[*Bioorg. Med. Chem.* **19**, 1721-1728 (2011)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Synthesis and Evaluation of Cyclic RGD-boron Cluster Conjugates to Develop Tumor-selective Boron Carriers for Boron Neutron Capture Therapy.

Sadaaki KIMURA, Shin-ichiro MASUNAGA, Tomohiro HARADA, Yasuo KAWAMURA, Satoshi UEDA, Kensuke OKUDA and Hideko NAGASAWA*

Boron-containing agents play a key role in successful boron neutron capture therapy (BNCT). Icosahedral boron cluster-Arg-Gly-Asp (RGD) peptide conjugates were designed, synthesized, and evaluated for the biodistribution to develop tumor-selective boron carriers. Integrin αvβ3 is an attractive target for anti-tumor drug delivery because of its specific expression in proliferating endothelial and tumor cells of various origins. Preparation of o-carborane derivatives involved microwave irradiation, and resulted in high yields in a short time. An in vitro cell adhesion assay and biodistribution experiments indicated that GPU-201 showed comparable tumor uptake and a significantly longer retention in tumors compared with BSH.

[*Bioorg. Med. Chem.* **19**, 5392-5401 (2011)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Isolation, Identification, and Biological Evaluation of HIF-1-modulating Compounds from Brazilian Green Propolis.

Hisanori HATTORI, Kensuke OKUDA, Tetsuji MURASE, Yuki SHIGETSURA, Kosuke NARISE, Gregg L. SEMENZA and Hideko NAGASAWA*

The tumor microenvironment is characterized by hypoxia, low-nutrient levels, and acidosis. Five compounds, such as baccharin (3), beturetol (4), kaempferide (5), isosakuranetin (6), and drupanin (9), that modulate HIF-1-dependent luciferase activity were identified from Brazilian green propolis using reporter assay. Compounds 3, 9 and 5 reduced HIF-1-dependent luciferase activity. They inhibited expression of the HIF-1alpha protein and HIF-1 downstream target genes including VEGFA. They also exhibited significant anti-angiogenic effects. These small molecules screened from Brazilian green propolis may be useful as lead compounds for the development of novel therapies against ischemic cardiovascular disease and cancer.

[*Heterocycles* **83**, 1315-1328 (2011)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 70: Synthesis of 5-Amino-1,2-dihydrofuro[2,3-*b*]pyrido[3',2':4,5]thieno[3,2-*d*]pyridines and Related Compounds. Evaluation of Effects on Lipoprotein Lipase mRNA Expression.

Kensuke OKUDA*, Hideyasu TAKECHI, Takashi HIROTA and Kenji SASAKI

Reaction of 3-(3-cyanopropoxy)thieno[2,3-*b*]pyridine-2-carbonitriles with potassium *tert*-butoxide gave 5-amino-1,2-dihydrofuro[2,3-*b*]pyrido[3',2':4,5]thieno[3,2-*d*]pyridines *via* a Truce-Smiles rearrangement. The 5-amino group was transformed to the chloro derivatives which were allowed to react with various nucleophiles. Effects of the newly synthesized compounds on lipoprotein lipase mRNA expression were also evaluated. The previously unreported parent compound, furo[2,3-*b*]pyrido[3',2':4,5]thieno[3,2-*d*]pyridine, was also synthesized.

[*J. Heterocycl. Chem.* **48**, 715-719 (2011)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 66: Synthesis of *N*-[2-([1,2,4]Oxadiazol-5-yl)cyclopenten-1-yl]formamide Oximes and Their Evaluation as Inhibitors of Platelet Aggregation.

Kensuke OKUDA*, Ying-Xue ZHANG, Hiromi OHTOMO, Takashi HIROTA and Kenji SASAKI

N-[2-([1,2,4]Oxadiazol-5-yl)cyclopenten-1-yl]formamide oximes were synthesized by fusion of (6,7-dihydro-5*H*-cyclopenta[1,2-*d*]pyrimidin-4-yl)amidines and/or their amide oximes with hydroxylamine hydrochloride through a subsequent rearrangement reaction. Assay of the products for anti-platelet aggregation activity revealed that certain of them showed promising inhibitory effect on arachidonic acid-induced platelet aggregation.

[*J. Heterocycl. Chem.* **48**, 1407-1413 (2011)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 67: Reaction of 6,7-Substituted *N*-(Quinazolin-4-yl)amidine Derivatives with Hydroxylamine Hydrochloride: Formation of *in Vitro* Inhibitors of Pentosidine.

Kensuke OKUDA*, Hideki MUROYAMA and Takashi HIROTA

Reactions of *N*-(quinazolin-4-yl)amidines and their amide oximes with hydroxylamine hydrochloride gave cyclization products that were formed by an initial ring cleavage of the pyrimidine component followed by a ring closure formation of 1,2,4-oxadiazole to give *N*-[2-([1,2,4]oxadiazol-5-yl)phenyl]formamide oximes. All isolated products were evaluated for in vitro inhibitory activity on the formation of pentosidine, which is one of representative advanced glycation end products. Some products exhibited significant inhibitory activity against pentosidine formation.

[*Synth. Commun.* **41**, 812-819 (2011)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 68: Reactions of 3-(2-Bromoethyl)quinazolin-4(3*H*)-one and 3-(2-Bromoethyl)-5,6,7,8-tetrahydroquinazolin-4(3*H*)-one with Primary Alkylamines via a Dimroth-type Rearrangement.

Kensuke OKUDA*, Hiromi OHTOMO, Tsuyoshi TAGATA, Takashi HIROTA and Kenji SASAKI

The reaction of 3-(2-bromoethyl)quinazolin-4(3*H*)-one with ethyl- and *n*-propylamine gave abnormal fused 3-alkyl-4-alkyliminoquinazolines via a Dimroth-type rearrangement, as well as normal substituted 3-(2-alkylaminoethyl) derivatives in methanol. The reaction of 3-(2-bromoethyl)-5,6,7,8-tetrahydroquinazolin-4(3*H*)-one with primary alkylamines was also investigated for the scope of this rearrangement reaction.

[*Bull. Chem. Soc. Jpn.* **84**, 386-394 (2011)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Design and Synthesis of Fluorescent Probe for Polyhistidine Tag Using Macrocyclic Nickel(II) Complex and Fluorescein Conjugate.

Masayasu TAKI, Fumiyoshi ASAHI, Tasuku HIRAYAMA* and Yukio YAMAMOTO

We report a newly designed polyhistidine tag (His-tag) targeting fluorescent probe, $NiL^{O}DCF$, based on the fluorophore displacement mechanism. A macrocyclic nickel(II) complex (NiL^{O}) was employed as a novel binding site for a His-tag motif, and we chose 2-4-dichlorofluorescein (DCF) as the fluorophore. A hypochromic absorption shift of $NiL^{O}DCF$ from the metal-unbound form ($L^{O}DCF$) suggested that the phenolic oxygen atom of DCF interacted directly with the NiL^{O} complex, resulting in efficient fluorescence quenching. When a model peptide having a hexahistidine sequence (H6Y1) was added to the solution of $NiL^{O}DCF$, a significant fluorescence enhancement in the emission was observed. These results indicate that NiL^{O}can serve as a novel binding site for the polyhistidine sequence and that $NiL^{O}DCF$ would be applicable to a switchable fluorescent probe for such His-tagged proteins.

[*J. Mol. Biol.* **408**, 18-25 (2011)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Human Spire Interacts with the Barbed End of the Actin Filament.

Takuto ITO, Akihiro NARITA, Tasuku HIRAYAMA*, Masayasu TAKI, Shohei IYOSHI, Yukio YAMAMOTO, Yuichiro MAEDA and Toshiro ODA

Spire is an actin nucleator that initiates actin polymerization at a specific place in the cell. Similar to the Arp2/3 complex, spire was initially considered to bind to the pointed end of the actin filament when it generates a new actin filament. Subsequently, spire was reported to be associated with the barbed end (B-end); thus, there is still no consensus regarding the end with which spire interacts. Here, we report direct evidence that spire binds to the B-end of the actin filament, under conditions where spire accelerates actin polymerization. Using electron microscopy, we visualized the location of spire bound to the filament by gold nanoparticle labeling of the histidine-tagged spire, and the polarity of the actin filament was determined by image analysis. In addition, our results suggest that multiple spires, linked through one gold nanoparticle, enhance the acceleration of actin polymerization.

[*J. Mol. Biol.* **408**, 29-30(2011)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Electron Microscopic Visualization of the Filament Binding Mode of Actin-Binding Proteins.

Takuto ITO, Tasuku HIRAYAMA*, Masayasu TAKI, Shohei IYOSHI, Shuheng DAI, Shuichi TAKEDA, Chieko KIMURA-SAKIYAMA, Toshiro ODA, Yukio YAMAMOTO, Yuichiro MAEDA and Akihiro NARITA

A large number of actin-binding proteins (ABPs) regulate various kinds of cellular events in which the superstructure of the actin cytoskeleton is dynamically changed. Thus, to understand the actin dynamics in the cell, the mechanisms of actin regulation by ABPs must be elucidated. Moreover, it is particularly important to identify the side, barbed-end or pointed-end ABP binding sites on the actin filament. However, a simple, reliable method to determine the ABP binding sites on the actin filament is missing. Here, a novel electron microscopic method for determining the ABP binding sites is presented. This approach uses a gold nanoparticle that recognizes a histidine tag on an ABP and an image analysis procedure that can determine the polarity of the actin filament. This method will facilitate future study of ABPs.

[*Tetrahedron* **67**, 1158–1165 (2011)] [Lab. of Organic Chemistry]

Halogen-Deuterium Exchange Reaction Mediated by Tributyltin Hydride using THF-d_8 as the Deuterium source.

Tomonobu MUTSUMI, Hiroki IWATA, Kazuo MARUHASHI, Yasunari MONGUCHI and Hironao SAJIKI*

A regioselective deuteration method for a wide variety of aromatic compounds using the halogen–deuterium exchange reaction initiated by Bu_3SnH using THF-d_8 as the deuterium source was developed.

[*Eur. J. Org. Chem.* 3361–3367 (2011)] [Lab. of Organic Chemistry]

Pyridine-N-oxide Mediated Oxidation of Diarylalkynes with Palladium on Carbon.

Yoshinari SAWAMA, Masato TAKUBO, Shigeki MORI, Yasunari MONGUCHI and Hironao SAJIKI*

Pyridine *N*-oxide works as an effective oxidant of 1,2-diarylalkynes at 120 °C to form benzil derivatives under Pd/C-catalyzed solvent-free conditions, and Pd/C could be reused up to five times after simple filtration.

[*Chem. Lett.* **40**, 910–912 (2011)] [Lab. of Organic Chemistry]

Pd/C-Catalyzed and Water-Mediated Hiyama Cross-Coupling Reaction using an Electron-Deficient Phosphine Ligand.

Takayoshi YANASE, Shigeki MORI, Yasunari MONGUCHI and Hironao SAJIKI*

The Pd/C-catalyzed Hiyama cross-coupling reaction between a variety of aryl halides and aryltriethoxysilanes was developed. Since only small amounts of the 10% Pd/C (0.5 mol %) and phosphine ligand (1.0 mol %) are required for efficient reaction, the protocol would be practical for the construction of biphenyl derivatives.

[*Tetrahedron* **67**, 8628–8634 (2011)] [Lab. of Organic Chemistry]

Palladium on Carbon-Catalyzed Solvent-Free and Solid-Phase Hydrogenation and Suzuki–Miyaura Reaction.

Yasunari MONGUCHI, Yuki FUJITA, Shota HASHIMOTO, Mariko INA, Tohru TAKAHASHI, Ryo ITO, Kei NOZAKI, TomohiroMAEGAWA and Hironao SAJIKI*

The solvent-free and solid-phase hydrogenation of various reducible functionalities was efficiently catalyzed by heterogeneous palladium on carbon (Pd/C) under ambient hydrogen pressure and temperature. The Pd/C-catalyzed Suzuki–Miyaura coupling reaction between solid aryl bromides and solid arylboronic acids to generate the corresponding solid biaryls was also achieved under the totally solid-phase conditions.

[*ChemCatChem* **3**, 1624–1628 (2011)] [Lab. of Organic Chemistry]

Facile Hydrogenation of Ketones Catalyzed by Platinum on Carbon under Ordinary Pressures and Temperatures.

Yuta FUJIWARA, Youhei IWASAKI, Tomohiro MAEGAWA, Yasunari MONGUCHI and Hironao SAJIKI*

An efficient and practical procedure for the hydrogenation of aliphatic and aromatic ketones under mild reaction conditions is established. The method is highly effective even for the hydrogenation of sterically hindered ketones. Furthermore, the selective hydrogenation of the carbonyl group of aromatic ketones was achieved with the aromatic nuclei and the resulting secondary benzyl alcohol moiety still intact on addition of catalytic amount of pyridine.

[*Angew. Chem. Int. Ed.* **50**, 12232–12235 (2011)] [Lab. of Organic Chemistry]

Reversing the Reactivity of Carbonyl Functions with Phosphonium Salts: Enantioselective Total Synthesis of (+)-Centrolobine.

Hiromichi FUJIOKA, Kenzo YAHATA, Ozora KUBO, Yoshinari SAWAMA*, Tomohito HAMADA and Tomohiro MAEGAWA

Step saver: Carbonyl groups with lower reactivities can be transformed in the presence of more reactive ones by treatment with PPh_3 (or PEt_3) and TMSOTf prior to the reaction (see scheme; TMS=trimethylsilyl, Tf=trifluoromethanesulfonyl). This methodology can be applied to reduction and alkylation reactions, and enabled the short asymmetric total synthesis of (+)-centrolobine with the highest overall yield reported to date.

[*Chem. Commun.* **47**, 9894–9896 (2011)] [Lab. of Organic Chemistry]

An Unusual Reaction of a-Alkoxyphosphonium Salts with Grignard Reagents under an O_2 Atmosphere.

Hiromichi FUJIOKA , Akihiro GOTO , Kazuki OTAKE , Ozora KUBO , Yoshinari SAWAMA* and Tomohiro MAEGAWA

An unusual and novel reaction of α-alkoxyphosphonium salts, generated from *O*,*O*-acetals and Ph_3P, with Grignard reagents under an O_2 atmosphere afforded alcohols in moderate to high yields. It was clarified by isotopic labelling experiments that the reaction proceeded *via* a novel radical pathway.

[*Tetrahedron Lett,* **52**, 3821-3824 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

Tandem Oxidation/Bromination of Ethyl Aromatics to α,α-Dibromoacetophenones with Molecular Oxygen under Visible Light Irradiation.

Norihiro TADA, Kazunori BAN, Takafumi ISHIGAMI, Tomoya NOBUTA, Tsuyoshi MIURA and Akichika ITOH*

A facile synthesis of α,α-dibromoacetophenones from ethyl-substituted aroms. by aerobic photooxidn. was developed. This synthetic method achieved oxidative dibromination of arom. Et groups by using inexpensive and easily handled Br sources, harmless visible light, and O_2.

[*Chem. Pharm. Bull.* **59**, 906-908 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

Oxidative Photodecarboxylation of α-Hydroxycarboxylic Acid Derivatives with FSM-16 under Visible Light Irradiation of Fluorescent Lamp.

Norihiro TADA, Yoko MATSUSAKI, Tsuyoshi MIURA and Akichika ITOH*

Hydroxycarboxylic acids were converted to the corresponding carbonyl compounds under aerobic photo-oxidative conditions in the presence of FSM-16 under visible light irradiation by a fluorescent lamp. This synthetic protocol is the first example of FSM-16 functioning as a photocatalyst by visible light.

[*Org. Lett.* **13**, 2576-2579 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

One-pot Metal-free Syntheses of Acetophenones from Styrenes through Aerobic Photo-oxidation and Deiodination with Iodine.

Tomoya NOBUTA, Shin-ichi HIRASHIMA, Norihiro TADA, Tsuyoshi MIURA and Akichika ITOH*

A one-pot synthetic protocol of acetophenones from styrenes with mol. oxygen, visible light, and mol. iodine is reported. This procedure involves aerobic photo-oxidn. and deiodination in one pot and provides the first report of metal-free direct syntheses of acetophenones from styrenes.

[*Green Chem.* **13**, 1669-1671 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

Facile Aerobic Photooxidation of Methyl Group in the Aromatic Nucleus in the Presence of an Organocatalyst under VIS Irradiation.

Norihiro TADA, Kasumi HATTORI, Tomoya NOBUTA, Tsuyoshi MIURA and Akichika ITOH*

We report a useful method for a facile synthesis of carboxylic acids from Me aroms. by aerobic photooxidn. using VIS irradn. and easily handled 2-chloroanthraquinone as org. catalysts under mild conditions such as an air atm. and ambient pressure and temp. This is a more environmentally benign oxidn. than previous methods, which require drastic reaction conditions.

[*Tetrahedron Lett.* **52**, 875-877 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

Facile Aerobic Photo-oxidative Synthesis of α-Diketones from Alkynes.

Tomoya NOBUTA, Norihiro TADA, Kasumi HATTORI, Shin-ichi HIRASHIMA, Tsuyoshi MIURA and Akichika ITOH*

We report a useful method for facile aerobic photo-oxidative synthesis of α-diketones from alkynes with $MgBr_2 \cdot OEt_2$. This procedure provides a practical synthetic method of α-diketones using easily handled bromine sources, harmless visible light, and mol. oxygen as terminal oxidant.

[*Green Chem.* **13**, 2347-2350 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

Efficient Synthesis of *Gem*-dihydroperoxides with Molecular Oxygen and Anthraquinone under Visible Light Irradiation with Fluorescent Lamp.

Lei CUI, Norihiro TADA, Hiroaki OKUBO, Tsuyoshi MIURA and Akichika ITOH*

An efficient dihydroperoxidn. protocol of various carbonyl compds. with mol. oxygen and anthraquinone in 2-propanol under visible light irradn. with a fluorescent lamp, which produced corresponding *gem*-dihydroperoxides in high yields, is reported.

[*Synlett*. 1381-1384 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

Direct Synthesis of α-Keto Esters from Ethylbenzenes using 48% Aqueous HBr by Aerobic Visible Light Photooxidation.

Norihiro TADA, Kazunori BAN, Tomoya NOBUTA, Shin-ichi HIRASHIMA, Tsuyoshi MIURA and Akichika ITOH*

We report that ethylbenzenes can be directly oxidized to the corresponding α-keto esters with mol. oxygen in the presence of 48% aq. HBr under visible light irradn. This synthetic procedure is the first example for direct prepn. of the corresponding α-keto esters from ethylbenzenes.

[*Tetrahedron: Asymmetry* **22**, 1028-1034 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

Direct Asymmetric Aldol Reactions in Water with a β-Aminosulfonamide Organocatalyst.

Tsuyoshi MIURA*, Mariko INA, Kie IMAI, Kosuke NAKASHIMA, Yumi YASAKU, Naka KOYATA, Yasuoki MURAKAMI, Nobuyuki IMAI, Norihiro TADA and Akichika ITOH

β-Aminosulfonamide organocatalyst promoted the direct asym. aldol reactions of aldehydes with ketones in brine or in the presence of water to afford the corresponding *anti*-aldol products in moderate to excellent yields with up to 97% ee.

[*Tetrahedron* **67**, 6340-6346 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

Direct Asymmetric Aldol Reactions in Brine with Recyclable Fluorous β-Aminosulfonamide Organocatalysts.

Tsuyoshi MIURA*, Kie IMAI, Hikaru KASUGA, Mariko INA, Norihiro TADA, Nobuyuki IMAI and Akichika ITOH

Fluorous organocatalyst I promotes direct asym. aldol reactions of ketones with aryl aldehydes in brine, leading to the synthesis of the corresponding *anti*-aldol products in high yields with up to 96% ee. Fluorous organocatalyst I is easily recovered by solid-phase extn. using fluorous silica gel and can be reused up to five times without purifn.

[*Synlett* 2896-2900 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

Catalytic Oxidative Cleavage of 1,3-Diketones to Carboxylic Acids by Aerobic Photooxidation with Iodine.

Norihiro TADA, Motoki SHOMURA, Lei CUI, Tomoya NOBUTA, Tsuyoshi MIURA and Akichika ITOH*

The catalytic oxidative cleavage of 1,3-diketones to the corresponding carboxylic acids by aerobic photooxidn. with iodine under irradn. with a high-pressure mercury lamp was reported.

[*Tetrahedron: Asymmetry* **22**, 1605-1609 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

Asymmetric Michael Reactions of α,α-Disubstituted Aldehydes with Maleimides Using a Primary Amine Thiourea Organocatalyst.

Tsuyoshi MIURA*, Akira MASUDA, Mariko INA, Kosuke NAKASHIMA, Shohei NISHIDA, Norihiro TADA and Akichika ITOH

Primary amine thiourea organocatalyst (*S*)-3,5-$(F_3C)_2C_6H_3NHCSNHCH(CH_2Ph)CH_2NH_2$ was used to promote Michael addns. of bulky α,α-disubstituted aldehydes, such as isobutyraldehyde with maleimides to afford the adducts in high to excellent yields and with up to 91% ee.

[*Tetrahedron Lett.* **52**, 4158-4160 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

Asymmetric Michael Additions of Aldehydes to Maleimides Using a Recyclable Fluorous Thiourea Organocatalyst.

Tsuyoshi MIURA*, Shohei NISHIDA, Akira MASUDA, Norihiro TADA and Akichika ITOH

Fluorous thiourea organocatalyst I promoted the Michael reaction of aldehydes with maleimides to afford the corresponding adducts in high yields with ≤99% ee. Organocatalyst I could be easily recovered as an insol. ppt. from the reaction mixt. by simple filtration and could be reused without significant loss of catalytic activity.

[*Chem. Commun.* **47**, 1875-1877 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

An Effective and Catalytic Oxidation Using Recyclable Fluorous IBX.

Tsuyoshi MIURA*, Kosuke NAKASHIMA, Norihiro TADA and Akichika ITOH

Oxidn. of alcs. in the presence of a catalytic amt. of fluorous IBX and Oxone as a co-oxidant resulted in the corresponding carbonyl compds. in good to high yields. The fluorous IBX is readily recovered as insol. fluorous IBA from the reaction mixt. by simple filtration, and can be reused without significant loss of catalytic activity.

[*Synlett* 410-414 (2011)] [Lab. of Pharmaceutical Synthetic Chemistry]

β-Aminosulfonamide-catalyzed Direct Asymmetric Aldol Reaction in Brine.

Tsuyoshi MIURA*, Mariko INA, Kie IMAI, Kosuke NAKASHIMA, Akira MASUDA, Norihiro TADA, Nobuyuki IMAI and Akichika ITOH

Direct asym. aldol reactions of aldehydes RCHO (R = Ph, 4-$O_2NC_6H_4$, 3-$MeOC_6H_4$, etc.) with ketones in the presence of a catalytic amt. of β-aminosulfonamide $PhCH_2CH(NHTf)CH_2NH_2$ (I) and trifluoroacetic acid in brine gave the corresponding *anti*-aldols, e.g. II, in high yields with up to 96% enantiomeric excess. The *anti*-aldol products obtained by using the β-aminosulfonamide catalyst have the opposite abs. configuration to those obtained using the sulfonamide catalyst $PhCH_2CH(NH_2)CH_2NHTf$ previously reported by the authors.

[*Chem. Pharm. Bull.* **59**, 239-248(2011)] [Lab. of Pharmacognosy]

Occurrence of *C*-Glucoside of Resveratrol Oligomers in *Hopea parviflora*.

Naohito ABE, Tetsuro ITO, Masayoshi OYAMA, Ryuichi SAWA, Yoshikazu TAKAHASHI, Veliah CHELLADURAI and Munekazu IINUMA*

Investigation of the highly polar chemical constituents in the stem of *Hopea parviflora* (Dipterocarpaceae) resulted in the isolation of four new resveratrol derivatives, hopeasides A and B (resveratrol pentamers), C (resveratrol trimer), and D (resveratrol dimer) together with nine known resveratrol oligomers. The new structures have a common partial structure of the 1-hydroxy-1-(3,5-dihydroxy-2-*C*-glucopyranosylphenyl)-2-(4-hydroxyphenyl)ethane-2-yl group after oxidative condensation of (*E*)-resveratrol-10-*C*-β-glucopyranoside. The structures were determined by spectroscopic analysis including 2D-NMR and computer-aided molecular modeling. The biogenetic relationship of the isolates and NMR characteristics caused by steric hindrance are also discussed in this paper.

[*Chem. Pharm. Bull.* **59**, 452-457(2011)] [Lab. of Pharmacognosy]

Resveratrol Derivatives from *Vatica Albiramis*.

Naohito ABE, Tetsuro ITO, Masayoshi OYAMA, Ryuichi SAWA, Yoshikazu TAKAHASHI and Munekazu IINUMA*

Three new stilbene derivatives, albiraminols A (resveratrol hexamer), B (resveratrol dimer), and vatalbinoside F (mono-glucoside of resveratrol dimer), along with malibatol were isolated from acetone soluble portions of the stem of *Vatica albiramis*. The structures of the isolates were established on the basis of spectroscopic analyses, including a detailed NMR spectroscopic investigation. The biosynthetic aspects of the isolates are discussed in this paper. Albiraminol A is composed of tetrameric resveratrol (vaticanol B) and dimeric resveratrol and is the first instance of the resveratrol derivative bearing a 5,6,11,12-tetrahydro-5,11-epoxydibenzo[*a*,*e*][8]annulene ring system. Albiraminol B possesses a novel 4,5-dihydro-13-oxabenzo[3,4]azuleno[7,8,1-*jkl*]phenanthrene skeleton in the framework.

[*Heterocycles* **83**, 571-580(2011)] [Lab. of Pharmacognosy]

Resveratrol Dimers with an Oxabicyclo Ring in *Vatica Albiramis*.

Naohito ABE, Tetsuro ITO, Masayoshi OYAMA, Ryuichi SAWA, Yoshikazu TAKAHASHI and Munekazu IINUMA*

Investigation of the chemical constituents in the stem of *Vatica albiramis* (Dipterocarpaceae) resulted in the isolation of six stilbenoid derivatives, albiraminols C, D, and vatalbinosides G-J. We determined their structures by spectroscopic anal. including two- dimensional NMR, comparison of the NMR data based on isomerism, and computer-aided molecular modeling. They had two resveratrol units and are the first instance of resveratrol derivatives bearing an oxabicyclo[3.2.2]nonadiene ring system.

[*Nippon Shokuhin Kagaku Gakkaishi* **18**, 71-76(2011)] [Lab. of Pharmacognosy]

Purification of Antioxidant from Cherry Leaf by High Speed Counter-current Chromatography and On-line HPLC/DPPH Radical Scavaging Assay.

Koichi INOUE, Tomomi KIMURA, Hiroyuki KOJIMA, Masayoshi OYAMA, Munekazu IINUMA*, Hisao OKA and Tomoaki HINO

In this study, the identification and purification of antioxidant compound from cherry leaf was proposed by a novel strategy of high-speed countercurrent chromatography (HSCCC) purification for the efficient and effective discovery of antioxidant from natural product based on online HPLC method with radical scavenging assay. The purification of this antioxidant form cherry leaf extract was performed by HSCCC with optimal two-phase solvent system. Using mass spectrometric and NMR analyses, this antioxidant was identified to 3-*O*-caffeoylquinic acid. Due to the advantages derived from online HPLC with DPPH radical scavenging assay and HSCCC technique, a efficient and effective strategy has been developed for the discovery of antioxidants from natural products.

[*Food Chem.* **126**, 289-294(2011)] [Lab. of Pharmacognosy]

Inhibitory Effects of Flavonoid Glycosides Isolated from the Peel of Japanese Persimmon (*Diospyros Kaki* Fuyu) on Antigen-stimulated Degranulation in Rat Basophilic Leukaemia RBL-2H3 Cells.

Tomohiro ITOH, Kenji OHGUCHI, Chizuru NAKAJIMA, Masayoshi OYAMA, Munekazu IINUMA*, Yoshinori NOZAWA, Yukihiro AKAO and Masafumi ITO

We found that two distinct flavonoid glycosides isolated from the peel of Japanese persimmon (*Diospyros kaki* Fuyu), isoquercitrin (Isq) and hyperin (Hyp), are capable of inhibiting antigen-stimulated degranulation in rat basophilic leukemia RBL-2H3 cells. In order to elucidate the underlying mechanisms, we examined effects of Isq and Hyp on cellular responses induced by antigen stimulation. These results indicate that inhibition of antigen-stimulated degranulation by Isq and Hyp is mainly due to suppression of intracellular Ca^{2+} elevation. Our findings suggest that Isq and Hyp would be beneficial for alleviating symptoms of type I allergy.

[*Heterocycles* **83**, 1603-1610(2011)] [Lab. of Pharmacognosy]

New Furanocoumarins from the Fruits of *Melicope Triphylla*.

Ken-ichi NAKASHIMA, Masayoshi OYAMA, Tetsuro ITO, Hiroko MURATA and Munekazu IINUMA*

Four new furanocoumarins were isolated from the fruits of *Melicope triphylla* (Rutaceae), together with two known coumarins, nine flavonoids, two alkaloids, and methyl *p*-geranyloxy-*trans*-cinnamate. The structures of the newly identified compounds were determined by extensive 1D- and 2D-NMR spectroscopic analyses to be linear-types of furanocoumarins bearing a hydroxyl or a hydroperoxy group on the geranyloxy side chain.

[*Tetrahedron Lett.* **52**, 4694-4696(2011)] [Lab. of Pharmacognosy]

Melicodenines A and B, Novel Diels-Alder Type Adducts Isolated from *Melicope Denhamii*.

Ken-ichi NAKASHIMA, Masayoshi OYAMA, Tetsuro ITO, Joko Ridho WITONO, Dedy DARNAEDI, Toshiyuki TANAKA, Jin MURATA and Munekazu IINUMA*

Two novel Diels-Alder type adducts, melicodenines A and B, were isolated from the leaves of *Melicope denhamii* (Seem.) T. G. Hartley and their structures were established by spectroscopic analyses, including extensive 2D NMR experiments. Melicodenine A is a bisquinolinone alkaloid comprised of two *N*-methylflindersines, while melicodenine B is the first naturally occurring quinolinone-acetophenone conjugate composed of *N*-methylflindersines and an evodionol methyl ether.

[*BMC Med.* **9**, 69-87(2011)] [Lab. of Pharmacognosy]

α-Mangostin Extracted from the Pericarp of the Mangosteen (*Garcinia Mangostana* Linn) Reduces Tumor Growth and Lymph Node Metastasis in an Immunocompetent Xenograft Model of Metastatic Mammary Cancer Carrying a p53 Mutation.

Masa-Aki SHIBATA, Munekazu IINUMA*, Junji MORIMOTO, Hitomi KUROSE, Kanako AKAMATSU, Yasushi OKUNO, Yukihiro AKAO and Yoshinori OTSUKI

Mammary tumors, induced by inoculation of BALB/c mice syngeneic with metastatic BJMC3879luc2 cells, were subsequently treated with α-mangostin using mini-osmotic pumps and histopathologically examined. The antimetastatic activity of α-mangostin as detected in mammary cancers carrying a p53 mutation in the present study may have specific clinical applications. In addition, α-mangostin may have chemopreventive benefits and/or prove useful as an adjuvant therapy, or as a complementary alternative medicine in the treatment of breast cancer.

[*Anal. Sci.* **27**, 217-220 (2011)] [Lab. of Pharmaceutical Analytical Chemistry]

Functional Preconcentration Tip of Total Volume Injection for ESI/MS Analysis of DNA Adducts.

Hiroya MURAKAMI, Mio KOGUCHI, Yukihiro ESAKA, Bunji UNO* and Yasushi ISHIHAMA

We have developed a simple method to significantly improve the sensitivity in the LC/MS analysis of DNA adducts. A preconcentration tip for the selective recovery of DNA adducts was prepared. Using this tip, the total amount of DNA adducts in a treated DNA sample was injected in a one-shot manner into an LC/MS system. We were able to improve the sensitivity by more than one order of magnitude in concentration. This method will be a useful tool for the quantitative determination of trace DNA adducts.

[*Chem. Lett.* **40**, 268-269 (2011)] [Lab. of Pharmaceutical Analytical Chemistry]

Oxidation of Guanosine to the Imidazolone Derivative via Proton-coupled Electron Transfer to Hydroperoxy Radical Derived from Superoxide.

Hiroya MURAKAMI, Yukihiro ESAKA, Tatsushi NAKAYAMA and Bunji UNO*

Oxidation of guanosine (G) with electrochemically generated superoxide ($O_2^{\bullet-}$) leads to the imidazolone (Iz) derivative as a single-electron oxidation product of G. A crucial step in the mechanism of the oxidation is the proton-coupled electron transfer (PCET) from G to the hydroperoxy radical ($HO_2^{\bullet}$) that is derived from $O_2^{\bullet-}$.

[*Anal. Sci.* **27**, 315-320 (2011)] [Lab. of Pharmaceutical Analytical Chemistry]

Structural and Spectral Characteristics of the Cross-linked Dimer Derived from Electrooxidation of Cyclic 1,N^2-Propanoguanosine.

Hiroya MURAKAMI, Yukihiro ESAKA and Bunji UNO*

The acetaldehyde-derived cyclic propano adduct of 2′-deoxyguanosine was easily oxidized electrochemically into the cross-linked dimer as an oxidative product. The structural and spectroscopic characteristics of the dimer were investigated by MS, NMR, UV, and DFT calculations. The dimer formation was inferred from the chemical formula as $C_{28}H_{36}N_{10}O_{12}$ provided by the high-resolution ESI-MS results. The C2–N5 linkage between the two monomers in the dimer was deduced from the 1D-NMR spectral results. In addition, the correlations in the 2-D NMR spectra were consistently explained by the structure of the C2–N5 cross-linked dimer. UV spectral measurements also support the C2–N5 linking in the dimer formation. The formation of the cross-link dimer is expected to interfere with DNA replication and to contribute to acetaldehyde-mediated genotoxicity.

[*Clin. Exp. Pharmacol. Physiol.* **38**, 658-665 (2011)] [Lab. of Pharmaceutical Analytical Chemistry]

Cilostazol Protects the Heart against Ischaemia Reperfusion Injury in a Rabbit Model of Myocardial Infarction: Focus on Adenosine, Nitric Oxide and Mitochondrial KATP Channels.

Yushan BAI, Muqier, Hiroya MURAKAMI, Masamitsu IWASA, Shohei SUMI, Yoshihisa YAMADA, Hiroaki USHIKOSHI, Takuma AOYAMA, Kazuhiko NISHIGAKI, Genzou TAKEMURA, Bunji UNO* and Shinya MINATOGUCHI

The present study examined whether or not cilostazol reduces the myocardial infarct size, and investigated its mechanism in a rabbit model of myocardial infarction. Japanese white rabbits underwent 30 min of coronary occlusion, followed by 48 h of reperfusion. Cilostazol or vehicle was given intravenously 5 min before ischaemia. 8SPT, l-NAME or 5-HD was given intravenously 5 min before cilostazol injection. The findings in this study show that cilostazol reduces the myocardial infarct size by increasing adenosine and NOx levels. Cilostazol might provide a new strategy for the treatment of coronary heart disease.

[*J. Control. Release* **149**, 81-88 (2011)] [Lab. of Pharmaceutical Engineering]

Mucoadhesive Nanoparticles for the Simultaneous Delivery of Macromolecules and Permeation Enhancers to the Intestinal Mucosa: *In vitro* and *In vivo* Evaluation.

Abdallah MAKHLOF, Martin WERLE, Yuichi TOZUKA and Hirofumi TAKEUCHI*

The feasibility of combining safe permeation enhancers in a mucoadhesive particulate system for the oral delivery of peptide drugs was investigated. Polyelectrolyte complex nanoparticles (NPs) were prepared by ionic interaction of spermine (SPM) with polyacrylic acid (PAA) polymer. The cellular transport of fluorescein isothiocyanate dextran (FD4) showed higher permeation enhancing profiles of SPM–PAA NPs, as compared to SPM solution or PAA NPs prepared by ionic gelation with $MgCl_2$. Confocal microscopy images of rats' small intestine confirmed previous findings in Caco-2 cells and revealed a strong and prolonged penetration of FD4 from the mucosal to the basolateral side of the intestinal wall.

[*Eur. J. Pharm. Biopharm.* **76**, 238-244 (2010)] [Lab. of Pharmaceutical Engineering]

In vitro and *in vivo* Evaluation of WGA-Carbopol Modified Liposomes as Carriers for Oral Peptide Delivery.

Abdallah MAKHLOF, Martin WERLE, Yuichi TOZUKA and Hirofumi TAKEUCHI*

Surface modification of liposomal nanocarriers with a novel polymer–lectin conjugate was proposed for enhancing the systemic uptake of encapsulated peptide and protein therapeutics after oral administration. Wheat germ agglutinin (WGA) was covalently attached to carbopol (CP) using the carbodiimide method. The uptake of WGA–CP liposomes by Caco-2 cells was significantly higher than that of non-modified or CP liposomes.The involvement of active transport mechanism for the cellular uptake of the modified liposomes, mediated mainly by binding of WGA to its specific cell membrane receptors. The pharmacological efficacy of calcitonin, a model peptide drug, was enhanced by more than 20- and 3-fold following peroral administration of calcitonin-loaded WGA–CP liposomes when compared to non-modified and CP liposomes, respectively.

[*Eur. J. Pharm. Sci.* **42**, 445-451 (2011)] [Lab. of Pharmaceutical Engineering]

A pH-Sensitive Chitosan Nanoparticulate System for the Peroral Delivery of Insulin.

Abdallah MAKHLOF, Yuichi TOZUKA and Hirofumi TAKEUCHI*

In the current study, chitosan nanoparticles (CS NPs) were formulated by ionic cross-linking with hydroxypropyl methylcellulose phthalate (HPMCP) as a pH-sensitive polymer and evaluated for the oral delivery of insulin. In vitro results revealed a superior acid stability of CS/HPMCP NPs with a significant control over insulin release and degradation in simulated acidic conditions with or without pepsin. After s.c. injection to rats, no significant difference in the hypoglycemic effect of insulin solution or insulin-loaded CS/HPMCP NPs was observed, confirming the physico-chemical stability and biological activity of the entrapped peptide. Following peroral administration, CS/HPMCP NPs increased the hypoglycemic effect of insulin by more than 9.8 and 2.8-folds as compared to oral insulin solution and insulin-loaded CS/tripolyphosphate (TPP) NPs, respectively.

[*Drug Delivery* **18**, 562–569 (2011)] [Lab. of Pharmaceutical Engineering]

N-trimethyl Chitosan-Modified Liposomes as Carriers for Oral Delivery of Salmon Calcitonin.

Aiwen HUANG, Abdallah MAKHLOF, Qineng PING, Yuichi TOZUKA and Hirofumi TAKEUCHI*

The aim of this work was to investigate the role of N-trimethyl chitosan- (TMC-) coated liposomes in the oral administration of calcitonin. TMC-coated liposomes containing calcitonin were prepared and characterized as having a particle size of 262 nm, zeta potential of 35.8 mV and high entrapment efficiency (89.1%). The results in confocal laser microscopy showed that TMC-coated liposomes prolonged the residence time and increased the penetration effect of the liposomal system compared to non-coated liposomes. The study of pharmacological effects confirmed that TMC-coated liposomes increased the area above the blood calcium concentration-time curves (AAC) from 3.13 ± 20.50 to 448.84 ± 103.56 compared to the calcitonin solution. These results support the feasibility of TMC-coated liposomes as a new oral delivery system for peptide and protein drugs.

[*Asian. J. Pharm.Sci.* **6**, 101-108 (2011)] [Lab. of Pharmaceutical Engineering]

Completely Dry Process for the Desired Release Profile of Poorly Water Insoluble Drugs by a Temperature-controllable Twin Screw Kneader.

Yohei HOASHI, Yuichi TOZUKA and Hirofumi TAKEUCHI*

Completely dry process was performed using a twin-screw kneader with hydrophilic porous silica in order to make solid dispersions of indomethacin, risperidone and fenofibrate at melting temperature of each drug. On the powder X-ray diffraction, nitrogen adsorption and differential scanning calorimetry analyses, indomethacin and risperidone in solid dispersion changed to an amorphous state to adsorb onto silica pores. However, crystalline form of fenofibrate in solid dispersion was maintained in spite of the adsorption of fenofibrate onto silica pores. A remarkable dissolution enhancement of the drugs from kneaded products was achieved by making the solid dispersion system with porous silica. Melt kneading process to make solid dispersion with porous silica was found to be an effective technology to enhance the dissolution rate.

[*Biol. Pharm. Bull.* **34**, 894-897 (2011)] [Lab. of Pharmaceutical Engineering]

Fluorescence Investigation of the Retinal Delivery of Hydrophilic Compounds via Liposomal Eyedrops.

Kohei HIRONAKA, Takuya FUJISAWA, Hitoshi SASAKI, Yuichi TOZUKA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA,Hideaki HARA and Hirofumi TAKEUCHI*

We examined the feasibility of using submicron-sized liposomes (ssLips) for retinal delivery of hydrophilic compounds, which would also have a wide range of applications. To evaluate the uptake into conjunctival cell line and the intraocular behavior of hydrophilic compound-containing ssLips after eyedrop application, fluorometric investigation was carried out by using a hydrophilic fluorescence probe, 5(6)-carboxyfluorescein (CF). CF being entrapped within the liposomes markedly enhanced both the uptake of CF into conjunctival cells and CF-oriented emission in the retina in mice after eyedrop application. ssLips of appropriate composition were considered to have good potential to carry hydrophilic compounds into the retina.

[*Eur. J. Pharm. Biopharm.* **79,** 119-125 (2011)] [Lab. of Pharmaceutical Engineering]

Edaravone Loaded Liposome for Retinal Protection against Oxidative Stress-Induced Retinal Damage.

Kohei HIRONAKA, Yuta INOKUCHI, Takuya FUJISAWA, Hiroki SHIMAZAKI, Mai AKANE, Yuichi TOZUKA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Hideaki HARA and Hirofumi TAKEUCHI*

To optimize the retinal protective effects of submicron-sized liposomes (ssLips) containing edaravone for intravitreal administration, we investigated the effects of liposomal formulation on the pharmacological effects. Edaravone-loaded EPC-ssLip, by a calcium acetate gradient method, scavenged intracellular H_2O_2 radical more strongly than DSPC-ssLip. The edaravone-loaded EPC-ssLip significantly reduced NMDA-induced ganglion cell layer (GCL) cell death compared with free edaravone. These results may be related to the release profile of the edaravone from ssLips across the inner layers of the retina including GCL, indicating effective retinal protection of EPC-ssLip compared to that of DSPC-ssLip.

[*Int. J. Pharm.* **410**, 114-117 (2011)] [Lab. of Pharmaceutical Engineering]

α-Glucosyl Hesperidin Induced Improvement in Bioavailability of Pranlukast Hemihydrate Using High-Pressure Homogenization.

Hiromasa UCHIYAMA, Yuichi TOZUKA, Fusatoshi ASAMOTO and Hirofumi TAKEUCHI*

The α-glucosyl hesperidin (Hsp-G)-induced improvement of both the dissolution and absorption properties of pranlukast hemihydrate (PLH) was achieved by means of a high-pressure homogenization (HPH) processing. The amount of dissolved PLH gradually increased with the pass number of HPH processing, and was extremely higher than the PLH solubility after the HPH processing. The amount of PLH that had permeated through the Caco-2 cell monolayers was improved in the case of HPH-processed PLH/Hsp-G (1/10). The bioavailability of PLH from HPH-processed PLH/Hsp-G (1/10) showed a 3.9- and 2.2-fold improvement over the PLH crystal in terms of Cmax and AUC values, respectively. High-pressure homogenization provides a good opportunity for molecular-level interaction of PLH and the associated structure of Hsp-G to occur.

[*Eur. J. Pharm. Sci.* **43**, 71-77 (2011)] [Lab. of Pharmaceutical Engineering]

Fluorescence Investigation of a Specific Structure Formed by Aggregation of Transglycosylated Stevias: Solubilizing Effect of Poorly Water-Soluble Drugs.

Hiromasa UCHIYAMA, Yuichi TOZUKA, Fusatoshi ASAMOTO and Hirofumi TAKEUCHI*

We investigated the solubility-enhancing effect of Stevia-G towards hydrophobic materials by using fluorescence spectroscopy. The plot of the pyrene I_1/I_3 ratio versus the Stevia-G concentration showed a sigmoidal curve as a function of the Stevia-G concentration, suggesting the existence of a hydrophobic environment around pyrene molecules under high Stevia-G concentrations. The critical micelle concentration calculated from the pyrene I_1/I_3 plot was about 16 mg/mL. Based on results from the static quenching plots, the micellar aggregation number of Stevia-G was estimated as ca.15. Therefore, the hydrophobic steviol-skeleton of Stevia-G made a hydrophobic core around a hydrophobic molecule. This specific structure formed by Stevia-G molecules led to an enhancement of the apparent solubility of poorly water-soluble drugs.

[*Eur. J. Pharm. Biopharm.* **79**, 559-565 (2011)] [Lab. of Pharmaceutical Engineering]

A Novel Application of α-Glucosyl Hesperidin for Nanoparticle Formation of Active Pharmaceutical Ingredient by Dry-Grinding.

Yuichi TOZUKA, Masaaki IMONO, Hiromasa UCHIYAMA and Hirofumi TAKEUCHI*

The effectiveness of α-glucosyl hesperidin (Hsp-G) as a novel grinding aid for the preparation of drug nanoparticles by dry grinding was investigated. Poorly water-soluble drugs and Hsp-G were mixed at a weight ratio of 1/5 and ground for 60 min by a vibrational ball mill. Administration of the ground mixture of glibenclamide/Hsp-G to rats resulted in a significantly higher rate of decrease in blood glucose levels than that of untreated glibenclamide. The area above the time-curve of plasmaglucose concentrations using the ground mixture of glibenclamide/Hsp-G was 6-fold higher than that using untreated glibenclamide. The improved dissolution rate due to nanoparticle formation of glibenclamide, induced by co-grinding with Hsp-G, was responsible for this improvement.

[*J. Pharm. Sci.* **100**, 4421-4431 (2011)] [Lab. of Pharmaceutical Engineering]

NMR Investigation of a Novel Excipient, α-Glicosylhesperidin as a Suitable Solubilizing Agent for Poorly Water-Soluble Drugs.

Junying ZHANG, Yuichi TOZUKA*, Hiromasa UCHIYAMA, Kenjirou HIGASHI, Kunikazu MORIBE, Hirofumi TAKEUCHI and Keiji YAMAMOTO

α-Glucosylhesperidin (Hsp-G), a functional food additive, significantly enhances the solubility and bioavailability of poorly water-soluble drugs despite little surface activity. Herein, we present investigations into the underlying mechanism by nuclear magnetic resonance techniques. Dynamic light scattering and two-dimensional nuclear Overhauser effect spectroscopy measurements demonstrated that Hsp-G molecules self-associated into particular small micelles, with the flavanone skeleton forming a hydrophobic core, and surrounding sugar groups working as a shell. Solubility enhancement was due to the incorporation of drugs into Hsp-G micelle. Hsp-G micellization process with little loss of surface tension is a unique observation in surface and interface science.

[*Chem. Pharm. Bull*. **59**, 1299-1302 (2011)] [Lab. of Pharmaceutical Engineering]

Molecular States of *p*-Dimethylaminobenzonitrile Coground with β-Cyclodextrin Investigated Using Solid-state Fluorescence Spectroscopy.

Yutaka INOUE, Nana HASEGAWA, Yuichi TOZUKA*, Etsuo YONEMOCHI, Toshio OGUCHI, Kenjirou HIGASHI, Kunikazu MORIBE and Keiji YAMAMOTO

Changes in molecular states of *p*-dimethylaminobenzonitrile (DMABN) coground with β-cyclodextrin (β-CD) were examined using solid-state fluorescence measurements. Solid-state fluorescence measurements revealed emission by DMABN crystals in a twisted intermolecular charge-transfer state at 473 nm. DMABN in the DMABN/β-CD coprecipitate had a fluorescence emission peak at 393 nm due to its planar structure. In contrast, DMABN in a DMABN/β-CD ground mixture had an emission peak at 473 nm due to its twisted structure.

[*Int. J. Pharm.* **420**, 191-197 (2011)] [Lab. of Pharmaceutical Engineering]

Guest Molecular Size-Dependent Inclusion Complexation of Parabens with Cholic Acid.

Kunikazu MORIBE, Miyuki MASAKI, Ryo KINOSHITA, Martin WERLE, Junying ZHANG, Waree LIMWIKRANT, Kenjirou HIGASHI, Yuichi TOZUKA*, Toshio OGUCHI and Keiji YAMAMOTO

Effects of p-hydroxybenzoate (paraben) ester chain length on the stoichiometry and structure of grindinginduced inclusion complexes with cholic acid (CA) were investigated. Ethyl-, n-propyl-, and isopropyl-parabens formed equimolar inclusion complexes with CA, and the complex structures were of the β-trans bilayer type. In contrast, the stoichiometry of the CA-paraben complex was 2:1, and the structure was of the α-gauche bilayer type when isobutylparaben was used as a guest molecule. Although the stoichiometries and structures of the complexes differed, solid-state NMR showed that the molecular states of parabens in the complexes were similar and independent of the ester chain length. Mechanical forces and thermal activation by grinding were important factors in the mechanism of CA-paraben complex formation.

[*J. Photopolym. Sci. Thechnol.* **24**, 467–470 (2011)] [Lab. of Pharmaceutical Physical Chemistry]

Immobilization of Proteins onto the Self-Assembled Phospholipid Layer Fabricated by Plasma-Assisted Method.

Shin-ichi KONDO*, Yasushi SASAI, Yukinori YAMAUCHI and Masayuki KUZUYA

In this paper, we studied the effect of the concentration of phosphatidyl choline (PC) suspension containing stearic acid (StA) on the thermal stability of self-assembled phospholipid layer. The thermal stability of the self-assembled phospholipid layer incorporating StA depended on the concentration of PC suspension. We immobilized three kinds of enzymes as model proteins on the self-assembled phospholipid layer incorporating StA. The maximum value of surface density of enzyme tended to be inversely proportional to the molecular weight. We also studied the activity of β-galactosidase (BG) immobilized onto the self-assembled phospholipid layer incorporating StA. The specific activity of BG immobilized was higher than that of BG directly immobilized on a polymer surface. It was suggested that the self-assembled phospholipid layer would act as a good bio-interface.

[*J. Photopolym. Sci. Thechnol.* **24**, 417–420 (2011)] [Lab. of Pharmaceutical Physical Chemistry]

Fabrication of Scaffold for Cell Adhesion on Plasma-irradiated Polystyrene.

Yasushi SASAI*, Yuko TANAKA, Shin-ichi KONDO, Yukinori YAMAUCHI and Masayuki KUZUYA

In this study, to fabricate a versatile platform for the immobilization of bioactive molecules on chemically inert polystyrene (PS) substrate, vinylmethylether maleic acid copolymer (VEMAC) was immobilized on plasma-irradiated PS petri dish through a coupling reaction of hydroxyl group indroduced on PS substrate by Ar plasma-irradiation with carboxyl group of VEMAC. For cell culture application, cell adhesive peptide "GRGDS" was conjugated with VEMAC immobilized on PS. The results indicated that GRGDS peptide conjugated with VEMAC immobilized on plasma-irradiated PS was specifically recognized by cell surface of NIH3T3 and stimulated cell adhesion and proliferation.

[*J. Photopolym. Sci. Thechnol.* **24**, 475–478 (2011)] [Lab. of Pharmaceutical Physical Chemistry]

Surface Functionalization of DLC Thin Films.

Yukinori YAMAUCHI, Masayuki KUZUYA, Yasushi SASAI and Shin-ichi KONDO*

The Diamond-like carbon (DLC) thin films have been widely used in a variety of industrial fields due to the attractive properties, such as high hardness, low friction coefficient, optical transparency, chemical inertness, and high electrical resistivity. In this study, we attempted to construct the high functional surface of DLC thin films for industrial and biological needs by plasma surface treatment. The graft polymerization underwent on the surface of DLC films by adding a monomer under an aerobic condition, so that the treated DLC films possessed highly-functionalized surface. It was suggested that the present procedure would be one of the fundamental methods to fabricate the advanced DLC film with long-acting functional surface.

[*Chem. Pharm. Bull.* **59**, 1200–1202 (2011)] [Lab. of Pharmaceutical Physical Chemistry]

Characterization of Novel pH-Sensitive Polymeric Micelles Prepared by the Self-Assembly of Amphipilic Block Copolymer with Poly-4-vinylpyridine Block Synthesized by Mechanochemical Solid-State Polymerization.

Shin-ichi KONDO*, Keitarou YAMAMOTO, Yuka SAWAMA, Yasushi SASAI, Yukinori YAMAUCHI and Masayuki KUZUYA

We fabricated novel pH-sensitive polymeric micelles consisting of amphiphilic block copolymer containing pyridyl groups as side chains in the hydrophobic block. A decrease in pH resulted in deformation of the polymeric micelles over a very narrow pH range (between pH 5.7 and 5.6). Interestingly, micellization and demicellization occurred reversibly in this narrow pH range. Polymeric micelles incorporating 5-fluorouracil (5FU) were also prepared. Decreasing the pH of this polymeric micelle solution from 7 to 5.5 resulted in the rapid release of 5FU at pH 5.6; the drug was completely released within 30 min.

[*J. Phar. Nutri. Sci.* **1**, 124–129 (2011)] [Lab. of Pharmaceutical Physical Chemistry]

Novel Synthesis of Macromonomers by Mechanochemical Reaction for Application to Polymeric Micelles.

Shin-ichi KONDO*, Masashi TUKAMOTO, Yasushi SASAI, Yukinori YAMAUCHI and Masayuki KUZUYA

We have presented the first example of the synthesis of macromonomers by mechanochemical reaction of polymethylmethacrylate (PMMA) and maleic anhydride (MA). The ESR spectrum of the fractured sample of PMMA and MA showed a broad singlet, which was apparently different from the spectrum of PMMA mechanoradical. We underwent the UV-labeling of the fractured samples of PMMA and MA to confirm the formation of macromonomers. The gel permeation chromatograms of UV-labeled compounds derived from this fractured sample showed a broad peak in a polymer region with refractive index detector and UV detector, which indicates that macromonomers bounding MA would be produced. This method seems to be applicable for a wide variety of polymers to synthesize macromonomers possessing MA.

[*J. Toxicol. Sci.* **35**, 209-215 (2011)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

Cadmium Toxicity is Caused by Accumulation of p53 through the Down-regulation of Ube2d Family Genes *in vitro* and *in vivo*.

Maki TOKUMOTO, Yasuyuki FUJIWARA, Akinori SHIMADA, Tatsuya HASEGAWA, Yoshiyuki SEKO, Hisamitsu NAGASE* and Masahiko SATOH

Cadmium (Cd) causes renal dysfunction with damage to kidney proximal tubule cells; however, the precise mechanisms of the toxicity remain unclear. To investigate the mechanisms of Cd-induced renal toxicity, we examined the effects of Cd on the ubiquitin-proteasome system, particularly the expression and function of *Ube2d* family members in the NRK-52E cells and mice. The results suggest that the Cd-induced accumulation of p53 may be due to inhibition of p53 degradation through the down-regulation of *Ube2d* family genes, and that Cd induces p53-dependent apoptosis in renal tubular cells. Moreover, Ube2d family members may be one of the critical targets of renal toxicity caused by Cd.

[*J. Toxicol. Sci.* **35**, 209-215 (2011)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

DNA Microarray Analysis of Normal Rat Kidney Epithelial Cells Treated with Cadmium.

Maki TOKUMOTO, Tomoaki OHTSU, Akiko HONDA, Yasuyuki FUJIWARA, Hisamitsu NAGASE* and Masahiko SATOH

In order to elucidate the transcriptional response of kidney epithelial cells to cadmium, the gene expression pattern was examined in normal rat kidney epithelial cells (NRK-52E cells) exposed to 50 μM cadmium for 4 hr using DNA microarray. Cadmium was found to increase the expression of 73 genes and decrease the expression of 42 genes in NRK-52E cells before the development of cytotoxicity.

[*Biomaterials* **32**, 4185-4193 (2011)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

Enhanced *in vivo* Gene Transfer into the Placenta Using RGD Fiber-mutant Adenovirus Vector.

Kazufumi KATAYAMA, Rie FURUKI, Hideaki YOKOYAMA, Makoto KANEKO, Masashi TACHIBANA, Ichiro YOSHIDA, Hisamitsu NAGASE, Keiichi TANAKA, Fuminori SAKURAI, Hiroyuki MIZUGUCHI, Shinsaku NAKAGAWA and Tsuyoshi NAKANISHI*

In the current study, as a part of a thorough evaluation of the fiber-mutant adenovirus vector carrying the Arg-Gly-Asp (RGD) peptide sequence (Ad-RGD) in preclinical studies, we designed an experiment to investigate in detail the distribution of Ad-RGD compared with conventional adenovirus vector (WT-Ad) in pregnant mice. As a result, Ad-RGD had substantial placental tropism, at 10–100 times that of WT-Ad. Ad-RGD showed high levels of transduction efficiency in *in vitro*-differentiated trophoblast stem cells, in which higher expression of αvβ3 integrin than in undifferentiated cells was observed. Our results suggest that the use of Ad-RGD or another RGD-mediated targeting strategy holds promise for drug delivery to the placenta.

[*J. Environ. Sci.* **23**, 125-132 (2011)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

Screening of Agonistic Activities against Four Nuclear Receptors in Wastewater Treatment Plants in Japan Using a Yeast Two-hybrid Assay.

Daisuke INOUE, Koki NAKAMA, Kazuko SAWADA, Taro WATANABE, Hisae MATSUI, Kazunari SEI, Tsuyoshi NAKANISHI* and Michihiko IKE

In the current study, we examined the agonistic activities on four NRs (estrogen receptor α, thyroid hormone receptor α, retinoic acid receptor α and retinoid X receptor α) of untreated and treated wastewater from municipal wastewater treatment plants (WWTPs) in Japan using a yeast two-hybrid assay. Investigation of the influent and effluent of seven WWTPs revealed that agonistic activities against NRs were always detected in the influents and partially remained in the effluents. These results indicate that municipal wastewater in Japan commonly contains endocrine disrupting chemicals that exert agonistic activities on NRs, and that some of these chemicals are released into the aquatic environment. Further study is required to assess their possible risks in detail.

[*Biochim. Biophys. Acta.* **1809**, 56-62 (2011)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

The Zinc-sensing Transcription Factor MTF-1 Mediates Zinc-induced Epigenetic Changes in Chromatin of the Mouse Metallothionein-I Promoter.

Fumika OKUMURA, Yong LI, Norio ITOH, Tsuyoshi NAKANISHI*, Masakazu ISOBE, Glen K. ANDREWS and Tomoki KIMURA

Metallothionein (MT) is a cysteine-rich protein active in zinc homeostasis, cadmium detoxification, and so on. *MT-I* gene transcription is regulated by metal response element-binding transcription factor-1 (MTF-1), which is recruited to the promoter by zinc. In the current study, we examined alterations in the chromatin structure of the *MT-I* promoter associated with enhanced transcriptional activation. We demonstarated that rapid disruption of nucleosome structure at the *MT-I* promoter is mediated by zinc-responsive recruitment of an active MTF-1-coactivator complex.

[*J. Toxicol. Sci.* **36**, 173-180 (2011)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

Chromium (VI) Inhibits Mouse Metallothionein-I Gene Transcription by Modifying the Transcription Potential of the Co-activator p300.

Tomoki KIMURA, Fumika OKUMURA, Akira ONODERA, Tsuyoshi NAKANISHI*, Norio ITOH and Masakazu ISOBE

The production of metallothioneins (MTs), is induced by heavy metals such as Zn and Cd. MTs maintain Zn homeostasis and attenuate heavy metal-induced cytotoxicity by sequestering these metals and lowering their intracellular concentrations. In the current study, we have shown that the inhibition by Cr(VI) was partially overcome by the overexpression of p300 or MTF-1 in an *MT-I* promoter-driven luciferase reporter assay system and have determined *MT-I* mRNA levels. Our results suggest that the inhibitory effects of Cr(VI) on *MT-I* transcription may be due to its effects on the histone acetyltransferase (HAT)-independent transactivation ability rather than the HAT-dependent, histone deacetylase release-related transactivation ability of p300.

[*Biomed. Res.* **32**, 1-7 (2011)] [Lab. of Molecular Biology]

Caffeic Acid Phenethyl Ester Reduces Spinal Cord Injury-evoked Locomotor Dysfunction.

Masaki KASAI, Hidefumi FUKUMITSU, Hitomi SOUMIYA and Shoei FURUKAWA*

Caffeic acid phenethyl ester (CAPE) is a component of propolis, which is a substance taken from the hives of honeybees, and is known to exhibit an anti-inflammatory activity. In the present study, we evaluated the effect of CAPE on functional locomotor recovery after spinal cord injury (SCI) caused by hemi-transection, because inflammatory responses are a major cause of the secondary injury observed following SCI and play a pivotal role in regulating the pathogenesis of acute and chronic SCI. When CAPE was i.p.-administered at a dosage of 10 μmol/kg, it enhanced the recovery of locomotor function and reduced the lesion size while suppressing the expression of the mRNAs for a pro-inflammatory cytokine interleukin-1β and the inflammatory enzymes, inducible nitric oxide synthase and cyclooxygenase-2. These results suggest CAPE to be a promising therapeutic tool for reducing the secondary neuronal damage following primary physical injury to the spinal cord.

[*J.Neurosci.Res.* **89**, 1342-1350 (2011)] [Lab. of Molecular Biology]

Prenatal Immune Challenge Compromises Development of Upper-Layer but not Deeper-Layer Neurons of the Mouse Cerebral Cortex.

Hitomi SOUMIYA, Hidefumi FUKUMITSU* and Shoei FURUKAWA

Maternal infection during pregnancy is an environmental risk factor for the offspring to develop severe brain disorders, including schizophrenia. However, little is known about the neurodevelopmental mechanisms underlying the association between prenatal exposure to infection and emergence of cognitive dysfunctions later in life. By injecting the viral mimetic polyriboinosinic-polyribocytidylic acid (Poly I:C) into mice, we investigated the influence of maternal immune challenge (MIC) during pregnancy on the development of the cerebral cortex. Without affecting the cell number or density of the cortical neurons, MIC significantly compromised gene-expression profiles and synaptic development in the upper- but not in deeper-layer neurons. These abnormalities in the upper-layer neurons may underlie the development of psychiatric brain emerging after MIC.

[*J. Neurosci. Res.* **89**, 1575-1585 (2011)] [Lab. of Molecular Biology]

Prenatal Immune Challenge Compromises the Normal Course of Neurogenesis during Development of the Mouse Cerebral Cortex.

Hitomi SOUMIYA, Hidefumi FUKUMITSU* and Shoei FURUKAWA

Our previous study showed that MIC compromised the expression properties and the synaptogenesis of cortical upper-layer neurons. The objective of the current study was to examine further whether MIC has an influence on the cellular-biological features of the cortical progenitors that generate distinct cortical neuronal subtypes. We found the following abnormalities in the cortex of mice given the prenatal Poly I:C injection during later stages of cortical neurogenesis. First, proliferative activity and the expression of Pax6, a master regulatory gene for cortical progenitors, were significantly decreased in the cortical progenitors. Second, the laminar allocation and gene expression were significantly altered in the daughter neurons generated at the same birth dates. These results demonstrate that specific abnormalities in the cortical progenitors preceded deficits in neuronal phenotypes after MIC.

[*Toxicol. Appl. Pharmacol.***257**, 385-395 (2011)] [Lab. of Molecular Biology]

A Superoxide Anion-scavenger, 1,3-Selenazolidin-4-one Suppresses Serum Deprivation-induced Apoptosis in PC12 Cells by Activating MAP Kinase.

Atsuyoshi NISHINA, Hirokazu KIMURA, Kunihisa KOZAWA, Geoffroy SOMMEN, Takao NAKAMURA, Heinz HEIMGARTNER, Mamoru KOKETSU and Shoei FURUKAWA*

Synthetic organic selenium compounds, such as ebselen, may show glutathione peroxidase-like antioxidant activity and have a neurotrophic effect. We synthesized 1,3-selenazolidin-4-ones, new synthetic organic selenium compounds to study their possible applications as antioxidants or neurotrophic-like molecules. 2-[3-(4-Methoxyphenyl)-4-oxo-1,3-selenazolidin-2-ylidene] malononitrile (compound **b**) showed the strongest superoxide anion-scavenging activity among the 6 of 2-methylene-1,3-selenazolidin-4-ones examined. The compound **b** induced the phosphorylation of MAP kinase in PC12 cells; the activity was equivanlent to NGF, indicated that the compound **b** suppressed serum deprivation-induced apoptosis via activation of MAP kinase.

[*Int. J. Toxico.* ***30***, 690-699 (2011)] [Lab. of Molecular Biology]

3-(2,6-Dimethylphenyl)-2-selenoxo-1,3-thiazolidin-4-one Suppresses Hydrogen Peroxide-Induced Cytotoxicity on PC12 Cells via Activation of MAPK.

Atsuyoshi NISHINA, Hirokazu KIMURA, Kunihisa KOZAWA, Geoffroy SOMMEN, Francesco FAVERO, Heinz HEIMGARTNER,, Mamoru KOKETSU and Shoei FURUKAWA*

We newly synthesized organic selenium compounds (5-membered ring compounds) including 2-selenoxo-1,3-thiazolidin-4-ones (compounds A) and 3-alkoxy-4,5-dihydro-5-selenoxo-1H-1,2,4-triazole-1-carboxylates (compounds B). The O(2) (-)-scavenging activities were markedly different among compounds; 3-(2,6-Dimethylphenyl)-2-selenoxo-1,3- thiazolidin-4-one (compound Aa) exhibited the strongest activity. Compound Aa activated ERK1/2 of the PC12 cell, as did ebselen, and suppressed hydrogen peroxide-induced cytotoxicity more potently than ebselen. In addition, the toxicity of compound Aa was less than that of ebselen. These results indicate that compound Aa is a candidate drug to prevent oxidative stress-induced cell death.

[*J. Cell. Biochem.* **112**, 244-255 (2011)] [Lab. of Clinical Pharmaceutics]

Extracellular-Superoxide Dismutase Expression during Monocytic Differentiation of U937 Cells.

Tetsuro KAMIYA*, Junya MAKINO, Hirokazu HARA, Naoki INAGAKI and Tetsuo ADACHI

We observed the reduction of extracellular-superoxide dismutase (EC-SOD) and Cu,Zn-SOD during the differentiation of U937 cells induced by 12-*O*-tetradecanoylphorbol acetate (TPA). The reduction of EC-SOD and Cu,Zn-SOD was attenuated by pretreatments with GF109203X (an inhibitor of protein kinase C (PKC)), diphenyleneiodonium (an inhibitor of NADPH oxidase (NOX)) and U0126 (an inhibitor of mitogen-activated protein kinase kinase (MEK)/extracellular-signal regulated kinase (ERK)). We also determined the involvement of newly synthesized protein and the instability of mRNA in the reduction of EC-SOD. Overall, our results suggest that the expression of EC-SOD is decreased by TPA through intracellular signaling consisting of PKC, NOX-derived reactive oxygen species and MEK/ERK.

[*Neurochem. Int.* **58**, 35-43 (2011)] [Lab. of Clinical Pharmaceutics]

Endoplasmic Reticulum Stress Inducers Provide Protection against 6-Hydroxydopamine-Induced Cytotoxicity.

Hirokazu HARA*, Tetsuro KAMIYA and Tetsuo ADACHI

In this study, we investigated whether ER stress exerts preconditioning effects on 6-OHDA-induced cytotoxicity in SH-SY5Y cells. Pretreatment with ER stress inducers protected against the cytotoxicity. We also found that thapsigargin (Tg) induced the expression of the antioxidant gene HO-1. Flow cytometric analysis revealed that reactive oxygen species generated by 6-OHDA were suppressed in cells pretreated with Tg. Moreover, the specific eIF2α phosphatase inhibitor salubrinal augmented Tg-induced HO-1 expression. The reporter assay revealed that Tg stimulated the antioxidant response element (ARE) that is located in regulatory regions of antioxidant genes. Taken together, our data suggest that preconditioning effects induced by Tg mediate an adaptive response to 6-OHDA-induced cytotoxicity via phosphorylation of eIF2α and activation of the ARE.

[*Jpn. J. Pharm. Health Care Sci.* **37**, 179-186 (2011)] [Lab. of Clinical Pharmaceutics]

A Scheme for the Safety Management of Cancer Chemotherapy –Effective Use of Aseptic Preparation Records of Anticancer Agents Attached with Pharmaceutical Management Information–.

Tomokazu FUJII, Kenichi NOMURA, Naoki SAWAYANAGI, Haruhiko NAKAMURA, Sadatoshi IWASE, Tetsuo ADACHI* and Tsuneyuki KAMIYA

Pharmacists are expected to play an important role in the safety management of cancer chemotherapy by checking prescriptions. We prepared aseptic preparation records with pharmaceutical management information (PMI) using the comment function of Microsoft Office Excel to facilitate quick referencing concerning items needed for prescription checking for patients undergoing cancer chemotherapy. The active use of the PMI with these records allowed inquiries to be made earlier and more precisely, facilitating important changes in prescriptions such as dose reductions and regimen changes, and helping prevent any discomfort or inconvenience to patients.

[*Free Radic. Res.* **45**, 692-698 (2011)] [Lab. of Clinical Pharmaceutics]

ER Stress Inducer, Thapsigargin, Decreases Extracellular-Superoxide Dismutase through MEK/ERK Signaling Cascades in COS7 Cells.

Tetsuro KAMIYA*, Aya OBARA, Hirokazu HARA, Naoki INAGAKI and Tetsuo ADACHI

We demonstrated that thapsigargin, an endoplasmic reticulum (ER) stress inducer, decreased extracellular-superoxide dismutase (EC-SOD) expression, whereas the expression of Cu,Zn-SOD and Mn-SOD was not changed. On the other hand, another ER stress inducer, tunicamycin, did not affect the expression of EC-SOD. Further, we showed that thapsigargin has the ability to activate extracellular-signal regulated kinase (ERK), but tunicamycin does not. Moreover, pretreatment with U0126, an inhibitor of mitogen-activated protein kinase kinase (MEK)/ERK, suppressed thapsigargin-triggered EC-SOD reduction, suggesting that MEK/ERK signaling should play an important role in the regulation of EC-SOD in COS7 cells under ER stress conditions.

[*Biol. Pharm. Bull.* **34**, 1297-1300 (2011)] [Lab. of Clinical Pharmaceutics]

Effect of Hypoxia Mimetic Cobalt Chloride on the Expression of Extracellular-Superoxide Dismutase in Retinal Pericytes.

Tetsuo ADACHI*, Kazunari AIDA, Hiroko NISHIHARA, Tetsuro KAMIYA and Hirokazu HARA

The initial clinical stage of diabetic retinopathy (DR) is characterized by the development of intraretinal microvascular abnormalities. The increased formation of reactive oxygen species (ROS) is thought to be a key event in the pathogenesis of DR. Treatment with cobalt chloride ($CoCl_2$) decreased the expression of extracellular-superoxide dismutase (EC-SOD) but not other SOD isozymes in pericytes accompanied with an increase of intracellular ROS production. We observed the activation of caspase-3 and DNA fragmentation as signs of apoptotic process by $CoCl_2$ treatment. The decrease in EC-SOD expression accompanied with elevation of ROS level in pericytes under hypoxia might induce and/or promote the ROS-triggered apoptosis of pericytes and the development of pathogenesis in DR.

[*Free Radic. Res.* **45**, 1083–1092 (2011)] [Lab. of Clinical Pharmaceutics]

Endoplasmic Reticulum Stress Induces Retinal Endothelial Permeability of Extracellular-Superoxide Dismutase.

Tetsuo ADACHI*, Hiroyuki YASUDA, Shinsuke NAKAMURA, Tetsuro KAMIYA, Hirokazu HARA, Hideaki HARA and Tsunehiko IKEDA

The aim of this study was to determine the reasons why the intravitreal level of extracellular-superoxide dismutase (EC-SOD) increases in proliferative diabetic retinopathy patients by the investigation of two possibilities: firstly, change of EC-SOD expression in retina; secondly, leakage of EC-SOD through endothelial monolayer by the treatment with endoplasmic reticulum (ER) stress inducers because ER stress is known to be involved in the vascular impairment in diabetic retinopathy. Our observations suggest that ER stress leads to the down-regulation of claudin-5 among tight junction proteins and may induce the elevation of endothelial permeability and leakage of EC-SOD into vitreous body.

[*Biol. Pharm. Bull.* **34**, 1443-1447 (2011)] [Lab. of Clinical Pharmaceutics]

Extracellular-Superoxide Dismutase Expression in COS7 Cells Exposed to Cadmium Chloride.

Aya OBARA, Tetsuro KAMIYA*, Misato IZUMI, Hirokazu HARA, Harutaka YAMADA and Tetsuo ADACHI

In this study, exposure to cadmium chloride ($CdCl_2$) enhanced intracellular reactive oxygen species (ROS) generation and induced COS7 cell death. Moreover, exposure to $CdCl_2$ decreased the expression of extracellular-superoxide dismutase (EC-SOD) at mRNA and protein levels, but not of other SOD isozymes, Cu,Zn-SOD and Mn-SOD. The reduction of EC-SOD and cell viability was partially attenuated by pretreatment with an antioxidant, N-acetylcysteine. Further, we determined the involvement of p38-mitogen-activated protein kinase (p38-MAPK) in the reduction of EC-SOD. From these observations, p38-MAPK signaling cascades activated by ROS play a pivotal role in the reduction of EC-SOD, and it is concluded that the reduction of EC-SOD leads to a decrease in the resistance to oxidative stress of $CdCl_2$-exposed COS7 cells.

[*Bioorg. Med. Chem.* **19**, 5559-5568 (2011)] [Lab. of Clinical Pharmaceutics]

Inhibitory Effects of Chalcone Glycosides Isolated from *Brassica Rapa* L. 'Hidabeni' and Their Synthetic Derivatives on LPS-Induced NO Production in Microglia.

Hirokazu HARA*, Yoko NAKAMURA, Masayuki NINOMIYA, Ryosuke MOCHIZUKI, Tetsuro KAMIYA, Elias AIZENMAN, Mamoru KOKETSU and Tetsuo ADACHI

In this study, we examined the effects of chalcone glycosides isolated from *Brassica rapa* L. 'hidabeni' on lipopolysaccharide (LPS)-induced NO production using rat immortalized microglia HAPI cells. Compound **A2** inhibited LPS-induced inducible NO synthase (iNOS) expression and NO production. However, **A2** did not affect nuclear factor-κB and mitogen-activated protein kinase pathways. **A2** suppressed LPS-induced phosphorylation and nuclear translocation of STAT1. These results indicate that the inhibitory effect of **A2** is due to the prevention of STAT signaling.

[*Immunol. Lett.* **135**, 144-150 (2011)] [Lab. of Clinical Pharmaceutics]

Allograft Inflammatory Factor-1 is Overexpressed and Induces Fibroblast Chemotaxis in the Skin of Sclerodermatous GVHD in a Murine Model.

Aihiro YAMAMOTO, Eishi ASHIHARA, Yoko NAKAGAWA, Hiroshi OBAYASHI, Mitsuhiro OHTA, Hirokazu HARA*, Tetsuo ADACHI, Takahiro SENO, Masatoshi KADOYA, Masahide HAMAGUCHI, Hidetaka ISHINO, Masataka KOHNO, Taira MAEKAWA and Yutaka KAWAHITO

Allograft inflammatory factor (AIF)-1 is thought to be involved in the immune response. We demonstrated that immunoreactive AIF-1 and IL-6 were significantly expressed in infiltrating mononuclear cells and fibroblasts in thickened skin of Scl GVHD mice. Wound healing assay revealed that rAIF-1 increased the migration of normal human dermal fibroblasts directly, but cell growth assay did not show that rAIF-1 increased the proliferation of them. These findings suggest that AIF-1, which can induce the migration of fibroblasts and the production of IL-6 in affected skin tissues, is an important molecule promoting fibrosis in GVHD.

[*Hypertens. Res.* **34**, 686-692 (2011)] [Lab. of Clinical Pharmaceutics]

Olmesartan Improves Endothelial Function in Hypertensive Patients: Link with Extracellular Superoxide Dismutase.

Shunichi TAKIGUCHI, Makoto AYAORI, Harumi UTO-KONDO, Maki IIZUKA, Makoto SASAKI, Tomohiro KOMATSU, Bonpei TAKASE, Tetsuo ADACHI*, Fumitaka OHSUZU and Katsunori IKEWAKI

Endothelial dysfunction in essential hypertension is an independent predictor for future cardiovascular events. We therefore performed a prospective, randomized crossover trial in which an angiotensin II type 1 receptor antagonist, olmesartan and calcium channel blocker, amlodipine, were compared in 31 essential hypertensive patients. Results showed that olmesartan, but not amlodipine, significantly improved endothelial function as evaluated by flow-mediated vasodilation (FMD) in the brachial artery. Finally, although overall changes in plasma extracellular superoxide dismutase (EC-SOD) levels were not modulated by either treatment, for olmesartan there was a positive correlation between changes in FMD and those in EC-SOD levels.

[*Seitaishiryobunseki* **34**, 151–158 (2011)] [Lab. of Clinical Pharmaceutics]

Pathologic Background to Apo E- and TG-rich Lipoproteins Revealed by Discrimination via Linear Regression.

Naoko IKOSHI, Akira TANAKA, Yojiro MAEHATA, Masaichi-Chang-il LEE, Eisuke MAEHATA, Matsuo TANIYAMA, Takahiro IMAZATO, Kazunari MATSUMOTO, Noriko ISHIDA, Tsuyoshi NAKAMURA, Hiroji SHIMOMURA, Teruo SHIBA, Naoya KISHIKAWA, Naotada KURODA, Minoru INOUE, Ikukatsu SUZUKI and Tetsuo ADACHI*

We analyzed the clinical data for Apo E and triglyceride (TG) using discrimination via linear regression. The individuals above the regression line had significantly higher Apo E and TG levels. Apo E significantly correlated with increases in insulin resistance (HOMA-R) and serum amyloid A (SAA) and decrease in HDL-C and adiponectin. The results suggest that an Apo E- and TG-rich status may constitute a risk of atherosclerosis in metabolic syndrome (MS) and impaired glucose tolerance (IGT).

[*Redox Rep.* **15**, 131-137 (2010)] [Lab. of Clinical Pharmaceutics]

The Effect of Hypoxia Mimetic Cobalt Chloride on the Expression of EC-SOD in 3T3-L1 Adipocytes.

Tetsuro KAMIYA*, Hirokazu HARA, Naoki INAGAKI and Tetsuo ADACHI

It is well known that hypoxic adipocytes are in an increased oxidative stress. Extracellular-superoxide dismutase (EC-SOD) is an anti-inflammatory enzyme that protects cells from reactive oxygen species (ROS). Previous reports showed that plasma EC-SOD levels in type 2 diabetes patients were significantly and inversely related to the body mass index, homeostasis model assessment-insulin resistance index; however, the mechanisms of EC-SOD and adiponectin reductions during hypoxia remain poorly understood. Here, we demonstrate that cobalt chloride ($CoCl_2$) decreases EC-SOD and adiponectin in 3T3-L1 adipocytes by intracellular ROS-independent, but tumor necrosis factor-α (TNF-α) and c-jun N-terminal kinase-dependent mechanisms. From these results, it is possible that TNF-α is a key regulator of the reduction of EC-SOD and adiponectin in $CoCl_2$-treated 3T3-L1 adipocytes, and we speculated that the reduction of EC-SOD and adiponectin would lead to and/or promote metabolic disorders.

[*Redox Rep.* **15**, 250-258 (2010)] [Lab. of Clinical Pharmaceutics]

Regulation of Extracellular-superoxide Dismutase in Rat Retina Pericytes.

Tetsuo ADACHI*, Hiroyuki YASUDA, Kazunari AIDA, Tetsuro KAMIYA, Hirokazu HARA, Ken-ichi HOSOYA, Tetsuya TERASAKI and Tsunehiko IKEDA

Diabetic retinopathy (DR) is regarded as a disease of the retinal microvascular system and metabolic abnormalities that are characteristic of oxidative stress and endoplasmic reticulum (ER) stress have been identified in the retina. Treatment with own conditioned medium significantly decreased EC-SOD expression in pericytes, while the expression of VEGF and TNF-α were elevated. Moreover, the cell viability of pericytes changed in a manner similar to that of EC-SOD expression. Continuous flow of culture media neutralized the ER-stress triggered decrease of EC-SOD expression. The stagnation of factors related to ER-stress around pericytes might reduce EC-SOD expression under pathophysiological conditions such as retinal edema, and this could induce and/or promote the intraretinal microvascular impairment and development of athogenesis in DR.

[*J. Jpn. Soc. Hosp. Pharm.* **46**, 1377-1380 (2010)] [Lab. of Clinical Pharmaceutics]

Pharmacists' Efforts to Evaluate Safety Management of Cancer Chemotherapy.

Tomokazu FUJII, Kenichi NOMURA, Naoki SAWAYANAGI, Haruhiko NAKAMURA, Sadatoshi IWASE, Tetsuo ADACHI* and Tsuneyuki KAMIYA

At Kouseiren Atsumi Hospital, pharmacists' sphere of activity was stepwise widened. Pharmacists manage regimens for cancer chemotherapy and perform the aseptic preparation of all anticancer agents at present. In this study, we found the percentage of inquiries and prescription changes were increased and the number of incident was decreased with expansion of pharmacists' sphere of activity. In addition inquiries about the results of blood tests on the day of administration and about the premedication based on individualized information about each patient were increased. Furthermore, the number of incident concerning preparation and administration were decreased. From these results, it is suggested that our approach contributed to safety management of cancer chemotherapy.

[*Endocr J.* **57**, 423-430 (2010)] [Lab. of Clinical Pharmaceutics]

Effect of Pioglitazone on Various Parameters of Insulin Resistance Including Lipoprotein Subclass according to Particle Size by a Gel-permeation High-performance Liquid Chromatography in Newly Diagnosed Patients with Type 2 Diabetes.

Koji NAKANO, Goji HASEGAWA, Michiaki FUKUI, Masahiro YAMASAKI, Kiyoshi ISHIHARA, Tooru TAKASHIMA, Yoshihiro KITAGAWA, Aya FUJINAMI, Mitsuhiro OHTA, Hirokazu HARA, Tetsuo ADACHI*, Masakazu OGATA, Hiroshi OBAYASHI and Naoto NAKAMURA

Pioglitazone, an insulin-sensitizing agent has been reported to have anti-arteriosclerotic effects. The aim of this study was to obtain a better understanding of the mechanism involved in the insulin sensitizing effect of pioglitazone. The results in this study suggest that the hypoglycemic effect of pioglitazone is achieved mainly through improvement of hepatic insulin resistance, and that pioglitazone may have an antiatherosclerotic effect by decreasing serum atherogenic modified-LDL and by increasing adiponectin.

[*Arch. Biochem. Biophys.**515**, 80-88* (2011)] [Lab. of Pharmaceutics]

AMP-Activated Protein Kinase Modulates the Gene Expression of Aquqporin 9 via Forkhead Box a2.

Yuichi YOKOYAMA, Kazuhiro IGUCHI, Shigeyuki USUI and Kazuyuki HIRANO*

We investigated the transcriptional regulation of the AQP9 gene by AMP-activated protein kinase (AMPK). An AMPK activator, AICAR, was observed to suppress the expression of the AQP9 gene in HepG2 cells by promoting the phosphorylation of AMPK and AKT/PKB. Forkhead box a2 (Foxa2) was speculated to be one of the transcriptional regulators of AQP9 gene expression repressed by AICAR from the results of a reporter gene assay and knock-down of the Foxa2 gene by a specific siRNA. AICAR was determined to induce the phosphorylation and nuclear exclusion of Foxa2. Leptomycin B prevented nuclear export of Foxa2 triggered by AICAR. These results suggest that the activated AMPK by AICAR causes suppression of the gene expression of AQP9 through transcriptional regulation by Foxa2.

[*Tumour Biol.* **32**, 1097–1102 (2011)] [Lab. of Pharmaceutics]

Androgen Receptor W741C and T877A Mutations in AIDL cells, an Androgen-independent Subline of Prostate Cancer LNCaP cells.

Takashi OTSUKA, Kazuhiro IGUCHI, Kazuhiro FUKAMI, Kenichiro ISHII, Shigeyuki USUI, Yoshiki SUGIMURA and Kazuyuki HIRANO*

The androgen-independent LNCaP (AIDL) cell line was generated by maintaining prostate cancer LNCaP cells in a hormone-deprived medium. The aim of this study was to clarify the mechanisms underlying androgen sensitivity in AIDL cells. AR protein levels were induced by R1881 and DHT in LNCaP cells, but not in AIDL cells. AIDL cells harbored a missense substitution (TGG → TGT) in the AR gene, which caused a point mutation at codon 741 (W741C). Double T877A and W741C AR mutants have been previously reported to exhibit reduced androgen sensitivity. Hence, the low-androgen-sensitive responses of AIDL cells may be explained, at least in part, by AR gene mutations.

[*J. Androl.* **32**, 144–150 (2011)] [Lab. of Pharmaceutics]

Castration- and Aging-induced Changes in the Expression of Zinc Transporter and Metallothionein in Rat Prostate.

Kazuhiro IGUCHI, Naoaki MORIHARA, Shigeyuki USUI, Minoru HAYAMA, Yoshiki SUGIMURA and Kazuyuki HIRANO*

To clarify the mechanisms underlying zinc homeostasis in prostate, we examined zinc content and the expression of zinc transporters and metallothioneins in the prostates of aged or castrated rats. The expression of the zinc transporter Slc30a2 (Znt2) in ventral prostate (VP) of aged rats (21 months) was approximately 21-fold higher than that in VP of young rats (4 months), and zinc levels in VP of young rats increased significantly compared with that in aged rats. Decreased metallothionein-3 (Mt3) expression was observed in LP of castrated rats, and this reduction was prevented by testosterone replacement. Zinc content and Mt3 expression levels correlated significantly in rat LP. Our findings suggest that Mt3 could play a critical role in zinc homeostasis in rat LP.

[*J Neurol Sci.* **303**, 95-99 (2011)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Patterns of levels of biological metals in CSF differ among neurodegenerative diseases.

Isao HOZUMI* , Tatsuya HASEGAWA , Akiko HONDA , Kazuhiro OZAWA , Yuichi HAYASHI , Kazunori HASHIMOTO , Megumi YAMADA , Akihiro KOUMURA , Takeo SAKURAI , Akio KIMURA , Yuji TANAKA , Masahiko SATOH and Takashi INUZUKA

We measured the levels of some biological metalsin the cerebrospinal fluid (CSF) in patients with neurodegenerative diseases (52 patients with amyotrophic lateral sclerosis (ALS)), 21 patients with Alzheimer's disease (AD), and 20 patients with Parkinson's disease (PD) by inductively coupled plasma mass spectrometry (ICP-MS). In ALS, the levels of Mg ($p<0.01$ significant difference), Fe, Cu ($p<0.05$), and Zn ($p<0.10$) in CSF were higher than those in controls. In AD, the levels of Cu and Zn in CSF were significantly higher in patients with late-onset AD ($p<0.01$). In PD, we found significantly increased levels of especially Cu and Zn in particular ($p<0.01$) and Mn ($p<0.05$) in CSF. These findings suggest that Cu and Zn in particular play important roles in the onset and/or progression of ALS, AD, and PD.

[*Intern Med.* **50**, 2021-2024 (2011)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Diffuse skeletal muscles uptake of [(18)f] fuluorodeoxyglucose on positron emission tomography in primary muscle peripheral T-cell lymphoma.

Yuji TANAKA , Yuichi HAYASHI , Junichi KATO , Megumi YAMADA , Akihiro KOUMURA , Takeo SAKURAI , Akio KIMURA , Isao HOZUMI* , Yuichiro HATANO , Yoshinobu HIROSE , Tsuyoshi TAKAMI , Hiroshi NAKAMURA , Senji KASAHARA , Hisashi TSURUMI , Hisataka MORIWAKI and Takashi INUZUKA

A 40-year-old man presented with weakness of neck extensor muscles. Primary skeletal muscle non-Hodgkin's lymphoma of T-cell immunophenotype is extremely rare and fluorodeoxyglucose-positron emission tomography demonstrated striking fluorodeoxyglucose uptake in multiple skeletal muscles and served as a quite useful modality for the diagnosis of this patient.

[*J Neurol.* **258**, 421-426 (2011)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Is there a delayed gastric emptying of patients with early-stage, untreated Parkinson's disease? : An analysis using the 13C-acetate breath test.

Yuji TANAKA , Tomohiro KATO, Hiroshi NISHIDA , Megumi YAMADA , Akio KIMURA , Takeo SAKURAI , Yuichi HAYASHI , Akihiro KOUMURA , Isao HOZUMI* , Hiroshi ARAKI , Masahiko MURASE, Masahito NAGAKI , Hisataka MORIWAKI and Takashi INUZUKA

During the pre-symptomatic stage of Parkinson's disease (PD), the idiopathic PD related abnormal synuclein immunostaining is confined to the medulla oblongata and olfactory bulb, according to Braak. Delayed gastric emptying may be one of markers of the pre-clinical stage of PD.

[*J Neuroimmunol.* **233**, 175-180 (2011)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Identification of antibodies as biological markers in serum from multiple sclerosis patients by immunoproteomic approach.

Takeo SAKURAI , Akio KIMURA , Megumi YAMADA , Akihiro KOUMURA , Yuichi HAYASHI ,Yuji TANAKA , Isao HOZUMI* and Takashi INUZUKA

We identified the antibody against mitochondrial heat shock protein 70 (mtHSP70) in serum from multiple sclerosis (MS) patients by proteomics-based analysis. Results of our study suggest that not only the anti-PGAM1 antibody but also the anti-mtHSP70 antibody is good diagnostic markers of MS and the combination of both these antibodies is useful for a more specific diagnosis of MS.

[*J Pharmacol Exp Ther*. **338**, 337-344 (2011)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Fasudil and ozagrel in combination show neuroprotective effects on cerebral infarction after murine middle cerebral artery occlusion.

Akihiro KOUMURA , Junya HAMANAKA , Kazuhiro TSURUMA , Masamitsu SHIMAZAWA , Isao HOZUMI* , Takashi INUZUKA and Hideaki HARA

Rho kinase (ROCK), one of the serine/threonine kinases, is involved in pathologic conditions, and its activation causes neuronal cell death. The findings indicate that the combination treatment of fasudil and ozagrel may be useful as a potential therapeutic strategy for the treatment of stroke.

[*Neuroscience* .**189**, 293-298 (2011)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Metallothionein-III prevents neuronal death and prolong life span in amyotrophic lateral sclerosis model mice.

Kazunori HASHIMOTO , Yuichi HAYASHI , Kazuhiko WATABE , Takashi INUZUKA and Isao HOZUMI*

We explored the expression and effects of MT-III on the motor neurons of spinal cords of ALS model mice (G93A Cu/Zn superoxide dismutase (SOD-1) mutant-transgenic (Tg) mice) using a retrograde viral delivery system. Once-weekly injection of the adenovirus encoding LacZ or MT-III gene was started at the age of 20 weeks, which was the mean age of ALS onset. Gene expression was detected in the motor neurons of the lumbar spinal cord. The mean life spans were 163.20±7.72 days, 159.50±3.27 days, and 178.14±12.97 days in the untreated group, LacZ group, and MT-III group, respectively. We demonstrated that MT-III prevents the loss of motor neurons of ALS model mice and prolongs the life span, even when the administration is started at the time of onset.

[*J. Virol.* **85**, 4606-4611 (2011)] [Lab. of Microbiology]

A Tryptophan-rich Motif in the Human Parainfluenza Virus Type 2 V Protein is Critical for the Blockade of Toll-like Receptor 7 (TLR7)- and TLR9-dependent Signaling.

Yoshinori KITAGAWA, Mayu YAMAGUCHI, Min ZHOU, Takayuki KOMATSU, Machiko NISHIO, Tsuyoshi SUGIYAMA*, Kenji TAKEUCHI, Masae ITOH and Bin GOTOH

Plasmacytoid dendritic cells (pDCs) do not produce alpha interferon (IFN-α) unless viruses cause a systemic infection or overcome the first-line defense provided by conventional DCs and macrophages. We show here that even paramyxoviruses, whose infections are restricted to the respiratory tract, have a V protein able to prevent Toll-like receptor 7 (TLR7)- and TLR9-dependent IFN-α induction specific to pDCs. Mutational analysis of human parainfluenza virus type 2 demonstrates that the second Trp residue of the Trp-rich motif (Trp-X3-Trp-X9-Trp) in the C-terminal domain unique to V, a determinant for IRF7 binding, is critical for the blockade of TLR7/9-dependent signaling.

[*PLoS One* **6**, e26526. (2011)] [Lab. of Microbiology]

Specific Egg Yolk Immunoglobulin as a New Preventive Approach for Shiga-toxin-mediated Diseases.

Paola NERI, Shunji TOKORO, Ryo KOBAYASHI, Tsuyoshi SUGIYAMA, Kouji UMEDA, Takeshi SHIMIZU, Takao TSUJI, Yoshikatsu KODAMA, Keiji OGUMA and Hiroshi MORI*

In this study, we immunized chickens with formalin-inactivated Stx-1 or Stx-2, and obtained immunoglobulin Y (IgY) from the egg yolk. Anti-Stx-1 IgY and anti-Stx-2 IgY recognized the corresponding Stx A subunit and polymeric but not monomeric B subunit. Anti-Stx-1 IgY and anti-Stx-2 IgY suppressed the cytotoxicity of Stx-1 and Stx-2 to HeLa 229 cells, without cross-suppressive activity. In vivo, the intraperitoneal or intravenous administration of these IgY rescued mice from death caused by intraperitoneal injection of the corresponding toxin at a lethal dose. Moreover, oral administration of anti-Stx-2 IgY reduced the mortality of mice infected intestinally with EHEC O157:H7. Our results therefore suggest that anti-Stx IgY antibodies may be considered as preventive agents for Stx-mediated diseases in EHEC infection.

[*Bioorg. Med. Chem. Lett.* **21**, 801-804 (2011)] [Lab. of Biochemistry]

Structure of Rat Aldose Reductase-like Protein AKR1B14 Holoenzyme: Probing the Role of His269 in Coenzyme Binding by Site-directed Mutagenesis.

Krithika SUNDARAM, Urmi DHAGAT, Satoshi ENDO, Roland CHUNG, Akira HARA* and Ossama EL-KABBANI

Rat aldose reductase-like protein (AKR1B14) is the ortholog of mouse vas deferens protein (AKR1B7) playing roles in detoxification of reactive aldehydes and synthesis of prostaglandin $F_{2\alpha}$. The crystal structure of the binary complex (AKR1B14-NADPH) was determined at 1.86Å resolution, and showed that the adenine ring and the 2'-phosphate group of the coenzyme formed π-stacking and electrostatic interactions with the imidazole ring and ND1 atom, respectively, of His269, which is not conserved in other aldose reductase-like proteins. The interactions were supported by site-directed mutagenesis of His269 to Arg, Phe and Met, which increased the K_m for NADPH by 4, 7 and 127-fold, respectively. This is the first report of the tertiary structure of a rodent AKR1B7 ortholog, which describes the role of a novel dual interaction for the non-conserved His269 in coenzyme binding.

[*Bioorg. Med. Chem. Lett.* **21**, 2564-2567 (2011)] [Lab. of Biochemistry]

Probing the Inhibitor Selectivity Pocket of Human 20α-Hydroxysteroid Dehydrogenase (AKR1C1) with X-Ray Crystallography and Site-directed Mutagenesis.

Ossama EL-KABBANI, Urmi DHAGAT, Midori SODA, Satoshi ENDO, Toshiyuki MATSUNAGA and Akira HARA*

Human 20α-hydroxysteroid dehydrogenase (AKR1C1) is an important drug target due to its role in the development of lung and endometrial cancers, premature birth and neuronal disorders. We report the crystal structure of AKR1C1 complexed with the first structure-based designed inhibitor 3-chloro-5-phenylsalicylic acid (CPSA, K_i=0.86 nM) bound in the active site. The binding of CPSA to AKR1C1 resulted in a conformational change in the side chain of Phe311 to accommodate the bulky phenyl ring substituent at the 5-position of the inhibitor. The contributions of the nonconserved residues Leu54, Leu306, Leu308 and Phe311 to the binding were further investigated by site-directed mutagenesis, and the effects of the mutations on the K_i value were determined. The L54V and L306A mutations resulted in 6- and 81-fold increases, respectively, in K_i values compared to the wild-type enzyme.

[*J. Nat. Prod.* **74**, 1201-1206 (2011)] [Lab. of Biochemistry]

Selective Inhibition of the Tumor Marker Aldo-keto Reductase Family Member 1B10 by Oleanolic Acid.

Mayuko TAKEMURA, Satoshi ENDO, Toshiyuki MATSUNAGA, Midori SODA, Hai-Tao ZHAO, Ossama EL-KABBANI, Kazuo TAJIMA, Munekazu IINUMA and Akira HARA*

AKR1B10 was recently suggested as a therapeutic target in the treatment of several types of cancer. Selective inhibition of AKR1B10 compared with AKR1B1 is required for the development of anticancer agents. In this study, we have examined AKR1B10 inhibition by seven pentacyclic triterpenes that show potential anticancer properties. Among them, oleanolic acid (OA) was found to be the most potent competitive inhibitor (K_i=72 nM) with the highest AKR1B10/AKR1B1 selectivity ratio of 1370. OA also inhibited the cellular metabolism by AKR1B10 (IC_{50}, 4 μM) and decreased mitomycin C tolerance of colon cancer HT29 cells. Thus, the selective and potent inhibition of AKR1B10 by OA may be related to a possible cancer inhibitory role.

[*Anal. Chem.* **8**, 1131-1136 (2011)] [Lab. of Biochemistry]

Geometrical Separation Method for Lipoproteins Using Bioformulated-fiber Matrix Electrophoresis: Size of High-density Lipoprotein Does not Reflect Its Density.

Mari TABUCHI, Makoto SEO, Takayuki INOUE, Takeshi IKEDA, Akinori KOGURE, Ikuo INOUE, Shigehiro KATAYAMA, Toshiyuki MATSUNAGA*, Akira HARA and Tsugikazu KOMODA

In this study, we describe a novel geometrical electrophoretic separation technique incorporating recently developed nanotechnology (Nata de Coco) to analyze high-density lipoprotein (HDL), a beneficial component of the cholesterol fraction. A dyslipidemia patient given a 1-month treatment of fenofibrate showed an inverse relationship between HDL density and size. Direct microscopic observation and morphological observation of fractionated HDL particles confirmed a lack of relationship between particle density and size. This new technique may improve diagnostic accuracy and medical treatment for lipid related diseases.

[*Anti-Cancer Drugs* **22**, 402-408 (2011)] [Lab. of Biochemistry]

Involvement of the Aldo-keto Reductase, AKR1B10, in Mitomycin-c Resistance through Reactive Oxygen Species-Dependent Mechanisms.

Toshiyuki MATSUNAGA*, Yumi YAMANE, Keiko IIDA, Satoshi ENDO, Yoshiko BANNO, Ossama EL-KABBANI and Akira HARA

The human aldo-keto reductase (AKR) 1B10 is suggested as a tumor marker in various solid tumors. Using colon cancer cells, we found that AKR1B10 was induced with acquisition of resistance to the anticancer drug mitomycin-c (MMC). In the resistant cells, treatment with an AKR1B10 inhibitor decreased their MMC tolerance. In the nonresistant cells, overexpression and silencing of AKR1B10 decreased and increased, respectively, susceptibility to cytotoxic effects of MMC and 4-hydroxy-2-nonenal, which was formed as a product of lipid peroxidation by MMC treatment. These results suggest a role of AKR1B10 in the development of MMC resistance, which may be mediated by its ability to detoxify cytotoxic aldehydes including 4-hydroxy-2-nonenal.

[*Chem. Biol. Interact.* **191**, 364-370 (2011)] [Lab. of Biochemistry]

Protective Effect of Rat Aldo-keto Reductase (AKR1C15) against Endothelial Cell Damage Elicited by 4-Hydroxy-2-nonenal.

Toshiyuki MATSUNAGA*, Yuhki SHINODA, Yukari INOUE, Satoshi ENDO, Ossama EL-KABBANI and Akira HARA

We here investigated whether aldo-keto reductase (AKR) 1C15 acts as a protective factor against endothelial damage elicited by 4-hydroxy-2-nonenal (HNE) and oxidized lipoproteins. Treatment of endothelial cells with HNE provoked apoptosis through reactive oxygen species formation, mitochondrial dysfunction and caspase activation. AKR1C15 converted HNE into less toxic 1,4-dihydroxy-2-nonene, and its overexpression markedly decreased the HNE susceptibility. The AKR1C15 overexpression also suppressed the viability loss caused by oxidized low-density lipoprotein and its lipidic fraction. Collectively, these data indicate an anti-atherogenic function of AKR1C15 through the protection of endothelial cells from damage elicited by toxic lipids such as HNE.

[*Free Radic. Res.* **45**, 848-857 (2011)] [Lab. of Biochemistry]

Aldo-keto Reductase 1C15 as a Quinone Reductase in Rat Endothelial Cells Involved in Redox Cycling of 9,10-Phenanthrenequinone.

Toshiyuki MATSUNAGA*, Yuuki SHINODA, Yukari INOUE, Yuki SHIMIZU, Mariko HAGA, Satoshi ENDO, Ossama EL-KABBANI and Akira HARA

9,10-Phenanthraquinone (PQ) damaged rat endothelial cells via induction of CCAAT/enhancer-binding protein-homologous protein (CHOP), an apoptotic factor derived from endoplasmic reticulum stress. The PQ-mediated CHOP induction was strengthened by a proteasome inhibitor (MG132) and the MG132-induced PQ sensitization was abolished by inhibitor of reactive oxygen species (ROS), suggesting that ROS generation and proteasomal dysfunction are responsible for the PQ-induced CHOP upregulation. PQ provoked the aldo-keto reductase (AKR) 1C15 upregulation and the PQ-induced damage was augmented by the enzyme overexpression, suggesting the presence of a negative feedback loop exacerbating the quinone toxicity in rat endothelial cells.

[*Chem. Biol. Interact.* **191**, 261-268 (2011)] [Lab. of Biochemistry]

Roles of Rat and Human Aldo-keto Reductases in Metabolism of Farnesol and Geranylgeraniol.

Satoshi ENDO*, Toshiyuki MATSUNAGA, Chisato OHTA, Midori SODA, Ayano KANAMORI, Yukio KITADE, Satoshi OHNO, Kazuo TAJIMA, Ossama EL-KABBANI and Akira HARA

Farnesol (FOH) and geranylgeraniol (GGOH) are produced from the mevalonate pathway, and catabolized into the carboxylic acids. We investigated the intracellular distribution, sequences and properties of the oxidoreductases responsible for the metabolic steps in rat tissues. The oxidation of FOH and GGOH into their aldehyde intermediates were mainly mediated by alcohol dehydrogenases 1 and 7, and the subsequent step into the carboxylic acids was catalyzed by a microsomal aldehyde dehydrogenase. In addition, the major reductase catalyzing the aldehyde intermediates into alcohol forms was identified as AKR1C15 in rats and AKR1B10 and AKR1C3 in humans. The overall metabolism from FOH to farnesoic acid in cultured cells was significantly increased by addition of AKR1C3 inhibitors. Thus, AKRs may play an important role in controlling the bioavailability of FOH and GGOH.

[*Biochimie* **93**, 1476-1486 (2011)] [Lab. of Biochemistry]

Activation of Aldo-keto Reductase Family Member 1B14 (AKR1B14) by Bile Acids: Identification of the Bile Acid-binding Site by Site-directed Mutagenesis.

Satoshi ENDO*, Toshiyuki MATSUNAGA, Anna FUJITA, Tsukasa KURAGANO, Midori SODA, Krithika SUNDARAM, Urmi DHAGAT, Kazuo TAJIMA, Ossama EL-KABBANI and Akira HARA

AKR1B14 is involved in the synthesis of prostaglandin $F_{2\alpha}$ and detoxification of 4-oxononenal formed by lipid peroxidation. The NADPH-linked reductase activity of AKR1B14 was activated by various bile acids. Kinetic analyses of the activation by glycochenodeoxycholic acid, together with fluorescence changes and protection against 4-oxononenal-induced inactivation by bile acid, indicate that the bile acid binds to the enzyme and its coenzyme binary complex as a non-essential activator. Moreover, molecular docking studies and site-directed mutagenesis suggest that His269 plays a key role in significant activation through its electrostatic interaction with the carboxyl group of bile acid, facilitating the release of $NADP^+$.

[*Environ. Toxicol.* **26**, 224-232 (2011)] [Lab. of Pharmacology]

Characterization of Skin Inflammation Induced by Repeated Exposure of Toluene, Xylene and Formaldehyde in Mice.

Asaka SAITO, Hiroyuki TANAKA*, Haruki USUDA, Tomonori SHIBATA, Sayaka HIGASHI, Hirotaka YAMASHITA, Naoki INAGAKI and Hiroichi NAGAI

Volatile organic compounds (VOCs) are considered the main cause of sick building syndrome; however, the toxic threshold and the mechanisms of cutaneous reaction induced by long-time VOC exposure have not been clarified. In the present study, we investigated the effect of repeated painting of VOCs onto mouse skin. Various concentrations of toluene, xylene, and formaldehyde (FA) were applied once a week for 5 weeks. While FA solution (2-10%) induced remarkable ear swelling and caused evident infiltration of inflammatory cells, high concentrations of toluene and xylene (50 or 100%) evoked mild ear swelling and marginal inflammatory cell invasion. These findings demonstrate that FA has more potent irritancy against skin than toluene or xylene.

[*Int. Arch. Allergy Immunol.* **157**, 194-201 (2011)] [Lab. of Pharmacology]

Induction of Thymic Stromal Lymphopoietin Production by Xylene and Exacerbation of Picrylchloride-Induced Allergic Inflammation in Mice.

Nozomi SATOU, Kenji ISHIHARA, Masahiro HIRATSUKA, Hiroyuki TANAKA*, Yasuo ENDO, Saburo SAITO, Yoichiro IWAKURA, Warren J LENARD and Noriyasu HIRASAWA

Some chemical compounds in the environment worsen allergic inflammation. Here, we examined whether organic solvents induce the production of thymic stromal lymphopoietin (TSLP) which elicits Th2-type immune responses. Organic solvents were painted on the earlobes of BALB/c mice. Among the aromatic compounds, xylene and trimethylbenzene caused apparent TSLP production. The TSLP level in the xylene-treated earlobes reached a maximum at 24 h, and TSLP was expressed in epithelial tissues. Xylene promoted the picryl chloride-induced thickening of the ear and IL-4 production, which were reversed in TSLP receptor knockout mice, demonstrating that TSLP production induced by xylene is responsible for the exacerbation of allergic inflammation.

[*Eur. J. Pharmacol.* **667**, 389-395 (2011)] [Lab. of Pharmacology]

Role of Hematopoietic Prostaglandin D Synthase in Biphasic Nasal Obstruction in Guinea Pig Model of Experimental Allergic Rhinitis.

Daisuke KAJIWARA, Hiroki AOYAGI, Kazuhiko SHIGENO, Michinori TOGAWA, Katsunao TANAKA, Naoki INAGAKI* and Kazuhisa MIYOSHI

We investigated the role of hematopoietic prostaglandin D synthase (H-PGDS) in biphasic nasal obstruction in guinea pig allergic rhinitis using a new specific inhibitor (TAS-204). Treatment with oral TAS-204 for 15 days during the period of antigen challenges suppressed increases in nasal airway resistance in both phases. The late phase nasal obstruction was almost completely abrogated by inhibiting H-PGDS alone. Eosinophil infiltration in nasal lavage fluid and nasal hyperresponsiveness to histamine was also reduced by TAS-204 administration. These findings suggest that H-PGDS plays a critical role in the development of allergic rhinitis, especially in the induction of late phase nasal obstruction.

[*Proc. Natl. Acad. Sci. USA* **108**, 10349-10354 (2011)] [Lab. of Molecular Pharmacology]

On－off System for PI3-Kinase－Akt Signaling Through *S*-Nitrosylation of Phosphatase with Sequence Homology to Tensin (PTEN).

Naoki NUMAJIRI, Kumi TAKASAWA, Tadashi NISHIYA, Hirotaka TANAKA, Kazuki OHNO, Wataru HAYAKAWA, Mariko ASADA, Hiromi MATSUDA, Kaoru AZUMI, Hideaki KAMATA, Tomohiro NAKAMURA, Hideaki HARA*, Masabumi MINAMI, Stuart A. LIPTON and Takashi UEHARA

Low concentrations of nitric oxide (NO), as found in the penumbra, preferentially S-nitrosylate phosphatase with sequence homology to tensin (PTEN), whereas higher concentrations of NO, known to exist in the ischemic core, also S-nitrosylate Akt. In the penumbra, inhibition of PTEN (but not Akt) activity by S-nitrosylation would be expected to contribute to cell survival. In contrast, in the ischemic core, S-nitrosylation of Akt formation would inhibit this neuroprotective pathway. Thus, we identify unique sites of PTEN and Akt regulation by means of S-nitrosylation, resulting in an "on-off" pattern of control of Akt signaling.

[*Eur. J. Neurosci.* **33**, 843-855 (2011)] [Lab. of Molecular Pharmacology]

Involvement of Endoplasmic Reticulum Stress on Neuronal Cell Death in the Lateral Geniculate Nucleus in the Monkey Glaucoma Model.

Yasushi ITO, Masamitsu SHIMAZAWA, Yuta INOKUCHI, Hajime YAMANAKA, Kazuhiro TSURUMA, Kazuyuki IMAMURA, Hirotaka ONOE, Yasuyoshi WATANABE, Makoto AIHARA, Makoto ARAIE and Hideaki HARA*

We investigated whether endoplasmic reticulum (ER) stress was involved in the pathophysiological mechanisms underlying neuronal death of the lateral geniculate nucleus (LGN) after intraocular pressure (IOP) elevation. In the LGN region, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)-positive cells, polyubiquitin, phosphorylation of eukaryotic initiation factor 2α and C/EBP-homologous protein were also detected at 11-24 weeks after the laser photocoagulation treatment. These findings indicate that ER stress may play a pivotal role in neuronal death of the LGN after IOP elevation.

[*CNS Neurosci. Ther.* **17**, 294-304 (2011)] [Lab. of Molecular Pharmacology]

Apoptosis-Inducing Factor and Cyclophilin a Cotranslocate to the Motor Neuronal Nuclei in Amyotrophic Lateral Sclerosis Model Mice.

Hirotaka TANAKA, Hiroki SHIMAZAKI, Masataka KIMURA, Hiroshi IZUTA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

We investigated the process of motor neuron degeneration in amyotrophic lateral sclerosis (ALS) and to determine whether the cyclophilin A (CypA)- apoptosis-inducing factor (AIF) complex would play a role in inducing motor neuronal cell death in mutant superoxide dismutase 1 (SOD1)(G93A)ALS model mice. In the spinal cords of SOD1(G93A) mice, the expressions of CypA and AIF were detected in the motor neurons, and CypA and AIF cotranslocated to the motor neuronal nuclei with CypA. Furthermore, the expression of CypA was detected in GFAP-positive astrocytes, but not in CD11b-positive microglial cells. These results suggest that CypA and AIF may play cooperative and pivotal roles in motor neuronal death in the murine ALS model.

[*Curr. Neurovasc. Res.* **8**, 86-94 (2011)] [Lab. of Molecular Pharmacology]

Blockade of Phosphodiesterase-III Protects against Oxygen-glucose Deprivation in Endothelial Cells by Upregulation of VE-Cadherin.

Mitsunori ISHIGURO, Yukiya SUZUKI, Keisuke MISHIRO, Mamoru KAKINO, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Shinichi YOSHIMURA, Toru IWAMA and Hideaki HARA*

We examined whether cilostazol might promote expression of adhesion molecules in endothelial cells, thereby preventing deterioration of endothelial barrier functions. Human brain microvascular endothelial cells were exposed to 6-h oxygen-glucose deprivation (OGD). Cilostazol and db-cAMP prevented OGD-stress injury in endothelial cells by promoting VE-cadherin expression, but not PECAM-1. Cilostazol promotes VE-cadherin expression through cAMP/protein kinase A-dependent pathways in brain endothelial cells; thus, cilostazol effects on adhesion molecule signaling may provide protection against OGD stress in endothelial cells.

[*Neuroscience* **185**, 116-124 (2011)] [Lab. of Molecular Pharmacology]

Forebrain Specific Heparin-binding Epidermal Growth Factor-like Growth Factor Knockout Mice Show Exacerbated Ischemia and Reperfusion Injury.

Atsushi OYAGI, Nobutaka MORIMOTO, Junya HAMANAKA, Mitsunori ISHIGURO, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

In this study, we investigated the possible role of Heparin-binding epidermal growth factor-like growth factor (HB-EGF) in ischemia and reperfusion injury following a middle cerebral artery occlusion (MCAO). The levels of HB-EGF mRNA in the cerebral cortex of wild-type (WT) mice were significantly increased 3-24 h after MCAO and reperfusion. Cerebral infraction in HB-EGF knockout mice was aggravated at 1 day and 6 days after MCAO and reperfusion compared with WT mice. These results indicate that HB-EGF may play a pivotal role in ischemia and reperfusion injury and that endogenously synthesized HB-EGF is necessary for both the neuroprotective effect and for regulation of cell proliferation in the subventricular zone.

[*Brain Res.* **1419**, 97-104 (2011)] [Lab. of Molecular Pharmacology]

Heparin-Binding EGF-Like Growth Factor is Required for Synaptic Plasticity and Memory Formation.

Atsushi OYAGI, Shigeki MORIGUCHI, Atsumi NITTA, Kenta MURATA, Yasuhisa OIDA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Kohji FUKUNAGA and Hideaki HARA*

In this study, we assessed the role of heparin-binding epidermal growth factor-like growth factor (HB-EGF) in learning and memory by testing HB-EGF conditional knock-out mice (KO) in two different learning tasks, and evaluated the long-term potentiation (LTP) in hippocampus slices from these mice. The HB-EGF KO mice were impaired in spatial memory in the Morris water maze and in fear learning in a passive avoidance test. HB-EGF KO mice also showed an impaired LTP, and reduction in activity of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and phosphorylated GluR1. These results confirm the importance of the HB-EGF in synaptic plasticity and memory formation.

[*Pharmacol. Pharm.* **2**, 10-16 (2011)] [Lab. of Molecular Pharmacology]

Restraint-induced Expression of Endoplasmic Reticulum Stress-related Genes in the Mouse Brain.

Mitsue ISHISAKA, Takashi KUDO, Masamitsu SHIMAZAWA, Kenichi KAKEFUDA, Atsushi OYAGI, Kana HYAKKOKU, Kazuhiro TSURUMA and Hideaki HARA*

Previously, increases in an endoplasmic reticulum (ER) stress-related protein were reported in the temporal cortex of subjects with major depressive disorder who had died by suicide. The present study was designed to investigate whether acute stress could affect the ER stress response. Mice were immobilized for a period of 6 hr and then expression of ER stress response-related genes was measured by real-time PCR. After a 6 hr restraint stress, mRNA levels of ER stress-related genes, such as the 78-kilodalton glucose regulated protein (GRP78), the 94-kilodalton glucose regulated protein (GRP94), and calreticulin, were increased in the cortex, hippocampus, and striatum of mouse brain. These results suggest that acute stress may affect ER function and that ER stress may be involved in the pathogenesis of restraint stress, including the development of depression.

[*Biol. Pharm. Bull.* **34**, 1481-1486 (2011)] [Lab. of Molecular Pharmacology]

Luteolin Shows an Antidepressant-like Effect *via* Supressing Endoplasmic Reticulum Stress.

Mitsue ISHISAKA, Kenichi KAKEFUDA, Mika YAMAUCHI, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Akifumi TSURUTA and Hideaki HARA*

In the present study, we investigated the effects of luteolin on endoplasmic reticulum stress-induced neuronal cell death. Luteolin significantly suppressed tunicamycin-induced cell death at 1 to 10 μM in human neuroblastoma cells. Luteolin increased in the expression of the 78 kDa glucose-regulated protein and 94 kDa glucose-regulated protein and decreased in the cleavage activation of caspase-3. Additionally, to investigate whether chronic luteolin treatment has an antidepression effect, we performed some behavioral tests. Chronic luteolin treatment showed antidepressant-like effects in behavioral tests and, luteolin attenuated the expression of endoplasmic reticulum stress-related proteins in the hippocampus of corticosterone-treated depression model mice. These findings indicate that luteolin has antidepressant-like effects, partly due to the suppression of endoplasmic reticulum stress.

[*Life Sci.* **88**, 411-417 (2011)] [Lab. of Molecular Pharmacology]

Automated Experimental System Capturing Three Behavioral Components During Murine Forced Swim Test.

Etsuko HAYASHI, Midori SHIMAMURA, Kazuyoshi KURATANI, Mine KINOSHITA and Hideaki HARA*

An automated experimental system applying a commercially available video image analyzer was developed for the simultaneous detection and measurement of three behavioral components; immobility, swimming (horizontal movements) and climbing (vertical movements) that occur in the murine forced swim test (FST). In 2-4 min time span analysis, all four antidepressants reduced immobility and increased climbing significantly, desipramine and bupropion increased swimming significantly, while imipramine and fluvoxamine did not. The automated experimental system enabled efficient and accurate analysis of the three murine behaviors during FST at once. Climbing could be more sensitive parameter to detect anti-depressant-like effect than immobility in this system.

[*J. Pharmacol. Toxicol. Methods.* **64**, 119-123 (2011)] [Lab. of Molecular Pharmacology]

An Automated Evaluation System for Analyzing Antinociceptive Effects on Intracolonic Capsaicin-induced Visceral Pain-related Licking Behavior in Mice.

Etsuko HAYASHI, Tomohiro KOBAYASHI, Yasuteru SHIROSHITA, Kazuyoshi KURATANI, Mine KINOSHITA and Hideaki HARA*

The development of automated detection systems for animal behaviors is increasing, and SCLABA® (Noveltec Inc., Kobe, Japan) is a commercially available analysis system originally developed for analyzing scratching behaviors in rodents, based on distances between points in videotaped images. Here, we used this software to automate analysis of abdominal licking behavior associated with visceral pain in mice. We demonstrated that visceral pain-related licking behaviors after intracolonic capsaicin treatment can be automatically detected by applying commercially available image analysis software. This automated experimental system is very efficient and useful to evaluate antinociceptive effect of a test compound on visceral pain.

[*Arterioscler. Thromb. Vasc. Biol.* **31**, 1041-1048 (2011)] [Lab. of Molecular Pharmacology]

Tissue Kallikrein Inhibits Retinal Neovascularization *via* the Cleavage of Vascular Endothelial Growth Factor-165.

Shinsuke NAKAMURA, Nobutaka MORIMOTO, Kazuhiro TSURUMA, Hiroshi IZUTA, Yoshika YASUDA, Noriaki KATO, Tsunehiko IKEDA, Masamitsu SHIMAZAWA and Hideaki HARA*

To identify the role of tissue kallikrein in retinal neovascularization, we investigated the antiangiogenic effect by using an *in vitro* and *in vivo* angiogenesis model. Tissue kallikrein in vitreous fluid was markedly elevated in proliferative diabetic retinopathy patients,.and it inhibited vascular endothelial growth factor-165 ($VEGF_{165}$)-induced tube formation, proliferation, and migration *in vitro* angiogenesis model. Tissue kallikrein reduced the pathological vascular changes in retinal neovascularization. These findings indicate that tissue kallikrein is partly involved in pathogenesis of proliferative diabetic retinopathy and may be a promising therapeutic agent that could cleave $VEGF_{165}$ itself when administered by a peripheral route.

[*Invest. Ophthalmol. Vis. Sci.* **52**, 7289-7297 (2011)] [Lab. of Molecular Pharmacology]

Edaravone-Loaded Liposome Eyedrops Protect against Light-induced Retinal Damage in Mice.

Hiroki SHIMAZAKI, Kohei HIRONAKA, Takuya FUJISAWA, Kazuhiro TSURUMA, Yuichi TOZUKA, Masamitsu SHIMAZAWA, Hirofumi TAKEUCHI and Hideaki HARA*

To investigate the pharmacologic effects of eyedrops containing liposomes loaded with edaravone against light-induced retinal damage in mice. Eyedrop administration of edaravone-loaded submicron-sized liposomes (ssLips) significantly prevented both the retinal dysfunction and the shrinkage of the outer nuclear layer compared with the control group (treated with empty ssLips) after 5 days of light exposure. This marked protection was not found in the group treated with free edaravone. Edaravone-loaded ssLips showed a stronger inhibition of in vitro light-induced reactive oxygen species production and cell death than did free edaravone. These finding suggest that edaravone-loaded ssLips protect against light-induced retinal dysfunction by eyedrop administration, and liposomal eyedrops may become one of the therapeutic candidates for drug delivery to posterior eye segments.

[*Invest. Ophthalmol. Vis. Sci.* **52**, 9710-9720 (2011)] [Lab. of Molecular Pharmacology]

Ligation of the Pterygopalatine and External Carotid Arteries Induces Ischemic Damage in the Murine Retina.

Hiromi OGISHIMA, Shinsuke NAKAMURA, Tomohiro NAKANISHI, Shunsuke IMAI, Mamoru KAKINO, Fumiya ISHIZUKA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

This study aimed to characterize the functional and morphologic changes in a murine model of ocular ischemic disease caused by vascular occlusion. The ligation of both the pterygopalatine artery (PPA) and the external carotid artery (ECA) significantly reduced ocular blood flow and narrowed the blood vessels. Five hours of ischemia induced the retinal dysfunction and retinal damages. Edaravone, a free radical scavenger, significantly reduced the retinal ischemic damage. These findings indicate that the murine model in which both the PPA and the ECA are ligated may be useful to clarify the pathologic mechanisms of retinal ischemic diseases and to evaluate neuroprotective drugs that target retinal ischemic injury.

[*Curr. Neurovasc. Res.* **8**, 25-34 (2011)] [Lab. of Molecular Pharmacology]

An Arylidene-thiazolidinedione Derivative, GPU-4, without PPARγ Activation, Reduces Retinal Neovascularization.

Shinsuke NAKAMURA, Kei HAYASHI, Haruka TAKIZAWA, Tetsuji MURASE, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Hiroki KAKUTA, Hideko NAGASAWA and Hideaki HARA*

We investigated whether GPU-4, 5-arylidene-2,4-thiazolidinedione derivative, has anti-angiogenic activity regarding human retinal microvascular endothelial cells (HRMECs) and retinal neovascularization in a mouse model of retinopathy of prematurity. GPU-4 inhibited the vascular endothelial growth factor-induced radicals, proliferation, and migration in HRMECs without a PPARγ-mediated effect. Furthermore, systemic administration of GPU-4 inhibited the development of retinal neovascularization in a murine oxygen-induced retinopathy model. These findings indicate that GPU-4 suppressed *in vitro* and *in vivo* retinal neovascularization partly by a radical scavenging effect.

[*J. Neurosci. Res.* **89**, 1783-1794 (2011)] [Lab. of Molecular Pharmacology]

Involvement of Bid and Caspase-2 in Endoplasmic Reticulum Stress- and Oxidative Stress-induced Retinal Ganglion Cell Death.

Rumi UCHIBAYASHI, Kazuhiro TSURUMA, Yuta INOKUCHI, Masamitsu SHIMAZAWA and Hideaki HARA*

We investigated the mechanisms of endoplasmic reticulum (ER) stress- and oxidative stress-induced RGC death *in vitro* and *in vivo*. In an *in vitro* study, both Bid and caspase-2 inhibitors protected against cultured retinal ganglion cells (RGC-5) death from ER stress or oxidative stress. A caspase-2 inhibitor did not inhibit BH3-interacting domain death agonist (Bid) cleavage, although a Bid inhibitor reduced the increase of caspase-2 activity in ER stress-induced RGC-5 death. A Bid inhibitor also reduced the increase of caspase-2 activity in oxidative stress-induced RGC-5 death. In an *in vivo* study, a Bid inhibitor inhibited *N*-methyl-D-aspartate (NMDA)- or ER stress-induced mouse retinal damage. These findings indicate that a common mechanism through Bid and caspase-2 exists in both ER stress- and oxidative stress-induced RGC death.

[*Mol. Vision* **17**, 3556-3565 (2011)] [Lab. of Molecular Pharmacology]

Unoprostone Reduces Oxidative Stress- and Light-induced Retinal Cell Death, and Phagocytotic Dysfunction, by Activating BK Channels.

Kazuhiro TSURUMA, Yuka TANAKA, Masamitsu SHIMAZAWA, Yukihiko MASHIMA and Hideaki HARA*

The current study investigated the effects and the underlying mechanism of action of unoprostone on oxidative stress- and light irradiation-induced damage in photoreceptor and retinal pigment epithelial cultures. Unoprostone and its major metabolite, M1, protected against light- or H_2O_2-induced cell death in a mouse retinal cone cell line, 661W cells, and against light-induced phagocytotic dysfunction in a human retinal pigment epithelial cell line (ARPE-19) cells. Additionally, iberiotoxin, a selective inhibitor of BK channel, inhibited the protective effects of unoprostone and M1. These findings indicate that unoprostone has protective effects on oxidative stress- and light irradiation-induced damage *in vitro* and that these effects are mediated by activation of BK channels.

[*Eur. J. Pharmacol.* **650**, 110-119 (2011)] [Lab. of Molecular Pharmacology]

Crocetin Prevents Retinal Degeneration Induced by Oxidative and Endoplasmic Reticulum Stresses *via* Inhibition of Caspase Activity.

Mika YAMAUCHI, Kazuhiro TSURUMA, Shunsuke IMAI, Tomohiro NAKANISHI, Naofumi UMIGAI, Masamitsu SHIMAZAWA and Hideaki HARA*

In this study, we investigated the effects of crocetin, a carotenoid that is the aglicone of crocin, on retinal damage. Crocetin at a concentration of 3 μM showed the inhibitory effect of 50-60% against tunicamycin- and H_2O_2-induced RGC-5 cell (a retinal cell line) death and inhibited increase in caspase-3 and -9 activity. Moreover, crocetin inhibited the enzymatic activity of caspase-9 in a cell-free system. Crocetin at 100 mg/kg, p.o. significantly inhibited photoreceptor degeneration and retinal dysfunction in mice after excessive light exposure. These results indicate that crocetin has protective effects against retinal damage *in vitro* and *in vivo*, suggesting that the mechanism may inhibit increase in caspase-3 and -9 activities after retinal damage.

[*Curr. Eye Res.* **36**, 1153-1163 (2011)] [Lab. of Molecular Pharmacology]

Increased Expression of Tight Junctions in APRE-19 Cells Under Endoplasmic Reticulum Stress.

Tadanobu YOSHIKAWA, Nahoko OGATA, Hiroshi IZUTA, Masamitsu SHIMAZAWA, Hideaki HARA* and Kanji TAKAHASHI

To investigate the effects of endoplasmic reticulum (ER) stress on the tight junctions of the retinal pigment epithelial (RPE) cells *in vitro*. The expressions of the mRNAs and/or proteins of ER-stress releted factors were significantly increased in ARPE-19 cells, a human RPE cell line, under ER stress induced by tunicamycin (TM) and thapsigargin (TG). The mRNAs of VEGF were also increased by both TM and TG. The proteins and mRNAs of occludin and claudin-1 were significantly increased by TM and TG, and that of zonula occludens (ZO)-1 was significantly increased by TG. Immunohistochemistry showed that the staining of ZO-1, occludin and claudin-1 under ER stress was stronger than that of the control. A significant increase of cell permeability measured by transepithelial electrical resistance was observed after exposure to TM and TG.

[*Neurosci. Lett.* **488**, 87-91 (2011)] [Lab. of Molecular Pharmacology]

SUN N8075, a Novel Radical Scavenger, Protects against Retinal Cell Death in Mice.

Mai AKANE, Masamitsu SHIMAZAWA, Yuta INOKUCHI, Kazuhiro TSURUMA and Hideaki HARA*

In this study, we examined the effect of SUN N8075, a radical scavenger with neuroprotective properties, on murine retinal damage induced by intravitreous injection of *N*-methyl-D-aspartate (NMDA) or high-intraocular pressure (IOP). In both models, systemic administration of SUN N8075 decreased the cell loss in the ganglion cell layer (GCL) after retinal damage occurred. Moreover, SUN N8075 reduced the number of apoptotic cells and the expression of an oxidative stress marker in GCL in the NMDA model. These findings suggest that SUN N8075 has a neuroprotective effect against retinal damage, presumably via the radical scavenging effect.

[*J. Agric. Food Chem.* **59**, 528-536 (2011)] [Lab. of Molecular Pharmacology]

Purple Rice Extract and Anthocyanidins of the Constituents Protect against Light-induced Retinal Damage *in Vitro* and *in Vivo*.

Junji TANAKA, Tomohiro NAKANISHI, Kenjirou OGAWA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Hiroshi SHIMODA and Hideaki HARA*

This study evaluated the protective effects of purple rice (*Oryza sativa* L.) bran extract (PRE) and its major anthocyanidins (cyanidin and peonidin) against light-induced retinal damage. In an *in vitro* experiment, viability of 661W (a cultured murine photoreceptor cell line) after light treatment was improved by PRE, cyanidin, and peonidin. PRE, peonidin, and cyanidin exhibited radical scavenging activities. In an *in vivo* mouse experiment, intravitreous injection of PRE significantly suppressed photoreceptor degeneration induced by exposure to light. These findings suggest that PRE and its anthocyanidins possess protective effects with antioxidation mechanism in both *in vitro* and *in vivo* models of retinal diseases.

[*Phytother. Res.* **25**, 1160-1165 (2011)] [Lab. of Molecular Pharmacology]

The Protective Effect and Action Mechanism of *Vaccinium Myrtillus* L. on Gastric Ulcer in Mice.

Kenjirou OGAWA, Atsushi OYAGI, Junji TANAKA, Saori KOBAYASHI and Hideaki HARA*

Vaccinium myrtillus L. anthocyanoside (VMA) is used as a folk medicine to treat diseases related to gastric ulcers in northern Europe. However, the effects of VMA and its detailed mechanism on gastric ulcer have not been investigated sufficiently. Therefore, the aim of the present study was to investigate the protective effects of VMA on gastric mucosal damage in a murine gastric ulcer model. VMA (10, 30 and 100 mg/kg, p.o.) significantly protected gastric mucosa against HCl/ethanol-induced gastric ulcers. Furthermore, VMA inhibited lipid peroxide levels in a concentration-dependent manner and showed high scavenging activity against the superoxide anion radical ($\cdot O_2^{-}$) and the hydroxyl radical ($\cdot OH$). Anthocyanidins also showed scavenging activity against the $\cdot O_2^{-}$, while only delphinidin showed high scavenging activity against the $\cdot OH$. These findings indicate that the protective effects of VMA on HCl/ethanol-induced gastric mucosal injury may be partially due to the antiperoxidative effects of anthocyanidins.

[*BioFactors* **37**, 25-30 (2011)] [Lab. of Molecular Pharmacology]

The Aantianxiety-like Effect of Astaxanthin Extracted from *Paracoccus Carotinifaciens*.

Yasushi NISHIOKA, Atsushi OYAGI, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Takashi ISHIBASHI and Hideaki HARA*

Astaxanthin is a red carotenoid pigment and is widely found in living organisms. In this study, to investigate the effects of astaxanthin on anxiety and depression, we performed some behavioral trials including the elevated plus maze test, hole-board test, forced swim test, and tail suspension test. Astaxanthin (100 and 300 mg/kg/day for 10 days, p.o.) significantly increased the time spent in open arms in the elevated plus maze test and increased the head-dipping count and duration in the hole-board test. On the other hand, astaxanthin (10, 100, 300, and 500 mg/kg/day for 10 days, p.o.) did not change the immobility time in the forced swim test or the tail suspension test. In conclusion, in mice, astaxanthin exerted anxiolytic-like effects, but not antidepressant-like effects.

[*Chem.Res. Toxicol.* **24**, 1845–1852 (2011)] [Lab. of Clinical Pharmacy]

The Pivotal Role of Intracellular Calcium in Oxaliplatin-Induced Inhibition of Neurite Outgrowth but Not Cell Death in Differentiated PC12 Cells.

Miki TAKESHITA, Yoshiko BANNO, Mitsuhiro NAKAMURA, Mayuko OTSUKA, Hitomi TERAMACHI, Teruo TSUCHIYA* and Yoshinori ITOH

To elucidate the molecular mechanisms of oxaliplatin-induced neurotoxicity and the effects of Ca/Mg against this toxicity, we examined the effect of Ca/Mg on oxaliplatin-induced inhibition of neurite outgrowth in PC12 cells, a commonly used neuronal cell model. We suggest that the inhibition of neurite outgrowth but not tumor cell death induced by oxaliplatin is partly associated with reductions in [Ca2+] i and GAP-43 expression, and this inhibition was suppressed by the addition of Ca/Mg. Therefore, it may be assumed that Ca/Mg is useful for protecting against oxaliplatin-induced neurotoxicity without reducing the antitumor activity of oxaliplatin.

[*YAKUGAKU ZASSHI*. **131**,587-595 (2011)] [Lab. of Clinical Pharmacy]

Development of Skill Scale for Communication Skill Measurement of Pharmacis.

Hitomi TERAMACHI*, Natsuki KOMADA, Katsuya TANIZAWA, Yumi KUZUYA and Teruo TSUCHIYA

To purpose of this study was to develop the pharmacist communication skill scale. A 38 items scale was made and 283 pharmacists responded. The original questionnaire consisted of 38 items, with 1-5 graded Likert scale. Completed responses of 228 pharmacists data were used for testing the reliability and the validity of this scale. From factor analysis, four factors were chosen among the 31 items as follows: patient respect reception skill, problem discovery and solution skill, positive approach skill, feelings processing skill. The correlation coefficient between this original scale and the KiSS-18 (Social Skill) received high score (r=0.694). The reliability of this scale showed high internal consistency (Cronbach α coefficient=0.951), so the result of test for the validity of this scale supports high content validity. Thus we propose adoption pharmacist communication skill scale to carry a brief eponymous name as TePSS-31.The above findings indicate that this developed scale possess adequate validity and reliability for practical use.

[*Jpn. J.Pharm.Health Care Sci*. **37**, 195-201 (2011)] [Lab. of Clinical Pharmacy]

Questionnaire Survey and Analysis for Lecture "Feelings Management in the Communication".

Hitomi TERAMACHI*, Kennosuke TSUBOI, Natsuki KOMADA, Yumi KUZUYA, Kunihiro HISADA and Teruo TSUCHIYA

We examined for "Feelings management in the Communication" of this lecture with the KiSS-18 standard, introduced by Akio Kikuchi, which is a social skill measurement standard. We also examined the link with the social skill and the feelings management in the communication of the pharmacists. We carried out questionnaire survey on pharmacists (n=254) who attended the lecture of November 29, 2009. The collection rate was 92.1%, and the response rate was 79.9%. Three factors ("Trouble processing skill", "Smooth conversation skill", and "Problem solution skill") were extracted for a factor analysis for 18 questions of the KiSS-18 standard. The covariance structure analysis, proposed in the casual model infers that "Smooth conversation skill" affects the total factor, and it is possible to cope with trouble management, and the succeeding steps in the model.

[*Jpn. J.Pharm.Health Care Sci*. **37**, 535-541 (2011)] [Lab. of Clinical Pharmacy]

Questionnaire Survey on Usage of Patient Drug Information Leaflets.

Yumi KUZUYA, Hitomi TERAMACHI, Kennosuke TSUBOI, Masahiro YASUDA, Takashi MIZUI, Katsutoshi GOTO, Kazuhumi YONEDA and Teruo TSUCHIYA*

With the inclusion of a fee for providing drug information in the medical fee charging structure in 1996, the provision of information on drug information leaflets (hereinafter simply called "leaflets") became more prominent. However, as some patients have said they do not read leaflets or they do not need them, we lelt that the drug information provided on leaflets should be reevaluated to have it better reflect the fee charged.To do this, we conducted a survey of patients (n=195, age: 17 - 93 year) on their usage of leaflets. Almost all of patients read leaflets and kept them. We also found that patients had a keen sense of the necessity of leaflets and were satisfied with them. In addition, level of need for leaflets and opinions on the way information should be provided on them varied with age group.

[*Jpn. J.Pharm.Health Care Sci*. **37**, 653-660 (2011)] [Lab. of Clinical Pharmacy]

Development of a Measurement Scale for Cancer Patients Communication Skill for Pharmacists.

Hitomi TERAMACHI*, Natsuki KOMADA, Hitomi SHIGA, Kento TAMURA and Teruo TSUCHIYA

We have developed a pharmacist communication skill scale for cancer patients. First, we formulated 41 questions for improving the communication skill needed for reception of the cancer patients. Then we carried out a questionnaire (41 questions with 1-5 graded Likert scale), survey on pharmacists (n=584) during August 2010. The collection and the response rate was 36.3%. Finally, five factors ("Problem solution skill", "Patient psychology understanding skill", "self-control skill", "End period reception skill", and "Reporting skill to a patient and the family") were extracted for a factor analysis from 29 questions. The correlation coefficient between this original scale and the KiSS-18 (Social Skill) received high score (r=0.618). The reliability of this scale showed high internal consistency (Cronbach α coefficient=0.916) ; thus the result of test for the validity of this scale supports high content validity. We propose adoption of this pharmacist communication skill scale to carry a brief name as Topics-29.

[*Jpn. J.Pharm.Health Care Sci*. **37**, 681-692 (2011)] [Lab. of Clinical Pharmacy]

Development and Evaluation of a 'Practical Training Management Evaluation Web System'.

Hitomi TERAMACHI*, Yumi KUZUYA, Yukihiro YAMAMOTO and Teruo TSUCHIYA

We have developed 'practical training management evaluation web system' with the web browser to conduct a more effective and smooth the long term practical training for 6-year curriculum of pharmaceutical education at the Gifu Pharmaceutical University. We used this web system for long term practical training in 2010. At the end of the training, a questionnaire survey was carried out for students, pharmacists and university teachers. Particularly, the pharmacists working at the pharmacy highly evaluated many questions, including the questions for necessity and satisfactions of this web systems, than the hospital pharmacist. We infer that this web system was very useful for pharmacists working at the pharmacy on the support of the long term practical training. Generally, many students, pharmacists and university teachers evaluated that this web system is highly necessary and offer high satisfaction for the long term practical training. We conclude that we are successful in developing this web system.

[*J. Pharm. Commun*. **8**, 3-11 (2011)] [Lab. of Clinical Pharmacy]

Questionnaire Survey and Analysis for Five Years to Early Exposure (Pharmacy Visit) Program Use at Gifu Pharmaceutical University Pharmacy.

Hitomi TERAMACHI*, Eiji SAKAI and Teruo TSUCHIYA

At Gifu Pharmaceutical University, a visit to the Gifu Pharmaceutical University pharmacy as a part of the early exposure program was implemented for first year students from 2006 to 2010. After this community pharmacy visit, a questionnaire survey was conducted among the students who had participated in this program, about this addition to the curriculum. The pharmacists (n=6) who had conducted training this program, also answered a different questionnaire. Due to active participation of most students during the visit, a significantly higher level of satisfaction with the program than that was previously expected was achieved. Furthermore, many students commented that their experience in this program increased their motivation for future training. Almost all students considered it to be useful. These results indicate that an early exposure increased the interest of students in medical care.

[*J. Pharm. Commun*. **9**(1), 5-16 (2011)] [Lab. of Clinical Pharmacy]

Construction and Evaluation of Medical Communication Lessons Utilizing Pharmacist Trainer®.

Hitomi TERAMACHI*, Sayaka HIGASHI, Yuzo TAKAHASHI and Teruo TSUCHIYA

We developed a pharmacist simulator (Pharmacist Trainer®) as education stratagem in medical communication for pharmaceutical education. Pharmacist Trainer® had teaching materials in which a learner gains correspondence training along with a simulated patient model of computer graphic. First time interview had three cases and provision of information had four cases. About video, first time interview had three cases and provision of information had five cases. With these two stratagem, we offered medical communication lessons for 4th- year students (n = 76) at our university. After lesson, a questionnaire survey was conducted. The results suggested that students could learn communication knowledge and master communication skills (ratios more than grade 4: 77%, 71%). Covariance structure analysis shows that students were interested in curriculum additions that make use of Pharmacist Trainer® and videos, as it helps their self learning and learning communication skills.

[*J. Gifu Byoyaku* **53,** 11-16 (2011)] [Lab. of Clinical Pharmacy]

Questionnaire Survey and Analysis for Five Years to Early Exposure (Hospital Visit) Program Use at Gifu Pharmaceutical University Pharmacy.

Hitomi TERAMACHI*, Eiji SAKAI, Shoei FURUKAWA, Shingo KATSUNO and Teruo TSUCHIYA

At Gifu Pharmaceutical University, hospital visits were introduced as an early exposure program for first year students (2006: n=123, 2007: n=126, 2009: n=144, 2010: n=129, 201: n=140) from 2006 to 2011. After this hospital visits, a questionnaire survey was conducted among the students who had participated in this program, about this addition to the curriculum. Many students commented that their experience in this program increased their motivation for future training. Almost all students considered it to be useful. These results indicate that an early exposure increased the interest of students in medical care.

[*Palliative Care Research* **6**, 109-118 (2011)] [Lab.of Pharmacy Practice & Social Science]

Effectiveness of a Group Seminar on Opioids for Lung Cancer Patients.

Makoto NAKASHIMA, Hiromitsu KATO, Takuya GOTO, Syuichi MATSUMOTO, Sayo ISHII, Toshitaka SUZUKI, Kimiyasu SANO, Tatsuo KATO, Masumi SUZUI and Tadashi SUGIYAMA*

We conducted a group seminar for lung cancer patients. The purpose of the group seminar was to eliminate the negative notions about opioids and to impart the right knowledge about these drugs. After completion of the group seminar, the understanding and knowledge about opioids increased, as indicated by the responses of the patients to all of the questions. Complete elimination of the negative notion about opioids could not be achieved through the group seminar. However, the group seminar was thought to be one of the useful methods of educating patients. If a patient resists treatment with opioids, control of pain may be delayed. This in turn would hamper improvement of the quality of life. Therefore, we consider that it is necessary that a patient has the right knowledge about opioids beforehand.

[*Jpn. J. Pharm. Health Care Sci.* **37**, 419-424 (2011)] [Lab.of Pharmacy Practice & Social Science]

Development of a Checking Method in Preparation of the Liquid Cancer Chemotherapeutic Agents.

Makoto NAKASHIMA, Akira TAKAHASHI, Takuya GOTO, Mie NOMURA, Tomomi SUZUKI, Yukiko SHIBATA, Kimio WAKABAYASHI, Hiromitsu KATO, Takahiro KUMAGAI, Syuichi MATSUMOTO, Masumi SUZUI and Tadashi SUGIYAMA*

We devised the cheking system to see if preparation did precisely. The checking procedure is measurement of weight of vials and ampules that include drug before and after preparation, then we compare net different weight to theoretical different weight. By this checking method, we could detect that net different weight was out of tolerance level reliably when net different weight was +4% or -8% away for theoretical different weight. To do the check, preparation time was extended, but the difference was not statistically significant. Therefore, regarding to get the correctness in preparation of liquid cancer chemotherapeutic agents, we consider this checking method is useful.

[*J. Jpn. Soc. Health care Manag.***12**, 85 - 89(2011)] [Lab.of Pharmacy Practice & Social Science]

The Approach of Chemotherapy Committee about the Reduction of Medicine Costs by the Proper Use of the Antiemetics.

Makoto NAKASHIMA and Tadashi SUGIYAMA*

The preventive administration of the antiemetics which selected based on expression risk level of nausea and emesis occurred by cancer chemotherapy is effective method. In the Nagara medical center, chemotherapy committee reviewed usage of the antiemetic in reference to a National Comprehensive Cancer Network guideline. We compared medicine cost of the antiemetics which administered after a change with the estimation in cases where we did not change it. The medicine cost fell by 49.5% a year. In addition, the increase of the incidence of nausea and emesis was not showed by deletion of the granisetron. By selecting antiemetic appropriately in reference from a guideline, medicine costs fell without letting incidence of nausea and emesis increase, and we able to reduce the burden of the patient.

[*Jpn. J. Pharm. Health Care Sci.* **37**, 721-727 (2011)] [Lab.of Pharmacy Practice & Social Science]

Development of Anticancer Chemotherapy Support Software and its Evaluation.

Makoto NAKASHIMA, Takuya GOTO, Sachika MAETA, Mie NOMURA, Yukiko SHIBATA, Kimio WAKABAYASHI, Hiromitsu KATO, Takahiro KUMAGAI, Nobuyuki MISHIWA, Masumi SUZUI and Tadashi SUGIYAMA*

We developed a software package using Microsoft Excel for supporting works related to cancer chemotherapy. This support software facilitates management of the dosage schedule of anticancer agents and accurate count of the anticancer agent to be used and creation of worksheets for various purposes such as selecting the most cost-effective vials and ampoules, calculating the quantity of medicinal solution to be prepared. We believe that this support software is extremely useful in the efficient and safe management of works related to cancer chemotherapy.

[*Biochim. Biophys. Acta*. **1811**, 119–128 (2011)] [Lab. of Drug Informatics]

Heterogeneous Sphingosine-1-phosphate Lyase Gene Expression and its Regulatory Mechanism in Human Lung Cancer Cell Lines.

Hiromi ITO, Kayo YOSHIDA, Masashi MURAKAMI, Kazumi HAGIWARA, Noriko SASAKI, Misa KOBAYASHI, Akira TAKAGI, Tetsuhito KOJIMA, Sayaka SOBUE, Motoshi SUZUKI, Keiko TAMIYA-KOIZUMI, Mitsuhiro NAKAMURA*, Yoshiko BANNO, Yoshinori NOZAWA and Takashi MURATE

The role of sphingolipid metabolic pathway has been recognized in determining cellular fate. Although sphingolipid degradation has been extensively studied, gene expression of human sphingosine 1-phosphate lyase (SPL) catalyzing sphingosine 1-phosphate (S1P) remains to be determined. Among 5 human lung cancer cell lines examined, SPL protein levels paralleled the respective mRNA and enzyme activities. High SPL transcription of H1155 cells was regulated by Sp1 and GATA-4/Sp1 complex formation, both of which bind to Sp1 sites of the 5'-SPL promoter.

[*J. Chromatogr. B.* **879**, 1029-1032 (2011)] [Lab. of Drug Informatics]

Direct-injection HPLC Method of Measuring Micafungin in Human Plasma using a Novel Hydrophobic/Hydrophilic Hybrid ODS Column.

Hiroaki URANISHI, Mitsuhiro NAKAMURA*, Hiroki NAKAMURA, Yukari IKEDA, Mayuko OTSUKA, Zenichiro KATO, Teruo TSUCHIYA

A direct-injection HPLC-based method has been developed for determining amounts of micafungin in human plasma using a novel hydrophobic/hydrophilic hybrid ODS column. The method is easy to perform and requires only 10 ìL of a filtered plasma sample. The chromatographic separations were carried out with a gradient mode. The fluorescence detection wavelengths of excitation and emission were set at 273 nm and 464 nm, respectively. The calibration curve of micafungin showed good linearity in the range of 0.5-20.0 ìg/mL (r(2)=1.00). The inter-day accuracy ranged from -9.8 to 1.5%. The precisions were less than 10%. This method is useful for the determination of micafungin in human plasma.

[*Mutagenesis.* **26**(2), 323–330 (2011)] [Lab. of Radiochemistry]

Effect of Cigarette Smoke on Mutagenic Activation of Environmental Carcinogens by Cytochrome P450 2A8 and Inactivation by Glucuronidation in Hamster Liver.

Kenjiro TATEMATSU*, Akihiro KOIDE, Masao HIROSE, Akiyoshi NISHIKAWA and Yukio MORI

To elucidate the mechanism underlying suppression of *N*-nitrosobis(2-oxopropyl)amine (BOP)-induced hamster pancreatic carcinogenesis by cigarette smoke (CS), hepatic levels of metabolic enzymes and mutagenic activation were assayed in hamsters exposed to CS for 4 weeks. CS exposure enhanced the levels of cytochrome P450 (CYP) 1A2 and 2A8 and mutagenicities of several mutagens with liver S9. However, no CS-induced alterations in the level of CYP2B, which is associated with BOP activation, and the mutagenic activities of BOP and other pancreatic carcinogens, were observed. CS enhanced UDP-glucuronyltransferase (UDPGT) activities towards 4-nitrophenol (4-NP). These results indicate that suppression of BOP-induced pancreatic carcinogenesis by CS might be attributed to increased detoxification by 4-NP UDPGT and not decreased CYP2B activation.

[*Shoyakugaku Zasshi* **65**, 108-113 (2011)] [Lab. of Herbal Garden]

A Quantitative Analysis of Schizandrin and Gomisin A in Schisandrae Fructus.

Takaomi Tagami, Keiko Arimoto, Michiho Ito, Yuko Osumi, Mamoru Okasaka, Tomonari Kanaya, Eiji Sakai*, Yasuo Shimada, Yoshitaka Takai, Takaomi Tagami, Kayoko Tokura, Kenichi Nakajima, Mamoru Noguchi, Takashi Hashizume, Yoichi Hista, Gisho Honda, Masataka Moriyasu, Yutaka Yamamoto and Tsuguo Yokokura.

Schisandrae Fructus is the fruit of *Schisandra chinensis* Baillon (*Schisandraceae*), whichi has been used as an ingredient of kampo formula such as Shoseiryuto. In order to control quality of this crude drug, several methods to analyze schizandrin and gomisin A in Schisandrae Fructus have been in reported. They use harmful solvents and acetonitrile; however acetonitrile has been short supply recently. A method for quantitative analysis using HPLC without acetonitrile or harmful solvents eas developed and was applied to 50 market samples for comparison. Schizandrin and gomisin A were both found in much greater quantities in the seeds than in the flesh of the fruits.

[*Chemistry & Biodiversity.* **8**, 476-482 (2011)] [Lab. of Herbal Garden]

Three New Constituents from the Roots of *Erythrina variegata* and Their Antibacterial Activity against Methicillin-Resistant *Staphylococcus aureus*.

Hitohi Tanaka, Ikunori Atsumi, Osamu Shirota, Setsuko Sekita, Eiji Sakai*, Masaru Sato, Jin Murata, Hiroko Murata, Dedy Darnaedi and Ih-Sheng Chen

Two new isoflavonoids, eryvarins V and W (1 and 2, resp.), and a new chromen-4-one derivative, eryvarin X (3), along with three known isoflavonoids, were isolated from the roots of *Erythrina variegata*. Their structures were established by spectroscopic analyses. Compound 1 is a rare naturally occurring isoflavanone which possesses a OH group at C(3). Among the new compounds 1-3, 2 exhibited a potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) strains.

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