Masami YAMAMOTO, Chie FURIHATA, Toshiaki OGIU, Tetsuya TSUKAMOTO, Ken-ichi INADA, Kazuyuki HIRANO,* and Masae TATEMATSU

Strain differences in susceptibility regarding stomach carcinogenesis due to N-methyl-N-nirosourea were examined in males of six strains of mice: BALB, C57BL6, CBA, C3H, DBA/2, and ICR. The frequency of pepsinogen-altered pyloric glands (PAPGs), putative precancerous lesions, was highest in the BALB and lowest in the ICR mice. Incidences of adenocarcinomas at week 52 were 59.3% and 18.5%, respectively. Intestinal alkaline phosphatese-positive intestinal type cells were observed heterogeneously in some hyperplasias, adenomas, and adenocarcinomas consisting of gastric type cells. The data suggest a direct histogenetic role for the PAPGs, a useful preneoplastic marker lesion in mouse strains.

[*J. Biochem* ., **131**, 469-475 (2002)] [Lab. of Pharmaceutics]

Activation of Caspase-3, Proteolytic Cleavage of DFF and No Oligonucleosomal DNA Fragmentation in Apoptotic Molt-4 Cells.

Kazuhiro IGUCHI, Kazuyuki HIRANO,* and Ryoji ISHIDA

DNA fragmentation factor (DFF) is one of the endonucleases responsible for DNA fragmentation. Since an oligonucleosomal DNA Ladder is not induced in apoptotic Molt-4 cells, we investigated whether or no the absence of ladder formation is related to an inability of DFF endonucleases in the cells. Semiquantitative RT-PCR analysis showed that the mRNA level of DFF-40 and DFF-45 in Molt-4 cells was approximately the same, compared with in other cells, which exhibit different levels of the fragmentation in apoptosis. When Molt-4 cells were induced to undergo apoptosis by neocarzionostatin (NCS) treatment, both caspase-3 activation and DFF-45 cleavage were observed. Furthermore, DFF immunoprecipitated from Molt-4 cells exhibited DNA degradation activity. These results suggest that functional expression of DFF is not sufficient for the induction of DNA fragmentaion in Molt-4 cells..

[*J. Reprod. Dev.*,**48**, 461-468 (2002)] [Lab. of Pharmaceutics]

In Vitro Evaluation of Acrosomal Status and Motility in Rat Epididymal Spermatozoa Treated with α-Chlorohydrin for Predicting Their Fertilizing Capacity.

Masashi KATO, Sachiko MAKINO, Hitoshi KIMURA, Takao OTA, Tadakazu FURUHASHI, Yoichi NAGAMURA, and Kazuyuki HIRANO*

Rat cauda epididymal spermatozoa treated with a-chlorohydrin (CH) under conditions that support *in vitro* fertilization were used to evaluate the effects of acrosomal status and sperm motility in predicting their fertilizing capacity. Acrosomal status was assessed by FITC-Con A assay in combination with supvital stain using CAM and EthD-1. Sperm motility was also examined with a computer assisted sperm analysis (CASA) system at the same time points as acrosomal status. A time-related increase in the percentage of live acrosome lost sperm were observed in the control and 0.01 mM CH treated group, but a low percentage and no time-related increase in live acrosome lost sperm without degenerative acrosome loss in dead sperm were observed in the 0.1 and 1.0 mM CH treated groups. On the basis of these results, rat spermatozoa labeled with FITC-Con A combined wit CAM and EthD-1 can have their acrosomal status clearly distinguished.

[*J. Androl.*, **23**, 819-824 (2002)] [Lab. of Pharmaceutics]

High-Level Expression of Zinc Transporter-2 in the Rat Lateral and Dorsal Prostate.

Kazuhiro IGUCHI, Shigeyuki USUI, Takahiro INOUE, Yoshiki SUDIMURA, Masae TATEMATSU, and Kazuyuki HIRANO*

Zinc is present at high concentrations in the prostate gland, however, the zinc-retention system in the prostate remains obscure. In this study, we investigated the expression of zinc transporters in the rat prostate and found that zinc transporter-2 (ZnT2), which sequeaters zinc to the lysosome-like compartment, is expressed at high levels in the lateral prostate (LP) and dosal prostate (DP), and that these areas contain higher levels of zinc than other tissues such as the ventral prostate (VP), liver, and kidney. Zinc levels in LP from castrated rats were lower than those in sham-operated rats. However, expression of ZnT2 in LP and DP was unaffected by castration. Expressio of other zinc transporters did not correlate with zinc levels. These results suggest that factors that regulate homeostasis other than zinc transportors are involved in lowering zinc content after castration in rat prostate.

[*Apoptosis*, **7**, 519-525 (2002)] [Lab. of Pharmaceutics]

Imidazole-Induced Cell Death, Associated with Intracellular Acidification, Caspase-3 Activation, DFF-45 Cleavage, But Not Oligonucleosomal DNA Fragmentation.

Kazuhiro IGUCHI, Shigeyuki USUI, Ryoji ISHIDA, and Kazuyuki HIRANO*

In this study, we found that in HL-60 cells imidazole induces cell death, associated with intracellular acidification, caspase-3 activation and DFF-45 cleavage, but not oligonucleosomal DNA fragmentation. A caspase inhibitor prevented cell death but not intracellular acidification. When pH*i* was neutralized by changing from imidazole-containing medium to fresh medium, oligonucleosomal DNA fragmentation and increased caspase-3 activity was observed in the imidazole-treated HL-60 cells. Furthermore, the DNA fragmentation induced by intracellular neutralization was inhibited by caspase inhibitor treatment. These results indicate that imidazole induces caspase-dependent cell death, and suggest that maintaining pH*i* in the neutral range is essential for the induction of oligonucleosomal DNA fragmentation in the execution phase of apoptosis.

[*Biol. Pharm. Bull.*, **25**, 1546-1549 (2002)] [Lab. of Hygienics]

Magnolol has the Ability to Induce Apoptosis in Tumor Cells.

Koji IKEDA and Hisamitsu NAGASE *

We previously found that magnolol inhibited tumor metastasis *in vivo* and the anti-metastatic effect was due to the inhibition of the tumor cell invasion. The purpose of this study was to clarify the inhibitory mechanism of magnolol on the growth of tumor cells, and we expect that magnolol may have the ability to induce apoptosis in tumor cells. In an *in vitro* proliferation assay, 100 μ M of magnolol inhibited the proliferation of B16-BL6, THP-1, BAE and HT-1080 cells, but 30 μ M of magnolol did not affected cells proliferation. In addition, 100 μ M of magnolol induced apoptotic cell death within 24 hr in three tumor cell lines, B16-BL6, THP-1 and HT-1080, not BAE cells, and then up-regulated the activity of caspase-3 and caspase-8. The up-regulation of caspases activity by 100 μ M of magnolol was suppressed by the inhibitor of all caspases, z-VAD-fmk. These data suggest that magnolol possesses ability to inhibit tumor growth, and the ability is due to the induction of apoptosis with the activation of caspases.

[*J. Liq. Chrom. Rel. Technol.*, **25**, 1283-1294 (2002)] [Lab. of Hygienics]

A Reverse-phase Thin-layer Chromatography/Scanning Densitometric Method for the Analysis of Red Cabbege Color in Food.

Yuko ITAKURA, Naoko OZEKI, Hisao OKA, Yuko ITO, Eiji UENO, Tomomi GOTO, Tomoko HAYASHI,Hitomi OHNO, Yasuhisa SASAKI, Masakuni MUKOYAMA, Hiroshi MATSUMOTO and Hisamitsu NAGASE*

A tequnique for the analysis of red cabbage color using reversed-phase TLC andscanning densitometory is described. The tequnique involves the following three steps. 1) clean up of the color with C18 cartridge, 2) separation of the colors on the reversed-phase C18-TLC using acetonitrile-0.2 mol/L trifluoroacetic acid (1:2) as the solvent system, and 3) measurement of visible absorption spectra of yje color using scanning densitometry without isolation of the color. In order to investigatethe capability of the present method, 45 commercial foods were analysed. The obtained separation and the spectra were not affected by coexisting substances in the foods, and spots always gave the same Rf values and spectra as the standards with good reproducibility.

[*Food Chem. Toxicol.*, **40**, 979-987 (2002)] [Lab. of Hygienics]

DNA Damage and the Effect of Antioxidants in Streptozotocin-treated Mice.

Aumune IMAEDA, Takeo KANEKO, Tomonori AOKI, Hisamitsu NAGASE *

We investigated that the effects of streptozotocin (STZ) on DNA damage in liver and kidney, as well as the protective effects of antioxidants, by using the alkaline single-cell gel electrophoresis assay and by measuring the ratio of 8-hydroxy-2'-deoxyguanosine (8-OHdG) to dG. A single intraperitoneal injection of STZ (150 mg/kg) increased serum levels of glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and blood urea nitrogen (BUN) and also caused DNA damage in liver and kidney, which recovered slowly with time. Antioxidants (ascorbic acid, trolox, and probucol) prevented the STZ-induced elevation of DNA damage in liver and kidney and inhibited the increase in serum levels of AST, ALT, and BUN. Thus the antioxidants protected the mice against STZ-induced DNA damage that might contribute to the development of hepatic or renal disease.

[*Food Chem. Toxicol.*, **40**, 1415-1422 (2002)] [Lab. of Hygienics]

Antioxidative Effects of Fluvastatin and Its Metabolites against DNA Damage in Streptozotocin-treated Mice.

Atsumune IMAEDA, Takeo KANEKO, Tomonori AOKI, Yasushi KONDO, Naoto NAKAMURA, Hisamitsu NAGASE *, Tosikazu YOSHIKAWA

Streptozocin (STZ) is a potential source of oxidative stress that induces genotoxicity. In order to elucidate the antioxidative effects of fluvastatin, a 3-hydroxy-3-methylgkutaryl coenzyme A reductase inhibitor, in vivo, we investigated the effects of 7-day treatment with flavastatin and its metabolites (M2, M3, and M4). Protective effects against DNA damage in the liver and kidnek from STZ-trated micewere assessed by the sigle-cell gel electrophoresis assay, and by detecting 8-Hydoroxy-2'-deoxyguanosine. A single intraperitoneal injection of STZ (150 mg/kg) inceased serum levels of glucose, asparutate aminotransferase (AST) , alanine aminotransferase (ALT) and blood urea nitrogen nitrogen (BUN), and also caused DNAdamage in the liver and kidney. Fluvastatin and its metabolites showed protective effects against DNA damage as potent as that of referrence antioxidants (ascorbic acid, trolox, and probucol). Flubastatin protected the mice against STZ-induced DNA damage, and may reduce the risk of oxidative stress *in vivo*.

[*Environ. Mutagen Res.*, **24**, 29-35 (2002)] [Lab. of Hygienics]

Antioxidative Effect of Fluvastatin and Its Metabolites in Cultured Humane Endothelial Cells Using Single Cell Gel Electrophoresis,.

Tomonori AOKI, Atsumune IMAEDA, Hisamitsu NAGASE *

In the present study, we investigated the antioxidative effects of fluvastatin, a 3-hydroxy-3-methylgkutaryl coenzyme A reductase inhibitor, and its metabolites (M2, M3, and M4) on oxidative DNA damage in cultured humane umbilical vein endothelial cells (HUVEC), as wellas the effects of other inhibitors of this enzyme, pravastatin and simvastatin. Single cell gell electrophoresis was used to evaluate the protective effecys of fluvaststin on reactive oxygen species (ROS)-induced DNA damage in HUVEC exposed to eithert-butylhydroperoxide or hydrogen peroxide. Fluvastatin and its metabolites showed protective effects on DNA damage as potent as the reference antioxidants ascorbic acid, trolox, and probucol. It was suggested that flavastatin did not exhibit clear protective effects. Fluvastatin may contribute to reduce ROS in humans and also ROS related diseases.

[*J. Mol. Biol.*, **318**, 985-997 (2002)] [Lab. of Hygienics]

Inhibition Mechanism of Cytokine Activity of Human Autocrine Motility Factor Examined by Crystal Structure Analyses and Site-Directed Mutagenesis Studies

Nobutada TANAKA, Arayo HAGA*, Hiroshi UEMURA, Hiroko AKIYAMA, Tatsuyoshi FUNASAKA, Hisamitsu NAGASE, Avraham RAZ, and Kazuo T. NAKAMURA

The crystal structures of the inhibitor-free open form and the inhibitor (erythrose 4-phosphate, E4P, a strong inhibitor of AMF¬s cytokine activity)-bound close form of human AMF have been determined at 1.9 Å and 2.4 Å resolution, respectively. Upon E4P binding, local conformation changes (open to close) occur around the inhibitor-binding site. The E4P-bound structure shows that the location of the inhibitor (of cytokine activity) binding site of human AMF is very similar to those of the inhibitor (of enzymatic activity) binding site of PHIs.

[*Biochem. Biophys. Res. Commun.*, **293**, 192-200 (2002)] [Lab. of Hygienics]

Tumor Autocrine Motility Factor Induces Hyperpermeability of Endothelial and Mesothelial Cells Leading to Accumulation of Ascites Fluid.

Tatsuyoshi FUNASAKA, Arayo HAGA*, Avraham RAZ, and Hisamitsu NAGASE

The response of endothelial or mesothelial cellular morphological alternation to AMF leads motile enhancement and vascular permeability. Tumor AMF induces gaps in a endothelial or mesothelial monolayer by stimulating a cellular movement, and accelerates the ascites accumulation. And treatment experiment with anti-AMF antibody succeeded in the reduction of the ascites accumulation, which renders AMF to the target molecule.

[*Int. J. Cancer*, **101**, 217-223 (2002)] [Lab. of Hygienics]

Autocrine Motility Factor Secreted by Tumor Cells Up-regulates Vascular Endothelial Growth Factor Receptor (Flt-1) Expression in Endothelial Cells.

Tatsuyoshi FUNASAKA, Arayo HAGA*, Avraham RAZ, and Hisamitsu NAGASE

The signaling of AMF-AMFR in the host endothelial cells induces expression of a vascular endothelial growth factor receptor (VEGFR) Flt-1 and AMFR feedback which is regulated at the transcriptional level. AMF-exposure stimulated the Flt-1 expression on human umbilical endothelial cells (HUVECs) surface, and this AMF-treated cells showed high-responsibility against VEGF.

[*Biol. Pharm. Bull.*, **25**, 441-445 (2002)] [Lab. of Biochemistry]

Substrate specificity of human 3(20)α-hydroxysteroid dehydrogenase for neurosteroids and its inhibition by benzodiazepines.

Noriyuki USAMI*, Tomohiro YAMAMOTO, Syunichi SHINTANI, Shuhei ISHIKURA, Yu HIGAKI, Yoshihiro KATAGIRI, Akira HARA

In this report, we compared kinetic constants and products in the reduction of the neurosteroids, 3α,5α-THP and 3α,5α-THDOC, and their precursors, 5α-DHP, 5α-DHDOC and progesterone, by three isoenzymes (AKR1C1, AKR1C2 and AKR1C3) of human 3α-hydroxysteroid dehydrogenase. The results suggest that AKR1C2 is involved in the neurosteroid synthesis, but AKR1C1 decreases the neurosteroid concentrations in human brain by inactivating 3α,5α-THP and eliminating the precursors from the synthetic pathways. In addition, we found that the several benzodiazepines inhibited the three isoenzymes noncompetitively with respect to the substrate. Although cloxazolam was a potent and specific inhibitor of AKR1C3, diazepam, estazolam, flunitrazepam, medazepam and nitrazepam, that inhibited AKR1C1 and AKR1C2, may influence the neurosteroid metabolism.

[*J. Biol. Chem.*, **277**, 17883-17891 (2002)] [Lab. of Biochemistry]

Molecular characterization of mammalian dicarbonyl/L-xylulose reductase and its localization in kidney.

Junichi NAKAGAWA, Shuhei ISHIKURA, Jun ASAMI, Tomoya ISAJI, Noriyuki USAMI, Akira HARA*, Takanobu SAKURAI, Katsuki TSURITANI, Koji ODA, Masayoshi TAKAHASHI, Makoto YOSHIMOTO, Noboru OTSUKA, Kunihiro KITAMURA

In this report, we first cloned a cDNA for a protein that is highly expressed in mouse kidney and then isolated its counterparts in human, rat hamster, and guinea pig by polymerase chain reaction-based cloning. The substrate specificity and kinetic constants of DCXRs for dicarbonyl compounds and sugars are similar to those of mammalian diacetyl reductase and L-xylulose reductase, respectively, and the identity of the DCXRs with these two enzymes was demonstrated by their co-purification from hamster and guinea pig livers and by protein sequencing of the hepatic enzymes. The results imply that P34H and diacetyl reductase are identical to L-xylulose reductase, which is involved in the uronate cycle of glucose metabolism, and the unique localization of the enzyme in kidney suggests that it has a role other than in general carbohydrate metabolism.

[*Acta Crystallogr. D Biol. Crystallogr.*, **58**, 1379-1380 (2002)] [Lab. of Biochemistry]

Crystallization and preliminary crystallographic analysis of human L-xylulose reductase.

Ossama EI-KABBANI, Roland P.-T. CHUNG, Shuhei ISHIKURA, Noriyuki USAMI, Jyunichi NAKAGAWA, Akira HARA*

Human L-xylulose reductase was crystallized from buffered polyethylene glycol solutions using the hanging-drop vapour-diffusion method. The crystals diffract to 2.1 Å resolution and belong to the orthorhombic P222 space group, with unit-cell parameters a = 72.9, b = 74.1, c = 87.9 Å. This is the first crystallization report of a L-xylulose reductase that is identical to diacetyl reductase.

[*Drug Metabol. Pharmacokin.*, **17**, 348-336 (2002)] [Lab. of Biochemistry]

Molecular charcterization of two monkey dihydrodiol dehydrogenases.

Yu HIGAKI, Takeshi KAMIYA, Syunichi SHINTANI, Shuhei ISHIKURA, Ikuo YAMAMOTO, Noriyuki USAMI, Akira HARA*

Japanese monkey liver contains multiple forms of DD with 3(20)α-HSD activity. The monkey DD1s and DD4s showed the highest sequence identity (94%) with AKR1C1 and AKR1C4, respectively, of four isoenzymes of human 3(20)α-HSD, which belongs to the AKR family. The substrate specificity and inhibitor sensitivity of the purified recombinant *Cynomolgus* monkey DD1 and Japanese monkey DD4 were also essentially identical to those of the recombinant AKR1C1 and AKR1C4, respectively, indicating that DD1 and DD4 are homologues of human AKR1C1 and AKR1C4, respectively. The results suggest a difference in the metabolism of steroids and xenobiotics mediated by 3(20)α-HSD isoenzymes between monkeys and humans.

[*Biol. Pharm. Bull.*, **25**, 318-322 (2002)] [Lab. of Pharmacology]

Effect of antiallergic drugs on interleukin 5-induced eosinophil infiltration of rat airways.

Toshiaki TAKIZAWA, Naoki KAWADA, Hiroyuki TANAKA and Hiroichi NAGAI*

Interleukin (IL)-5 is thought to play important roles in asthma and to be potential therapeutic target. An intratracheal injection of murine recombinant IL-5 (3-30 μg/animal) induced a dose-dependent increase in the number of eosinophils in the bronchoalveolar lavage fluid of Brown Norway (BN) rats 24h after administration. Bovine serum albumin (30 μg/animal), used as reference material, did not cause any change. The reaction was not observed in F344 rats. The increase in the number of eosinophils did not accompany with bronchial hyperreactivity in BN or F344 rats. Prednisolone (3-10 mg/kg, i.p.) and emedastine (30 mg/kg, p.o.) reduced the increased number of eosinophils induced by the IL-5 challenge. These results suggest that IL-5 is a potent inducer of eosinophils in the airway of BN rats. Prednisolone and emedastine are effective against IL-5-induced eosinophilia.

[*Biochem. Biophyls. Res. Commun.*, **292**, 689-696 (2002)] [Lab. of Pharmacology]

Arachidonate release and eicosanoid generation by group IIE phospholipase A_2.

Makoto MURAKAMI, Kumiko YOSHIHARA, Satoko AHIMBARA, Gérard LAMBEAU, Alan SINGER, Michael H. GELB, Masatsugu SAWADA, Naoki INAGAKI, Hiroichi NAGAI* and Ichiro KUDO

The heparin-binding group II subfamily of secretory phospholipase A_2s (sPLA$_2$s), such as sPLA$_2$s-IIA and –IID, augments stimulus-induced arachidonic acid (AA) release through the cellular heparan sulfate proteoglycan (HSPG)-dependent pathway when transfected into HEK293 cells. Here we show that the closet homolog, sPLA$_2$s-IIE, also promoted stimulus-induced AA release and prostaglandin production similar to those elicited by HSPG-dependent sPLA$_2$s. Confocal laser microscopic analysis demonstrated the location of sPLA$_2$s-IIE in cytoplasmic puncate compartments. sPLA$_2$s-IIE also enhanced leukotriene production and granule exocytosis by RBL-2H3 mastocytoma cells. Expression of sPLA$_2$s-IIE was highly upregulated in mice injected with lipopolysaccharide and in mice with experimental atopic dermatitis. These observations suggest that this enzyme plays a role in the inflammatory process, as proposed for other group II subfamily sPLA$_2$s.

[*J. Biol. Chem.*, **277**, 19145-19155 (2002)] [Lab. of Pharmacology]

Cellular arachidonate-releasing function and inflammation-associated expression of group IIF secretory phospholipase A_2.

Makoto MURAKAMI, Kumiko YOSHIHARA, Satoko AHIMBARA, Gérald LAMBEAU, Michael H. GELB, Alan G. SINGER, Masatsugu SAWADA, Naoki INAGAKI, Hiroichi NAGAI*, Motoko ISHIHARA, Yukiko ISHIKAWA, Toshiharu ISHII and Ichiro KUDO

We report the cellular arachidonate (AA)-releasing function of group IIF secretory phospholipase A_2 (sPLA$_2$-IIF), uniquely containing a longer C-terminal extension. sPLA$_2$-IIF increased spontaneous and stimulus-dependent release of AA. sPLA$_2$-IIF also enhanced interleukin 1-stimulated expression of cyclooxygenase-2 and microsomal prostaglandin E synthase. AA release by sPLA$_2$-IIF was facilitated by oxidative modification of cellular membranes. Cellular actions of sPLA$_2$-IIF occurred independently of the heparan sulfate proteoglycan glypican. The unique C-terminal extension was crucial for its plasma membrane localization and cellular functions. sPLA$_2$-IIF expression was increased in various inflammatory tissues of animal models and humans. These results suggest that sPLA$_2$-IIF is a potent regulator of AA metabolism and participates in the inflammatory process under certain conditions.

[*Eur. J. Biochem.*, **269**, 2698-2707 (2002)] [Lab. of Pharmacology]

Group IID heparin-binding secretory phospholipase A_2 is expressed in human colon carcinoma cells and human mast cells and up-regulated in mouse inflammatory tissues.

Makoto MURAKAMI, Kumiko YOSHIHARA, Satoko AHIMBARA, Masatsugu SAWADA, Naoki INAGAKI, Hiroichi NAGAI* , Mikihiko NAITO, Takashi TSURUO, Tae Churl MOON, Hyeun Wook CHANG and Ichiro KUDO

Group IID secretory phospholipase A_2 (sPLA$_2$-IID) augments stimulus-induced cellular arachidonate release in a manner similar to sPLA$_2$-IIA. Here we identified the residues of sPLA$_2$-IID that are responsible for heparanoid binding, and are therefore essential for cellular function. Mutating four cationic residues in the C-terminal portion of sPLA$_2$-IID resulted in abolition of its ability to associate with cell surface heparan sulfate and to enhance stimulus-induced delayed arachidonate release, cyclooxygenase-2 induction, and prostaglandin generation in 293 cell transfectants. sPLA$_2$-IID, but not other sPLA$_2$ isozymes, was expressed in human cord blood-derived mast cells. The expression of sPLA$_2$-IID was significantly altered in several tissues of mice with experimental inflammation. These results indicate that sPLA$_2$-IID may be involved in inflammation in cell- and tissue-specific manners.

[*Inflamm. Res.*, **51**, 307-316 (2002)] [Lab. of Pharmacology]

Time course study on the development of allergen-induced airway remodeling in mice: the effect of allergen avoidance on established airway remodeling.

Hiroyuki TANAKA, Taisei MASUDA, Shota TOKUOKA, Yoshimasa TAKAHASHI, Masato KOMAI, Koichi NAGAO and Hiroichi NAGAI*

We carried out a time course study on the development of allergen-induced airway remodeling in a murine model of allergic asthma. Moreover, we examined the effect of allergen avoidance on the established airway remodeling. The number of inflammatory leukocytes in the airways and the percentage of goblet cells in the epithelium, Th2 cytokine production, IgE production, collagen deposition beneath the basement membrane and bronchial responsiveness to acetylcholine were all markedly increased after repeated antigen challenge for 1-3 weeks. In contrast, after cessation of antigen exposure, goblet cell hyperplasia, inflammatory infiltrates and bronchial hyperresponsiveness were gradually attenuated and had almost resolved 4 weeks after cessation, but subepithelial fibrosis was still observed at this time point.

[*Allergol. Int.*, **51**, 197-203 (2002)] [Lab. of Pharmacology]

Role of protein kinase A in the inhibition of human mast cell histamine release by β-adrenergic receptor agonists.

Toshinobu KATO, Masahiro KIMATA, Toshikazu TSUJI, Michitaka, SHICHIJO, Masayuki MURATA, Toru MIURA, Isao SERIZAWA, Naoki INAGAKI and Hiroichi NAGAI*

The aim of the present study was to investigate the inhibitory mechanisms of β-adrenergic receptor agonists for human mast cell histamine release. Human cultured mast cells (HCMCs) were sensitized with human myeloma IgE and stimulated with antihuman IgE. Stimulation of MCs induced the elevation of intracellular cytosolic free Ca^{2+} concentrations ($[Ca^{2+}]i$) and the translocation of protein kinase C (PKC) from the cytosol to the cell membrane, followed by the release of stored histamine. Isoproterenol, salbutamol and dibutyryl cAMP inhibited both the histamine release and PKC translocation, whereas they failed to affect the elevation of $[Ca^{2+}]i$. H-89, a protein kinase A (PKA) inhibitor, abrogated the inhibition. The present results suggest that PKA activation induced by β-adrenergic receptor agonists plays a crucial role in inhibition IgE-mediated histamine release through suppressing PKC translocation.

[*Eur. J. Pharmacol.*, **448**, 175-183 (2002)] [Lab. of Pharmacology]

Involvement of unique mechanisms in the induction of scratching behavior in BALB/c mice by compound 48/80.

Naoki INAGAKI, Katsuhiro IGETA, Jon Fan KIM, Masafumi NAGAO, Noriko SHIRAISHI, Nobuaki NAKAMURA and Hiroichi NAGAI*

Compound 48/80 (48/80) induced scratching behavior (SB) in BALB/c mice, and the role of mast cell mediators in this behavior was examined. 48/80 increased the incidence of SB and scratching time in a dose-dependent manner. Dibucaine and μ-opioid receptor antagonists inhibited the SB. Although histamine H_1 receptor antagonists potently inhibited the vascular permeability increase, they did not affect the SB. Methysergide inhibited the SB slightly without affecting the vascular permeability increase, whereas cyproheptadine inhibited both. High doses of serotonin induced SB less frequently than did 48/80. Furthermore, mast cell-deficient mice exhibited frequent SB after injection of 48/80. These results clearly indicate that 48/80 can induce SB in mice independent of mast cell mediators.

[*Br. J. Pharmacol.*, **137**, 315-322 (2002)] [Lab. of Pharmacology]

Augmentation of allergic inflammation in prostanoid IP receptor deficient mice.

Yoshimasa TAKAHASHI, Shota TOKUOKA, Taisei MASUDA, Yosuke HIRANO, Masafumi NAGAO, Hiroyuki TANAKA, Naoki INAGAKI, Shu NARUMIYA and Hiroichi NAGAI*

To evaluate the role of prostaglandin I_2 (PGI_2) in allergic inflammation were studied in prostanoid IP receptor (IP) deficient mice (IP KO). The vascular leakage caused by passive cutaneous anaphylaxis, substance P and serotonin was markedly increased in the skin of IP KO, compared with comparably treated wild-type mice (WT). The inhalation of antigen in sensitized mice resulted in increased serum antigen specific IgE, total IgE and IgG levels. The magnitude of the elevations of each immunoglobulin level in IP KO is notably higher than that in WT. Moreover, antigen-induced IL-4 production by spleen cells from sensitized IP KO was almost 3 times greater than that in WT. On the contrary, the anti-CD3 antibody-induced interferon-γ production by $CD4^+$ T cells from non-sensitized IPKO was significantly lower than that in WT. These findings suggest a regulatory role of PGI_2 in allergic inflammation.

[*Pharmacology*, **67**, 21-31 (2002)] [Lab. of Pharmacology]

Effect of synthetic retinoid, TAC-101, on experimental autoimmune disease.

Naoki MIYAGAWA, Takeyasu HOMMA, Hiroyuki KAGECHIKA, Koichi SHUDO and Hiroichi NAGAI*

The effect of 4-[3,5-bis(trimethylsilyl)benzamido] benzoic acid (TAC-101), one of the synthetic retinoids, on collagen-induced arthritis (CIA) in mice and experimental autoimmune encephalomyelitis (EAE) in rats was studied. TAC-101 at doses of 5 and 20 mg/kg clearly inhibited the development of CIA in terms of the swelling of fore- and hind-limbs and bone destruction in knee joints. TAC-101 also suppressed the production of anti-type II collagen (CII) IgG antibody and delayed type hypersensitivity (DTH) against CII. In addition, TAC-101 delayed the onset and development of EAE but did not affect the maximum symptom of EAE in rats. The elevation of serum anti-myelin basic protein (MBP) antibody and DTH to MBP on day 13 were clearly suppressed by TAC-101 in EAE rats. Moreover, TAC-101 inhibited the IL-1β-induced PGE_2 production by MG-63 cells, human osteoblast-like cells, through the suppression of cyclooxygenase II mRNA expression. These findings suggest that TAC-101 inhibits CIA in mice and EAE in rats due to the suppression of immune response to auto-antigen and the production of PGE_2.

[*Blood*, **100**, 3861-3868 (2002)] [Lab. of Pharmacology]

Marked increase in CC chemokine gene expression in both human and mouse mast cell transcriptomes following Fcε receptor I corss-linking: an interspecies comparison.

Toshiharu NAKAJIMA, Naoki INAGAKI, Hiroyuki TANAKA, Akane TANAKA, Mamoru YOSHIKAWA, Mayumi TAMARI, Koichi HASEGAWA, Kenji MATSUMOTO, Hiroshi TACHIMOTO, Motohiro EBISAWA, Gozoh TSUJIMOTO, Hiroshi MATSUDA, Hiroichi NAGAI* and Hirohisa SAITO

We examined the expression of more than 10,000 distinct genes in human and mouse cultured mast cells (MC) using high-density oligo-nucleotide probe arrays to find molecules similarly regulated and expressed by the 2 MC types. After stimulation via high-affinity Fcε receptor I (FcεRI), the transcriptional levels of several CC chamokines were markedly increases, and I-309 (CCL1), macrophage inflammatory protein-1α (MIP-1α) (CCL3) and MIP-1β (CCL4) were found among the 10 most increased human and mouse transcripts from approximately 12,000 genes. Interspecies comparison studies at the whole genome expression level should be useful for the interpretation of experimental data obtained in animal models of human pathobiology.

[*Chem. Pharm. Bull.*, **50**, 1086-1090 (2002)] [Lab. of Pharmacognosy]

Regulation of Lithospermic Acid B and Shikonin Production in *Lithospermum erythrorhizon* Cell Suspension Cultures.

Hirobumi YAMAMOTO, Ping ZHAO, Kazufumi YAZAKI and Kenichiro INOUE *

Cell suspension cultures of *Lithospermum erythrorhizon* produced a large amount of lithospermic acid B, a caffeic acid tetramer, as well as shikonin derivatives (each ca. 10% of dry wt.) when cultured in shikonin production medium M-9. Various culture factors for increasing the production of lithospermic acid B were investigated. Lithospermic acid B production was inhibited by 2,4-D or NH_4^+, whereas it was stimulated by Cu^+. These regulatory patterns were similar to those for the production of shikonin derivatives in these cell cultures, suggestive of close relations and similar metabolic regulation between the production of these compounds. Cultivation under light illumination, however, showed that these metabolisms were independently regulated. In particular, blue light showed, a stimulatory effect on lithospermic acid B production, while shikonin production was strongly inhibited, indicative of an effective condition for lithospermic acid B production.

[*J. Food. Sci. Technol.*, 39, 345-352 (2002)] [Lab. of Pharmacognosy]

Formation of Red Pigment Produced from Geniposidic Acid and Amino Compound.

Nobuharu MORITOME, Kouichi NAKASHIMA, Kenichiro INOUE* and Tetsurou SHINGU

The formation of red pigment from geniposidic acid and an amino compound was studied by monitering its reaction. The double bond at C-7 in geniposidic acid was essential for the colouration and the hydroxymethyl group at C-8 was required for the development of a reddish colour. The key reaction of the red-colouration was decarboxylation of C-4 carboxyl group of geniposidic acid aglucon. It was suggested that the decarboxylated aglucon formed with evolution of carbon dioxide under acidic conditions reacted with primary amines under anaerobic conditions. The intermediates polymerized on heating subsequently and deepened bathochromically at the same time. The NMR spectrum of the red pigment implied that the pigment had heterogeneity and high molecularity.

[*Phytochemistry*,**60**, 263-267 (2002)] [Lab. of Pharmacognosy]

Origin of Two Isoprenoid Units in a Lavandulyl Moiety of Sophoraflavanone G from *Sophora flavescens* Cultured Cells.

Hirobumi YAMAMOTO, Ping ZHAO and Kenichiro INOUE *

Cell suspension cultures of *Sophora flavescens* produced large amounts of sophoraflavanone G, an 8-lavandulylated flavanone and lupalbigenin, a 6,3'-di-dimethylallylated isoflavone, by the simultaneous addition of cork tissues and methyl jasmonate. The labeling pattern of the isoprene units resulting after administration of [1-^{13}C] glucose into the cell cultures in the presence of the above additives revealed that two isoprene units in the lavandulyl group of sophoraflavanone G and two dimethylallyl groups of lupalbigenin were biosynthesized via the 1-deoxy-D-xylulose -5-phosphate pathway.

[*Plant Biotechnology*, **19**, 295-301 (2002)] [Lab. of Pharmacognosy]

Iridoid Biosynthesis: 7-Deoxyloganetic Acid 1-*O*-Glucosyltransferase in Cultured *Lonicera japonica* cells.

Hirobumi YAMAMOTO, Manyoung SHA, Yoshie KITAMURA,
Misako YAMAGUCHI, Nobuyuki KATANO and Kenichiro INOUE*

Iridoid 1-O-glucosylation enzyme activities of crude cell-free extracts prepared from loganin-producing plants and nonproducing cultured cells were compararatively examined. Crude cell-free extracts from *Lonicera japonica* cell suspension cultures glucosylated 7-deoxyloganetic acid, 7-deoxyloganetin, and loganetin, but not iridotrial, an intermediate just preceding 7-deoxyloganetic acid. Crude cell-free extractes from *Hydrangea macrophylla* young leaves also glucosylated 7-deoxyloganetin and loganetin, whereas those from cultured cells induced from iridoid-nonproducing plants did not show any iridoid glucosylation activity. The partially purified glucosyltransferse from *L. japonica* cells showed the highest glucosylation activity for loganetin. However, kinetic studies showed that *Km* values for 7-deoxyloganetic acid, 7-deoxyloganetin, and loganetin were 106 μM, 561 μM, and 660 μM, respectively, indicating that the enzyme had the highest affinity to 7-deoxyloganetic acid. These data suggest that the presence of a pathway for the biosynthesis of loganin, in which 7-deoxyloganetic acid is glucosylated at the 1-*O*-position to 7-deoxyloganic acid, which further hydroxylated and methylated to produce loganin.

[*Brain Res.*,**935**, 24-31 (2002)] [Lab. of Molecular Biology]

Alterations in hippocampal GAP-43, BDNF, and L1 following sustained cerebral ischemia.

Keiko MIYAKE, Wataru YAMAMOTO, Mina TADOKORO, NorioTAKAGI, Kyoko SASAKAWA , Atsumi NITTA,
Shoei FURUKAWA* and Satoshi TAKEO

Alterations in factors involved in the regeneration of the neuronal network in the hippocampus of rats with microsphere embolism (ME) were examined. Hematoxylin-eosin staining showed progressive and severe degeneration of the hippocampus after ME. The protein levels of BDNF, GAP-43, and L1 in the ipsilateral hippocampus of the ME animal, determined by Western blot analysis or enzyme immunoassay, were increased, unaltered, and decreased, respectively. In contrast, the immunohistochemical study showed increases in GAP-43, and BDNF, and a decrease in an L1. These results suggest that some factors for regeneration of the neuronal network in the ischemic penumbra responded to sustained cerebral ischemia for a certain period, although functional network of the nerve cells in the microsphere-injected hemisphere would be unlikely established after ME.

[*Neurosci Lett.*,**317**, 21-24 (2002)] [Lab. of Molecular Biology]

Brain-derived neurotrophic factor alters cell migration of particular progenitors in the developing mouse cerebral cortex.

Makoto OHMIYA, Toshihiro SHUDAI, Atsumi NITTA, Hiroshi NOMOTO,
Yoshiko FURUKAWA and Shoei FURUKAWA*

Effects of brain-derived neurotrophic factor (BDNF) on ventricular neural progenitor cell migration in developing mouse cerebral cortex were examined. BDNF was injected into the brain ventricle of 13- or 14-day-old embryos (E13 or E14) after the intraperitoneal administration of 5-bromodeoxyuridine (BrdU) to pregnant mice. BDNF injection at E13 increased the number of BrdU+ cells migrated into the CP until E15, and caused them to become localized in much deeper layers (V-VI) than expected (IV-V, as in the vehicle-treated mice) by postnatal day 1. However, when the injections were made at E14, BrdU+ cells predominantly migrated to layers II/III irrespective of BDNF administration. These results demonstrate that BDNF affects particular progenitors at limited stages, and suggest the presence of a Reelin-independent mechanism(s) to regulate cell migration.

[*J.Neurosci.Res.*,**70**, 335-339 (2002)] [Lab. of Molecular Biology]

4-Methylcatechol stimulates phosphorylation of Trk family neurotrophin receptors and MAP kinases in cultured rat cortical neurons.

Ayako SOMETANI, Hiroshi NOMOTO, Atsumi NITTA, Yoshiko FURUKAWA and Shoei FURUKAWA*

4MC stimulated tyrosine phosphorylation of various proteins of molecular weight from 10-300 kDa including Trks. The phosphorylation of MAPK/ERK was unaffected, however, in the presence of a protein synthesis inhibitor, suggesting that the effect of newly synthesized BDNF was negligible on this event. These results suggest that 4MC primarily activates multiple signal transduction molecules such as tyrosine kinases, including Trks. A significant increase in the survival rate of cortical neurons in the presence of 10 or 100 nM 4MC supported this idea, because the concentrations were much lower than those for stimulation of BDNF synthesis. Our results strongly suggest that the neurotrophic actions of 4MC found so far are mediated predominantly by direct activation of some intracellular signals including MAPK/ERK rather than by neurotrophin synthesis.

[*J.Neurosci.Res.*,**69**, 653-661 (2002)] [Lab. of Molecular Biology]

Role of low-affinity p75 receptor in nerve growth factor-inducible growth arrest of PC12 cells.

Hisanori ITO, Hiroshi NOMOTO and Shoei FURUKAWA*

Mutant PC12 cell clones (PC84 cells) were obtained by transfection with nerve growth factor (NGF) cDNA. The inhibition of TrkA by K252a diminished the short processes of PC84 cells but had no effect on their fast proliferation. The expression level of TrkA in PC84 cells was comparable to that in PC12 cells; whereas that of another NGF receptor, p75, was significantly lower. These data suggest that the decrease of p75 contributed to the continuous growth of PC84 cells, which was confirmed by suppressing p75 activity of PC12 cells with the antisense oligonucleotide of p75 or with anti-p75 neutralizing antibody. Our results suggest that NGF signaling via TrkA affects the differentiation characteristics of PC12 cells but that an additional signaling via p75 is necessary for the growth arrest of the cells.

[*J Neurol Sci.*,**198**, 63-69 (2002)] [Lab. of Molecular Biology]

Accumulation of nerve growth factor protein at both rostral and caudal stumps in the transected rat spinal cord.

Yutaka MURAKAMI, Shoei FURUKAWA*, Atsumi NITTA and Yoshiko FURUKAWA

Change in the NGF content in the rat spinal cord after traumatic spinal cord injury was examined by using a two-site EIA system and an immunohistochemical technique. The NGF level started to increase in the rostral and caudal stumps nearest to the injury site at 2 and 4 days, respectively. The NGF level of the caudal side returned to the original level by 2 weeks, but that of the rostral side remained high even 3 weeks, after the injury. At 4 days after the injury, NGF-like immunoreactivity in both stumps was predominantly localized in the axon-like structures of the white matter and in cells morphologically resembling immune cells. These observations suggest that the NGF was transported within the spinal tracts, and that NGF secreted from immune cells that had invaded into the injured spinal cord had accumulated around the transection site.

[*Neurotoxicol Teratol.*,**24**, 695-701 (2002)] [Lab. of Molecular Biology]

Diabetic neuropathies in brain are induced by deficiency of BDNF.

Atsumi NITTA, Rina MURAI, Norio SUZUKI, Hisanori ITO, Hiroshi NOMOTO, Gunshiroh KATOH, Yoshiko FURUKAWA, Shoei FURUKAWA*

Diabetes is known to be one of the risk factors for dementia; however, neuropathic changes in the brain of patients with the disease have not been completely revealed. So in the present study, we investigated the brain function of rats with diabetes induced by streptozotocin (STZ). In the diabetic rats, immediately working memory performance was impaired in the Y-maze task. Furthermore, morphological observation by Golgi staining showed a decrease in the number of basal dendrites and abnormality of spine structure. Next, we measured the content of brain-derived neurotrophic factor (BDNF) in the diabetic brain. In the diabetic brains, both protein and mRNA levels of BDNF were severely reduced. These results suggest that, in diabetes, synapse dysfunction is, at least in part, caused by a failure of BDNF synthesis in the brain.

[*Explore.*, **11**, 46-51 (2002)] [Lab. of Molecular Biology]

The Inducer of Synthesis of Nerve Growth Factor From Lion's Mane (*Hericium erinaceus*).

Hirokazu KAWAGISHI, Shoei FURUKAWA*, Cun ZHANG and Rika YUNOKI

Nerve growth factor (NGF) is closely related to Alzheimer's dementia, and studies have suggested that the disease may be prevented or its symptoms may be improved when NGF is given into the brain directly. However, since NGF is a protein it usually cannot pass through the blood-brain barrier. Recently, researchers have targeted on the substances that could pass through the blood-brain barrier and induce NGF synthesis in the brain. Some compounds with lower molecular weight have been found to have such bioactivity. Among these bioactive compounds, hericenones and erinacines, which were isolated from an edible mushroom called as Lion's Mane (Hericium erinaceus), showed remarkable activity of stimulating the synthesis of NGF. They could be developed as a dietary supplement or medicine to be used for treating Alzheimer's dementia. This article offers an introdection to the isolation meyhod, bioactivity assay and chemical structure analysis of hericenones and erinacines.

[*Cell Transplant.*, **11**, 459-464 (2002)] [Lab. of Molecular Biology]

Implantation of BDNF-producing packaging cells into brain.

Hidefumi FUKUMITSU, Sayaka TAKASE-YODEN, Shoei FURUKAWA*, Kiyomitsu NEMOTO
Tomio IKEDA and Rihito WATANABE

In order to invent a screening system to check in vivo gene function and the efficiency of gene transfer mediated by a retroviral vector system, we established a novel packaging cell, PacC6/A8, transplantable to rat brains. A vector that expresses BDNF tagged by c-Myc (LxA/bdmh) was constructed. After transfection of LxA/bdmh to PacC6/A8, a cloned cell line, PacC6/A8/bmh, was established. For a control, a retroviral cell line that expresses EGFP tagged by c-Myc was also created (PacC6/A8/gfmh). These cells were injected to the brain of newborn rats. A histological study revealed that the transferred BDNF gene was expressed in the brain injected with PacC6/A8/bmh cells, but not with PacC6/A8/gfmh cells. Interestingly, many activated microglia had migrated into the tumor induced by PacC6/A8/bmh cells, and expressed a high amount of BDNF.

[*Cell Transplant.*, **11**, 471-473 (2002)] [Lab. of Molecular Biology]

Cell transplantation to the brain with microglia labeled by neuropathogenic retroviral vector system.

Rihito WATANABE, Sayaka TAKASE-YODEN, Hidefumi FUKIMITSU* and Kazuyuki NAKAJIMA

A8 virus (A8-V) is a molecular clone of the neuropathogenic FrC6 virus. We constructed a gene transfer system with the A8-V gene. Pseudotyped virus carrying the surface protein of A8-V (A8-SU) transduced the beta-glactosidase gene incorporated in the retroviral vector efficiently to cultured microglial cells derived from newborn rats. Ex vivo gene transferred microglial cells were then injected into the right hemisphere of 3-day-old and 3-week-old rat brains. All of the rats examined at 4 weeks after the injection contained the labeled microglial cells in the brain (7/7 and 5/5 of the rats injected at 3 days and 3 weeks, respectively). None of the rats showed pathological changes in the whole body investigated, including the central nervous system, 4 weeks after transplantation of the labeled microglial cells.

[*Neuropathology.*, **22**, 280-289 (2002)] [Lab. of Molecular Biology]

Neuropathology of experimental autoimmune encephalomyelitis modified by retroviral infection.

Hidefumi FUKUMITSU*, Sayaka TAKASE-YODEN, Rihito WATANABE

The A8 virus is a molecular clone of the neuropathogenic FrC6 virus derived from the Friend murine leukemia virus (F-MuLV). To elucidate the effects of A8 virus-infection on immune-mediated diseases in the central nervous system, we investigated the development of acute and monophasic experimental autoimmune encephalomyelitis (EAE) in A8 virus-infected Lewis rats. In EAE rats after A8 virus infection (A8-EAE), many inflammatory cells were found in the gray matter including the frontal lobe, where almost no inflammatory cells were found in rats with EAE alone. The modified distribution of inflammatory cells was not dependent on the ages of A8 virus-infected rats, although the frequency of the modified distribution was reduced in older rats. The chimeric virus Rec2, which contains the pol and env genes of 57 virus on the background of A8 and does not induce spongiform degeneration in the CNS, caused the same distributional modification of inflammatory cells in the rats with EAE as in A8-EAE rats. Furthermore, the incidence and intensity of spongiform degeneration, thymoma and splenomegaly caused by A8 virus were reduced by the induction of EAE.

[*Organic Letters*, **4**, 355-357 (2002)] [Lab. of Microbiology]

Molecular Design and Biological Potental of Galacto-Type Trehalose as a Nonnatural Ligand of Shiga Toxins.

Hirofumi DOHI, Yoshihiro NISHIDA, Yuki FURUTA, Hirotaka UZAWA, Shin-ichiro YOKOYAMA, Saori ITO, Hiroshi MORI * and Kazukiyo KOBAYASHI

Galacto-type trehalose, a "C-4 epimer of trehalose", possesses a sterochemical structure around the α(1-1)-linkage analogous to that of the globosyl α(1-4)-linkage in Gb_2 and Gb_3 ceramides known as the ligands of Shiga toxins produced by enterohemorrhagic *Escherichia coli*. The glycoside α(1-1)-linkage is highly tolerant to enzymatic (mammalian α-glycosidase) degradation compared with glycosyl α(1-4)-linkages. Galacto-trehalose conjugated acrylamido copolymer inhibited biological activity of Shiga toxin 1. This paper presents evidence supporting the new idea of using a trehalosyl α(1-1)-linkage as a substiture for the galactobiosyl α(1-4)-linkage.

[*Biol. Pharm. Bull*, **25**, 986-990 (2002)] [Lab. of Microbiology]

New Rapid Enzyme-linked Immunosorbent Assay to detect Antibodies agaist Bacterial Surface Antigens Using Filtration Plates.

Saori ITOH, Masako KARIYA, Keiji NAGANO, Shin-ichiro YOKOYAMA, Toshio FUKAO, Yoshihiro YAMAZAKI and Hiroshi MORI*

A new ELISA system, Filtration ELISA, to detect antibodies against bacterial surface antigens was developed using a 96-well filtration plate fitted with a 0.22 μm membrane (MultiScreen®-GV, Millipore). The technique was evaluated by assaying antibodies to Shiga-toxin producing *E. coli* O157:H7 (STEC) and the other bacteria in fecal extracts of 157 children who had eaten school lunches contaminated with STEC in comparison with 25 age-matched control children. The lunch group showed significantly higher IgA antibody titers against STEC than the control group ($p<0.0005$), but not against *Lactobacillus acidphilus*. This technique is widely applicable to assay antibodies in various samples including serum and fecal extract against various kinds of bacteria.

[*Biomaclomolecules.*,**3**, 411-414 (2002)] [Lab. of Mocrobiology]

A quartz Crystal Microbalance Method for Papid Detection and Differentiation of Shiga Toxins by Applying a Monoalkyl Globobioside as the Toxin Ligand.

Hirotaka UZAWA, Shoko KAMIYA, Norihiko MINOURA, Hirofumi DOHI, Yoshihiro NISHIDA, Kazuhiro TAGUCHI, Shin-ichiro YOKOYAMA, Hiroshi MORI*, Toshimi SHIMIZU and Kazukiyo KOBAYASHI

A globobiosyl (Gb2) ceramide mimic carring a monoalkyl chain (C18) was applied for a monolayer Langmuir-Blodgett (L-B) technique to detect Shiga toxins (Stxs) by a quartz crystal microbalance (QCM) method. The glycolipid, synthesized from penta-*O*-acetyl-D-galactopyranose via a conventional glycosidation pathway, was developed for the monolayer film formation. The film was transferred onto a QCM cell surface modified with alkanethiols. Upon the addition of each Stx-1 and Stx-2, the decrease of frequency reached saturation within 45 min at few nanogram per quartz cell with little difference of Ka values. In the presence of an acrylamido Gb2 copolymer as a competitive inhibitor, the two toxins showed a large difference in the binding behavior to the L-B monolayer.

[*Natural Medicines*, **56**, 40-46 (2002)] [Lab. of Herbal Garden]

Studies on the Chinese Crude Drug "Luoshiteng"(3): The Plant Origins of Luoshiteng on the Market and their Identification.

Sansei NISHIBE,* Han Ying MEI, Yukari NOGUCHI, Eiji SAKAI and Toshihiro TANAKA

The identification for the plant origins of Lyoshiteng distributed on the market in Asia, North America and England were phytochemically investigated by HPLC method using the respective marker compounds. Luoshiteng of *Trachelospermum jasminoides* (Lindl.) Lem. (Apocynaceae) was clearly distinguishable from Luoshitengs of *Ficus pumila* L. (Moraceae) or of *Psychotria serpens* L. (Rubiaceae), respectively by the presence of apigenin 7-O-neohespheridoside as marker compound in HPLC of the leaf part, and presence of trachelogenin as marker compound in HPLC of the stem part. In addition to phytochemical examination, the plant origins of Luoshitengs on the market were also morphologically examined.

[*Natural Medicines*, **56**, 90-96(2002)] [Lab. of Herbal Garden]

Pharmacognostical Studies on the Genus Datura Plants (3): Morphological Characteristics of the Fruits and the Seeds.

Tomoko KAWAMURA,* Kazuyo OKUDA, Youichi HISATA, Yukio NORO, Eiji SAKAI, Kyouko OGAWA and Toshihiro TANAKA

A comparative anatomical study was carried out on the fruits and seeds of 14 Datura taxa belonging to the section Datuta 6 taxa, Stramonium 6 taxa and Brugmansia 2 taxa. The results showed that fruits of 14 taxa cold be distinguished from each other by the following characteristics: shape of the capsule and calyx; nodding or erect capsules; breaking irregularly or regularly into 4 parts at maturity; and spiny, tubercular or smooth on the outer surface. The seeds were characterized morphologically by shape, color and figure of the surface, and anatomically, size, of epidermal cell lumen, shape of basal and outer cell wall of epidermis, shape of superior processes of epidermal cell in surface view and others.

[*Natural Medicines*, **56**, 198-199 (2002 [Lab. of Herbal Garden]

Diversity of the Quality of Phellodendron Barks from Different Habitats (2): Content Variation of Limonoids.

Tomoko KAWAMURA,* Misato YOKOYAMA, Youichi HISATA, Kazuyo OKUDA, Yukio NORA, Shigeharu YAMAGUCHI, Toshihiro TANAKA, Sansei NISHIBE and Keiji WADA

Obakunon and limonin of the Phellodendron barks collected from various habitats were measured by HPLC. There were regional variations in limonoid content. Japanese barks showed the highest content value of obacunon, and barks from China and Korea mainly contained limonin. The barks from Northeast China, Korea and Hokkaido Japan contained a large amount of limonoids, however they contained less berberine than in the previous paper.

[*中药材*, **25**, 89-94 (2002)] [Lab. of Herbal Garden]

Study on Cross-section Morphology of Flos Chrysanthemi from Zhejiang Province and Comparison with Other Kinds of Flos Chrysanthemi.

Xu JUANHUA* and Toshihiro TANAKA

The cross-section morphology of each part of Hangbaiju was studied and compared with other 4 kinds of Flos Chrysnthemi. The characters of cross-section morphology of Hangbaiju were obtained. And it was found that cross-section morphology of the commodity Flos Chrysanthemi was different. It provided evidences for the identification of Hangbaiju with other 4 kinds of Flos Chrysanthemi.

[*Jpn. J.Cancer Res.*, **93**, 24-31 (2002)] [Lab. of Radiochemistry]

Enhancement by cigarette smoke exposure of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline-induced rat hepatocarcinogenesis in close association with elevation of hepatic CYP1A2.

Akiyoshi Nishikawa, Fumio Furukawa, Makoto Miyauchi, Hwa-Young Son, Kazushi Okazaki, Akihiro Koide, Yukio Mori* and Masao Hirose

The modifying effects of cigarette smoke (CS) on 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx)-induced carcinogenesis were investigated in male F344 rats. The development of GST-P^+ foci was significantly greater in rats treated with CS than in rats fed 300 ppm MeIQx alone for 16 weeks. The mean number of colonic aberrant crypt foci per animal was increased by CS exposure regardless of MeIQx feeding. The hepatic CYP1A2 level was remarkable increased by CS exposure for 16 weeks, and CS also increased the mutagenic activities of six HCAs including MeIQx compared to sham smoke control. Thus, our results clearly indicate that CS enhances hepatocarcinogenesis when given in the initiation phase via increasing intensity of metabolic activation for MeIQx and possibly colon carcinogenesis when given in the post-initiation phase in rats treated with MeIQx.

[*Oncology Reports*, **9**, 1069-1073 (2002)] [Lab. of Radiochemistry]

Weak enhancing effects of simultaneous ethanol administration on chemically induced rat esophageal tumorigenesis.

Masahiro Kaneko, Keiichirou Morimura, Takayuki Nishikawa, Hideki Wanibuchi, Harushi Osugi, Hiroaki Kinoshita, Akihiro Koide, Yukio Mori* and Shoji Fukushima

In order to clarify the effect of ethanol on *N*-nitrosomethylbenzylamine (NMBA)-induced rat esophageal tumorigenesis, animals were treated with 0.1 or 0.5 mg/kg NMBA with 10% ethanol in the drinking water for 5 and 24 weeks. In 0.1 mg/kg NMBA-initiation groups, the incidence of esophageal papillary hyperplasias was significantly increased by 24-week ethanol administration. Ethanol showed a tendency to increase the incidence and multiplicity of tumors and the multiplicity of papillary hyperplasias induced by 0.5 mg/kg NMBA. Proliferating cell nuclear antigen positive indices tended to be increased in tumors by 5- and 24-week ethanol administration, but cyclin D1 expression was not affected. These data suggest that simultaneous ethanol administration have weak enhancing effects, and also promoting effects in post-initiation phase on NMBA-induced rat tumorigenesis.

[*Mutagenesis*, **17**, 251-256 (2002)] [Lab. of Radiochemistry]

Effect of ethanol treatment on metabolic activation and detoxification of esophagus carcinogenic *N*-nitrosamines in rat liver.

Yukio Mori,* Akihiro Koide, Yoshinori Kobayashi, Keiichirou Morimura, Masahiro Kaneko and Shoji Fukushima

A mechanism underlying enhancement by ethanol of *N*-nitrosodiethylamine (DEN)- or *N*-nitrosomethylbenzylamine (NMBA)-induced esophageal tumorigenesis was investigated in F344 rats. Ethanol treatment (10% in the drinking water) induced hepatic CYP2E1, but not CYP2B1/2, 1A1/2 and 3A2, while NMBA treatment (0.5 mg/kg) showed no significant alterations on levels of these CYP species. Ethanol also elevated mutagenic activities of *N*-nitrosodimethylamine, DEN and *N*-nitrosopyrrolidine up to 2.1-, 1.6- and 2.3-fold above each control, respectively, but not four other *N*-nitrosamines including NMBA. However, ethanol did not affect UDPGT activities towards 4-nitrophenol, bilirubin and testosterone. Consequently, this suggests that enhancement by ethanol of DEN-induced esophageal carcinogenesis can be attributed to increase in the hepatic activation but that of the NMBA-induced tumorigenesis is not attributable to both metabolic activation and inactivation via glucuronidation in liver.

[*Biol. Pharm. Bull.*, **35**, 147-148 (2002)] [Lab. of Instrumental Center]

Vaticanol C, a Novel Resveratrol Tetramer, Inhibits Cell Growth through Induction of Apoptosis in Colon Cancer Cell Lines.

Tetsuro ITO, Yukihiro AKAO, Toshiyuki TANAKA, Munekazu IINUMA* and Yoshinori NOZAWA

A novel resveratrol tetramer, vaticanol C, isolated from the stem bark of *Vatica rassak* (Dipterocarpaceae) markedly suppressed cell growth through induction of apoptosis, which was characterized by nuclear changes and DNA ladder formation, in three different human colon cancer cell lines. The findings in the current study suggest the possible chemopreventive and chemotherapeutic ability of vaticanol C.

[*Chem. Pharm. Bull.*, **50**, 796-801 (2002)] [Lab. of Instrumental Center]

Stilbene Derivatives from Two Species of Gnetaceae.

Ibrahim ILIYA, Toshiyuki TANAKA, Munekazu IINUMA,* Zulfiqar ALI, Miyuki FURUAWA, Ken-ichi NAKAYA, Yoshiaki SHIRATAKI, Jin MURATA and Dedy DARNAEDI

Five new stilbene oligomers (gnemonols A, B and C, gnemonoside E and gnetol) were isolated together with 2b-hydroxylampelopsin F and gnetin E from *Gnetum gnemon* and *G. gnemonoides* (Gnetaceae). Gnemonol A is a trimer of resveratrol, and gnemonols B and C are tetramers of resveratrol. Gnemonoside E was characterized as ε-viniferin 4a,4b-*O*-β-diglucopyranoside. The structures were elucidated on the basis of spectral evidence.

[*Heterocycles*, **57**, 1057-1062 (2002)] [Lab. of Instrumental Center]

Dimeric Stilbenes from Stem Lianas of *Gnetum africanum*.

Ibrahim ILIYA, Toshiyuki TANAKA, Munekazu IINUMA,* Zulfiqar ALI, Miyuki FURUSAWA and Ken-ichi NAKAYA

Two new stilbene dimers, gneafricanins A, B and bisisorhapontigenin B were isolated from the stem lianas of *Gnetum africanum* (Gnetaceae) along with eight known stilbenoids: longusol A, gnetin C, gnetin D, gnetin E, gnetofolin E, gnetol, isorhapontigenin and resveratrol. Gneafricanin A was composed of an oxyresveratrol unit and an isorhapontigenin unit. Bisisorhapontigenin B previously synthesized by oxidative coupling of isorhapontigen was first isolation from nature.

[*J. Health Sci.*, **48**, 273-276 (2002)] [Lab. of Instrumental Center]

Antibacterial Activity of Extracts Prepared from Tropical and Subtropical Plants on Methicillin-Resistant *Staphylococcus aureus*

Tomoto NITTA, Takashi ARAKI, Hiromu TAKAMATSU, Yuka INATOMI, Hiroko MURATA, Munekazu IINUMA,* Toshiyuki TANAKA, Teturo ITO, Fujio ASAI, Iliya IBRAHIM, Tsutomu NAKANISHI and Kazuhitao WATANABE

The antibacterial activity of the extracts prepared from 181 species (75 families) of tropical and subtropical plants was screened against various types of pathogenic bacteria. Among the 505 extracts tested, 53 of them inhibited the growth of methicillin-resistant *Staphylococcus aureus* (MRSA). The active extracts obtained from barks of *Shorea hemsleyana* and roots of *Cyphostemma bainessi* were separated to their components, some of which greatly reduced the viable cell number of MRSA. These active compounds were all identified as stilbene derivatives. Hemsleyanol D, one of stilbene tetramer isolated from *S. hemsleyana*, was the most effective compound and has MIC of 2μg/ml.

[*Heterocyles*, **57**, 1507-1512 (2002)] [Lab. of Instrumental Center]

Four Dimeric Stilbenes in Stem Lianas of *Gnetum africanum*.

Ibrahim ILIYA, Toshiyuki TANAKA, Munekazu IINUMA,* Zulfiqar ALI, Miyuki FURUSAWA, Ken-ichi NAKAYA, Nobuyuki MATSUURA and Makoto UBUKATA

From the stem lianas of *Gnetum africanum* Welw. (Gnetaceae), four new stilbenoid dimers (gneafricanins C, D, E and F) were isolated along with four known compounds. The structure of gneafricanin F is composed of two isorhapontigenin units in symmetrical form. The occurrence of gneafricanins D and E in this species is of chemotaxonomic importance as to the similarity of *Welwitschia*. Gneafricanins C, D and E showed inhibition in lipid peroxide and scavenging super oxide.

[*Nat. Med.*, **56**, 139-142 (2002)] [Lab. of Instrumental Center]

Stilbene Oligomers in Roots of *Sophora moorcroftiana*.

Yoshiaki SHIRATAKI, Toshiyuki TANAKA, Masayoshi OHYAMA, Shizuo TODA and Munekazu IINUMA*

Further investigation of chemical constituents in roots of *Sophora moorcroftiana* (Leguminosae) afforded five resveratrol oligomers including a new compound. The new one named sophorastilbene A is a resveratrol trimer with a nine-member ring. As known compounds, (-)-ε-viniferin, (+)-α-viniferin, miyabenol C and *cis*-miyabenol C were identified.

[*Helv. Chim. Acta*, **85**, 2394-2402 (2002)] [Lab. of Instrumental Center]

Five Stilbene Glucosides from *Gnetum gnemonoides* and *Gnetum africanum*.

Ibrahim ILIYA, Toshiyuki TANAKA, Munekazu IINUMA,* Miyuki FURUSAWA, Zulfiqar ALI, Ken-ichi NAKAYA, Jin MURATA and Dedy DARNAEDI

Five new stilbene glucosides, gnemonosides F, G, H, I and J, were isolated from the stem lianas of *Gnetum gnemonoides* Brongn and *Gnetum africanum* Wele along with nine known stilbenoids. The structures of the new compounds were elucidated as gnetin E 4a,4b-*O*-β-triglucopyranoside, gnetin E 4a,4b-*O*-β-diglucopyranoside, gnetin C 4a,4b,11a-*O*-β-triglucopyranoside, gnetin D 4a,4b-*O*-β-diglucopyranoside and gnetuhainin A 4a,4b-*O*-β-diglucopyranoside, respectively on the basis of spectroscopic evidence.

[*Helv. Chim. Acta*, **85**, 2538-2546 (2002)] [Lab. of Instrumental Center]

Four New Stilbene Oligomers in the Root of *Gnetum gnemon*

Ibrahim ILIYA, Zulfiqua ALI, Toshiyuki TANAKA, Munekazu IINUMA,* Miyuki FURUSAWA, Ken-ichi NAKAYA, Jin MURATA and Dedy DARNAEDI

Four new stilbene oligomers, gnemonols G, H, I and J, were isolated from acetone extract of the root of *Gnetum gnemon* (Gnetaceae) along with five known stilbenoids, ampelopsin E, *cis*-ampelopsin E, gnetins C, D and E. Gnemonol G is a resveratrol dimer with two furan rings and gnetins H-J are resveratrol trimers with a seven-member ring. *cis*-Ampelopsin E is the first instance of an enatiomer isolated from nature, which has been isolated as a racemate from Vitaceaceous plant.

[*Heterocycles*, **57**, 2175-2177 (2002)] [Lab. of Instrumental Center]

A New Flavonol Glucoside from Onion

Miyuki FURUSAWA, Toshiyuki TANAKA, Ken-ichi NAKAYA, Munekazu IINUMA* and Hironori TSUCHIYA

Onion (*Allium cepa*) is well known as rich source of flavonoids. For the purpose of further utilization of onion and application to the prevention of life-style-related diseases, the chemical constituents in the outer bulb of onions were examined. A new oxidative quercetin, which is condensed by two molecules through its A and B ring, was isolated as a glucoside. The oxidative quercetin derivative will be composed by radical scavenging reaction.

[*Atherosclerosis*, **163**, 223-228 (2002)] [Lab. of Clinical Pharmaceutics]

The Expression of Extracellular-Superoxide Dismutase is Increased by Lysophosphatidylcholine in Human Monocytic U937 cells.

Masayuki YAMAMOTO, Hirokazu HARA and Tetsuo ADACHI *

Lysophosphatidylcholine (lysoPC) is generated during oxidation of low-density lipoprotein (LDL) and is located within atherosclerotic plaques. Recently, lysoPC has been reported to induce transcription of a variety of cellular genes. In this study, we observed that lysoPC significantly increased the expression of EC-SOD mRNA and protein in human monocytic U937 cells, but not those of Cu,Zn-SOD or Mn-SOD. Induced EC-SOD by lysoPC had a high affinity for heparin, and may bind to endothelial cell surface. Very recently, it has been reported that exogenous addition of EC-SOD or overexpression of EC-SOD prevented endothelial cell-mediated oxidative modification of LDL. Therefore, it is speculated that EC-SOD is induced by lysoPC-stimulated monocytes as a feedback mechanism in vascular homeostasis.

[*Mol. Pharmacol.*, **61**, 194-200 (2002)] [Lab. of Clinical Pharmaceutics]

Contribution of Hepatocyte Nuclear Factor-4 to Down-Regulation of *CYP2D6* Gene Expression by Nitric Oxide.

Hirokazu HARA* and Tetsuo ADACHI

Nitric oxide (NO) released under inflammatory and infectious conditions has been implicated in the down-regulation of many cytochrome P450 genes, but its mechanism of action remains unknown. NOR4, a NO donor decreased the expression of CYP2D6 mRNA. Using a CYP2D6 promoter-luciferase construct, we found that NOR4 and another NO donor, GSNO, reduced the luciferase activity. Deletion analysis of the CYP2D6 promoter revealed that the -80 to +65 region, which contains the nuclear receptor hepatocyte nuclear factor-4 (HNF4) binding site, was responsible for the suppression of CYP2D6 promoter activity by NO. The DNA-binding activity of HNF4 was directly inhibited by NO donors. Mutation of the HNF4 binding site in the CYP2D6 promoter partially restored the suppression of the promoter activity by NO donors. These results demonstrated that NO down-regulates *CYP2D6* gene expression, at least in part, by directly inhibiting HNF4 binding to the CYP2D6 promoter.

[*Biochem. Biophys. Res. Commun.*, **296**, 182-188 (2002)] [Lab. of Clinical Pharmaceutics]

Alteration of Cellular Phosphorylation State Affects Vitamin D Receptor-mediated CYP3A4 mRNA Induction in Caco-2 Cells.

Hirokazu HARA,* Yoko YASUNAMI and Tetsuo ADACHI

Expression of cytochrome P450 3A4 (CYP3A4) is induced by 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) in Caco-2 cells. However, since a typical vitamin D responsive element has not been found in the 5'-flanking region of CYP3A4 gene, the mechanism of $1,25(OH)_2D_3$-induced CYP3A4 mRNA expression is poorly understood. In the present study, we demonstrated that vitamin D receptor (VDR) is a critical factor for the induction. In addition, we found that treatment of Caco-2 cells with the protein kinase C (PKC) inhibitors and the tyrosine kinase inhibitors suppressed CYP3A4 mRNA induction by $1,25(OH)_2D_3$. These findings suggest that the change in the phosphorylation state via PKC and tyrosine kinase might, at least in part, modulate $1,25(OH)_2D_3$-induced CYP3A4 mRNA expression via VDR.

[*FEBS Lett.*, **519**, 77-81 (2002)] [Lab. of Clinical Pharmaceutics]

Extracellular Superoxide Dismutase and Glomerular Mesangial Cells: Its Production and Regulation.

Harutaka YAMADA, Tetsuo ADACHI,* Atsushi FUKATSU, Sachiko MISAO, Yasukazu YAMADA, Takanari AOKI, Naoto MIURA, Masato SAKUMA, Kazuhiro NISHIKAWA, Arao FUTENMA and Shinichi KAKUMU

Extracellular superoxide dismutase (EC-SOD) is synthesized in mesenchymally derived cells and prevents the oxygen radical-induced injury. We studied whether kidney mesangial cells (MCs) produce EC-SOD and how its production is associated with chemokine secretion. Under unstimulated condition, MCs produced EC-SOD, and its production was correlated positively with cyclic adenosine monophosphate (cAMP), but negatively with interleukin (IL)-6 or IL-8 production. By prednisolone or phorbol myristate acetate treatment, EC-SOD levels were correlated negatively with levels of IL-6 and IL-8. The presence of adenylate cyclase inhibitor 2',3'-dideoxyadenosine lost the prednisolone effect. The stimulation of EC-SOD production might be one of the important effects of prednisolone via cAMP pathway in MCs.

[*Jpn. J. Pharm. Health Care Sci.*, **28**, 164-171 (2002)] [Lab. of Clinical Pharmaceutics]

The Use of a "Medicine-Pocketbook" in the Pharmacy of Gifu Pharmaceutical University.

Tetsuo ADACHI,* Koji NIWA, Masashi TAGASHIRA, Masafumi KUBOTA, Hisayo KAMATA, Hirokazu HARA and Kazuyuki HIRANO

The development of drug information on prescribed medicine using a "medicine-pocketbook" was evaluated as one of the methods for drug information offered due to the revision in the medical treatment fees and pharmacy remuneration that went into effect in April 2000. The patients need to carry and present a medicine-pocketbook to the medical institution and pharmacy, and fill in the points personally noticed in the pocketbook to prevent the occurrence of side effects and duplicated prescriptions, which are the main purposes for this medicine-pocketbook. We conducted a questionnaire survey of patients to discover the possession and usage of this medicine-pocketbook. We have to explain the usage and advise the patients so that they can better use the pocketbook at other medical institutions and pharmacies, and this will lead to the prevention of side-effects and duplicated prescriptions.

[*J. Jpn. Soc. Hospital Pharmacists*, **38**, 1171-1174 (2002)] [Lab. of Clinical Pharmaceutics]

Integration of Pharmaceutical Guidance to the Clinical Pathway.

Takashi NIWA, Yoko YASUNAMI, Hirotoshi TANAKA, Michiko SETTA, Chitoshi GOTO, Tadashi SUGIYAMA, Tetsuo ADACHI * and Yoshihiro KATAGIRI

Clinical pathways were recently introduced in order to carry out an appropriate drug control and medication instructions. We integrated the pharmaceutical guidance to the clinical pathway for patients who had undergone coronary angiography (CAG). Prior to the start of the integration of pharmaceutical guidance to the clinical pathway, the pharmacists performed pharmaceutical consultation for 62% of the CAG patients. After that, the pharmacists instructed almost all of CAG patients. Careless omissions of consultation were eliminated because all instruction items were listed and they were confirmed by a check off system. Moreover, the check off system was useful to standardize the pharmaceutical guidance.

[*Anal. Sci.*, **18**, 371-372 (2002)] [Lab. of Information Processing Science]

Crystal Structure of *o*-(*p*-*N*,*N*-Dimethylaminobenzoyl)benzoic Acid.

Soh-ichi KITOH, Takanori MATSUSHIMA, Naoyuki MATSUMOTO, Hitoshi SENDA, Ko-ki KUNIMOTO, Akio. KUWAE, Akihiro NOGUCHI* and Kazuhiko HANAI

The C=O stretching bands of the carboxylic acid and keton in the IR spectra of *o*-(*p*-*N*,*N*-dimethylaminobenzoyl)benzoic acid (DMABBA) in the solid state are observed at 1732 and 1627 cm^{-1},respectively. These frequencies are deviated to a higher and a lower frequency by 39 and 49 cm^{-1} respectively, from those of *o*-benzoylbenzoic acid (BBA). The X-ray analysis of DMABBA was undertaken. The carboxylic acid OH group is intermolecularly hydrogen bonded to the ketone oxygen of an adjacent molecule. The carboxylic acid C=O group does not participate in either inter- or intramolecular hydrogen bonding. The hydrogen bonding pattern differs distinctly from that of other BBA derivatives. The differences correspond to differences in the IR frequencies.

[*J. Org. Chem.*, **67**, 668-673 (2002)] [Lab. of Medicinal Informatics]

Novel Photodegradation of the Antifungal Antibiotic Pyrrolnitrin in Anhydrous and Aqueous Solvents.

Magoichi SAKO,* Toshiyuki KIHARA, Mihoko TANISAKI, Yoshifumi MAKI, Akira MIYAMAE, Toshio AZUMA, Shigetaka KOHDA, Takashi MASUGI

The UV irradiation of pyrrolnitrin (**1**), which is an antibiotic clinically useful against dermatophytosis and possesses a unique 2-(pyrrol-3-yl)nitrobenzene moiety in the molecule, in an anhydrous solvent resulted in the exclusive formation of transient 7,4'-dichlorospiro[1,3-dihydrobenzo(*c*)isoxazole-3,3'-pyrrolin-2'-one] (**2**) *via* the intramolecular oxidation of the juxtaposed pyrrole ring by the triplet-excited nitro group. The irradiation in an aqeous aprotic solvent, however, allowed the concurrent occurrence of intramolecular cyclization by the singlet-excited nitro group in **1** and the hydroxylation at the 2-position of the pyrrole ring by water to afford 3,7-dicholoro-8-hydroxy-8,8a-dihydropyrrolo[2,3-*b*]indol-2-one (**3**), accompanied by the the formation of **2**. Elongation of the irradiation time in these photoreactions caused a rapid consumption of the products, **2** and **3**, to give undetermined polar products. These results indicate that the photodegradation of **1** is significantly influenced by the presence of water in the reaction media and by the nature of its excited state. Thus, the loss of the antifungal activities by the photosensitive antibiotic **1** was chemically proved.

[*Tetrahedron Lett.*, **43**, 6701-6703 (2002)] [Lab. of Medicinal Informatics]

Smooth and Selective Formation of the Cyclic 1,N^2-Propano Adducts in the Reactions of Guanine Nucleosides and Nucleotides with Acetaldehyde.

Magoichi SAKO,* Isamu YAEKURA, Yoshihiro DEYASHIKI

Chemical modifications of nucleosides and nucleotides by acetaldehyde (AA) have been investigated in connection with the toxicity, mutagenicity, and carcinogenicity of AA commonly existing as exogeneous and endogenous sources, such as the primary metabolite of ethanol, a component in tobacco smoke, and a metabolic intermediate of sugars, in the human environment. The treatment of guanine nucleosides and nucleotides with excess AA in pH 8.0 phosphate buffer containing a basic amino acid such as arginine and lysine resulted in the smooth and selective formation of the corresponding cyclic 1,N^2-propano adducts even under mild conditions. The present results are interesting in connection with the molecular mechanisms explaining the toxic, mutagenic, and carcinogenic effects of AA.

[*Tetrahedron*, **58**, 8413-8416 (2002)] [Lab. of Medicinal Informatics]

A Convenient Preparative Method for the 1,N^2-Cyclic Adducts of Guanine Nucleosides and Nucleotides with Crotonaldehyde.

Magoichi SAKO * and Isamu YAEKURA

The treatment of guanine nucleosides and nucleotides with excess crotonaldehyde (CA) in pH 8.0 phosphate buffer containing an equimolar amount of L-arginine at 50°C for 2 h resulted in the selective formation of the corresponding cyclic 1,N^2-propano adducts as a mixture of its diastereomers. CA is a mutagen and carcinogen commonly existing in the human environment as a component of mobile source emissions, tobacco smoke, and other thermal degradation products, and as metabolite of lipid peroxidations. Recently, it has been documented that a 1,N^2-cyclic adduct of 2'-deoxyguanosine with CA has been detected in tissues of laboratory animals in the range of three adduct/10^9 nucleotides after intake of CA *via* food followed by enzymatic treatment. The present method is very useful for the preparation of a variety of the cyclic 1,N^2-propano adducts of the guanine nucleosides and nucleotides to study the molecular mechanisms explaining the mutagenic and carcinogenic effects of CA.

[*J. J. A. Phys. M. Baln. Clim.*, **65**, 93-101 (2002)] [Lab. of Health and Physical Education]

Survey on the Bathing Situation and the Drowning Accidents in the Bathtub among the Club Member of the Aged Persons.

Ryoich INABA, Haruo SUGIURA, * Yukisada KATSUSE, Junich KUROKAWA, and Hirotoshi IWATA

A self-administered survey on bathing and nearly drowning accidents in the bathtub was conducted among subjects consisting of 216 members of an aged person's club for the purpose of utilizing the results for guidance in bathing for elderly persons. The subjects were bathing almost every day. The total bathing time was 20 min and the time spent in the bathtub was 11 min. The ratios of the subjects in this survey who had nearly drowned in the bathtub while bathing at home was 4.3%. A nearly drowning accident occurred primarily when the subject got fatigued (44.4%) or physical condition of the subject was bad (44.4%). The ratio of the subjects who had nearly drowned in a large bathtub of a hotel was 1.4%. No subjects had experienced a nearly drowning accident in the bathtub of a public bath. These results suggested that old persons, particularly males, bath under rather unsafe conditions.

[*J Educ Health Sci*, **47**,380-388(2002)] [Lab. of Health and Physical Education]

Effects of Growth History and Dietary Habits on the Calcaneal Osteo-Sono Assessment Index in Kindergarten Boys.

Hiroyuki NISHIDA*, Kaei WASHINO, Yasufumi TAKEMOTO, Kohsho KASUGA
Tsuyoshi YOKOYAMA, Haruo SUGIURA and Masaru NAKAGAMI

This study was undertaken to determine factors that affect the osteo-sono assessment index (OSI) in kindergarten boys. Quantitative ultrasound is a radiation-free method found to be capable of obtaining consistent measurements of bone density on children when an adapter and cardboard are used to position the child's heel exactly. As such, quantitative ultrasound is useful and safe for screening tests of bone condition from infancy. It was established that there is no difference in OSI between three, four and five year old boys. There was also no connection made between OSI and events in the boys' growth history. However, OSI was affected by the boys' eating habits, especially by the level of dietary calcium-rich dairy products. In addition, there seems to be a connection between the boys' OSI and the level of calcium ingested by their mothers when pregnant with those boys. Families and kindergartens should cooperate to ensure that the importance of diet and exercise to healthy bone condition is recognized,especially for children with a low OSI. Well-balanced eating habits and exercise programs,including play,should be instituted and adhered to from early in life.

[*Acta Physiol. Scand.*, **174**, 247-256 (2002)] [Lab. of Health and Physical Education]

Immunomodulatory Action of Chronic Exercise on Macrophage and Lymphocyte Cytokine Production in Mice.

Haruo SUGIURA, * Hroyuki NISHIDA, Hiroko SUGIURA, and Seyed M MIRBOD

This study was designed to evaluate the effects of 8-week voluntary running exercise on cytokine production of macrophage and lymphocytes. Exercise consisted of spontaneous running wheels for 3 days per week over 8weeks. Seven-week-old-male BALB/c inbred mice were divided into two groups (exercise group and control group). The level of nitric oxide and interleukin-(IL) 1β production by LPS-stimulated peritoneal macrophages from the exercise group was significantly higher than that in the control group. The exercise group showed a significant increase in the splenic lymphocyte production of IL-2 stimulated by Con A. IL-4 production of splenocytes stimulated by Con A in the exercise was higher than that in the control group;however,the difference was not statistically signifycant. These results suggest that 8-week voluntary running exercise effectively enhanced macrophage and lymphocyte functions in mice.

[*BMC Women's Health*, 3, 1-8 (2002)] [Lab. of Health and Physical Education]

Effects of Long-Term moderate Exercise and Increase in Number of Daily Steps on Serum Lipids in Women: Randomized Controlled Trial.

Hiroko SUGIURA, Haruo SUGIURA, * Kazue KAJIMA, Seyed M MIRBOD,
Hirotoshi IWATA, and Toshio MATSUOKA

This study was designed to evaluate the effects of a 24-month period of moderate exercise on serum lipids in menopausal women. The subjects (40-60 y) were randomly divided into an exercise group and a control group. The women in the exercise group were asked to participate in a 90 min physical education class once a week and to record their daily steps as measured by pedometer for 24 months. A significant interaction between the exercise group and the control group in the changes of total cholesterol (TC), high-density lipoprotein cholesterol (HDLC) and TC : HDLC ratio could be observed. By multiple regression analysis, the number of daily steps was related to HDLC and TC : HDLC levels after 24 months, and the changes in TC and HDLC concentrations. These results suggest that daily exercise as wellas increasing the number of daily steps can improve the profile of serum lipids.

[*J Educ Health Sci*, **47**,320-327(2002)] [Lab. of Health and Physical Education]

The Factors Influencing Radius Bone Density in Women University Students: A Comparison between Athletes and Non-athletes.

Yasufumi TAKEMOTO, Hiroyuki NISHIDA*, Kaei WASHINO, Nobuharu KUWABARA
Tsuyoshi YOKOYAMA, Masaru NAKAGAMI and Shingo KATSUNO

This study was undertaken to determine the effect of various environmental factors on bone density. The dietary composition, past fitness and activity levels, aspects of life history and anthropometrical factors were investigated in relation to radius bone density in athletic and non-athletic students in a women's university. Radius bone density in athletic students was greater than that in non-athletic students. In addition, there was a contra-lateral difference in radius density in athletes whose sports are played using chiefly a dominant arm. The athletic students tended to be taller at birth than the non-athletes, and also began to crawl and walk at earlier ages. There was also a tendency for athletic students to be more active in kindergarten (ages 4-6) and elementary/junior high school (ages 7-14), before starting competitive sport. There was a delay in the onset of menstruation in the athletes who were highly physically active in childhood, and athletes who experienced irregular menstrual cycles tended to exhibit lower radius bone density. Non-athletic students consumed less seafood and dairy products than athletic students. Regarding the athletic students, the level of exercise during pre-adulthood might exert the greatest influence on radius bone density out of all the factors considered in this study.

[*J. Indian and Buddhist Studies*, 508-512 (2002)] [Lab.of English]

Amida Buddha and Shinjin with a Clue from Vedāntic Thought.

Shoun HINO

Shin Buddhism is Amida Buddha monism as much as we can say that Advaita Vedānta is Brahman monism. Pantheistically, we might say, 'all is the Brahman' in the case of Advaita and 'all is the Amida Buddha' in the case of Shin Buddhism, however, the religious contents of both differ due to their life attitudes, i.e. the former is the negation of the world and the latter the affirmation of the world. This paper will explain that the affirmation of the world of Shin Buddhism has its background in the ideas of both Amida Buddha monism and also Hoben 'skillful/provisional means', and a few points are referred to with regard to significances derived from true affirmation of the world.