[*Adv. Exp. Med. Biol.* **662**(4), 415–421 (2010)] [Lab. of Pharm. Med. Chemistry]

Design of Novel Hypoxia-targeting IDO Hybrid Inhibitors Conjugated with an Unsubstituted L-TRP as an IDO Affinity Moiety.

Hitomi NAKASHIMA, Kazuhiro IKKYU, Kouichiro NAKASHIMA, Keiichiro SANO, Yoshihiro UTO, Eiji NAKATA, Hideko NAGASAWA*, Hiroshi SUGIMOTO, Yoshitsugu SHIRO, Yoshinori NAKAGAWA and Hitoshi HORI

We presented here design, syntheses and inhibitory activities of novel hypoxia-targeting IDO hybrid inhibitors conjugated with an unsubstituted L-Trp as an IDO affinity moiety without inhibitor 1MT, such as L-Trp-TPZ hybrids 1 (TX-2274), 2 (UTX-3), 3 (UTX-4), and 4 (UTX-2). Among them TPZ-monoxide hybrid 1 have the strongest IDO inhibitory activity. It suggests that TPZ-monoxide hybrids 1 and 3 are able to bind the active site of IDO, TPZ hybrids 2 and 4 are able to bind the enzyme-substrate complex. We proposed the possible mechanism of action of TPZ hybrid 2 that may first affect as a hypoxic cytotoxin, and then metabolized to TPZ-monoxide hybrid 1, which may do as an IDO inhibitor more effectively than its parent TPZ hybrid 2.

[*Chem.bio.chem.* **11**(5), 673-680 (2010)] [Lab. of Pharm. Med. Chemistry]

Structural Investigation of the Binding of 5-Substituted Swainsonine Analogues to Golgi α-Mannosidase II.

Douglas A. KUNTZ, Shinichi NAKAYAMA, Kayla SHEA, Hitoshi HORI, Yoshihiro UTO, Hideko NAGASAWA* and David R. ROSE

Golgi alpha-mannosidase II (GMII) is a potential target for cancer chemotherapy. The natural product swainsonine is a potent inhibitor of GMII. In this paper we characterize the binding of 5alpha-substituted swainsonine analogues to the soluble catalytic domain of Drosophila GMII by X-ray crystallography. The phenyl groups of these analogues occupy a portion of the binding site not previously seen to be populated with either substrate analogues or other inhibitors and they form novel hydrophobic interactions. Approximately tenfold more active against the Golgi enzyme than the lysosomal enzyme, these inhibitors offer the potential of being extended into the N-acetylglucosamine binding site of GMII for the creation of even more potent and selective GMII inhibitors.

[*Chem. Comm.* **46**(14), 2462–2464(2010)] [Lab. of Pharm. Med. Chemistry]

Oxindole Synthesis by Palladium-catalysed Aromatic C-H Alkenylation.

Satoshi UEDA, Takahiro OKADA and Hideko NAGASAWA*

A strategy involving palladium-catalysed aromatic C-H functionalisation/intramolecular alkenylation provides a convenient and direct synthesis of 3-alkylideneoxindoles. In the presence of 5 mol% of PdCl(2)MeCN(2) and AgOCOCF(3), a wide variety of N-cinnamoylanilines gave 3-alkylideneoxindoles in moderate to good yield.

[*Radiat. Res.* **174**(4), 459-466 (2010)] [Lab. of Pharm. Med. Chemistry]

Evaluation of the Radiosensitivity of the Oxygenated Tumor Cell Fractions in Quiescent Cell Populations within Solid Tumors.

Shin-ichiro MASUNAGA, Hideko NAGASAWA*, Yong LIU, Yoshinori SAKURAI, Hiroki TANAKA, Genro KASHINO, Minoru SUZUKI, Yuko KINASHI and Koji ONO

Labeling of all proliferating cells in C57BL/6J mice bearing EL4 tumors was achieved by continuous administration of 5-bromo-2'-deoxyuridine (BrdU). Tumors were irradiated with gamma rays at a high dose rate or a reduced dose rate at 1 h after the administration of pimonidazole. Overall, the quiescent cell population showed significantly greater radioresistance and capacity to recover from radiation-induced damage than the total tumor cell population. Thus we believe that the subfraction of the quiescent tumor cell population that was not labeled with pimonidazole and that was probably oxygenated is a critical target in the control of solid tumors.

[*Tetrahedron Lett.* **51**(44), 5778-5780 (2010)] [Lab. of Pharm. Med. Chemistry]

Enantioselective Darzens Reaction Using Organoselenide-lithium Hydroxide Complexes.

Shin-ichi WATANABE, Risa HASEBE, Jun OUCHI, Hideko NAGASAWA* and Tadashi KATAOKA

Asymmetric Darzens reaction catalyzed by chiral selenides is described. A novel Lewis acid/Brønsted base catalyst formed by a C2 symmetric chiral selenide bearing isoborneol skeletons, which were readily prepared from (1S)-10-camphorsulfonic acid, and LiOH promoted the reaction of phenacyl bromide with aldehydes to afford the desired trans oxiranes with up to 62% ee.

[*Tetrahedron Lett.* **51**(6), 903–906 (2010)] [Lab. of Pharm. Med. Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 61: A Novel Smiles-truce Type Rearrangement Reaction of 4-(2-Cyanovinyloxy)butanenitriles to Give Cycloalkeno[1,2-*d*]furo[2,3-*b*]pyridines.

Kensuke OKUDA*, Norimasa WATANABE, Takashi HIROTA and Kenji SASAKI

The cycloalkeno[1,2-*d*]furo[2,3-*b*]pyridine skeleton was conveniently synthesized from fused 4-(2-cyanovinyloxy)butanenitriles in one step through sequential intramolecular Michael addition, β-elimination and intramolecular nucleophilic addition. This sequence thus consists of a novel Truce–Smiles type rearrangement followed by cyclization. The 5-amino derivatives were transformed further to lactams in good yields.

[*Chem. Pharm. Bull.* **58**(3), 369–374 (2010)] [Lab. of Pharm. Med. Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 62: Reaction of *N*-(Quinazolin-4-yl)amidine Derivatives with Hydroxylamine Hydrochloride and Anti-platelet Aggregation Activity of the Products.

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

The reactions of *N*-(5,6,7,8-tetrahydroquinazolin-4-yl)amidines and their amide oximes with hydroxylamine hydrochloride gave abnormal cyclization products *via* a ring cleavage of pyrimidine component accompanied with a ring closure of 1,2,4-oxadiazole to give *N*-[2-([1,2,4]oxadiazol-5-yl)cyclohexen-1-yl]formamide oximes. Similarly, *N*-(quinazolin-4-yl)amidines reacted with hydroxylamine hydrochloride gave the same results. The evaluation of inhibitory activities against platelet aggregation *in vitro* is also described to show one derivative has potent activity.

[*Chem. Pharm. Bull.* **58**(3), 363–368 (2010)] [Lab. of Pharm. Med. Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 63: Improved Synthesis of 5-Amino-1,2-dihydrofuro[2,3-*c*]isoquinolines *via* Truce–smiles Rearrangement and Subsequent Formation to Furo[2,3-*c*]isoquinoline.

Kensuke OKUDA*, Masahiko YOSHIDA, Takashi HIROTA and Kenji SASAKI

An improved synthesis of 5-amino-1,2-dihydrofuro[2,3-*c*]isoquinoline has been achieved using a slight modification of reaction conditions for the Truce–Smiles rearrangement. Acid treatment of the obtained 5-amino-1,2-dihydrofuro[2,3-*c*]isoquinolines gave unexpected ring-opened spiro ring compounds. The previously unreported parent compound, furo[2,3-*c*]isoquinoline, was also synthesized.

[*Chem. Pharm. Bull.* **58**(5), 685–689 (2010)] [Lab. of Pharm. Med. Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 64: Synthesis of 5-Amino-1,2,6,7-tetrahydrobenzo[*f*]furo[2,3-*c*]isoquinolines and Related Compounds. Evaluation of their Bronchodilator Activity and Effects on Lipoprotein Lipase mRNA Expression.

Kensuke OKUDA*, Hiroshi DEGUCHI, Setsuo KASHINO, Takashi HIROTA and Kenji SASAKI

Reaction of 1-(3-cyanopropoxy)-3,4-dihydronaphthalene-2-carbonitriles with potassium *tert*-butoxide gave 5-amino-1,2,6,7-tetrahydrobenzo[*f*]furo[2,3-*c*]isoquinolines via a Truce–Smiles rearrangement. The 5-amino group was transformed to the bromo derivatives which were allowed to react with aliphatic cyclic amines to produce amino derivatives. In contrast, a combination of imidazole and NaH gave a dihydrofuran ring cleaved product, the structure of which was confirmed by X-ray crystallographic analysis. Effects of the newly synthesized compounds on carbamylcholine chloride-induced contractions of trachea and lipoprotein lipase mRNA expression were also evaluated and found one promising bronchodilator.

[*Chem. Pharm. Bull.* **58**(5), 755–757 (2010)] [Lab. of Pharm. Med. Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 65: Ring Cleavage Reactions of Fused Furo[2,3-*c*]isoquinolines and Related Compounds with Various Nucleophiles.

Kensuke OKUDA*, Hiroshi DEGUCHI, Takashi HIROTA and Kenji SASAKI

Reaction of fused 2,3-dihydrofuro[2,3-*b*]pyridines with various nucleophiles (N and O) gave dihydrofuran ring cleaved products. The scope of this reaction was investigated in detail.

[*Acta Cryst*. E **66**(11), 2949 (2010)] [Lab. of Pharm. Med. Chemistry]

7-Chloro-1,2-dihydrofuro[2,3-*c*]isoquinolin-5-amine.

Kensuke OKUDA*, Kenji SASAKI, Takashi HIROTA and Hiroyuki ISHIDA

In the title compound, $C_{11}H_9ClN_2O$, the fused-ring system is essentially planar, with a maximum deviation of 0.0323 (16) Å. In the crystal, molecules are connected by N-H…O hydrogen bonds forming a zigzag chain along the *c* axis. Molecules are further stacked along the *a* axis through weak π-π interactions, the shortest distance between ring centroids being 3.6476 (8) Å.

[*J. Label. Compd. Pharm.* **53**, 686–692 (2010)] [Lab. of Organic Chemistry]

Synthesis of Deuterium-labelled Drugs by Hydrogen-deuterium (H-D) Exchange Using Heterogeneous Catalysis.

Nkaelang MODUTLWA, Tomohiro MAEGAWA, Yasunari MONGUCHI and Hironao SAJIKI*

Multi-deuterium incorporations into drugs, such as theophylline, caffeine, valpromide, phenytoin, and trimethoprim, were post-synthetically achieved by Pd/C-, Pt/C-, or/and Rh/C-catalyzed hydrogen-deuterium exchange reactions under neutral conditions using deuterium oxide as the deuterium source in the presence of hydrogen gas. The present study offers facile and convenient methods for the prepn. of highly deuterated medicines, which are expected to be used as long-lasting medicines as well as internal stds. for metabolic studies and for the quant. analyses of the parent drugs.

[*Tetrahedron*, **66**, 8654–8660 (2010)] [Lab. of Organic Chemistry]

Palladium on Charcoal-catalyzed Ligand-free Stille Coupling.

Yuki YABE, Tomohiro MAEGAWA, Yasunari MONGUCHI and Hironao SAJIKI*

An efficient ligand-free Stille coupling reaction catalyzed by palladium on charcoal was developed. Biaryls were obtained by the reaction of tetraphenyltin with aryl halides including aryl chlorides using LiCl as an additive. The reactions of tri-Bu tin compds. with aryl iodides were effectively expedited by the addn. of LiF. These reactions efficiently proceeded without a phosphine or arsenic ligand and no leached palladium was detected in the reaction mixt.

[*Org. Process Res. Dev.*, **14**, 1140–1146 (2010)] [Lab. of Organic Chemistry]

Pilot Plant Study of the PCB Degradation at Ambient Temperature and Pressure.

Yasunari MONGUCHI, Shinji ISHIHARA, Akiko IDO, Miki NIIKAWA, Koichi KAMIYA, Yoshinari SAWAMA, Hisamitsu NAGASE and Hironao SAJIKI*

A continuous pilot plant for the degrdn. of polychlorinated biphenyls (PCBs) by the palladium on carbon (Pd/C)-catalyzed hydrogenation in the presence of triethylamine was designed and constructed. Both undiluted PCBs obtained from a capacitor and dild. PCBs with desulfurized trans oil were smoothly decompd. at ambient temp. and pressure. Desulfurization of the trans oil was found to be essential for the efficient degrdn. due to the possible deactivation of the Pd/C by catalysis poisoning due to the sulfur-contg. materials in the oil. The combined use of the present degrdn. method and the catalytic desulfurization technol. for the purifn. of gasoline and kerosene could be used in practical applications.

[*Adv. Synth. Catal.*, **352**, 1630–1634 (2010)] [Lab. of Organic Chemistry]

Palladium on Carbon-catalyzed Synthesis of Benzil Derivatives from 1,2-Diarylalkynes with DMSO and Molecular Oxygen as Dual Oxidants.

Shigeki MORI, Masato TAKUBO, Takayoshi YANASE, Tomohiro MAEGAWA, Yasunari MONGUCHI and Hironao SAJIKI*

A palladium on carbon (Pd/C)-catalyzed synthetic method for the prepn. of benzil derivs. from 1,2-diarylalkynes has been established using DMSO and mol. oxygen as dual oxidants. Regardless of the elec. nature of the functional groups on the arom. rings, 1,2-diarylalkynes were oxidized to the corresponding benzil derivs. in high to excellent yields. Furthermore, the oxidn. could efficiently be catalyzed by both the dry and wet types of Pd/C under atm. conditions.

[*Org. Biomol. Chem.*, **8**, 3338–3342 (2010)] [Lab. of Organic Chemistry]

Palladium on Carbon-catalyzed Synthesis of 2- and 2,3-Substituted Indoles under Heterogeneous Conditions.

Yasunari MONGUCHI, Shigeki MORI, Satoka AOYAGI, Azusa TSUTSUI, Tomohiro MAEGAWA and Hironao SAJIKI*

A mild, efficient and LiCl-free synthetic method for indole derivs., e.g., I based on the heteroannulation of alkynes with 2-iodoanilines was achieved using palladium on carbon (Pd/C) and NaOAc in heated NMP. The N-tosyl protection of 2-iodoaniline expedited the reaction progress, while other protecting groups, such as tert-butoxycarbonyl, acetyl, and benzyloxycarbonyl groups, underwent deprotection under the present conditions. A variety of di- and monosubstituted alkynes could effectively react with N-tosyl-2-iodoaniline to give the corresponding indoles in good to high yields.

[*Chem. Eur. J.*, **16**, 7372–7375 (2010)] [Lab. of Organic Chemistry]

Copper-Midiated Reductive Amination of Aryl Halides with Trimethylsilyl Azide.

Yasunari MONGUCHI, Toshihide MAEJIMA, Shigeki MORI, Tomohiro MAEGAWA and Hironao Sajiki*

A variety of aryl halides react with $TMSN_3$ in the presence of copper species and an amine, in heated DMA, to give anilines as the sole products, without the formation of the corresponding aryl azides.

[*Chem. Commun.*, **46**, 4977–4979 (2010)] [Lab. of Organic Chemistry]

Regio-, Chemo- and Stereoselective Deuterium Labeling Method of Sugars Based on Ruthenium-catalyzed C-H Bond Activation.

Yuta FUJIWARA, Hiroki IWATA, Yoshinari SAWAMA, Yasunari MONGUCHI and Hironao SAJIKI*

An efficient and facile deuterium labeling of sugars has been achieved in a completely regio-, chemo- and stereoselective manner using the Ru/C-H_2-D_2O combination via C-H bond activation assisted by the coordination of Ru to the oxygen atom of the sugar-hydroxyl groups.

[*Adv. Synth. Catal.*, **352**, 718–730 (2010)] [Lab. of Organic Chemistry]

Ligand-free and Heterogeneous Palldium on Carbon-catalyzed Hetero-Suzuki-Miyaura cross-coupling.

Yoshiaki KITAMURA, Satoko SAKO, Azusa TSUTSUI, Yasunari MONGUCHI, Tomohiro MAEGAWA, Yukio KITADE and Hironao SAJIKI*

A ligand-free and heterogeneous palladium on carbon (Pd/C)-catalyzed hetero-Suzuki-Miyaura coupling reaction has been developed. The protocol enables the construction of both heterocyclic-alicyclic and heterocyclic-heterocyclic biaryl derivs. in good to excellent yields. Furthermore, Pd/C could be reused. The time-course study clarified that palladium was leached into the reaction media as the reaction proceeded and then completely deposited on the carbon support.

[*Heterocycles*, **80**, 537–555 (2010)] [Lab. of Organic Chemistry]

C-C Bond Formation on 5-Position of Uridine Ring by Morita-Baylis-Hillman Type Reaction.

Yasunari MONGUCHI, Kanoko YASUNAGA, Takashi TSUNODA, Takayuki ANDO, Tomohiro MAEGAWA, Kosaku HIROTA and Hironao SAJIKI*

A useful and efficient C-C bond formation reaction at the 5-position of uridine derivs. using a wide range of aldehydes was established on the basis of the Morita-Baylis-Hillman type reaction.

[*Synlett,* **14**, 2151–2155 (2010)] [Lab. of Organic Chemistry]

Regioselective Gold-catalyzed Allylative Ring Opening of 1,4-Epoxy-1,4-dihydronaphthalenes.

Yoshinari SAWAMA*, Koichi KAWAMOTO, Hiroyuki SATAKE, Norbert KRAUSE and Yasuyuki KITA

In the presence of a gold catalyst, the ring opening of 1,4-epoxy-1,4-dihydronaphthalenes, e.g. I (R^1 = MeO, Br, R^2 = H; R^1 = H, R^2 = Me, MeO), with allyltrimethylsilane affords allylnaphthalenes, e.g. II (R^1 = MeO, Br, R^2 = H; R^1 = H, R^2 = Me, MeO), in high yield. For unsym. substrates, high regioselectivity is obsd. in many cases. This reaction might proceed via tricyclic THF intermediates which are formed stereoselectively.

[*Pure Appl. Chem.*, **82**, 1529–1536 (2010)] [Lab. of Organic Chemistry]

Combined Coinage Metal Catalysis for the Synthesis of Bioactive Molecules.

Norbert KRAUSE, Özge AKSIN-ARTOK, Viola BREKER, Carl DEUTSCH, Birgit GOCKEL, Manojkumar POONOTH, Yoshinari SAWAMA*, Yuka SAWAMA, Tao SUN and Christian WINTER

A review. The use of the coinage metals copper, silver, and gold enables an efficient and stereoselective assembly of bioactive heterocycles via allenic intermediates. The synthesis of functionalized allenes by S_N2'-substitution or S_N2'-redn. is mediated or catalyzed by copper, whereas silver and gold are the catalysts of choice for subsequent 5- or 6-endo-cyclizations. Overall, this sequence proceeds with efficient center-to-axis-to-center chirality transfer.

[*Chem. Commun.*, **46**, 3976–3978 (2010)] [Lab. of Organic Chemistry]

Remarkable Effect of Phosphine on the Reactivity of *O*,*P*-Acetal-efficient Substitution Reaction of *O*,*P*-Acetal.

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

The structure and electronic nature of the phosphine have a significant influence on not only the formation, but also the subsequent transformation of *O*,*P*-acetals. The *O*,*P*-acetals generated from tris(*o*-tolyl)phosphine [(*o*-tol)$_3$P] underwent efficient substitution reactions with various nucleophiles.

[*Chem. Commun.*, **46**, 1772-1774 (2010)] [Lab. of Pharm. Synthetic Chemistry]

A facile Catalyst-free Synthesis of *Gem*-dihydroperoxides with Aqueous Hydrogen Peroxide.

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

gem-Dihydroperoxides were easily obtained from the corresponding carbonyl compounds in high yields through a catalyst-free method with aqueous H_2O_2 (35%) in 1,2-dimethoxyethane at room temperature.

[*Org. Lett.*, **12**, 1620-1623 (2010)] [Lab. of Pharm. Synthetic Chemistry]

Direct Asymmetric Aldol Reaction with Recyclable Fluorous Organocatalyst.

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

Direct asymmetric aldol reactions of aldehydes with ketones in the presence of a catalytic amount of fluorous sulfonamide **4** and trifluoroacetic acid result in the corresponding aldol products in high yields with up to 96% ee. The fluorous organocatalyst **4** can be readily recovered from the reaction mixture by fluorous solid-phase extraction and could be reused without a significant loss of the catalytic activity and enantioselectivity.

[*Org. Lett.*, **12**, 1948-1951 (2010)] [Lab. of Pharm. Synthetic Chemistry]

Tandem Oxidation/Rearrangement of β-Ketoesters to Tartronic Esters with Molecular Oxygen Catalyzed by Calcium Iodide under Visible Light Irradiation with Fluorescent Lamp.

Naohiko KANAI, Hiroki NAKAYAMA, Norihiro TADA and Akichika ITOH*

It was found that β-ketoesters were directly transformed to the corresponding α-hydroxymalonic esters, tartronic esters, with molecular oxygen catalyzed by calcium iodide under visible light irradiation from fluorescent lamp. This reaction includes tandem oxidation/rearrangement and has received much attention from the viewpoint of reduction of energy consumption, labor, and solvents.

[*Org. Lett.*, **12**, 3645-3647 (2010)] [Lab. of Pharm. Synthetic Chemistry]

Direct Aerobic Photo-oxidative Synthesis of Aromatic Methyl Esters from Methyl Aromatics via Dimethyl Acetals.

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

A useful method for facile synthesis of aromatic methyl esters from methyl aromatics via dimethyl acetals by aerobic photo-oxidation using inexpensive and easily handled CBr_4 as catalyst is reported. This is the first example for direct preparation of the corresponding aromatic methyl esters from methyl aromatics.

[*Adv. Synth. Catal.*, **352**, 2383-2386 (2010)] [Lab. of Pharm. Synthetic Chemistry]

An Efficient Synthesis of *Gem*-dihydroperoxides with Molecular Oxygen and Anthracene under Light Irradiation.

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

A new efficient dihydroperoxidation protocol of a wide variety of carbonyl compounds with molecular oxygen, anthracene, and 2-propanol under light irradiation afforded their corresponding *gem*-dihydroperoxides in high yields.

[*Org. Biomol. Chem.*, **8**, 4701-4704 (2010)] [Lab. of Pharm. Synthetic Chemistry]

Direct Synthesis of α-Bromoketones from Alkylarenes by Aerobic Visible Light Photooxidation.

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

The direct synthesis of α-bromoketones from alkylarenes by aerobic photooxidation with hydrobromic acid is reported. The key success for this direct oxidative reaction is due to control of bromination with acetic acid and ethanol, which are generated *in situ* by solvolysis of ethyl acetate in the course of the reaction.

[*Synlett*, 1979-1983 (2010)] [Lab. of Pharm. Synthetic Chemistry]

Direct Synthesis of 1,2-Diketones by Catalytic Aerobic Oxidative Decarboxylation of 1,3-Diketones with Iodine and Base under Irradiation of Fuorescent Light.

Norihiro TADA, Motoki Shomura, Hiroki NAKAYAMA, Tsuyoshi MIURA and Akichika ITOH*

We report a catalytic direct synthesis of 1,2-diketone from 1,3-diketone through iodine/base-catalyzed aerobic photooxidation under visible-light irradiation of fluorescent lamp.

[*Synlett*, 2335-2339 (2010)] [Lab. of Pharm. Synthetic Chemistry]

Facile Aerobic Photo-oidative Synthesis of Penacyl Iodides and Bromides from Styrenes Using I_2 or Aqueous HBr.

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

We report a useful method for facile synthesis of phenacyl iodides and bromides from styrene derivatives by aerobic photo-oxidation using I_2 or 48% aqueous HBr in the presence of water.

[*Tetrahedron Lett.*, ***51***, *4576-4578 (2010)*] [Lab. of Pharm. Synthetic Chemistry]

Facile Aerobic Poto-oxidative Syntheses of α,α-Dibromoacetophenones from Aromatic Alkynes with 48% aq HBr.

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

We report a useful method for facile aerobic photo-oxidative synthesis of α,α-dibromoacetophenones from aromatic alkynes with 48% aq HBr. This method provides the synthesis of α,α-dibromoacetophenones using inexpensive and easily handled bromine sources, harmless visible light, and molecular oxygen.

[*Tetrahedron Lett.*, **51**, 6098-6100 (2010)] [Lab. of Pharm. Synthetic Chemistry]

Aerobic Photooxidation of Benzylamide under Visible Light Irradiation with a Combination of 48% aq HBr and $Ca(OH)_2$.

Norihiro TADA, Kazunori BAN, Momoko YOSHIDA, Shin-ichi HIRASHIMA,
Tsuyoshi MIURA and Akichika ITOH*

Benzylamides were found to be oxidized to their corresponding diacylamines in the presence of molecular oxygen, catalytic 48% aq HBr, and $Ca(OH)_2$ under visible light irradiation of a fluorescent lamp.

[*J. Nat. Prod.*, **73**, 1499-1506 (2010)] [Lab.of Pharmacognosy]

Resveratrol Oligomers from *Vatica Albiramis*.

Naohito ABE, Tetsuro ITO, Kenji OHGUCHI, Minori NASU, Yuichi MASUDA, Masayoshi OYAMA,
Yoshinori NOZAWA, Masafumi ITO and Munekazu IINUMA*

Five new stilbenoids, vatalbinosides A-E, and 13 known compounds were isolated from the stem of Vatica albiramis. The effects of these new compounds on interleukin-1β-induced production of matrix metalloproteinase-1 in human dermal fibroblasts were examd. Three resveratrol tetramers, (-)-hopeaphenol, vaticanol C, and stenophyllol C, were identified as strong inhibitors of MMP-1 production.

[*Chem. Pharm. Bull.*, **58**, 1369-1378 (2010)] [Lab.of Pharmacognosy]

Chemical Constituents in the Leaves of *Vateria Indica*.

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

Comprehensive re-investigation of the chemical constituents in the leaves of Vateria indica (Dipterocarpaceae) resulted in the isolation of a novel resveratrol dimeric dimer having a C2-symmetrical structure, vateriaphenol F, and two new O-glucosides of resveratrol oligomers, vateriosides A (resveratrol dimer) and B (resveratrol tetramer), along with a new natural compound and 33 known compounds including 26 resveratrol derivatives. The absolute structures were elucidated by spectroscopic analysis, including two dimensional NMR and CD spectra.

[*Nutr. Metab.*, **7,** doi:10.1186/1743-7075-7-46 (2010)] [Lab.of Pharmacognosy]

Vaticanol C, a Resveratrol Tetramer, Activates PPARα and PPARβ/δ *in Vitro* and *in Vivo*.

Tomoko TSUKAMOTO, Rieko NAKATA, Emi TAMURA, Yukiko KOSUGE, Aya KARIYA,
Michiko KATSUKAWA, Satoshi MISHIMA, Tetsuro ITO, Munekazu IINUMA*, Yukihiro AKAO,
Yoshironi NOZAWA, Yuji ARAI, Shobu NAMURA and Hiroyasu INOUE

We evaluated the activation of PPARs by vaticanol C, a resveratrol tetramer, in cell-based reporter assays using bovine arterial endothelial cells, as well as the activation of SIRT1. Moreover, we tested the metabolic action by administering vaticanol C with the high fat diet to wild-type and PPARα-knockout male mice. We show that vaticanol C activates PPARα and PPARβ/δ in cell-based reporter assays, but does not activate SIRT1. Eight-week intake of vaticanol C with a high fat diet upregulates hepatic expression of PPARα-responsive genes and skeletal muscle expression of PPARβ/δ-responsive genes, but not PPARα-knockout mice. These findings indicate that activation of PPARα and PPARβ/δ by vaticanol C may be a novel mechanism.

[*Bioorg. Med. Chem.*, **18**, 3133-3139 (2010)] [Lab.of Pharmacognosy]

A Novel Kavalactone Derivative Protects Against H_2O_2-induced PC12 Cell Death via Nrf2/ARE Activation.

Arisa TANAKA, Nanako HAMADA, Yasunori FUJITA, Tomohiro ITOH, Yoshinori NOZAWA, Munekazu IINUMA* and Masafumi ITO

We synthesized a series of chemical-modified kavalactones and studied their effects on the ARE enhancer activity in rat pheochromocytoma PC12 cells. Among 81 compounds tested, a kavalactone derivatives, 2',6'-dichloro-5-methoxymethyl-5,6-dehydrokawain, exhibited the strongest ARE enhancer activity. The ARE activation and HO-1 protein induction by the compound 1 were higher than those by natural kavalactones. The experimantal results suggest that the compound 1 protects against oxidative stress-induced neuronal cell death via a preconditioning effect on the Nrf2/ARE activation.

[*Biol. Pharm. Bull.*, **33**, 122-124 (2010)] [Lab.of Pharmacognosy]

Inhibitory Effects of Flavonoid Glycosides Isolated from the Peel of Japanese Persimmon (*Diospyros Kaki* 'Fuyu') on Melanin Biosynthesis.

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

We found that the acetone extract of the peel of Japanese persimmon (*Diospyros kaki* 'Fuyu') inhibits melanin biosynthesis in mouse B16 melanoma cells. The activity-guided purification of the extract resulted in isolation of 2 active compounds, which were identified as flavonoid glycosides, isoquercitrin (quercetin-3-*O*-glucoside) and hyperin (quercetin-3-*O*-galactoside) by spectral analysis. Isoquercitrin and hyperin strongly inhibited the production of melanin (IC_{50}: 21.7 and 18.2 μM, respectively). The inhibitory effects were found to be mediated by suppression of tyrosinase expression.

[*Biol. Pharm. Bull.*, **33**, 714-716 (2010)] [Lab.of Pharmacognosy]

Allergy-preventive Effects of the Flowers of *Impatiens Textori*.

Emiko IWAOKA, Hisae OKU, Munekazu IINUMA* and Kyoko ISHIGURO

The allergy-preventive activity of a 35% EtOH extract of flowers of *Impatiens textori* Miq. was demonstrated in a continuing search for allergy-preventive substances from natural sources. The evaluation of its activity used an *in vivo* assay method for monitoring the blood flow decrease in the tail vein microcirculation of mice subjected to sensitization with hen-egg white lysozyme. Among the principal compounds, apigenin, luteolin, and luteolin 7-glucoside showed significant allergy-preventive effects.

[*Chem. Lett.*, **39**, 162-164 (2010)] [Lab. of Pharm. Anal. Chemistry]

Quinone-hydroquinone π-Conjugated Redox Reaction Involving Proton-coupled Electron Transfer Plays an Important Role in Scavenging Superoxide by Polyphenolic Antioxidants.

Tatsushi NAKAYAMA and Bunji UNO*

The proton-coupled electron transfer (PCET) from *p*-, *o*-, and *m*-dihydroxybenzenes (PQH_2, OQH_2 and MQH_2, respectively) to the hydroperoxy radical ($HO_2\cdot$) derived from superoxide ($O_2\cdot^-$) is investigated. It is demonstrated that PQH_2 and OQH_2 moieties are essential to scavenge $O_2\cdot^-$ via PCET. It is suggested that the antioxidant action of flavonoids relates to a planar preference of the ensuing radicals that allows extended electronic delocalization between adjacent rings. In this respect, natural polyphenolic antioxidants characterized by PQH_2 and OQH_2 moieties may have strong activity in scavenging $O_2\cdot^-$ in association with stabilization of $PQ\cdot^-$ and $OQ\cdot^-$ by adjacent rings.

[*Chromatographia*, **72**, 1043-1048 (2010)] [Lab. of Pharm. Anal. Chemistry]

Screening DNA Adducts by LC-ESI-MS-MS: Application to Screening New Adducts Formed from Acrylamide

Shinsuke INAGAKI, Haruo HIRASHIMA, Yukihiro ESAKA*, Tatsuya HIGASHI, Jun Zhe MIN, Toshimasa TOYO'OKA.

A method for screening DNA adducts with unknown chemical structures was developed; it involves the use of LC–ESI–MS–MS. In ESI product ion mass spectra of guanine adducts, fragment ions were observed at m/z 152 and 135. Precursor ion scan analysis of these fragment ions indicated that the screening of DNA adducts would be possible. The developed method was used for the analysis of DNA adducts derived from acrylamide, which is not only a constituent of many commonly consumed foods but also a carcinogenic compound. We successfully discovered new guanine adducts. The results of this study indicate that the developed method is useful for screening new DNA adducts.

[*Int. J. Pharm.*, **386**, 243-248 (2010)] [Lab. of Pharm. Engineering]

A Combinational Supercritical CO_2 System for Nanoparticle Preparation of Indomethacin.

Yuichi TOZUKA, Yuta MIYAZAKI and Hirofumi TAKEUCHI*

An improved system using both supercritical antisolvent precipitation and rapid expansion from supercritical to aqueous solution (RESAS) was proposed to overcome the problem of low solubility of medicinal substances in $scCO_2$. When the ethanol solution with IMC was sprayed into the vessel purged with $scCO_2$, no precipitation of IMC was observed if the CO_2 pressure was more than 15MPa at 40°C. This indicates that very small droplets of the ethanol solution with IMC could disperse in the high pressure CO_2. SEM images of freeze-dried sample showed that the suspension was composed of submicron particles with 300–500 nm. The freeze-dried sample of the IMC suspension after the treatment shows good redispersibility as a nanosuspension. This apparatus is found to be a promising way to produce fine crystals of IMC with a high yield.

[*Int. J. Pharm.*, **386**, 172-177 (2010)] [Lab. of Pharm. Engineering]

Release Profile of Insulin Entrapped on Mesoporous Materials via Freeze-thaw Method.

Yuichi TOZUKA, Eri SUGIYAMA and Hirofumi TAKEUCHI*

Adsorption profiles of insulin on porous materials and release profiles of insulin entrapped on folded sheet mesoporous silica (FSM) were studied. Three types of FSM with different pore sizes (3.0, 6.1, and 9.2 nm) were used as candidates. A simple technique of repeated freezing and thawing resulted in effective adsorption of insulin on mesoporous structures. The amount of adsorbed insulin, estimated by protein assay, increased with an increase in the pore sizes of FSM used. Nitrogen sorption analysis showed that the specific surface area and pore volume decreased according to the insulin adsorption. On the release profile of insulin, the smallest pore size of FSM (3.0) was found to be a suitable material for a fast release of insulin, whereas the medium-pore FSM (6.1) held the insulin inside the pores for a longer time. Consequently, the desired release of insulin could be achieved by selecting the appropriate pore size of FSM.

[*Int. J. Pharm.*,**397**, 92-95 (2010)] [Lab. of Pharm. Engineering]

Nanoparticles of Glycol Chitosan and Its Thiolated Derivative Significantly Improved the Pulmonary Delivery of Calcitonin.

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

A novel thiomer derivative of glycol chitosan (GCS) was synthesized by coupling with thioglycolic acid (TGA) and evaluated for the pulmonary delivery of peptides. Nanoparticles (NPs) based on GCS and GCS-TGA were obtained by the ionic gelation method and demonstrated a particle size in the range of 0.23-0.33μm with positive surface charge and high calcitonin entrapment. Fluorescent GCS-TGA NPs resulted in a 2-fold increase in mucoadhesion to lung tissue after intra-tracheal administration to rats as compared to non-thiolated NPs. Calcitonin-loaded GCS and GCS-TGA NPs resulted in a pronounced hypocalcemic effect for at least 12 and 24 h, and a corresponding pharmacological availability of 27 and 40%, respectively. These findings suggest that both GCS and its thiomer derivative are promising and safe carriers for pulmonary peptide delivery.

[*Adv. Powder. Tech.*, **21**, 305-309 (2010)] [Lab. of Pharm. Engineering]

Anomalous Dissolution Property Enhancement of Naringenin from Spray-dried Particles with α-Glucosyl Hesperidin.

Yuichi TOZUKA, Jyunichiro KISHI and Hirofumi TAKEUCHI*

Spray-dried particles were prepared with α-glucosyl hesperidin (Hsp-G), a hesperidin derivative with enhanced water solubility, to improve the solubility profile of naringenin (NRG). Naringenin was used as a model hydrophobic polyphenol. The spray-dried sample of NRG in the presence of Hsp-G formed 3-4μm spherical particles. The obtained powders dissolved immediately into the aqueous media and demonstrated dramatically high apparent solubility, over 60-fold greater than NRG crystals, when the loading ratio of NRG/Hsp-G was 1/20. The apparent solubility of NRG increased in proportion to the amount of Hsp-G loaded (R^2> 0.99). These results suggested that a specific molecular interaction was formed in spray-dried particles, resulting in the anomalous enhancement in the solubility of NRG.

[*Int. J. Pharm.*, **392**, 101-106 (2010)] [Lab. of Pharm. Engineering]

Improvement of Dissolution and Absorption Properties of Poorly Water-soluble Drug by Preparing Spray-dried Powders with α-Glucosyl Hesperidin.

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

The feasibility of α-glucosyl hesperidin (Hsp-G) to improve the dissolution and bioavailability of poorly water-soluble drug was investigated. A spray-dried powder (SDP) of Hsp-G and flurbiprofen (FP) was prepared by a spray-drying method. The SDPs of FP/Hsp-G resulted in pronounced improvement in both the dissolution rate and solubility of FP. The apparent solubility of FP in hydrochloric acid solution (pH 1.2) was improved by 10-fold more than untreated FP crystals when prepared as SDPs in Hsp-G. The bioavailability of FP from the prepared SDPs was evaluated *in vivo* after oral administration to rats, in comparison with the untreated FP crystals. The results revealed 2.5- and 2.8-fold improvement in the Cmax and AUC values, after oral administration of the SDPs of FP/Hsp-G. Hsp-G is a potentially safe material to enhance the dissolution and absorption of poorly water-soluble drugs.

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

Transglycosylated Stevia and Hesperidin as Pharmaceutical Excipients: Dramatic Improvement in Drug Dissolution and Bioavailability.

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

The capability of transglycosylated materials, α-glycosyltransferase-treated stevia (Stevia-G) and α-glycosyl hesperidin (Hsp-G), to enhance the bioavailability of poorly water-soluble drugs was investigated. Spray-dried particles (SDPs) of drug/transglycosylated material, such as, flurbiprofen (FP)/Stevia-G, probucol (PRO)/Stevia-G, FP/Hsp-G, and PRO/Hsp-G were prepared. All SDPs showed pronounced improvement in both dissolution rate and apparent drug solubility. Values for the AUC of untreated PRO, SDPs of PRO/Hsp-G and PRO/Stevia-G after oral administration to rats were 4.94±2.06, 26.08±4.52 and 48.79±9.97 μg h/mL, respectively. The effect on drug absorption enhancement may depend on the type of transglycosylated materials used. Stevia-G, a newly investigated material for this purpose, was found to have good potential for use as a pharmaceutical excipient.

[*Chem. Pharm. Bull.*, **58**, 214-218 (2010)] [Lab. of Pharm. Engineering]

Coloration Phenomenon of Mefenamic Acid in Mesoporous Silica FSM-16.

Kunikazu MORIBE, Ryo KINOSHITA, Kenjirou HIGASHI, Yuichi TOZUKA* and Keiji YAMAMOTO

Coloration of mefenamic acid (MFA) was investigated in the presence of mesoporous silica FSM-16 with 16.0Å (Oc) and 45.0Å (Doc) pore diameter. The color change of MFA/FSM-16 physical mixture from white to deep blue was observed by sealed-heating (SH) and the subsequent humidification (HU). The coloration and the color difference were caused by the changes of chroma and lightness. Powder X-ray diffraction data indicated that difference of the dispersed states of MFA molecules in FSM-16 mesopore affected the coloration. Solid-state ^{13}C-NMR showed that the molecular mobility of MFA was increased in the dispersed state in FSM-16 mesopores compared to the crystalline state. Structural changes of silanol groups in FSM-16 by humidification were observed by solid-state ^{29}Si-NMR. MFA adsorption in FSM-16 mesopore by SH as well as changes of the surface state of FSM-16 by HU affected the coloration of MFA.

[*Int. J. Pharm.*, **387**, 236-243 (2010)] [Lab. of Pharm. Engineering]

Ascorbyl Dipalmitate/PEG-lipid Nanoparticles as a Novel Carrier for Hydrophobic Drugs.

Kunikazu MORIBE, Sunao MARUYAMA, Yutaka INOUE, Sakiko KANEDA, Toyofumi SUZUKI, Kazuo TOMONO, Kenjirou HIGASHI, Yuichi TOZUKA* and Keiji YAMAMOTO

L-Ascorbyl 2,6-dipalmitate (ASC-DP), a fatty ester derivative of ascorbic acid, is poorly soluble in water and does not spontaneously form micelles or liposomal structures in water. We attempted to prepare an ASC-DP/surfactant nano-sized complex as a carrier for hydrophobic drugs. Several hydrophobic drugs were incorporated in the ASC-DP/DSPE-PEG nanoparticles. Stability, toxicity, and blood residence of the drug-containing ASC-DP/DSPE-PEG nanoparticles were evaluated using amphotericin B (AmB) as the model drug. When 2.0 mg/kg, FungizoneR was administered, the mice showed higher renal and hepatic toxicities. Intravenously administered AmB/ASCDP/DSPE-PEG nanoparticles demonstrated higher concentration in plasma than FungizoneR. Thus, the ASC-DP/DSPE-PEG nanoparticle system appears to be a promising delivery system for hydrophobic drugs.

[*J. Pharm. Sci.*, **99**, 4192-4200 (2010)] [Lab. of Pharm. Engineering]

Salicylic Acid/g-Cyclodextrin 2:1 and 4:1 Complex Formation by Sealed-heating Method.

Kenjirou HIGASHI, Yuichi TOZUKA*, Kunikazu MORIBE and Keiji YAMAMOTO

A novel complex of salicylic acid (SA) and γ-cyclodextrin (γ-CD) was obtained via the sealed-heating method. Quantitative determination of SA revealed that sealed-heated samples of SA and γ-CD with low water content (0.8–5.4%) formed the SA/γ-CD (2:1) complex, while the samples with high water content (8.5–11.5%) formed the SA/γ-CD (4:1) complex. The molecular arrangements of γ-CD in 2:1 and 4:1 complexes were determined by powder X-ray diffraction measurements to be in monoclinic-columnar and tetragonalcolumnar forms, respectively. The results of ^{13}C solid-state NMR measurements showed that two types of SA molecules resided in the 4:1 complex, whereas only one type of SA molecules existed in the 2:1 complex. The obtained 2:1 complex was assumed to contain two SA molecules per one γ-CD, with the SA molecules existing in the intermolecular spaces formed by the γ-CD columns.

[*J. Soc. Powder Technol. Jpn.*, **47**(6), 388-393 (2010)] [Lab. of Pharm. Engineering]

Evaluation of Availability of Sugar Ester (SE) Having Different Properties as a Lubricant.

Hitomi YAMAMOTO, Yuichi TOZUKA, Minoru UCHIDA and Hirofumi TAKEUCHI*

The lubricant property of sucrose esters of fatty acids (SEs) having different particle size and HLB was evaluated with a model formulation in tableting. The binding tendency of the model formulation composed of isomalt powder was depressed by adding SEs in the formulation. Tablets containing SE showed faster disintegration time compared to those containing magnesium stearate or calcium stearate, while the tablets containing SEs indicated a high tensile strength. Milled SE (S370-F) exhibited better tablet properties than original SE (S-370). The SEs having different HLB showed different effects on the resultant tablet properties such as the magnitude of tensile strength, disintegration time, and spreading properties. SEs are able to use as lubricants on tablet formulation by approximately controlling its particle size, content, and HLB.

[*J. Soc. Powder Technol. Jpn.*, **47(11)**, 773-778 (2010)] [Lab. of Pharm. Engineering]

Combination Processing for Preparing CoQ10/γ-CyD Nanoparticles Using both an Ultra High-pressure Homogenizer and a Pulse Combustion Dryer.

Akiko YAMAGUCHI, Yuichi TOZUKA and Hirofumi TAKEUCHI*

The aim of this study is to prepare fine particles of inclusion compound of CoQ_{10} and γ-Cyclodextrin (γ-CyD), in order to improve either solubility in aqueous media or bioavailability of CoQ_{10}. A pulse combustion drying system was used to prepare fine powders. The resultant powder shows n a formation of inclusion compound of CoQ_{10}/γ-CyD. When the suspension was passed through an ultra high-pressure homogenizer, prior to pulse combustion drying, the resultant powders could more easily form a dispersion of submicron particles in aqueous media. Besides, absorption property of CoQ_{10} could be improved significantly after administration of resultant powders. A combination processing using ultra high-pressure homogenizer and pulse combustion dryer was found to be a promising way to create fine particles of CoQ_{10}/γ-CyD complex with a good bioavailability.

[*Chem. Pharm. Bull.* **58**(11) 1521-1524 (2010)] [Lab. of Pharm. Engineering]

A Novel Rapid Quantitative Analysis of Drug Migration on Tablets Using Laser Induced Breakdown Spectroscopy.

Makoto YOKOYAMA, Martine TOURIGNY, Kenji MOROSHIMA, Junsuke SUZUKI, Miyako SAKAI, Kiyoshi IWAMOTO and Hirofumi TAKEUCHI*

We propose a novel, rapid, quantitative analysis of drug migration in tablets using laser induced breakdown spectroscopy (LIBS). Using manifold tablets, visual inspection, Fourier transform (FT)-IR mapping and LIBS analysis were carried out to evaluate the drug migration in the tablets. In this work, we compared the sample preparation, data analysis process and measurement time for visual inspection, FT-IR mapping and LIBS analysis. The results of the comparison between these methods demonstrated that LIBS analysis is the simplest and the fastest method for migration monitoring.

[*Chem. Pharm. Bull.* **58**(3) 432-434 (2010)] [Lab. of Pharm. Engineering]

Carbopol-lectin Conjugate Coated Liposomes for Oral Peptide Delivery.

Martin WERLE, Abdallah MAKHLOF and Hirofumi TAKEUCHI*

Within the current study, a delivery system based on a novel polymer-lectin conjugate (carbopol-lectin) was evaluated for the oral delivery of therapeutic peptides and proteins. It was demonstrated that covalent attachment of lectin to carbopol does neither decrease nor abolish the specific binding properties of lectin. Bioadhesion studies revealed that liposomes coated with carbopol lectin are more bioadhesive than liposomes coated with unmodified carbopol. Finally, the *in vivo* data suggest that carbopol-lectin conjugate coated liposomes are effective oral peptide delivery systems which are capable of increasing the pharmacological effect of orally administered calcitonin.

[*J. Photopolym. Sci. Thechnol,* **23**, 567-570 (2010)] [Lab. of Pharm. Physical Chemistry]

Activity Evaluation of Antibody Immobilized 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 discussed on the effect of the grafted alkyl groups on the thermal stability of self-assembled phospholipid layer based on their grafting ratio and structural characteristics. We also estimated the activity of immobilized cytochrome C antibody by sandwich enzyme linked immunosorbent assay (ELISA). The thermal stability of the self-assembled phospholipid layer depended on the density and structure of grafted alkyl groups. Cytochrome C antibody was immobilized onto self-assembled phospholipid layer containing stearic acid. It was suggested that the immobilization of antibody onto self-assembled phospholipid layer would be more useful than the direct immobilization onto polymer surface.

[*J. Photopolym. Sci. Thechnol,* **23**, 595-598 (2010)] [Lab. of Pharm. Physical Chemistry]

Plasma Surface Modification of Polymer Substrate for Cell Adhesion Control.

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

We have reported that the preparation of vinylmethylether-maleic acid copolymer (VEMAC)-immobilized polystyrene (PS) dish (PS/VEMAC) and the covalent immobilization of cell adhesive peptide “GRGDS” on the PS/VEMAC. In this study, the cell behaviors of mouse embroic fibroblast NIH3T3 on GRGDS-immobilized PS/VEMAC were examined under serum-free conditions. The result indicates that the promotional effects of immobilized GRGDS on cell adhesion and spreading are caused by a specific interaction of RGD peptide sequence and cell surface integlin receptor. The present study suggests that cell behaviors such as adhesion, spreading and proliferation can be controled on the GRGDS-immobilized PS/VEMAC under serum free conditions.

[*Thin Solid Films*, **518**, 3492-3496 (2010)] [Lab. of Pharm. Physical Chemistry]

Chemical Diagnosis of DLC by ESR Spectral Analysis.

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

Diamond-like carbon (DLC) films were deposited utilizing plasma enhanced chemical vapor deposition (PECVD) with four precursor gases such as methane, ethylene, acetylene and benzene in gas phase. Electron spin resonance (ESR) spectra showed that dangling-bond sites (DBSs) observed in all films were characterized by an isotropic broad single line. The DLC film with unsaturated precursor gases had the higher film growth rate and the higher DBS accumulative rate. Although the DBS in DLC films were quite stable at room temperature under anaerobic conditions, the DBS decayed rapidly to level off toward a limiting value when exposed to air. The stability and reactivity of the DBS in DLC film were assumed to depend on chemical structure of organic gas used as precursor.

[*J. Health Sci.*, **56**, 65-71 (2010)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Preventive Effect of Preinduction of Metallothionein on Mutagenicity Caused by Benzo[a]pyrene.

Masaki TAKAISHI, Masahiko SATOH, Junko S. SUZUKI and Hisamitsu NAGASE*

The effect of pretreatment with zinc (Zn) compounds on the mutagenicity of benzo[*a*]pyrene (B[*a*]P) was investigated using metallothionein (MT)-I/II null mice. B[*a*]P-induced micronucleus frequencies were reduced by Zn pretreatment in the wild-type mice but not in the MT-I/II null mice. Zn administration significantly increased the concentration of MT in the liver and bone marrow cells of wild-type mice, but the statuses of other cellular antioxidants, such as glutathione, catalase and superoxide dismutase, were unchanged. In addition, the activity of a major B[*a*]P metabolic activation enzyme, cytochrome P450 1A, was unchanged by Zn treatment in both MT-I/II null mice and wild-type mice. These results suggest that Zn pretreatment protects against the mutagenicity of B[*a*]P through the induction of MT synthesis.

[*J Toxicol Sci.,* **35**, 209-215 (2010)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Resistance of Metallothionein-III Null Mice to Cadmium-induced Acute Hepatotoxicity.

Akiko HONDA, Hiroaki KOMURO, Tatsuya HASEGAWA, Yoshiyuki SEKO, Akinori SHIMADA, Hisamitsu NAGASE*, Isao HOZUMI, Takashi INUZUKA, Hideaki HARA, Yasuyuki FUJIWARA, and Masahiko SATOH

We examined the sensitivity of metallothionein (MT)-III null mice to cadmium (Cd)-induced acute hepatotoxicity. Male MT-I/II null mice, MT-III null mice and wild-type mice were given s.c. injection of Cd and then the blood and liver were collected from each mouse under ether anesthesia at 2 days after the administration. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities elevated by injection of Cd were significantly higher in the MT-I/II null mice than in the wild-type mice. In the MT-III null mice, ALT and AST activities were not elevated following the injection of Cd. In the present study, it was clearly found that MT-III null mice were resistant to Cd hepatotoxicity, although MT-I/II null mice were sensitive to its toxicity. MT-III may be an accelerative factor in Cd-induced acute hepatotoxicity.

[*J Toxicol Sci.,* **35**, 225-230 (2010)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Involvement of Metallothionein (MT) as a Biological Protective Factor Against Carcinogenesis Induced by Benzo[a]pyrene (B[a]P).

Masaki TAKAISHI, Akinori SHIMADA, Junko S. SUZUKI, Masahiko SATOH and Hisamitsu NAGASE*

The purpose of this study was to examine whether intracellular metallothionein (MT) protects against benzo[a]pyrene (B[a]P)-induced forestomach and lung carcinogenesis. Ten-week-old male MT-I/II null mice and wild-type mice were orally administered B[a]P at a dose of 100 or 250 mg/kg twice a week for 4 weeks. The incidence of tumors in the forestomach and lung was 78.6% and 7.1% in the wild-type mice treated with 100 mg/kg B[a]P, respectively. In the MT-I/II null mice treated with B[a]P, tumor incidence in the forestomach and lung was 100% and 33.3%, respectively. The tumor area in the forestomach and lung in the MT-I/II null mice treated with B[a]P was greater than that of wild-type mice. These results suggest that MT acts as a biological protective factor against carcinogenesis induced by B[a]P.

[*J Toxicol Sci.,* **35**, 271-273(2010)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Microarray Analysis of the Liver in Metallothionein-III Null Mice Treated with Cadmium.

Akiko HONDA, Hiroaki KOMURO, Hisamitsu NAGASE*, Isao HOZUMI, Takashi INUZUKA, Hideaki HARA, Yasuyuki FUJIWARA, and Masahiko SATOH

In order to elucidate the effect of metallothionein (MT)-III on hepatic gene expression altered by cadmium (Cd), we examined gene expression patterns in the liver of MT-III null mice and wild-type mice after Cd injection using a DNA microarray containing 35,852 genes. In a comparison between Cd-injected MT-III null mice and Cd-injected wild-type mice, 9 genes were found to be up-regulated and 28 genes-including serum amyloid A1 (SAA-1) and SAA-2--were down-regulated.

[*J Toxicol Sci.,* **35**, 699-707 (2010)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Enhancement of Immediate Allergic Reactions by Trichloroethylene Ingestion via Drinking Water in Mice.

Ryo KOBAYASHI, Tadayoshi IKEMOTO, Makoto SEO, Masahiko SATOH, Naoki INAGAKI, Hiroichi NAGAI and Hisamitsu NAGSE*

BALB/c mice were treated with TCE dissolved in drinking water for 2 and 4 weeks, and the mice were immunized with ovalbumin (OVA)/aluminum hydroxide twice. On the final day of the TCE exposure period, we measured the active cutaneous anaphylaxis (ACA) reaction and the antigen-specific IgE level in serum as well as the histamine level at the allergic reaction site and assayed the proliferation rates of splenocytes collected from the animals. The ACA reaction was enhanced by TCE ingestion. The OVA specific IgE level was enhanced by TCE exposure. The proliferation rate of the splenocytes was enhanced by TCE ingestion. The enhancement of the ACA reaction by TCE ingestion via drinking water may be related to the increase in splenocyte proliferation.

[*Life Sci.,* **87**, 545-550 (2010)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Atteuation of Cadmium-induced Testicular Injury in Metallothionein-III Null Mice.

Akiko HONDA, Hiroaki KOMURO, Akinori SHIMADA, Tatsuya HASEGAWA, Yoshiyuki SEKO, Hisamitsu NAGASE*, Isao HOZUMI, Takashi INUZUKA, Hideaki HARA, Yasuyuki FUJIWARA, and Masahiko SATOH

In order to evaluate the role of metallothionein (MT)-III in cadmium (Cd)-induced testicular toxicity, we examined the sensitivity of MT-III null mice to severe testicular injury caused by Cd. Male MT-III null mice, MT-I/II null mice and wild-type mice were given a subcutaneous injection of CdCl(2) (15μmol/kg). The testis was collected from each mouse at 6, 12 and 24h after Cd administration. Testicular hemorrhages by evaluating morphology, hemoglobin content and histological parameters in the 3 types of mice were elevated by Cd injection. MT-III null mice were found to show attenuation of Cd-induced severe testicular toxicity. These results suggest the lack of MT-III contributes to protection of testis from Cd. In addition, regulation of Pnp2, Rd3, and Cdh24 mRNA levels may involve the sensitivity of MT-III null mice to Cd.

[*Water Res.,* **44**, 2409-2418 (2010)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Contamination with Retinoic Acid Receptor Agonists in Two Rivers in the Kinki Region of Japan.

Daisuke INOUE, Koki NAKAMA, Kazuko SAWADA, Taro WATANABE, Mai TAKAGI, Kazunari SEI, Min YANG, Junji HIROTSUJI, Jianying HU, Jun-ichi NISHIKAWA, Tsuyoshi NAKANISHI* and Michihiko IKE

This study was conducted to investigate the agonistic activity against human retinoic acid receptor (RAR) α in the Lake Biwa–Yodo River and the Ina River in the Kinki region of Japan. RARα agonistic activity was commonly detected in the surface water samples collected along two rivers at different periods. The results indicated that RARα agonists are always present and widespread in the rivers. Fractionation using high performance liquid chromatography (HPLC) directed by the bioassay found two bioactive fractions from river water samples, suggesting the presence of at least two RARα agonists in the rivers. Comparison of retention times in HPLC analysis and quantification with liquid chromatography–mass spectrometry analysis revealed that the major causative contaminants responsible for the RARα agonistic activity were not RAs (natural RAR ligands) and 4-oxo-RAs.

[*Biol Pharm Bull.,* **33**, 1878-1885 (2010)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

A Phagocytotic Inducer from Herbal Constituent, Pentagalloylglucose Enhances Lipoplex-mediated Gene Transfection in Dendritic Cells.

Shinichiro KATO, Keiichi KOIZUMI, Miyuki YAMADA, Akiko INUJIMA, Nobuhiro TAKENO, Tsuyoshi NAKANISHI*, Hiroaki SAKURAI, Shinsaku NAKAGAWA and Ikuo Saiki

Antigen-presenting cells are key vehicles for delivering antigens in tumor immunotherapy, and the most potent of them are dendritic cells (DCs). Here, we show that Paeoniae radix, herbal medicine, and the constituent, 1,2,3,4,6-penta-O-galloyl-β-D-glucose (PGG), have an attractive function to enhance phagocytosis in murine dendritic cell lines, DC2.4 cells. In particular, PGG in combination with lipofectin synergistically enhanced phagocytic activity. Hence, according to our data, PGG could be an effective aid in lipofection using dendritic cells. Furthermore, these findings provide an expectation that constituents from herbal plant enhance lipofection efficacy.

[*Acta Crystallographica,* **F66**, 333-336 (2010)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Crystallization and Preliminary X-ray Crystallographic Study of Phosphoglucose Isomerase from *Plasmodium Falciparum*.

Ken-ichi AOKI, Nobutada TANAKA, Yoshio KUSAKABE, Chiharu FUKUMI, Arayo HAGA*, Masayuki NAKANISHI, Yukio KITADE and Kazuo T. NAKAMURA

Phosphoglucose isomerase (PGI) is a key enzyme in glycolysis and glycogenesis that catalyses the interconversion of glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P). For crystallographic studies, PGI from the human malaria parasite Plasmodium falciparum (PfPGI) was overproduced in Escherichia coli, purified and crystallized using the hanging-drop vapour-diffusion method. X-ray diffraction data to 1.5 A ˚ resolution were collected from an orthorhombic crystal form belonging to space group P212121 with unit-cell parameters a = 103.3, b = 104.1, c = 114.6 A ˚ . Structural analysis by molecular replacement is in progress.

[*Cancer Res.,* **70**, 9483-9493 (2010)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Silencing of Autocrine Motility Factor Induces Mesenchymalto-epithelial Transition and Suppression of Osteosarcoma Pulmonary Metastasis.

Yasufumi NIINAKA, Kiyoshi HARADA, Masahiro FUJIMURO, Masamitsu ODA, Arayo HAGA*, Misa HOSOKI, Narikazu UZAWA, Naoya ARAI, Satoshi YAMAGUCHI, Masashi YAMASHIRO and Avraham RAZ.

The stable transfectant cells showed effective downregulation of AMF expression and subsequent abrogation of AMF secretion, which resulted in morphologic change with reduced growth, motility, and invasion. Silencing of AMF induced MET, in which upregulation of E-cadherin and cytokeratins, as well as downregulation of vimentin, were noted. The MET guided by AMF gene silencing induced osteosarcoma MG-63 to terminally differentiate into mature osteoblasts. Furthermore, MET completely suppressed the tumor growth and pulmonary metastasis of LM8 cells in nude mice. Thus, acquisition of malignancy might be completed in part by upregulation of AMF, and waiver of malignancy might also be controlled by downregulation of AMF.

[*Biomed. Res.,* **31**, 45-52 (2010)] [Lab. of Mol. Biology]

4-Methylcatechol-induced Heme Oxygenase-1 Exerts a Protective Effect against Oxidative Stress in Cultured Neural Stem/Progenitor Cells via PI3 Kinase/ Akt Pathway.

Yoshiko FURUKAWA, Tomomi URANO, Misato MINAMIMURA, Mitsunari NAKAJIMA, Satoshi OKUYAMA and Shoei FURUKAWA*

4-Methylcatechol (4MC), a stimulator of the synthesis of neurotrophin in various cells, was able to up-regulate the expression of heme oxygenase (HO)-1, a redox-sensitive inducible stress protein, in neural stem/progenitor cells (NS/PCs). When NS/PCs were pretreated with 4MC before exposure to hydrogen peroxide (H(2)O(2)), most of the cells were ERK, inhibited both the 4MC-induced HO-1 expression and neuroprotective effect, demonstrating that PI3K/Akt signaling pathway played a significant role in 4MC-induced HO-1 induction and neuroprotection. Taken together, our results suggest that 4MC activates the expression of HO-1 through the PI3K/Akt signaling pathway and that the HO-1 protein inhibits the death of NS/PCs induced by oxidative stress.

[*Spine*, **35**, 497-504 (2010)] [Lab. of Mol. Biology]

Targeted Retrograde Gene Delivery of Brain-derived Neurotrophic Factor Suppresses Apoptosis of Neurons and Oligodendroglia after Spinal Cord Injury in Rats.

Hideaki NAKAJIMA, Kenzo UCHIDA, Takafumi YAYAMA, Shigeru KOBAYASHI, Alexander Rodriguez GUERRERO, Shoei FURUKAWA* and Hisatoshi BABA

To investigate the neuroprotective effect of targeted retrograde AdV-BDNF gene transfection in the traumatically injured spinal cord. Retrograde AdV-BDNF gene transfection resulted in a significant decrease in the number of apoptotic cells, with significant promotion of NG2 expression in injured spinal cord, compared with control virus injection. Our results suggest that targeted retrograde BDNF gene delivery suppresses apoptosis of neurons and oligodendrocytes in the injured rat spinal cord.

[*Neuroscience*, **171**, 1377-1385 (2010)] [Lab. of Mol. Biology]

2-Decenoic Acid Ethyl Ester, a Derivative of Unsaturated Medium-chain Fatty Acids, Facilitates Functional Recovery of Locomotor Activity after Spinal Cord Injury.

Akihiro HIRAKAWA, Katsuji SHIMIZU, Hidefumi FUKUMITSU, Hitomi SOUMIYA, Munekazu IINUMA and Shoei FURUKAWA*

We found that exposure to trans-2-decenoic acid ethyl ester (DAEE) markedly activated extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) in cultured cortical neurons. Therefore, we examined the effect of DAEE treatment on a rat model of spinal cord injury. DAEE administered after hemisection of the spinal cord resulted in improved functional recovery, decreased the lesion size, increased the activation of ERK1/2, and enhanced the expression of bcl-2 and BDNF mRNA in the injury site of the spinal cord. Furthermore, it also increased neuronal survival after spinal cord injury. These results indicate that the possibility that DAEE will become a promising tool for reducing the secondary damage observed following primary physical injury to the spinal cord.

[*Int J Mol Sci*., **11**, 4114-4123 (2010)] [Lab. of Mol. Biology]

Estrogen Stimulates Proliferation and Differentiation of Neural Stem/Progenitor Cells through Different Signal Transduction Pathways.

Makiko OKADA, Akihisa MAKINO, Mitsunari NAKAJIMA, Satoshi OKUYAMA, Shoei FURUKAWA* and Yoshiko FURUKAWA

We showed that 17β-estradiol (E2) could rapidly activate extracellular signal-regulated kinases 1/2 (ERK 1/2), which was not inhibited by ICI-182,780. ICI-182,780 abrogated the stimulatory effect of these estrogens (E2 and BPA) on the proliferation of neural stem/progenitor cells (NS/PCs), but not their effect on the differentiation of the NS/PCs into oligodendroglia. Furthermore, E2-BSA mimicked the activity of differentiation from NS/PCs into oligodendroglia, but not the activity of proliferation. Our study suggests that (1) the estrogen induced proliferation of NS/PCs is mediated via nuclear estrogen receptors (ERs); (2) the oligodendroglial generation from NS/PCs is likely to be stimulated via putative membrane-associated ERs.

[*Biomed. Res.***31.** 379-386 (2010)] [Lab. of Mol. Biology]

2-Decenoic Acid Ethyl Ester Possesses Neurotrophin-like Activities to Facilitate Intracellular Signals and Increase Synapse-specific Proteins in Neurons Cultured from Embryonic Rat Brain.

Akihisa MAKINO, Munekazu IINUMA, Hidefumi FUKUMITSU, Hitomi SOUMIYA, Yoshiko FURUKAWA and Shoei FURUKAWA*

We found that 1) DAEE phosphorylated ERK1/2 via MEK activation without the involvement of tyrosine kinases of neurotrophin Trk receptors; 2) DAEE activated CREB predominantly through ERK1/2 activation, not through other pathways such as cAMP/protein kinase A; and 3) DAEE increased the expression of RNAs of Neurotrophins and the protein content of synapse-specific proteins. Based on these observations we propose that DAEE and some other derivatives of medium-chain fatty acids (MCFAs) having neurotrophin-like neurotrophic activities may become therapeutic tools for certain neurological or psychiatric disorders.

[*Neuroreport*, **21**, 1177-1181 (2010)] [Lab. of Mol. Biology]

Overexpression of Piccolo C2A Domain Induces Depression-like Behavior in Mice.

Yoko FURUKAWA-HIBI,Atsumi NITTA, Hidefumi FUKUMITSU, Hitomi SOUMIYA,Shoei FURUKAWA*, Toshitaka NABESHIMA and Kiyofumi YAMADA

Piccolo is one of the components of the active zone at chemical synapses and regulates the transport of synaptic vesicles. The piccolo C2A domain is an important calcium sensor and binds with phosphatidylinositol or synaptotagmin-1. Recently, clinical studies suggested that a single nucleotide polymorphism in the piccolo C2A domain might be a causal risk factor for major depression. To clarify the association of piccolo with depression, we produced a transgenic mouse overexpressing the C2A domain of piccolo, and investigated the behavior of these mice. The mice exhibited depression-like behavior in both forced swim and tail suspension tests, suggesting that piccolo might regulate the depressive behavior.

[*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.

[*J. Pharm. Pharmacol.* **62**, 477-484 (2010)] [Lab. of Pharmaceutics]

Up-regulation of the Lysyl Hydroxylase 2 gene by Acetaminophen and Isoniazid is Modulated by Transcription Factor c-Myb.

Masafumi KUBOTA, Aya SHINODA, Kazuhiro IGUCHI, Yukari TAKAHASHI, Shigeyuki USUI, Tadashi KIHO and Kazuyuki HIRANO*

Lysyl hydroxylase 2 (LH2), an isoform of hydroxylase, catalyses the hydroxylation of lysine residues in the telopeptide of collagen to form stable and irreversible cross-linkages in collagen. In this study, we found that the expression of LH2 was increased in HepG2 cells incubated with acetaminophen and isoniazid. This increase was accompanied by an increase in the expression of c-myeloblastosis viral oncogene homolog (Myb) mRNA. Over-expression of c-Myb in cells transfected with a c-Myb expression plasmid, pMbm I, caused an increase in the expression of LH2 mRNA. These results suggest that c-Myb modulates the expression of the LH2 gene in HepG2 cells incubated with drugs causing hepatic fibrosis.

[*Eur. J. Pharmacol.* **641**, 35-40 (2010)] [Lab. of Pharmaceutics]

Pamidronate Inhibits Antiapoptotic Bcl-2 Expression through Inhibition of the Mevalonate Pathway in Prostate Cancer PC-3 cells.

Kazuhiro IGUCHI, Yoshiki TATSUDA, Shigeyuki USUI and Kazuyuki HIRANO*

Bisphosphonates are expected to be efficacious to prevent the growth of metastatic cancer in bone tissue. In this study, we found that the mRNA expression of bcl-2, which is a potent antiapoptotic protein, was significantly inhibited by treatment with pamidronate in prostate cancer PC-3 cells. Simultaneous treatment with geranylgeraniol, an intermediate of the mevalonate pathway, significantly blocked inhibition by pamidronate, and treatment with geranylgeranyl transferase inhibitor GGTI-286 also suppressed bcl-2 mRNA expression. Furthermore, pamidronate inhibited the translocation of Rap1 protein to the membrane fraction. Finally, knockdown of Rap1 by siRNA resulted in the inhibition of bcl-2 expression. These results strongly indicate that bcl-2 reduction in bisphosphonate-treated PC-3 cells is dependent on inhibition of the mevalonate pathway.

[*Eur. J. Pharmacol.* **627**, 348-53 (2010)] [Lab. of Pharmaceutics]

Protein Kinase C is Inhibited by Bisphosphonates in Prostate Cancer PC-3 cells.

Yoshiki TATSUDA, Kazuhiro IGUCHI, Shigeyuki USUI, Masumi SUZUI and Kazuyuki HIRANO*

Bisphosphonates are expected to be effective at preventing tumor metastasis to bone tissue. Since protein kinase C (PKC) plays a crucial role in cancer progression, we examined the effect of bisphosphonates on PKC expression to clarify the mechanism behind the inhibition of the bone metastasis of prostate cancer by bisphosphonates. We found that pamidronate inhibits PKC protein expression and PKC activity in prostate cancer PC-3 cells. Urokinase-type plasminogen activator (uPA) is one of the critical proteins in tumor metastasis and decreased in bisphosphonate-treated PC-3 cells. We also showed that uPA expression was suppressed by PKC inhibitors (calphostin C and staurosporine) and induced by a PKC activator (PMA) in PC-3 cells, suggesting that the inhibition of uPA by bisphosphonates is involved in PKC inhibition. These results strongly suggest that one of the mechanisms behind the inhibitory effect of bisphosphonates on tumor bone metastasis is mediated by PKC inhibition.

[*Int. J. Med. Mushr.*, **12**, 205-211 (2010)] [Lab. of Pharmaceutics]

Effect of Polysaccharides and 70% Ethanol Extracts from Medicinal Mushrooms on Growth of Human Prostate Cancer LNCaP and PC-3 Cells.

Tadashi KIHO, Kazuhiro IGUCHI, Shigeyuki USUI and Kazuyuki HIRANO*

To find anticancer compounds in medicinal mushrooms (MM), we first examined the effect of polysaccharides and ethanol extracts of MM on cell growth. Some heteroglycans from MM and antitumor glucans significantly suppressed the growth of prostate cancer cell lines, LNCaP and PC-3 cells. The 70% ethanol extract of the fruiting bodies of Tremella aurantia (TA-70E) showed the most potent inhibitory effect on cell growth. In addition, the growth inhibition of LNCaP and PC-3 cells by the 70% ethanol extract was, at least in part, characterized by G2/M phase cell cycle arrest, as evidenced by FACS analysis. These results showed that the polysaccharides and 70% ethanol extract of mushrooms have growth-inhibitory activity in LNCaP and PC-3 cells.

[*Eur. J. Med. Chem.*, **45**, 1140-1145 (2010)] [Lab. of Biochemistry]

Structure of Aldehyde Reductase in Ternary Complex with a 5-Arylidene-2,4-thiazolidinedione Aldose Reductase Inhibitor.

Vincenzo CARBONE, Marco GIGLIO, Roland CHUNG, Trevor HUYTON, Julian ADAMS, Rosanna MACCARI, Rosaria OTTANA, Akira HARA* and Ossama EL-KABBANI

The structure of aldehyde reductase (ALR1) in ternary complex with the coenzyme NADPH and CMD, a potent inhibitor of aldose reductase (ALR2), was determined at 1.99 Å resolution. The partially disordered inhibitor formed a tight network of hydrogen bonds with the active site residues (Tyr50 and His113) and coenzyme. Pi-stacking interactions with active site tryptophan residues and hydrogen-bonding interactions with the non-conserved C-terminal residue are contributed to inhibitor selectivity. In particular for the potent inhibitor CMD, the rotameric state of the conserved residue Trp219 (Trp220 in ALR1) is important in forming a pi-stacking interaction with the inhibitor in ALR2 and contributes to the difference in the binding of the inhibitor to the enzymes.

[*Acta Crystallogr. D Biol. Crystallogr.*, **66**, 198-204 (2010)] [Lab. of Biochemistry]

Studies on a Tyr Residue Critical for the Binding of Coenzyme and Substrate in Mouse 3(17)α-Hydroxysteroid Dehydrogenase (AKR1C21): Structure of the Y224D Mutant Enzyme.

Urmi DHAGAT, Satoshi ENDO, Hiroaki MAMIYA, Akira HARA* and Ossama EL-KABBANI

Mouse 3(17)α-hydroxysteroid dehydrogenase (AKR1C21) is the only aldo-keto reductase that catalyzes the stereospecific reduction of 3- and 17-ketosteroids to the corresponding 3(17)α-hydroxysteroids. The Y224D mutation of AKR1C21 reduced the K_m value for NADP(H) and completely reversed the 17α stereospecificity of the enzyme. The crystal structure of the Y224D mutant at 2.3 Å resolution revealed that the mutation resulted in a change in the conformation of the flexible loop B, including the V-shaped groove, which is a unique feature of wild-type AKR1C21 and is formed by the side chains of Tyr224 and Trp227. Furthermore, mutations (Y224F and Q222N) of residues involved in forming the safety belt for binding of the coenzyme showed similar alterations in kinetic constants for 3α-hydroxy/3-ketosteroids and 17-hydroxy/ketosteroids compared with the wild type.

[*Eur. J. Med. Chem.*, **45**, 4354-4357 (2010)] [Lab. of Biochemistry]

Selectivity Determinants of Inhibitor Binding to the Tumour Marker Human Aldose Reductase-like Protein (AKR1B10) Discovered from Molecular Docking and Database Screening.

Hai-Tao ZHAO, Midori SODA, Satoshi ENDO, Akira HARA* and Ossama EL-KABBANI

AKR1B10 was recently identified to be linked with several types of cancers, while exhibiting high sequence identity with human aldose reductase (AKR1B1). In order to identify potential inhibitors of AKR1B10, the NCI database which contains approximately 250,000 chemical structures was screened using in silico techniques. One of the ligands, 9-methyl-2,3,7-trihydroxy-6-fluorone, showed an IC_{50} value of 0.4 μM with a 4-fold selectivity towards AKR1B10 relative to AKR1B1, and its inhibition was competitive with respect to the substrate, showing a K_i value of 0.2 μM. In addition, through molecular docking in both the AKR1B10-$NADP^+$ and AKR1B1-$NADP^+$ complexes, as well as site-directed mutagenesis, non-conserved residues which are involved in inhibitor binding to AKR1B10 were identified and included Lys125 and Gln303.

[*J. Mol. Biol.*, **401**, 906-920 (2010)] [Lab. of Biochemistry]

Dimeric Crystal Structure of Rabbit L-Gulonate 3-Dehydrogenase/Lambda-crystallin: Insights into the Catalytic Mechanism.

Yukuhiko ASADA, Chizu KUROISHI, Yoko UKITA, Rie SUMII, Satoshi ENDO, Toshiyuki MATSUNAGA, Akira HARA* and Naoki KUNISHIMA

L-Gulonate 3-dehydrogenase (GDH) is a bifunctional dimeric protein that functions not only as an NAD^+-dependent enzyme in the uronate cycle but also as a taxon-specific lambda-crystallin in rabbit lens. The present crystal structures of GDH in both apo form and NADH-bound holo form and amino acid mutagenesis assigned the role of active-site residues: catalytic base for His145 and substrate binding for Ser124, Cys125, Asn196, and Arg231. Notably, Arg231 participates in substrate binding from the other subunit of the GDH dimer, indicating the functional significance of the dimeric state. Proper orientation of the substrate-binding residues for catalysis is likely to be maintained by an interprotomer hydrogen-bonding network of Asn196, Gln199, and Arg231.

[*Bioorg. Med. Chem. Lett.*, **20**, 5274-5276 (2010)] [Lab. of Biochemistry]

Factorizing the Role of a Critical Leucine Residue in the Binding of Substrate to Human 20α-Hydroxysteroid Dehydrogenase (AKR1C1): Molecular Modeling and Kinetic Studies of the Leu308Val Mutant Enzyme.

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

A comparison of the structures and kinetic properties of AKR1C1 and its mutants (Leu308Val and Leu308Ala) indicates that Leu308 is a selectivity determinant for substrate binding. While the Leu308Val mutation improved the catalytic efficiency of AKR1C1 towards 5α-pregnane-3α,20α-diol (PregA) and 5β-pregnan-3α-ol-20-one (PregB), the Leu308Ala mutation rendered the enzyme inactive. The orientation of PregA was similar to that of 20α-hydroxyprogesterone in the crystal structure of the AKR1C1 complex. Meanwhile, PregB interacted with the catalytic residue and formed close contacts with Leu308, suggesting that the binding mechanism of 3α-hydroxysteroids in the active site of AKR1C1 is different from that of 20α-hydroxysteroids.

[*Eur. J. Med. Chem.*, **45**, 5309-5317 (2010)] [Lab. of Biochemistry]

Structure-based Optimization and Biological Evaluation of Human 20α-Hydroxysteroid Dehydrogenase (AKR1C1) Salicylic Acid-based Inhibitors.

Ossama EL-KABBANI, Peter J. SCAMMELLS, Tom DAY, Urmi DHAGAT, Satoshi ENDO, Toshiyuki MATSUNAGA, Midori SODA and Akira HARA*

The tertiary structure of the Leu308Val mutant of AKR1C1 in complex with the inhibitor 3,5-dichlorosalicylic acid (DCL) has been determined. Mutation of Leu308, indicating a selectivity determinant for inhibitor binding, to Val resulted in 13-fold reductions in the inhibitory potencies of DCL. The replacement of Leu308 with an alanine resulted in 473-fold reductions in the potencies for DCL. We synthesized 5-substituted 3-chlorosalicylic acid derivatives, of which the most potent compound, 3-chloro-5-phenylsalicylic acid (K_i = 0.86 nM), was 24-fold selective for AKR1C1 relative to the structurally similar AKR1C2. Furthermore, the compound inhibited the metabolism of progesterone in AKR1C1-overexpressed cells with an IC_{50} value equal to 100 nM.

[*Toxicology*, **268**, 191-197 (2010)] [Lab. of Biochemistry]

Nitric Oxide Mitigates Apoptosis in Human Endothelial Cells Induced by 9,10-Phenanthrenequinone: Role of Proteasomal Function.

Toshiyuki MATSUNAGA*, Marina ARAKAKI, Tetsuro KAMIYA, Mariko HAGA, Satoshi ENDO, Ossama EL-KABBANI and Akira HARA

Treatment of human aortic endothelial cells with 9,10-phenanthrenequinone (9,10-PQ) evoked a bell-shaped production of nitric oxide (NO), which was presumably due to activation of endothelial NO synthase. Exogenous NO decreased the susceptibility to 9,10-PQ, and retrieved from apoptotic signaling (reactive oxygen species generation and caspase activation) induced by 9,10-PQ. In addition, inhibition of NO production augmented the toxicity of 9,10-PQ. Interestingly, 9,10-PQ markedly decrease the proteasomal activities, which were partially abrogated by NO. These results indicate that proteasomal dysfunction by 9,10-PQ is ameliorated by NO, suggesting the protective role of NO in vascular damage caused by 9,10-PQ.

[*Cancer Chemother. Pharmacol.,* **66**, 517-526 (2010)] [Lab. of Biochemistry]

Toxicity against Gastric Cancer Cells by Combined Treatment with 5-Fluorouracil and Mitomycin c: Implication in Oxidative Stress.

Toshiyuki MATSUNAGA*, Yoshitaka TSUJI, Kairyu KAAI, Saki KOHNO, Renzo HIRAYAMA, David H. ALPERS, Tsugikazu KOMODA and Akira HARA

We examined the administration sequence of combining 5-fluorouracil (5FU) with mitomycin c (MMC) to maximize toxicity against human gastric cancer cell MKN45 cells and found that pretreatment for 24 h with 5FU synergistically augmented the toxic effect of MMC in MKN45 cells. The synergic effect was mediated mainly via ROS formation and the p53-dependent apoptotic pathway, leading to mitochondrial dysfunction and caspase activation. *In vitro* experiments using extracts of the treated cells showed superoxide anion generation in a redox cycle of MMC, involving alterations in superoxide dismutase. This observation will need confirmation in the clinical setting.

[*Anal. Chem.,* **82**, 1128-1132 (2010)] [Lab. of Biochemistry]

Direct and Simple Fluorescence Detection Method for Oxidized Lipoproteins.

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

We present a straightforward analytical method for direct quantitation of oxidized lipoproteins by fluorescence spectrometry with excitation in the UV (365 +/- 10 nm) or visible (470 +/- 10 nm) range and emission detected at 450 +/- 30 nm or 535 +/- 15 nm. This method can be readily applied for clinical measurement in patients with dyslipidemia using only 1 microg of lipoprotein and without the need for any expensive detection antibodies. Using this new technique, biological samples from patients with dyslipidemia showed higher fluorescence intensities than samples from normal subjects when detecting oxidized low-density lipoprotein (LDL) and light high-density lipoprotein (HDL) (d=1.063-1.125 g/mL), whereas samples from patients with dyslipidemia showed the lower intensities than samples from normal subjects when measuring oxidized heavy HDL (d=1.125-1.210 g/mL) levels.

[*Bioorg. Med. Chem.,* **18**, 2485-2490 (2010)] [Lab. of Biochemistry]

Chromene-3-carboxamide Derivatives Discovered from Virtual Screening as Potent Inhibitors of the Tumour Maker, AKR1B10.

Satoshi ENDO*, Toshiyuki MATSUNAGA, Kazuo KUWATA, Hai-Tao ZHAO, Ossama EL-KABBANI, Yukio KITADE and Akira HARA

A human aldose reductase-like protein, AKR1B10 in the aldo-keto reductase (AKR) superfamily, was recently identified as a therapeutic target in the treatment of several types of cancer. In order to identify potential leads for new inhibitors of AKR1B10, we adopted the virtual screening approach using the automated program icm, which resulted in the discovery of several chromene-3-carboxamide derivatives as potent competitive inhibitors. The most potent chromene-3-carboxamide derivative, which has one methoxy group at the position 4 on the 2-phenylimino moiety inhibited the reductase activity of AKR1B10 with a K_i value of 2.7 nM, and the metabolism of farnesal in the AKR1B10-overexpressed cells from 0.1 μM with an IC_{50} value equal to 0.8 μM.

[*Biol. Pharm. Bull.,* **33**, 886-890 (2010)] [Lab. of Biochemistry]

Selective Inhibition of the Tumor Marker AKR1B10 by Antiinflammatory N-Phenylanthranilic Acids and Glycyrrhetic Acid.

Satoshi ENDO*, Toshiyuki MATSUNAGA, Midori SODA, Kazuo TAJIMA, Hi-Tai ZHAO, Ossama EL-KABBANI and Akira HARA

AKR1B10 was recently identified as a tumor marker of several types of cancer. In this study, we compared the inhibitory effects of aldose reductase inhibitors (ARIs) on AKR1B10 and AR. However, ARIs showed lower inhibitory potency for AKR1B10 than for AR. Moreover, we found that NSAIDs competitively inhibited AKR1B10, showing K_i values of 0.35-2.9 μM and high selectivity to this enzyme. Molecular docking studies and site-directed mutagenesis suggest that the side chain of Val301 and a hydrogen-bonding network among Val301, Gln114 and Ser304 are important for determining the inhibitory potency and selectivity of the NSAIDs. Thus, the potent and selective inhibition may be related to the cancer chemopreventive roles of the drugs.

[*Arch. Biochem. Biophys.*, **503**, 230-237 (2010)] [Lab. of Biochemistry]

Properties and Tissue Distribution of a Novel Aldo-keto Reductase Encoding in a Rat Gene (Akr1b10).

Satoshi ENDO*, Toshiyuki MATSUNAGA, Tsukasa KURAGANO, Satoshi OHNO, Yukio KITADE, Kazuo TAJIMA, Ossama EL-KABBANI and Akira HARA

A recent rat genomic sequencing predicts a gene Akr1b10 that encodes a protein with 83% sequence similarity to human AKR1B10. In this study, we isolated the cDNA for the rat AKR1B10 (R1B10) from rat brain, and examined the enzymatic properties of the recombinant protein. R1B10 utilized NADPH as the preferable coenzyme, and reduced various aldehydes (including cytotoxic 4-hydroxy-2-hexenal and 4-hydroxy- and 4-oxo-2-nonenals) and α-dicarbonyl compounds. Among the substrates, 4-oxo-2-nonenal was the best substrate, and its cytotoxicity against bovine endothelial cells was decreased by the overexpression of R1B10. The mRNA for R1B10 was expressed highly in rat brain and heart, and at low levels in other rat tissues and skin fibroblasts. The results suggest that R1B10 functions as a defense system against oxidative stress and glycation in rat tissues.

[*Biol. Pharm. Bull.*, **33**, 1886-1890 (2010)] [Lab. of Biochemistry]

Rat Aldose Reductase-like Protein (AKR1B14) Efficiently Reduces the Lipid Peroxidation Product 4-Oxo-2-nonenal.

Satoshi ENDO*, Toshiyuki MATSUNAGA, Anna FUJITA, Kazuo TAJIMA, Ossama EL-KABBANI and Akira HARA

In this study, we examined the substrate specificity, inhibitor sensitivity and kinetic mechanism of a rat aldose reductase-like protein, which is named AKR1B14. The enzyme reduced reactive carbonyl compounds, xenobiotic aromatic aldehydes and some aromatic ketones. 4-Oxo-2-nonenal (ONE), the best substrate showing a K_m value of 0.16 μM, was reduced into less reactive ONE, and its cytotoxicity was attenuated by the overexpression of the enzyme in cultured cells. The enzyme also showed low K_m values for medium-chain aliphatic aldehydes (such as 4-hydroxynonenal, 1-hexenal and farnesal) and 3-deoxyglucosone. Kinetic analyses of the oxidoreduction and dead-end inhibition suggest that the reaction follows an ordered sequential mechanism.

[*Eur. J. Pharmacol.*, **626**, 283-289 (2010)] [Lab. of Pharmacology]

Depletion of Substance P, a Mechanism for Inhibition of Mouse Scratching Behavior by Tacrolimus.

Naoki INAGAKI*, Noriko SHIRAISHI, Katsuhiro IGETA, Masafumi NAGAO, John Fan KIM, Takao CHIKUMOTO, Tomokazu ITOH, Hideo KATOH, Hiroyuki TANAKA and Hiroichi NAGAI

Tacrolimus has been suggested to attenuate the itching in atopic dermatitis. However, the anti-itch mechanism of tacrolimus has not been well elucidated. Repeated 2, 4-dinitrofluorobenzene (DNFB) solution paintings onto the mouse ear caused a typical dermatitis accompanied by scratching behaviors. Both tacrolimus and dexamethasone given topically for 10 days inhibited the ear swelling, but only tacrolimus significantly inhibited the scratching behavior. Repeated DNFB challenge depleted substance P in the dermis, tacrolimus completely inhibited the recovery of substance P content. Because DNFB-induced ear swelling and scratching behavior were inhibited by tachykinin NK1 receptor antagonist, substance P seems to participate in the induction of ear swelling and scratching behavior, and tacrolimus contributes to depletion of substance P related with ear swelling and scratching behavior.

[*J. Pharmacol. Sci.*, **112**, 192-202 (2010)] [Lab. of Pharmacology]

Effect of Diesel Exhaust Particles on House Dust Mite-Induced Airway Eosinophilic Inflammation and Remodeling in Mice.

Go TAKAHASHI, Hiroyuki TANAKA*, Keiko WAKAHARA, Reishi NASU, Mikiko HASHIMOTO, Kosuke MIYOSHI, Hirohisa TAKANO, Hirotaka YAMASHITA, Naoki INAGAKI and Hiroichi NAGAI

Ambient particulate pollution is associated with the onset of asthma; however, the effect of diesel exhaust particles (DEP) on the development of allergen-induced airway remodeling has not been fully investigated. Therefore, we examined the effects of DEP on *Dermatophagoides farinae*-induced asthma-like phenotypes using mice. DEP aggravated the airway responsiveness and infiltration of eosinophils accompanied by Th2 cytokines. Furthermore, goblet cell hyperplasia and subepithelial fibrosis with increases in amount of hydroxyproline and transforming growth factor-beta1 in lung were aggravated. DEP can exhibit adjuvant activity for airway remodeling due to the enhancement of allergen sensitization and/or Th2 polarizing pathways.

[*J. Pharmacol. Sci.*, **112**, 203-213 (2010)] [Lab. of Pharmacology]

A Novel CC-Chemokine Receptor3 Antagonist, Ki19003, Inhibits Airway Eosinophilia and Subepithelial/Peribronchial Fibrosis Induced by Repeated Antigen Challenge in Mice.

Masato KOMAI, Hiroyuki TANAKA*, Koichi NAGAO, Masayuki ISHIZAKI, Disuke KAJIWARA, Toru MIURA, Hiroshi OHASHI, Tomoko HABA, Kazuki KAWAKAMI, Eiji SAWA, Osamu YOSHIE, Naoki INAGAKI and Hiroichi NAGAI

CC-chemokine receptor 3 (CCR3) is a chemokine receptor of chemotaxis for eosinophils. We evaluated the effect of a low molecular weight CCR3-receptor antagonist, Ki19003 on airway remodeling in murine asthma model. Ki19003 clearly inhibited increase in infiltration of eosinophils, and aggravation of subepithelial and peribronchial fibrosis with producing of transforming growth factor-β1 and hydroxyproline. These findings indicate that CCR3 antagonism prevents not only the infiltration of eosinophils but also the development of fibrosis. Therefore, a CCR3 antagonist may be useful for airway remodeling of allergic asthma.

[*Bio. Pharm. Bull.*, **33**, 1050-1053 (2010)] [Lab. of Pharmacology]

Role of Leukotriene B_4 in 5-Lipoxygenase Metabolite- and Allergy-induced Itch-associated Responses in Mice.

Fumio TSUJI, Hiroyuki AONO, Takashi TSUBOI, Tadahiro MURAKAMI, Hiroshi ENOMOTO, Keiko MIZUTANI and Naoki INAGAKI*

We investigated the role of leukotriene (LT) B_4 in 5-lipoxygenase metabolite and itch-associated responses using SA6541, an LTA4 hydrolase inhibitor. Itch-associated responses and passive cutaneous anaphylaxis were evaluated by intradermal injection of 5-hydroperoxyeicosatetraenoic acid (HPETE), a precursor of 5-lipoxygenase metabolites. 5-HPETE-induced scratching behavior was inhibited by SA6541; however, increase in vascular permeability was hardly attenuated. On the other hand, ketotifen fumarate, a histamine H1 antagonist, strongly inhibited the scratching behavior and the increase in vascular permeability. These results suggest that LTB4 is an endogenous itch mediator in the skin and is involved in the pruritus response in allergic reactions.

[*J. Pharmacol. Sci.*, **112**, 487-490 (2010)] [Lab. of Pharmacology]

Effects of SA13353, a Transient Receptor Potential Vanilloid 1 Agonist, on Leukocyte Infiltration in Lipopolysaccharide-induced Acute Lung Injury and Ovalbumin-induced Allergic Airway Inflammation.

Fumio TSUJI, Masaaki MURAI, Kenji OKI, Hiroyuki INOUE, Minoru SASANO, Hiroyuki TANAKA, Naoki INAGAKI* and Hiroyuki AONO

Transient receptor potential vanilloid (TRPV) 1 is located in airways and plays an important role in respiratory diseases. We investigated the effects of orally administered SA13353, a TRPV1 agonist, on lipopolysaccharide (LPS)-induced acute lung injury and ovalbumin-induced allergic airway inflammation. In LPS-induced lung injury, SA13353 attenuated neutrophil infiltration with tumor necrosis factor-alpha and cytokine-induced neutrophil chemotactic factor-1 levels. In the allergic airway inflammation, SA13353 attenuated interleukin (IL)-4 and IL-12p40 levels. TRPV1 may play an anti-inflammatory role in lung inflammation.

[*Microbio. Immunol.*, **54**, 523-533 (2010)] [Lab. of Pharmacology]

Orally Supplemented Lactobacillus Acidophilus Strain L-92 Inhibits Passive and Active Cutaneous Anaphylaxis as well as 2,4-Dinitroflurobenzene and Mite Fecal Antigen Induced Atopic Dermatitis-like Skin Lesions in Mice.

Mohammad Monir SHAH, Yoshihiro MIYAMOTO, Yoshihito YAMADA, Hirotaka YAMASHITA, Hiroyuki TANAKA, Takayuki EZAKI, Hiroichi NAGAI and Naoki INAGAKI*

Oral supplementation of lactic acid bacteria is a potential approach to the prevention of allergic diseases. We evaluated the effect of that heat-killed Lactobacillus acidophilus strain L-92 (L-92) on passive cutaneous anaphylaxis (PCA), active cutaneous anaphylaxis (ACA) and allergic dermatitis models by painting with hapten or antigen. Orally administrated L-92 significantly suppressed both PCA and ACA. Moreover, in the allergic dermatitis models, inflammations and scratching behaviors were moderately suppressed by L-92. Our data indicated oral administration of L-92 might be useful for alleviating allergic symptoms.

[*Int. Immunol.*, **22**, 739-747 (2010)] [Lab. of Pharmacology]

Endotoxin Tolerance Attenuates Airway Allergic Inflammation in Model Mice by Suppression of the T-cell Stimulatory Effect of Dendritic Cells.

Hidetomo MATSUSHITA, Shoichiro OHTA, Hiroshi SHIRAISHI, Shoichi SUZUKI, Kazuhiko ARIMA, Shuji TODA, Hiroyuki TANAKA*, Hiroichi NAGAI, Masao KIMOTO, Akira INOKUCHI and Kenji IZUHARA

Endotoxin tolerance (ET) impairs antigen presentation of dendritic cells (DCs) to T cells by down-regulating expression of MHC class II and co-stimulatory molecules. Some epidemiological studies have shown that endotoxin acts as a protective factor for allergic diseases. However, results from animal models are controversial. UT12, a monoclonal agonistic antibody against TLR4, exhibited more potent and sustained ET than does LPS on a murine asthma model. Administration of UT12 significantly suppressed airway allergic inflammation, and UT12 inhibited the capacity of DCs to expand Th2 and Th17 cells. Our data demonstrate that ET attenuates airway allergic inflammation through direct suppression of the T-cell stimulatory effect of DCs in a murine asthma model.

[*Eur. J. Pharmacol.*, **645**, 171-176 (2010)] [Lab. of Pharmacology]

Comparison of the Efficacy of Tacrolimus and Cyclosporine A in a Murine Model of Dinitrofluorobenzene-induced Atopic Dermatitis.

Hirotaka YAMASHITA, Tomokazu ITO, Hideo KATO, Shusei ASAI, Hiroyuki TANAKA, Hiroichi NAGAI and Naoki INAGAKI*

Tacrolimus and cyclosporine A (CysA) suppress immune system to interact with calcineurin (CaN). In contrast, steroidal anti-inflammatory drugs suppress via inhibition of NF-kappaB and AP-1 pathway. Previously, we reported that tacrolimus, but not dexamethasone, reduced scratching behavior in murine atopic dermatitis model. To elucidate the mechanism of the inhibition of scratching behavior, we compared tacrolimus with CysA. We found that CysA suppressed scratching behavior in the dermatitis model, as did tacrolimus, and attenuated increases in vascular permeability of passive cutaneous anaphylaxis with scratching behavior. Our data indicated that inhibition of the CaN pathway may play an important role in suppression of scratching behavior.

[*PLoS ONE*, **5(12)**; e15307 (2010)] [Lab. of Molecular Pharmacology]

An Inducer of VGF Protects Cells against ER Stress-induced Cell Death and Prolongs Survival in the Mutant SOD1 Animal Models of Familial ALS.

Masamitsu SHIMAZAWA, Hirotaka TANAKA, Yasushi ITO, Nobutaka MORIMOTO, Kazuhiro TSURUMA, Michinori KADOKURA, Shigeki TAMURA, Teruyoshi INOUE, Mitsunori YAMADA, Hitoshi TAKAHASHI, Hitoshi WARITA, Masashi AOKI and Hideaki HARA*

We identified a small molecule, SUN N8075, which has a marked protective effect on endoplasmic reticulum (ER) stress-induced cell death, in an *in vitro* cell-based screening, and its protective mechanism was mediated by an induction of VGF nerve growth factor inducible (VGF). Furthermore, SUN N8075 slowed disease progression and prolonged survival in mutant SOD1 transgenic mouse and rat models of amyotrophic lateral sclerosis (ALS). These data suggest that VGF plays a critical role in motor neuron survival and may be a potential new therapeutic target for ALS.

[*PLoS ONE*, **5(12)**; e15178 (2010)] [Lab. of Molecular Pharmacology]

Phosphodiesterase-III Inhibitor Prevents Hemorrhagic Transformation Induced by Focal Cerebral Ischemia in Mice Treated with tPA.

Mitsunori ISHIGURO, Keisuke MISHIRO, Yasuyuki FUJIWARA, Huayue CHEN, Hiroshi IZUTA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Shinichi YOSHIMURA, Masahiko SATOH, Toru IWAMA and Hideaki HARA*

The purpose of the present study was to investigate whether cilostazol, a phosphodiesterase-III inhibitor and antiplatelet drug, would prevent tPA-associated hemorrhagic transformation. Combination therapy with tPA plus cilostazol prevented development of hemorrhagic transformation, reduced brain edema, prevented endothelial injury via reduction MMP-9 activity, and prevented the blood-brain barrier opening by inhibiting decreased claudin-5 expression. The present study indicates that a phosphodiesterase-III inhibitor prevents the hemorrhagic transformation induced by focal cerebral ischemia in mice treated with tPA.

[*PLoS ONE*, **5(7)**; e11602 (2010)] [Lab. of Molecular Pharmacology]

Essential Role of Neuron-enriched Diacylglycerol Kinase (DGK), DGKβ in Neurite Spine Formation, Contributing to Cognitive Function.

Yasuhito SHIRAI, Takeshi KOUZUKI, Kenichi KAKEFUDA, Shigeki MORIGUCHI, Atsushi OYAGI, Kyoji HORIE, Shin-ya MORITA, Masamitsu SHIMAZAWA, Kohji FUKUNAGA, Junji TAKEDA,Naoaki SAITO and Hideaki HARA*

We developed DGKβ KO mice using the Sleeping Beauty transposon system, and found that its long-term potentiation in the hippocampal CA1 region was reduced, causing impairment of cognitive functions including spatial and long-term memories in Y-maze and Morris water-maze tests. The primary cultured hippocampal neurons from KO mice had less branches and spines compared to the wild type. These results demonstrate that membrane-localized DGKβ regulates spine formation by regulation of lipids, contributing to the maintenance of neural networks in synaptic transmission of cognitive processes including memory.

[*PLoS ONE*, **5(10)**; e13447 (2010)] [Lab. of Molecular Pharmacology]

Diacylglycerol Kinase β Knockout Mice Exhibit Lithium-sensitive Behavioral Abnormalities.

Kenichi KAKEFUDA, Atsushi OYAGI, Mitsue ISHISAKA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Koichi YOKOTA, Yasuhito SHIRAI, Kyoji HORIE, Naoaki SAITO, Junji TAKEDA and Hideaki HARA*

In the present study, we performed behavioral tests using DGKβ knockout (KO) mice to investigate the effects of DGKβ deficits on psychomotor behavior. DGKβ KO mice exhibited some behavioral abnormalities, such as hyperactivity, reduced anxiety, and reduced depression. Additionally, hyperactivity and reduced anxiety were attenuated by the administration of the mood stabilizer, lithium, but not haloperidol, diazepam, or imipramine. Moreover, DGKβ KO mice showed impairment in Akt-glycogen synthesis kinase (GSK) 3β signaling and cortical spine formation. These findings suggest that DGKβ KO mice exhibit lithium-sensitive behavioral abnormalities that are, at least in part, due to the impairment of Akt-GSK3β signaling and cortical spine formation.

[*CNS Neurosci. Ther.*, **16**, 103-114 (2010)] [Lab. of Molecular Pharmacology]

Lomerizine, a Ca^{2+} Channel Blocker, Protects against Neuronal Degeneration within the Visual Center of the Brain after Retinal Damage in Mice.

Yasushi ITO, Shinsuke NAKAMURA, Hirotaka TANAKA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Makoto ARAIE and Hideaki HARA*

We examied whether lomerizine, a Ca^{2+} channel blocker, protects against neuronal degeneration within the dorsal lateral geniculate nucleus (dLGN) and superior colliculus (SC) after the induction of retinal damage by intravitreal injection of *N*-methyl-D-aspartate (NMDA) in mice. Lomerizine reduced the retinal damage induced by NMDA and partially prevented the transsynaptic neuronal degeneration within dLGN and SC on the contralateral side. Moreover, lomerizine reduced the intravitreal NMDA induced decrease in the light-induced expression of c-Fos in the contralateral dLGN. These results indicate that lomerizine affords some protection against transsynaptic neuronal degeneration within the visual center of the mouse brain.

[*Neuroscience*, **171**, 258-267 (2010)] [Lab. of Molecular Pharmacology]

Toll-like Receptor 4 (TLR4), but not TLR3 or TLR9, Knock-out Mice Have Neuroprotective Effects against Focal Cerebral Ischemia.

Kana HYAKKOKU, Junya HAMANAKA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Hiroyuki TANAKA, Satosi UEMATSU, Shizuo AKIRA, Naoki INAGAKI, Hiroichi NAGAI and Hideaki HARA*

Toll-like receptors (TLRs) are signaling receptors in the innate immune system that is a specific immunologic response to systemic bacterial infection. We investigated whether cerebral ischemia induced by the middle cerebral artery occlusion (MCAO) for 2 h differed in mice that lack a functional TLR3, TLR4, or TLR9 signaling pathway. TLR4, but not TLR3 or TLR9, knock-out (KO) mice had significantly smaller infarct area and volume at 24 h after ischemia-reperfusion (I/R) compared with wild-type mice. In addition, TLR4 KO mice improved in neurological deficits after I/R compared with wild-type mice. These data suggest that TLR4 knockout, but not TLR3 or TLR9 knockout, may play a neuroprotective role in ischemic brain injury induced by MCAO in mice.

[*Exp. Transl. Stroke Med.,* **2 : 20** (2010)] [Lab. of Molecular Pharmacology]

Proteomic Approach with LCMS-IT-TOF Identified an Increase of Rab33B after Transient Focal Cerebral Ischemia in Mice.

Kana HYAKKOKU, Junya HAMANAKA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

We studied the changes in the ischemic brain proteome after focal cerebral ischemia, induced by middle cerebral artery occlusion (MCAO) in mice. LCMS-IT-TOF mass spectrometry was used to detect the changes in ischemic brain protein patterns after MCAO. Nine unique proteins were identified from the ischemic area at 10 h after ischemic insult. Among these proteins, we focused on Rab33b, a member of RAS oncogene family and we found that Rab33b was up-regulated in the ischemic striatum and the number of Rab33B-positive cells increased in a time-dependent manner. Rab33B colocalized with Iba-1 positive microglia in the ischemic area. These findings suggest that LCMS-IT-TOF is useful for identifying changes in proteins after cerebral ischemia and that Rab33B is partially related to the pathogenesis of transient cerebral ischemia in mice.

[*Neurosci. Lett.,* **484**, 43-46 (2010)] [Lab. of Molecular Pharmacology]

Post-treatment of a BiP Inducer Prevents Cell Death after Middle Cerebral Artery Occlusion in Mice.

Yasuhisa OIDA, Junya HAMANAKA, Kana HYAKKOKU, Masamitsu SHIMAZAWA, Takashi KUDO, Kazunori IMAIZUMI, Tadashi YASUDA and Hideaki HARA*

We previously reported the effect of a selective inducer of BiP (a BiP inducer X; BIX) after permanent middle cerebral artery occlusion (MCAO) in mice. However, in acute stroke, almost all drugs have been used clinically after the onset of events. We evaluated the effect of post-treatment of BIX after permanent MCAO in mice, and examined its neuroprotective properties in *in vivo* mechanism. BIX administered either at 5 min or 3 h after occlusion reduced both infarct volume and brain swelling, but at 6 h after occlusion there was no reduction. BIX protected against the decrease in a dose-dependent manner. These findings indicate that post-treatment with BIX after ischemia has neuroprotective effects against acute ischemic neuronal damage in mice even when given up to 3 h after MCAO. BIX may therefore be a potential drug for stroke.

[*Pharmacology,* **86**, 293-296 (2010)] [Lab. of Molecular Pharmacology]

Pharmacologically Distinctive Behaviors other than Burying Marbles during the Marble Burying Test in Mice.

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

In the marble burying test, we focused on the 5 distinctive behavioral parameters of mice other than burying marbles, i.e. digging, latency to the first digging, exploration around marbles, rearing and locomotor activity. Typical anxiolytics or antidepressants with different mechanisms, fluvoxamine, bupropion, imipramine and diazepam were used to examine whether these behavioral parameters are sensitive to pharmacological treatments. Each of the drugs demonstrated an individual action pattern on the 4 behavioral parameters other than digging. On the other hand, all 4 drugs reduced burying marbles and digging. These results suggest that the former 4 behavioral parameters are sensitive to pharmacological treatment and that pharmacological regulation mechanisms of them may be different from burying marbles and digging.

[*J. Neurochem.,* **113**, 1545-1554 (2010)] [Lab. of Molecular Pharmacology]

Induction of Amyloid Precursor Protein by the Neurotoxic Peptide, Amyloid-beta 25-35, Causes Retinal Ganglion Cell Death.

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

Patients with Alzheimer's disease (AD) show a significantly increased incidence of glaucoma. AD is also associated with the occurrence of the neurotoxic peptide amyloid beta (Aβ). Therefore, we investigated whether Aβ is associated with retinal cell death in a retinal ganglion cell line (RGC-5). Treatment with $A\beta_{25\text{-}35}$, a neurotoxic fragment of Aβ, induced cell death in RGC-5. The amount of amyloid precursor protein was increased by treatment of RGC-5 and primary culture of mouse cortical neurons with fibril $A\beta_{25\text{-}35}$ and $A\beta_{1\text{-}42}$. Amyloid precursor protein knockdown inhibited the cell death induced by $A\beta_{25\text{-}35}$. Treatment with $A\beta_{25\text{-}35}$ increased the amount of intracellular $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$, while β- and gamma-secretase inhibitors reduced cell death. Thus, the regulation of Aβ can be viewed as a new therapeutic target for glaucoma, especially in patients with coincident AD.

[*J. Pharmacol. Exp. Ther.*, **332**, 380-387 (2010)] [Lab. of Molecular Pharmacology]

A Novel Calpain Inhibitor, ((1S)-1-((((1S)-1-Benzyl-3-cyclopropylamino-2,3-di-oxopropyl)amino)carbonyl)-3-methylbutyl)carbamic Acid 5-Methoxy-3-oxapentyl Ester (SNJ-1945), Reduces Murine Retinal Cell Death *In Vitro* and *In Vivo*.

Masamitsu SHIMAZAWA, Shinsuke Suemori, Yuta Inokuchi, Nozomu MATSUNAGA, Yoshimi NAKAJIMA, Takayuki OKA, Tetsuya YAMAMOTO and Hideaki HARA*

We examined whether SNJ-1945, a new orally available calpain inhibitor, might reduce retinal cell death. In mouse retinas, SNJ-1945 significantly inhibited the cell loss in the ganglion cell layer and the thinning of the inner plexiform layer induced by *N*-methyl-D-aspartate (NMDA). Levels of cleaved α-spectrin products increased and p35 decreased 6 h after NMDA injection or later, and their effects were attenuated by SNJ-1945. *In vitro*, SNJ-1945 inhibited the OGD stress-induced reduction in cell viability. In conclusion, SNJ-1945 may afford valuable neuroprotection against retinal diseases.

[*J. Pharmacol. Exp. Ther.*, **335**, 645-652 (2010)] [Lab. of Molecular Pharmacology]

Calpain Inhibitor Protects Cells against Light-induced Retinal Degeneration.

Shunsuke IMAI, Masamitsu SHIMAZAWA, Tomohiro NAKANISHI, Kazuhiro TSURUMA and Hideaki HARA*

We investigated the protective effects of ((1S)-1-((((1S)-1-benzyl-3-cyclopropylamino-2,3-di-oxopropyl)amino)carbonyl)-3-methylbutyl)carbamic acid 5-methoxy-3-oxapentyl ester (SNJ-1945), a calpain inhibitor, against light-induced retinal degeneration in mice. SNJ-1945 inhibited the proteolysis of α-spectrin and p35 by light exposure and presented a decrease in the numbers of TUNEL-positive cells and outer nuclear layer atrophy. Furthermore, SNJ-1945 presented a decrease in a- and b-wave amplitude and caspase-3/7 activation induced by light exposure. These findings suggest that the activation of calpain plays a pivotal role in photoreceptor degeneration by light exposure, and SNJ-1945 may be a candidate for effectively treating diseases related to photoreceptor degeneration.

[*Invest. Ophthalmol. Vis. Sci.*, **51**, 3162-3170 (2010)] [Lab. of Molecular Pharmacology]

Physicochemical Properties Affecting Retinal Drug/Coumarin-6 Delivery from Nanocarrier Systems *via* Eyedrop Administration.

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

We elucidated the effect of physicochemical properties of nanocarrier systems on drug delivery efficiency to the retina by eyedrop administration in mice, rabbits, and monkeys. Submicron-sized liposomes (ssLips) of different particle size, cholesterol content, surface charge, and multilamellar vesicles were prepared by the hydration. The delivery efficiency of coumarin-6 to the retina was altered depending on particle size, constituents, and rigidity. ssLips with appropriate features would be promising drug carriers for retinal delivery through eyedrops.

[*Invest. Ophthalmol. Vis. Sci.*, **51**, 2575-2586 (2010)] [Lab. of Molecular Pharmacology]

Effect of Hypoxia on Susceptibility of RGC-5 Cells to Nitric Oxide.

Takaki SATO, Hidehiro OKU, Kazuhiro TSURUMA, Kozo KATSUMURA, Masamitsu SHIMAZAWA, Hideaki HARA*, Tetsuya SUGIYAMA and Tsunehiko IKEDA

We determined whether retinal neurons become more susceptible to injury by nitric oxide (NO) under hypoxic conditions. Exposure of RGC-5 cells to SNAP (100 μM) significantly decreased the number of living cells cultured under hypoxic conditions with or without glucose. Coadministration of carboxy-PTIO (1.0 μM) suppressed SNAP-induced cell death. SNAP-induced cell death of cells cultured under hypoxia with glucose was accompanied by increased expression of phosphatidylserine and hypodiploid DNAs. These findings indicated that death was mediated in part by apoptosis. In addition, loss of mitochondrial membrane potential, increase of superoxide formation, and activation of caspase was observed. These results indicate that RGC-5 cells become susceptible to SNAP under hypoxic conditions in which NO may have greater impact on mitochondrial function.

[*Exp. Eye Res.*, **90**, 137-145 (2010)] [Lab. of Molecular Pharmacology]

Ruboxistaurin, a PKCβ Inhibitor, Inhibits Retinal Neovascularization *via* Suppression of Phosphorylation of ERK1/2 and Akt.

Shinsuke NAKAMURA, Yuichi CHIKARAISHI, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

We assessed the efficacy of ruboxistaurin, a protein kinase C (PKC) β inhibitor, both *in vitro* and *in vivo* and also evaluated its anti-angiogenic mechanisms. Ruboxistaurin inhibited formation, proliferation, and migration of VEGF-induced human umbilical vein endothelial cells. It also inhibited the VEGF-induced phosphorylation of ERK1/2 and Akt. In *in vivo* retinal neovascularization experiments, ruboxistaurin significantly reduced pathologic vascular changes. These findings indicate that ruboxistaurin has anti-angiogenic effects both *in vitro* and *in vivo* that are exerted partly via suppressing the phosphorylation of ERK1/2 and Akt. Ruboxistaurin may be a candidate for treatment of angiogenesis in retinal neovascularization diseases.

[*Mol. Vision*, **16**, 130-136 (2010)] [Lab. of Molecular Pharmacology]

Proliferative Diabetic Retinopathy and Relations among Antioxidant Activity, Oxidative Stress, and VEGF in the Vitreous Body.

Hiroshi IZUTA, Nozomu MATSUNAGA, Masamitsu SHIMAZAWA, Tetsuya SUGIYAMA, Tsunehiko IKEDA and Hideaki HARA*

We investigated the relationships among antioxidant activities, oxidative stress, and vascular endothelial growth factor (VEGF) in the vitreous body and serum from proliferative diabetic retinopathy (PDR) patients. Both the potential antioxidant (PAO) and Nepsilon-hexanoyl-lysine (HEL) levels in the vitreous and serum were significantly higher in PDR patients than in those with macular hole (MH) (both $p<0.01$). Positive correlations were found between the PAO and VEGF concentrations and between the HEL and VEGF concentrations in the vitreous of both the PDR and the MH patients. These findings suggest that VEGF and lipid peroxide levels in the vitreous may play some role in the pathogenesis of PDR.

[*Eur. J. Pharmacol.*, **642**, 77-85 (2010)] [Lab. of Molecular Pharmacology]

Systemic Administration of a Free Radical Scavenger, Edaravone, Protects against Light-induced Photoreceptor Degeneration in the Mouse Retina.

Shunsuke IMAI, Yuta INOKUCHI, Shinsuke NAKAMURA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

We investigated the protective effects of edaravone against light-induced retinal damage in the mouse. Edaravone (3 mg/kg, i.p. and 1 mg/kg. i.v.) significantly protected against light-induced photoreceptor degeneration at 5 days after exposure to light. In ERG measurement, edaravone inhibited retinal dysfunction at 5days after exposure to light. These findings suggest that oxidative stress plays a pivotal role in light-induced retinal damage and that systemic administration of edaravone may slow the progression of photoreceptor degeneration.

[*Jpn. J. Ophthalmol.*, **54**, 615-621 (2010)] [Lab. of Molecular Pharmacology]

Effects of Timolol-related Ophthalmic Solutions on Cultured Human Conjunctival Cells.

Kazuhide KAWASE, Wenzhong LIN, Yumiko AOYAMA, Tetsuya YAMAMOTO, Masamitsu SHIMAZAWA and Hideaki HARA*

We investigated the inhibitory effects of drugs containing timolol on the proliferation of human conjunctival cells *in vitro*. Timoptol, Timoptol XE, Rysmon TG, and Timabak solutions were used. The effects of drugs containing benzalkonium chloride (BAK) as well as those of the Rysmon TG vehicle alone were also assessed. Cell activity decreased in a concentration-dependent manner after the addition of either Timoptol or Timoptol XE. Rysmon TG and Timabak showed significantly higher cell activity than Timoptol or Timoptol XE at both 1/100 and 1/30 dilutions. The cell count increased in a concentration-dependent manner in the BAK-free group, while cell activity decreased in a concentration-dependent manner in the cultures in the BAK-containing group. Compared with Timoptol and Timoptol XE, Rysmon TG and Timabak showed milder toxicity on human conjunctival cells *in vitro*.

[*Mol. Nutr. Food Res.*, **54**, 566-575 (2010)] [Lab. of Molecular Pharmacology]

Angiostatic Effects of Brazilian Green Propolis and Its Chemical Constituents.

Yuichi CHIKARAISHI, Hiroshi IZUTA, Masamitsu SHIMAZAWA, Satoshi MISHIMA and Hideaki HARA*

The aim of this study was to evaluate the anti-angiogenic effects of a water extract of Brazilian green propolis (WEP) and its constituents, caffeoylquinic acid derivatives, against angiogenic processes in human umbilical vein endothelial cells (HUVECs) *in vitro*, and against retinal neovascularization in a murine oxygen-induced retinopathy model *in vivo*. WEP and its constituents significantly suppressed vascular endothelial growth factor (VEGF)-induced HUVEC proliferation, migration, and tube formation *in vitro*. WEP and its caffeoylquinic acid derivatives suppressed VEGF-stimulated phosphorylation of mitogen-activated protein kinase in HUVECs. Moreover, WEP (300 mg/kg/day, subcutaneously for 5 days) significantly suppressed retinal neovascularization in the murine oxygen-induced retinopathy model. These findings suggest that WEP and its caffeoylquinic acid derivatives may represent candidates for preventive or therapeutic agents against diseases caused by angiogenesis.

[*BMC Complement. Altern. Med.*, **10 : 68** (2010)] [Lab. of Molecular Pharmacology]

Laxative Effects of Agarwood on Low-fiber Diet-induced Constipation in Rats.

Mamoru KAKINO, Shigemi TAZAWA , Hiroe MARUYAMA , Kazuhiro TSURUMA , Yoko ARAKI , Masamitsu SHIMAZAWA and Hideaki HARA*

Agarwood (*Aquilaria sinensis*), well known as incense in Southeast Asia, has been used as a digestive in traditional medicine. We investigated the laxative effects of an ethanol extract of agarwood leaves (EEA) in a rat model of low-fiber diet-induced constipation. Feeding of the animals with a low-fiber diet resulted in a decrease in stool weight, frequency, and water content and also delayed carmine egestion. EEA (300 and 600 mg/kg) significantly increased frequency, weight, and water content of the stools while accelerating carmine egestion in the constipated rats. Senna (150 and 300 mg/kg) produced similar effect as the higher doses of EEA but, in addition, induced severe diarrhea. These findings indicate that EEA has a laxative effect, without causing diarrhea, in a rat model of low-fiber diet-induced constipation.

[*Biosci. Biotech. Biochem.*, **74**, 1550-1555 (2010)] [Lab. of Molecular Pharmacology]

Agarwood Induced Laxative Effects *via* Acetylcholine Receptors on Loperamide-induced Constipation in Mice.

Mamoru KAKINO, Hiroshi IZUTA, Tetsuro ITO, Kazuhiro TSURUMA, Yoko ARAKI., Masamitsu SHIMAZAWA, Masayoshi OYAMA, Munekazu IINUMA and Hideaki HARA*

We investigated the laxative effects and mechanism of agarwood leaves extracted with ethanol (EEA-1, *Aquilaria sinensis*; EEA-2, *Aquilaria crasna*). EEA-1, EEA-2, the main constituents of EEAs (mangiferin, and genkwanin-5-*O*-primeveroside), and senna increased the frequency and weight of stools in loperamide-induced constipation model mice. Furthermore, the increase in frequency and weight of stools induced by EEA-1 were blocked by pre-administration of atropine in the model mice. These findings indicate that EEAs exerted a laxative effect *via* acetylcholine receptors in the mouse constipation model.

[*BMC Complement. Altern. Med.*, **10 : 45** (2010)] [Lab. of Molecular Pharmacology]

Protective Effects of a Gastrointestinal Agent Containing Korean Red Ginseng on Gastric Ulcer Models in Mice.

Atsushi OYAGI, Kenjirou OGAWA, Mamoru KAKINO and Hideaki HARA*

The purpose of this study was to assess the effects of a Korean red ginseng (KRG)-containing drug (KRGCD) on gastric ulcer models in mice. Stomach ulcers were induced by oral ingestion of hydrochloride (HCl)/ethanol or indomethacin. Treatment with KRGCD (30, 100, and 300 mg/kg, p.o.) occurred 1 hr before the ulcer induction. KRGCD (100 and 300 mg/kg, p.o.) significantly decreased ethanol- and indomethacin-induced gastric ulcer compared with the vehicle-treated (control) group. KRGCD (100 and 300 mg/kg) also decreased the level of thiobarbituric acid reactive substance (TBARS) and increased gastric mucosal blood flow compared with the control group. These results suggest that the gastroprotective effects of KRGCD on mice ulcer models can be attributed to its ameliorating effect on oxidative damage and improving effect of gastric mucosal blood flow.

[*Phytother. Res.*, **24**, S42-S47 (2010)] [Lab. of Molecular Pharmacology]

Inhibitory Actions of Bilberry Anthocyanidins on Angiogenesis.

Nozomu MATSUNAGA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Shigeru YOKOTA and Hideaki HARA*

The aim of this study was to examine the antiangiogenic properties and antioxidant activities of the main anthocyanidins (delphinidin, cyanidin and malvidin) found as constituents in *Vaccinium myrtillus* (bilberry) anthocyanosides (VMA). Each of these anthocyanidins concentration-dependently inhibited vascular endothelial growth factor (VEGF)-induced tube formation in a co-culture of human umbilical vein endothelial cells (HUVECs) and fibroblasts, the effect of each anthocyanidin being significant at 3 and/or 10 μM. Moreover, each anthocyanidin (0.3–10 μM) concentration-dependently scavenged the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The inhibitory effects against angiogenesis were similar among the anthocyanidins, as were those against the DPPH radical.. These findings indicate that the inhibitory effect of VMA on angiogenesis may depend on those of its main constituent anthocyanidins (delphinidin, cyanidin and malvidin), presumably via antioxidant effects.

[*eCAM*, **7**, 47-56 (2010)] [Lab. of Molecular Pharmacology]

Vaccinium myrtillus (Bilberry) Extracts Reduce Angiogenesis *In Vitro* and *In Vivo*.

Nozomu MATSUNAGA, Yuichi CHIKARAISHI, Masamitsu SHIMAZAWA, Shigeru YOKOTA and Hideaki HARA*

Vaccinium myrtillus (Bilberry) extracts (VME) were tested for effects on angiogenesis *in vitro* and *in vivo*. VME (0.3-30 μg ml^{-1}) concentration-dependently inhibited both tube formation and migration of human umbilical vein endothelial cells (HUVECs) induced by vascular endothelial growth factor-A (VEGF-A). In addition, VME inhibited VEGF-A-induced proliferation of HUVECs. VME inhibited VEGF-A-induced phosphorylations of extracellular signal-regulated kinase 1/2 (ERK 1/2) and serine/threonine protein kinase family protein kinase B (Akt), but not that of phospholipase Cγ (PLCγ). In an *in vivo* assay, intravitreal administration of VME inhibited the formation of neovascular tufts during oxygen-induced retinopathy in mice. Thus, VME inhibited angiogenesis both *in vitro* and *in vivo*, presumably by inhibiting the phosphorylations of ERK 1/2 and Akt. These findings indicate that VME may be effective against retinal diseases involving angiogenesis.

[*BMC Complement. Altern. Med.*, **10 : 30** (2010)] [Lab. of Molecular Pharmacology]

Anti-Inflammatory Effect of Bee Pollen Ethanol Extract from *Cistus* sp. of Spanish on Carrageenan-induced Rat Hind Paw Edema.

Hiroe MARUYAMA, Takashi SAKAMOTO, Yoko ARAKI and Hideaki HARA*

In this study, we aimed to investigate the anti-inflammatory effect of bee pollen from *Cistus* sp. of Spanish origin by a method of carrageenan-induced paw edema in rats, and to investigate the mechanism of anti-inflammatory action and also to elucidate components involved in bee pollen extracted with ethanol. The bee pollen bulk mildly suppressed the carrageenan-induced paw edema and the water extract showed almost no inhibitory activity, but the ethanol extract showed relatively strong inhibition of paw edema. The ethanol extract inhibited the NO production and COX-2 but not COX-1 activity, but the water extract did not affect the NO production or COX activities. Flavonoids were identified at least five flavonoids and their glycosides from the ethanol extract of bee pollen. The bee pollen would be beneficial not only as a dietary supplement but also as a functional food.

[*J. Bone Miner. Metab.*, **28**, 131-138 (2010)] [Lab. of Molecular Pharmacology]

Regulation of ER Molecular Chaperone Prevents Bone Loss in a Murine Model for Osteoporosis.

Shin-ichiro HINO, Shinichi KONDO, Kazuya YOSHINAGA, Atsushi SAITO, Tomohiko MURAKAMI, Soshi KANEMOTO, Hiroshi SEKIYA, Kazuyasu CHIHARA, Yuji AIKAWA, Hideaki HARA*, Takashi KUDO, Tomohisa SEKIMOTO, Taro FUNAMOTO, Etsuo CHOSA and Kazunori IMAIZUMI

We examined a possible correlation between osteoporosis and endoplasmic reticulum (ER) stress response. Bone specimens from 8 osteoporosis patients and 8 disease-controls were used for immunohistochemical analysis. ER molecular chaperones, such as BiP (immunoglobulin heavy-chain binding protein) and PDI (protein-disulfide isomerase) were down-regulated in osteoblasts from osteoporosis patients. Oral administration of BIX (BiP inducer X), selective inducer BiP, effectively improved decline in bone formation through the activation of folding and secretion of bone matrix proteins. Considering these results together, BIX may be a potential therapeutic agent for the prevention of bone loss in osteoporosis patients.

[*Bio. Pharm. Bull.*,**33,** 1907-1910 (2010)] [Lab. of Clinical Pharmacy]

Clonazepam Use for Prevention of Acute and Delayed Vomiting Induced by Cisplatin-based Chemotherapy for Lung Cancer.

Masahiko HAYASHI, Yusuke TAKAO, Chihiro HATA, Hitomi TERAMACHI* and Teruo TSUCHIYA

We investigated the efficacy of clonazepam as an antiemetic in cisplatin-based chemotherapy for lung cancer. Seven patients experienced cisplatin-induced vomiting despite antiemetic therapy including 5-HT3 antagonist and dexamethasone. Therefore, the antiemetic therapy including clonazepam, it was subsequently explored in the next course for the same patients. We administered clonazepam once a day orally for 5 days from day one prior to chemotherapy. The patients whose serum clonazepam concentrations were below the lower limit of detection (3.0 ng/ml) experienced vomiting in three of three courses, whereas the patients whose serum clonazepam concentrations were higher than 4.3 ng/ml experienced no vomiting in six of seven courses. We observed that the symptom of cisplatininduced delayed vomiting is controlled with serum clonazepam levels in the order of 10.0 ng/ml.

[*Jpn. J .Ther. Drug Monit.*, **27,** 1-9 (2010)] [Lab. of Clinical Pharmacy]

The Relationship between the Peak Plasma Concentration of Methotrexate (MTX) and the MTX Dose per Lean Body Mass on High-dose-MTX Rapid Infusion Method Used for Primary CNS Lymphoma Patients.

Masahiko HAYASHI, Haruo KITAMURA, Nobutaka YAMAKAWA, Masakazu FURUNO, Kouji NAKAO, Hitomi TERAMACHI*and Teruo TSUCHIYA

The purpose of this study is to investigate retrospectively the relationship between the apparent peak methotrexate(MTX) concentration(C0), 24-hour, 48-hour levels and the dose of MTX per LBM with 9 primary central nervous system lymphoma patients who received high-dose methotrexate rapid infusion method. The coefficient of determination, the mean prediction error, the mean absolute error and the root mean squared prediction error calculated by C0 and Cpre using the linear regression coefficient performed to characterize the relationship between the dose per LBM and C0 were marginally better than those of other parameters of body size.

[*Jpn. J.Pharm.Health Care Sci.*, **36,** 436-444 (2010)] [Lab. of Clinical Pharmacy]

Analysis of Factor Influencing "Interest in" and "Satisfaction with" Hospital Visit as Early Exposure Program.

Hitomi TERAMACHI*, Eiji SAKAI, Yumi KUZUYA and Teruo TSUCHIYA

At Gifu Pharmaceutical University, hospital visits were introduced as an early exposure program for first year students (n = 144) in 2009. On conducting a questionnaire survey of students who had gone on a hospital visit, the results suggested that pre-learning raised the students' interest in the hospital visit and that they were satisfied with it. We also conducted a factor analysis and covariance structure analysis to investigate a connection between "interest in hospital visit" and "level of satisfaction with hospital visit" and the learning structure. Covariance structure analysis showed that three factors. We inferred that this was linked with "hospital pharmacist desire" and "positive attitude". We also inferred that 3 factors - "desire to be a hospital pharmacist", "active pharmacist", and "positive attitude"- raised the "level of satisfaction with hospital visit".

[*Jpn. J.Pharm.Health Care Sci.*, **36,** 729-734 (2010)] [Lab. of Clinical Pharmacy]

A Retrospective Study on the Expression of the Myelosuppression in S-1 plus Cisplatin Combination Therapy for the Treatment of Advanced Gastric Cancer.

Katsumi TANIZAWA, Yoshiko TANAKA , Keiko TAGUCHI, Tomoyuki HIRASHITA, Hideharu ENDO, Hitomi TERAMACHI, Tadashi SUGIYAMA and Teruo TSUCHIYA*

We conducted a retrospective study on myelosuppression following the S-1+CDDP combination therapy in patients receiveing such therapy for advanced gastric cancer at Gifu Prefectural General Medical Center from June 2006 to April 2008 based on electronic records. We conducted the investigation on 119 courses of therapy in 36 patients, consisting of 22 men (70 courses) and 14 women (49 courses). Neutropenia occurred more frequently in female patient ($p = 0.0031$), those over 70 years old ($p = 0.0191$), and those having more than two courses ($p = 0.036$), and these finding were significant. It is therefore necessary to conduct blood tests from day 20 ? day29 in S-1+CDDP combination therapy.

[*Jpn. J.Pharm.Health Care Sci.*, **36,** 807-816 (2010)] [Lab. of Clinical Pharmacy]

Development of Teaching Materials for Instructing Students in Medical Communication and Their Evaluation-effectiveness of Videos and Pharmacist Simulator-.

Hitomi TERAMACHI*, Yumi KUZUYA, Takashi KATO, Hiroshi BABA, Yuzo TAKAHASHI and Teruo TSUCHIYA

We developed videos and a pharmacist simulator as methods of instructing students in medical communication in the pharmaceutical education curriculum. Using these 2 methods, 4th-year students (n=68) of our university received instruction in medical communication and then a questionnaire survey was conducted. The results indicated that the training had been effective in enhancing students' knowledge of medical communication and helping them improve their communication skills. As a result of the training, students' interest in this topic increased and they found it useful as a means of self-learning and as a way of learning communication skills. Covariance structure analysis showed that students were interested in additions to the curriculum that make use of videos and a simulator as they are useful in self-learning and learning communication skills.

[*J. Gifu Byoyaku*, **50,** 9-20 (2010)] [Lab. of Clinical Pharmacy]

“Medication Teaching Application” Lessons on Medical Communication and Analysis of Role-playing Presentation Evaluation.

Hitomi TERAMACHI*, Yumi KUZUYA and Teruo TSUCHIYA

We began a program “Medical Communication” to establish a training program for improving pharmaceutical communication skills to the 4th-year students of Gifu Pharmaceutical University in 2009. Students made communication sheet for pharmacists and scenario analysis sheet for patients, next students announced the role-playing every group. The result of a questionnaire survey for the class suggested that this class smoothly introduced students to the practice, was useful for their learning and that students learned activity. Analysis of the evaluation by students identified “interview” and “communication” as on item necessary to be improved to raise comprehensive evaluation regarding the question “Can you teach appropriate medication totally?”, “communication” was extracted from teachers as an improvement item required, and it is inferred that a basic communication skill is important.

[*J. Gifu Byoyaku*, **50,** 21-32 (2010)] [Lab. of Clinical Pharmacy]

Evaluation and Improvement for Pharmacy Practical Training Trial of Self Learning by Students.

Yumi KUZUYA*, Hitomi TERAMACHI, Masahiro YASUDA, Takashi MIZUI, Kenji KOBAYASHI, Makoto SAHASHI, Katsutoshi GOTO, Masamitsu SAKAIDA and Teruo TSUCHIYA

At Gifu Pharmaceutical University, using a support system for pharmacy practical training, we have been trying the construction of self learning program based on the “model core curriculum for practical training”. Making the revision of the training schedule, the exercises for specific behavioral objectives (SBOs) and introduction of small group discussion (SGD), we tried establishment of the training methods students learn for themselves. As a result, this system and schedule list was very useful tools, and the introduction of questions and SGD were very useful when students deepened understanding of SBOs.When, with customer satisfaction analysis, we extracted improvements, “the usability of the system” was given. We conclude that the improvement of “the usability of the system” help us to establish the practical training satisfy students.

[*Pharmaceuticals Monthly*, **52**, 771-774 (2010)] [Lab.of Pharmacy Practice and Social Science]

Development and Evaluation of a Recurrent Hands-on Training Program for Pharmacists : Establishing the Learning Methods of Vital Signs Examination.

Hiromi OKADA, Tadashi SUZUKI, Kazunori KIMURA, Tadashi SUGIYAMA*, Teruo TSUCHIYA, Noriyuki NAMIKI, Yoshiyuki KAGAWA and Satoshi FUJII

In recurrent educational programs for pharmacists in Tokai area we attempted to establish the learning method of measuring vital signs and assessing physical signs. The methods developed by us were based on measuring vital signs using common instruments easily obtainable in community pharmacy. We also used physical assessment model for developing additional skills. We conclude that this type of bedside training as recurrent pharmaceutical education was helpful to extend pharmacists’ skills and useful for consultations of patients.

[*J. Clin. Therap. Med.*,**26**, 721-726 (2010)] [Lab.of Pharmacy Practice and Social Science]

Development of a Simple Checking Method in Measurement of the Cancer Chemotherapeutic Agents.

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

To develop a useful checking method in measurement of the residual liquid amount of chemotherapeutic agents, we used the predictive net weight of the agents by calculating the average weight of the drugs. Calculation formula constructed in the Microsoft Excel computer program provides the predictive net weight by imputing dose level of the agent. When we check the residual liquid amount of chemotherapeutic agents by naked eyes, there are errors more than 20% in the precision. Therefore, the measurement of predictive net weight of the drug may be useful for evaluating the accuracy of the checking procedure of liquid cancer chemotherapeutic agents. The current method may provide a simple and effective system for preparing liquid mixtures of cancer chemotherapeutic agents.

[*Jpn. J. Pharm. Health.*, **36**, 25-30(2010)] [Lab. of Drug Informatics, Clinical Pharmaceutics and Clinical Pharmacy]

Analysis of Students' Practical Training Reports Using Text Mining.

Mitsuhiro NAKAMURA*, Hitomi TERAMACHI, Tetsuo ADACHI and Teruo TSUCHIYA

We have conductiong practical training for fourth-year students at Gifu Pharmaceutical University. At the end of the training, they write reports on their practical praining in the hospital pharmacy and practical training in the community pharmacy.

We worked out the frequency of keyowrds based on the word class information in the Japanese language morphologic analysis. The results showed that the students' awareness of "patient" tended to be higher in the clinical pharmacy report. Furthermore, the results of correspondence analysis showed that the characteristics of the reports on training in the hospital pharmacy and the reports on training in the community pharmacy were independent. We considered text-mining to be a useful approach in the evaluation of practical training.

[*Oncol.Lett..*, **1**, 273-278 (2010)] [Lab. of Radiochemistry]

Chemoprevention of 1,2-Dimethylhydrazine-Induced Colonic Preneoplastic Lesions in Fischer Rats by 6-Methylsulfinylhexyl Isothiocyanate, a Wasabi Derivative.

ToshiyaKUNO, Yoshinobu HIROSE, Yasuhiro YAMADA, Katsumi IMAIDA, Kenjiro TATEMATSU*, Yukio MORI and Hideki MORI

The preventive effect of a wasabi derivative 6-methylsulfinylhexyl isothiocyanate (6-MSITC) on 1,2-dimethylhydrazine (DMH)-induced colonic cancer was investigated in male F344 rats. Dietary administration of 6-MSITC during the initiation phase caused a significant reduction in the number and size of colonic cancer incidence compared to treatment with DMH alone. In addition, protein levels of hepatic cytochrome P-450 isozymes at 24 h after 6-MSITC exposure were significantly suppressed. The results indicated that 6-MSITC exerted chemopreventive effects in the present short-term colon carcinogenesis bioassay, through alterations in cell proliferation activity and drug metabolizing enzyme levels.

[*J Toxicol. Sci.*, **35**, 743-747 (2010)] [Lab. of Radiochemistry]

Testosterone-Lowering Activity of Canola and Hydrogenated Soybean Oil in the Stroke-Prone Spontaneously Hypertensive Rat.

Harumi OKUYAMA, Naoki OHARA, Kenjiro TATEMATSU*, Shinya FUMA, Tomoyuki NONOGAKI, Kazuyo YAMADA, Yuko ICHIKAWA, Daisuke MIYAZAWA, Yuko YASUI and Seijiro HONMA

Canola oil, hydrogenated soybean oil and some other types of oil unusually shorten the survival of stroke-prone spontaneously hypertensive rats (SHRSP). Since steroid hormone metabolism were suggested by a preliminary DNA microarray analysis as a reason for this, the steroid hormone levels in the serum and tissues of SHRSP fed different oils were investigated. The testosterone levels in the serum and the testes were found to be significantly lower in the canola oil and hydrogenated soybean oil groups than in the soybean oil group. The testosterone-lowering activities of these oils were considered in relation to factors possibly affecting the physiology of SHRSP.

[*Natural ProductCommumications*, **55**, 1781-1784 (2010)] [Lab. of Herbal Garden]

A New Biisoflavonoid from the Roots .of Erythrina Varieata.

Hitoshi Tanaka*, Masaru Sudo, Miyuki Hirata, Hideo Etho, Masaru Sato, Ryozo Yamaguchi, Eiji Sakai, Ih-Sheng Chen and Toshio Fukai

A new biisoflavonoid, biseryvarin A, together with two known compounds were isolated from the roots of Erythrina variegata. The structute of biseryvarin A was established on the basis of spectroscopic evidence. Biseryvarin A is the first dimeric isoflavonoid possessing isoprenoid groups from the genus Erythrina. Biseryvarin A showed low activity against methicillin-resistant Staphylococcus aurens (MRSA).

[*Planta Med.*, **76**, 916-919 (2010)] [Lab. of Herbal Garden]

Antibacterial Constituents from the Roots of Erythrina Herbaces against Methicillin-Resistant Staphylococcus Aureus.

Hitoshi Tanaka*, Masaru Sudo, Tomoko Kawamura, Masaru Sato, Ryozo Yamaguchi, Toshio Fukai, Eiji Sakai and Nobuyuki Tanaka

Two new compounds, erybacin A and erybacin B, together with 10 known compounds, were isolated from the roots of Erythrina herbacea. Their structures were esabulished on the basis of spectroscopic analyses. Erybacin A is a rare, naturally occurring -hydroxy-1,3-diphenylpropan-2-one derivative. The isolated compounds were evaluated for their antibacterial activity against 13 strains of merhicillin-resistant Staphylococcus aureus (MRSA). The new compound 2 showed a potent bactericidal activity against MRSA.

[*Shoyakugaku Zasshi*, **64**, 83-89 (2010)] [Lab. of Herbal Garden]

A Quantitative Analysis of Catalposide and p-Hydroxybenzoic Acid in CATALPAE FRUCTUS.

Mamoru Okasaka, Keiko Arimoto, Michiho Ito, Yuko Osumi, 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.

CATALPAE FRUCTUS, a traditional Japanese Medicines used as a diuretic whose origin described in the Japanese Pharmacopoeia Fifteenth Edition, is the fruit of *Catalpa ovata* G. Don and C. Bungei C. A. Mayer (Birnoniaceae). Detection of para-hydroxybenzoic acid and catalposide by thin layer chromatography (TLC) is an authorized method for identification of the drug; however, ctalposide cnnot be detected from some of the market samples by this method probably because of low content. In order to investigate catalposide content of the market samples, a method for quantitative analysys of the compound useing HPLC was developed. The content of ctalposide was shown to vary from 0% to 0.079% among the samples and seemed to be less in aged samples.

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