[*J. Am. Chem. Soc*. **181**(42),15080–15081 (2009)] [Lab. of Pharm. Chemistry]

Facile Synthesis of 1,2,4-Triazoles via a Copper-Catalyzed Tandem Addition-Oxidative Cyclization.

Satoshi UEDA and Hideko NAGASAWA*

A simple one-step synthesis of 1,2,4-triazole derivatives is provided by a copper-catalyzed oxidative coupling reaction under an atmosphere of air. The process should consist of sequential N-C and N-N bond-forming copper-catalyzed oxidative coupling reactions. Starting materials and the copper catalyst are readily available and inexpensive. A wide range of functional groups are tolerated to achieve chemical diversity.

[*J. Org. Chem*. **74**(11),4272–4277 (2009)] [Lab. of Pharm. Chemistry]

Copper-Catalyzed Synthesis of Benzoxazoles via a Regioselective C−H Functionalization/C−O Bond Formation under an Air Atmosphere.

Satoshi UEDA and Hideko NAGASAWA*

An efficient method for the synthesis of functionalized benzoxazoles is described that involves a copper(II)-catalyzed regioselective C-H functionalization/C-O bond formation protocol. The use of dichlorobenzene as a solvent at 160 degrees C allows the use of air as the terminal oxidant in the catalytic synthesis of benzoxazoles in a process that has high functional group tolerance. The presence of a directing group at the meta position markedly improves the reaction efficacy and a variety of 7-substituted benzoxazoles are selectively produced under mild reaction conditions. The mechanism of the reaction is also discussed in this report.

[*Cancer Chemother. Pharmacol.* **64**(5), 885–892 (2009)] [Lab. of Pharm. Chemistry]

Downregulation of matrix metalloprotease-9 and urokinase plasminogen activator by TX-1877 results in decreased tumor growth and metastasis on xenograft model of rectal cancer.

Kotaro MIYAKE, Mitsuo SHIMADA, Masanori NISHIOKA, Koji SUGIMOTO, Erdenebulgan BATMUNKH, Yoshihiro UTO, Hideko NAGASAWA*, and Hitoshi HORI

It is well known that hypoxic milieu is the primary cancer environment. Therefore, tumor hypoxia is considered to be a potential therapeutic target. In the present study, we investigated the antitumor and antimetastatic effect of hypoxic cell radiosensitizer, TX-1877 on xenograft model of rectal cancer. In subcutaneous model, tumor treated with TX-1877 and irradiation showed significant reductions in volume. Quantitative real-time reverse transcription-PCR and immunohistochemical analysis revealed that TX-1877 significantly inhibited expression of the MMP-9 and uPA. These data show that the treatment of TX-1877 with irradiation decreased growth of human rectal cancer and, furthermore, suppressed lymph node metastasis.

[*Adv. Exp. Med. Biol.* **645**,109–114 (2009)] [Lab. of Pharm. Chemistry]

A chemical biosynthesis design for an antiatherosclerosis drug by acyclic tocopherol intermediate analogue based on "isoprenomics".

Yoshihiro UTO, Daisuke KOYAMA, Mamoru OTSUKI, Naoki OTOMO, Tadashi SHIRAI, Chiaki ABE, Eiji NAKATA, Hideko NAGASAWA*, and Hitoshi HORI

We have achieved the biosynthesis-oriented design and synthesis of alpha- (TX-2254) and beta-(TX-2247) phytyl quinol, other gamma- (TX-2242) and delta-(TX-2231) phytyl quinol. Geometry optimization and Molecular orbital (MO) calculation of TX-2254 showed a unique right-angle structure; however, MO energy of TX-2254 and d-alpha-tocopherol were very similar. Radical reactivity of TX-2231 was equal to dl-alpha-tocopherol, whereas TX-2254, TX-2247, and TX-2231 showed lower reactivity than dl-alpha-tocopherol. All four phytyl quinols showed almost the same moderate inhibitory activity against low-density lipoprotein (LDL) oxidation. We proposed phytyl quinols were possible antioxidants in plants and animals, like vitamin E.

[*Mol. Nutr. Food Res.* **53**(5), 643–651 (2009)] [Lab. of Pharm. Chemistry]

Correlation between antiangiogenic activity and antioxidant activity of various components from propolis.

Mok-Ryeon AHN, Kazuhiro KUNIMASA, Shigenori KUMAZAWA, Tsutomu NAKAYAMA, Kazuhiko KAJI, Yoshihiro UTO, Hitoshi HORI, Hideko NAGASAWA*, and Toshiro OHTA

In this study, we examined the antiangiogenic and antioxidant activities of various components from propolis. Two propolis components, caffeic acid phenethyl ester, and quercetin, possessed strong inhibitory effects on tube formation and on endothelial cell proliferation and, coincidentally, showed strong antioxidant activity. From these results, we propose that components from propolis such as artepillin C, caffeic acid phenethyl ester, galangin, kaempferol, and quercetin might represent a new class of dietary-derived antioxidative compounds with antiangiogenic activities. These propolis components may have the potential to be developed into pharmaceutical drugs for the treatment of angiogenesis-dependent human diseases such as tumors.

[*J. Org. Chem*. **74**(6), 2609–2612 (2009)] [Lab. of Pharm. Chemistry]

Synthesis of a Fluorine-Substituted Puromycin Derivative for Brønsted Studies of Ribosomal-Catalyzed Peptide Bond Formation.

Kensuke OKUDA*, Takashi HIROTA, David A. KINGERY, and Hideko NAGASAWA

The mechanism by which the ribosome catalyzes peptide bond formation remains controversial. Here we describe the synthesis of a nucleoside that can be used in Brønsted experiments to assess the transition state of ribosome catalyzed peptide bond formation. This substrate is the nucleoside 3′-amino-3′-deoxy-3′-[(3″*R*)-3-fluoro-l-phenyl-alanyl]-N^6,N^6-dimethyladenosine, which was prepared from (1*R*,2*R*)-2-amino-1-phenylpropane-1,3-diol. This substrate is active in peptide bond formation on the ribosome and is a useful probe for Brønsted analysis experiments on the ribosome.

[*Chem. Pharm. Bull.* **57**(7), 755–758 (2009)] [Lab. of Pharm. Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 59: Rearrangement Reactions of Fused Tricyclic 3-(2-Bromoethyl)pyrimidin-4(3*H*)-ones with Primary Amines via a Dimroth-type Rearrangement.

Kensuke OKUDA*, Hiromi OHTOMO, Fumiaki TANAKA, Takashi HIROTA, and Kenji SASAKI

Reaction of some fused tricyclic 3-(2-bromoethyl)pyrimidin-4(3*H*)-ones with primary alkyl amines gave abnormal fused 3-alkyl-4-alkyliminopyrimidines *via* a Dimroth-type rearrangement, as well as normal substituted 3-(2-alkylaminoethyl) derivatives in methanol. This abnormal rearrangement reaction depended on reaction solvent.

[*Chem. Pharm. Bull.* **57**(11), 1296–1299 (2009)] [Lab. of Pharm. Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 60: Reactions of 3-(2-Cyanophenyl)quinazolin-4(3*H*)-ones with Primary Amines.

Kensuke OKUDA*, Tsuyoshi TAGATA, Setsuo KASHINO, Takashi HIROTA, and Kenji SASAKI

The reaction of 3-(2-cyanophenyl)quinazolin-4(3H)-one with various primary alkylamines gave 3-alkylquinazolin-4(3H)-ones *via* an addition of the nucleophile, ring opening, and ring closure (ANRORC) mechanism. This type of reaction required hydroxy group functionality in either the solvent or reagent. When hydroxylamine was used as nitrogen nucleophile, the intermediate of this reaction was isolated and found to be an amide oxime. When ethylenediamine was used as the nucleophile, the amidine moiety of the intermediate decomposed to give a benzanilide.

[*Chem. Eur. J.,* **15**, 834–837 (2009)] [Lab. of Organic Chemistry]

A Highly Active Heterogeneous Palladium Catalyst Supported on a Synthetic Adsorbent.

Yasunari MONGUCHI, Yuki FUJITA, Koichi ENDO, Shinobu TAKAO, Masatoshi YOSHIMURA, Yukio TAKAGI, Tomohiro MAEGAWA, and Hironao SAJIKI*

Highly dispersed 10% Pd/HP20 was readily prepd. from $Pd(OAc)_2$ and a com. synthetic adsorbent, DIAION HP20. The 10% Pd/HP20 has strong catalyst activity and was used for the hydrogenation and ligand-free Suzuki-Miyaura coupling reaction.

[*Chem. Eur. J,* **15**, 6953–6963 (2009)] [Lab. of Organic Chemistry]

Efficient and Practical Arene Hydrogenation by Heterogeneous Catalysts under Mild Conditions.

Tomohiro MAEGAWA, Akira AKASHI, Kiichiro YAGUCHI, Youhei IWASAKI, Masahiro SHIGETSURA, Yasunari MONGUCHI, and Hironao SAJIKI*

An efficient and practical arene hydrogenation procedure based on the use of heterogeneous platinum group catalysts has been developed. Rh/C is the most effective catalyst for the hydrogenation of the arom. ring, which can be conducted in iPrOH under neutral conditions and at ordinary to medium H2 pressures (<10 atm). A variety of arenes such as alkylbenzenes, benzoic acids, pyridines, furans, are hydrogenated to the corresponding cyclohexyl and heterocyclic compds. in good to excellent yields. The use of Ru/C, less expensive than Rh/C, affords an effective and practical method for the hydrogenation of arenes including phenols. Both catalysts can be reused several times after simple filtration without any significant loss of catalytic activity.

[*Adv. Synth. Catal.,* **351**, 2091–2095 (2009)] [Lab. of Organic Chemistry]

Development of Molecular Sieves-Supported Palladium Catalyst and Chemoselective Hydrogenation of Unsaturated Bonds in the Presence of Nitro Groups.

Tomohiro MAEGAWA, Tohru TAKAHASHI, Masatoshi YOSHIMURA, Hroyoshi SUZUKA, Yasunari MONGUCHI, and Hironao SAJIKI*

The chemoselective hydrogenation of unsatd. bonds and azide functionalities in the presence of nitro groups is achieved by a heterogeneous palladium catalyst supported on mol. sieves (MS3A). The present method shows a wide-range of applicability with regard to substrates and the catalyst can be easily prepd. and reused at least three times without any loss of activity..

[*Chem. Commun.,* 5159–5161 (2009)] [Lab. of Organic Chemistry]

A Simple and Efficient Oxidation of Alcohols with Ruthenium on Carbon.

Shigeki MORI, Masato TAKUBO, Kazuya MAKIDA, Takayoshi YANASE, Satoka AOYAGI, Yasunari MONGUCHI, and Hironao SAJIKI*

A simple, efficient, and environmentally-friendly heterogeneous Ru/C-catalyzed oxidn. of secondary and primary alcs. without additives under an atm. of oxygen has been established.

[*Amino Acids,* **36**, 493–499 (2009)] [Lab. of Organic Chemistry]

Novel Deprotection Method of Fmoc Group Under Neutral Hydrogenation Conditions.

Tomohiro MAEGAWA, Yuta FUJIWARA, Takashi IKAWA, Hideo HISASHI, Yasunari MONGUCHI, and Hironao SAJIKI*

Novel deprotection method of the Fmoc (Fmoc = 9-flurorenylmethoxycarbonyl) protective group under Pd/C-catalyzed hydrogenation conditions at room temp. was developed. The addn. of CH_3CN accelerated the deprotection of the Fmoc group, and also the application of H_2 pressure (3 atm) shows notable rate enhancement.

[*J.Mol.Catal.A,* **307**, 77–87 (2009)] [Lab. of Organic Chemistry]

Pd(0)-Polyethyleneimine Complex as a Partial Hydrogenation Catalyst of Alkynes to Alkenes.

Shigeki MORI, Tomoyuki OHKUBO, Takashi IKAWA, Akira KUME, Tomohiro MAEGAWA, Yasunari MONGUCHI, and Hironao SAJIKI*

A Pd(0)-polyethyleneimine [Pd(0)-PEI] complex for the selective partial hydrogenation of alkynes to alkenes was developed. Notably, Pd(0)-PEI catalyzed the partial hydrogenation of mono-substituted alkynes with an excellent selectivity (77-100%), which was very difficult to achieve even with the Lindlar catalyst. Moreover, the use of Pd(0)-PEI led to no redn. in the other reducible functionalities, such as the N-benzyloxycarbonyl, benzyl ester, benzyl ether and O-tert-butyldimethylsilyl protective groups; i.e., Pd(0)-PEI offers a concise synthetic route to a variety of functionalized alkenes.

[*Catal. Commun.,* **10**, 1161–1165 (2009)] [Lab. of Organic Chemistry]

Temperature-Dependent Suppression of Palladium on Carbon-Catalyzed Hydrogenations.

Utpal BORA, Kiichiro YAGUCHI, Akira KUME, Tomohiro MAEGAWA, Yasunari MONGUCHI, and Hironao SAJIKI*

Pd/C-catalyzed hydrogenation reactions are found to be highly temp.-dependent. The hydrogenation smoothly proceeds in the temp. range from ambient to the b.p. of the solvents, although the hydrogenation is suppressed at an elevated bath temp. of approx. 30 °C above the b.p. of the solvent under ambient hydrogen pressure.

[*Synthesis, 16,* 2674–2678 (2009)] [Lab. of Organic Chemistry]

Bimetallic Palladium-Platinum on Carbon Catalyzed H-D Exchange Reaction: Synergistic Effect on Multiple Deuterium Incorporation.

Tomohiro MAEGAWA, Nobuhiro ITO, Keiji OONO, Yasunari MONGUCH,I and Hironao SAJIKI*

Several activated carbon-supported bimetallic Pd-Pt catalysts (Pd-Pt/C) were prepd. using various reducing reagents, and their catalytic activities were examd. for the deuteration of alkyl-substituted arom. compds. Multiple deuterations catalyzed by Pt-Pd/C proceeded in D_2O at 180° under a H_2 atmosphere, and a synergistic effect was obsd. in relation to the incorporation of deuterium at sterically hindered positions on arom. rings.

[*Heterocycles,* **77**, 521–532 (2009)] [Lab. of Organic Chemistry]

Cu/HP20: Novel and Polymer-Supported Copper Catalyst for Huisgen Cycloaddition.

Yoshiaki KITAMURA, Kazumi TANIGUCHI, Tomohiro MAEGAWA, Yasunari MONGUCHI, Yukio KITADE, and Hironao SAJIKI*

A polymer-supported copper catalyst (Cu/HP20) is easily prepd. in water and effectively catalyzed the Huisgen cycloaddn. between azides and terminal alkynes.

[*Heterocycles,* **79**, 669–680 (2009)] [Lab. of Organic Chemistry]

Iodobenzene Diacetate-Promoted N-N And N-O Bond Formation for Pyrazolo-And Isoxazolopyrimidine Synthesis.

Yasunari MONGUCHI, Kazuyuki HATTORI, Tomohiro MAEGAWA, Kosaku HIROTA, and Hironao SAJIKI*

Pyrazolo[3,4-d]pyrimidine-4,6-dione derivs., i.e. I, were efficiently synthesized via the intramol. N-N bond coupling of 5-iminomethyl-6-aminouracil derivs. using iodobenzene diacetate. The oxidative coupling was also applied to the analogous N-O bond formation producing isoxazolo[3,4-d]pyrimidine-4,6-dione derivs. II (R = Me, Et, n-Pr).

[*Tetrahedron Lett.*, **50**, 4328–4330 (2009)] [Lab. of Pharm. Synthetic Chemistry]

Aerobic photo-oxidative cleavage of the C-C double bonds of styrenes

Shin-ichi HIRASHIMA, Yasuhisa KUDO, Tomoya NOBUTA, Norihiro TADA, Akichika ITOH*

The oxidative cleavage of the C-C double bonds of styrenes was carried out in the presence of CBr_4 under aerobic photoirradn. conditions. Oxidative cleavage of the various β-substituted styrenes produced benzoic acid in good yields. Since this reaction is found to be applicable to the α- or β-substituted styrenes, which showed very low reactivity under previously reported cleavage reaction conditions with FSM-16 and I2, it can be used complementarily.

[*Synlett*, 2017–2019 (2009)] [Lab. of Pharm. Synthetic Chemistry]

Acceleration of Norrish type I reaction with molecular oxygen and catalytic CBr_4

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

A useful method is reported for facile synthesis of arom. carboxylic acids from aryl ketones by aerobic photooxidn. using the inexpensive and easily handled CBr_4 as catalyst. This procedure is applicable to inert compds. under usual photo-irradn. conditions, and appears very attractive as expansion of the Norrish I reaction.

[*Synlett*, 3024–3026 (2009)] [Lab. of Pharm. Synthetic Chemistry]

Metal-free epoxidation of alkenes with molecular oxygen and benzaldehyde under visible light irradiation

Norihiro TADA, Hiroaki OKUBO, Tsuyoshi MIURA, Akichika ITOH*

A new convenient metal-free oxidn. protocol of a wide variety of alkenes with mol. oxygen and benzaldehyde under visible light irradn. of a fluorescent lamp afforded the corresponding epoxides in 49-99% yields.

[*Tetrahedron Lett.*, **50**, 2516–2520 (2009)] [Lab.of Pharmacognosy]

Absolute structures of *C*-glucosides of resveratrol oligomers from *Shorea uliginosa*.

Tetsuro ITO, Naohito ABE, Masayoshi OYAMA, and Munekazu IINUMA*

Two *C*-glucosides of resveratrol dimers [uliginoside A and hemsleyanoloside B] consisting of enantiomeric aglycons and two *C*-glucosides of resveratrol trimers [uliginosides B and C] consisting of diastereomeric aglycons were isolated from *Shorea uliginosa* (Dipterocarpaceae). These structures were elucidated by spectroscopic analyses including NMR expts., and their absolute configurations were determined based on CD data. Resveratrol oligomers of *C*-glucosides with a 1,2-diaryldihydrobenzofuran ring are produced with specific biogenetic routes.

[*Anticancer Res.*, **29**, 2485–2496 (2009)] [Lab.of Pharmacognosy]

Panaxanthone isolated from pericarp of *Garcinia mangostana* L. suppresses tumor growth and metastasis of a mouse model of mammary cancer.

Hitoshi DOI, Masa-Aki SHIBATA, Eiko SHIBATA, Junji MORIMOTO, Yukihiro AKAO, Munekazu IINUMA*, Nobuhiko TANIGAWA, and Yoshinori OTSUKI

The antitumor growth and antimetastatic activity of panaxanthone (approx. 80% alfa-mangostin and 20% gamma-mangostin) were studied in a mouse metastatic mammary cancer model that produces a metastatic spectrum similar to that seen in human breast cancer. In the *in vivo* study, tumor vols. were significantly suppressed in mice treated with 2,500 and 5,000 ppm panaxanthone in their diet. The multiplicity of lung metastasis was significantly lower in the 5,000 ppm group. Lymph node metastasis also tended to decrease in the 5,000 ppm group but not significantly. The antitumor effects of panaxanthone were associated with elevation of apoptotic cell death, antiproliferation and antiangiogenesis. The *in vitro* study demonstrated that alfa-mangostin induced apoptosis, as evidenced by increased nos. of TUNEL-pos. cells, elevated activities of caspases and a decrease in mitochondrial membrane potential, cell cycle arrest in the G_1-phase and decreases in the cell population in the S- and G_2/M-phases. These results suggest that the obsd. antimetastatic activity of panaxanthone may be of clinically significance as adjuvant therapy in metastatic human breast cancer, and may also be useful as a chemopreventative of breast cancer development.

[*Chem. Pharm. Bull.*, **57**, 516–519 (2009)] [Lab.of Pharmacognosy]

Two new resveratrol tetramers from *Upuna borneensis*.

Tetsuro ITO, Naohito ABE, Zulfiqar ALI, Masayoshi OYAMA, Toshiyuki TANAKA, Ryuichi SAWA, Yoshikazu TAKAHASHI, Jin MURATA, Dedy DARNAEDI, and Munekazu IINUMA*

Phytochemical investigation of an acetone ext. of *Upuna borneensis* (Dipterocarpaceae) resulted in the isolation of two new resveratrol tetramers, upunaphenols O and P. The structures were elucidated by spectroscopic analyses including NMR expts.

[*Helv. Chim. Acta*, **92**, 195–208 (2009)] [Lab.of Pharmacognosy]

Two novel resveratrol derivatives from the leaves of *Vateria indica*.

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

Two new resveratrol (5-[(*E*)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol) derivatives, vateriaphenols D and E, were isolated from the leaves of *Vateria indica* (Dipterocarpaceae), together with six known resveratrol oligomers, a isocoumarin (bergenin), and a benzophenone. The structures of the isolates were established on the basis of spectroscopic analyses, including a detailed NMR spectroscopic investigation. Vateriaphenol D is composed of three resveratrol units and a piceatannol (5-[(*E*)-2-(3,4-dihydroxyphenyl)ethenyl]benzene-1,3-diol) unit, and is the first instance of a heterogeneous coupled stilbene tetramer in dipterocarpaceaeous plants. Vateriaphenol E bears a rare 2,3-dihydrobenzofuran-2-ol skeleton in the framework.

[*Helv. Chim. Acta*, **92**, 1203–1216 (2009)] [Lab.of Pharmacognosy]

Two Novel Resveratrol Trimers from *Dipterocarpus grandiflorus*.

Tetsuro ITO, Naohito ABE, Masayoshi OYAMA, Toshiyuki TANAKA, Jin MURATA, Dedy DARNAEDI, and Munekazu IINUMA*

Two new resveratrol (5-[(*E*)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol) trimers, grandiphenols C (I) and D (II), were isolated from the stem of *Dipterocarpus grandiflorus* (Dipterocarpaceae). The structures of I and II were elucidated by spectral analysis including 1D- and 2D-NMR experiments and by computer-aided molecular modeling. The NMR characteristics caused by the steric hindrance and the biogenetic relationship of the isolates are also discussed in this work.

[*Bioorg. Med. Chem.*, **17**, 3189–3197 (2009)] [Lab.of Pharmacognosy]

Inhibitory effects of sesquiterpene lactones isolated from *Eupatorium chinense* L. on IgE-mediated degranulation in rat basophilic leukemia RBL-2H3 cells and passive cutaneous anaphylaxis reaction in mice.

Tomohiro ITOH, Masayoshi OYAMA, Norihiko TAKIMOTO, Chihiro KATO, Yoshinori NOZAWA, Yukihiro AKAO, and Munekazu IINUMA*

Sesquiterpene lactones (SQTLs) have been shown to suppress the degranulation as inferred by histamine release in rat basophilic leukemia RBL-2H3 cells. In this study, 9 kinds of SQTLs were isolated from *Eupatorium chinense* and the effects of these SQTLs on the degranulation in RBL-2H3 cells were examined. The chemical structures of two novel compounds (SQTL-3 and 8) were determined. All the SQTLs suppressed the degranulation from Ag-stimulated RBL-2H3 cells. To disclose the inhibitory mechanism of degranulation by SQTLs, the activation of intracellular signaling molecules such as Lyn, Syk, and PLC were examined. None of these SQTLs showed activation of Syk and PLC. The intracellular free Ca^{2+} concentration was elevated, but SQTLs treatment reduced the elevation of [Ca^{2+}] by suppressing Ca^{2+} influx. Thus, it was suggested that the suppression of Ag-stimulated degranulation by these SQTLs is mainly due to the decreased Ca^{2+} influx. Furthermore, in order to clarify the *in vivo* effect of SQTL-rich extract, SQTL-rich extract. was administered to type I allergic model mice and the passive cutaneous anaphylaxis (PCA) reaction induced by IgE-antigen complex was measured. The SQTLs remarkably suppressed PCA reaction in a dose-dependent manner. Thus, it was suggested that SQTLs would be a candidate as an anti-allergic agent.

[*Helv. Chim. Acta*, **92**, 1999–2008 (2009)] [Lab.of Pharmacognosy]

Novel Flavonoids in Dragon's Blood of *Daemonorops draco*.

Ken-ichi NAKASHIMA, Naohito ABE, Fumiko KAMIYA, Tetsuro ITO, Masayoshi OYAMA, and Munekazu IINUMA*

Three novel methylene bis[flavonoids], a novel 2-flavene, a new naturally occurring flavan, and a new retro-dihydrochalcone were isolated from dragon's blood (resin) of *Daemonorops draco* (Palmae), together with seven known compounds. The structures were elucidated by extensive 1D- and 2D-NMR spectroscopic analysis.

[*J. Nat. Med.*, **63**, 355–359 (2009)] [Lab.of Pharmacognosy]

Hydroxychavicol: a potent xanthine oxidase inhibitor obtained from the leaves of betel, *Piper betle*.

Kazuya MURATA, Kikuyo NAKAO, Noriko HIRATA, Kensuke NAMBA, Takao NOMI, Yoshihisa KITAMURA, Kenzo MORIYAMA, Takahiro SHINTANI, Munekazu IINUMA*, and Hideaki MATSUDA

The screening of Piperaceous plants for xanthine oxidase inhibitory activity revealed that the ext. of the leaves of *Piper betle* possesses potent activity. Activity-guided purification led us to obtain hydroxychavicol as an active principle. Hydroxychavicol is a more potent xanthine oxidase inhibitor than allopurinol, which is clinically used for the treatment of hyperuricemia.

[*Biol. Pharm. Bull.*, **32**, 308–310 (2009)] [Lab.of Pharmacognosy]

Effects of sesquiterpene lactones on melanogenesis in mouse B16 melanoma cells.

Kenji OHGUCHI, Masaaki ITO, Kouji YOKOYAMA, Munekazu IINUMA*, Tomohiro ITOH, Yoshinori NOZAWA and Yukihiro AKAO

In this study, we examined the effect of sesquiterpene lactones isolated from *Calea urticifolia* and *Tanacetum parthienium* (feverfew) on melanogenesis in mouse B16 melanoma cells. In response to 3-isobutyl-1-methylxanthin (IBMX), B16 melanoma cells underwent differentiation characterized by increased melanin biosynthesis. Treatment of sesquiterpene lactones at lower concentration significantly blocked IBMX-induced melanogenesis, but did not induce the inhibitory activity of cell growth. Among them, 2,3-epoxyjuanislamin exhibited a potent inhibitory effect on melanogenesis. Treatment of B16 cells with 2,3-epoxyjuanislamin elicited significant decreases in tyrosinase protein and *m*RNA levels. These results demonstrated that the inhibitory effects of sesquiterpene lactones on melanin biosynthesis may be due to the suppression of tyrosinase expression.

[*Chem.Pharm. Bull.,* **57**, 1434–1436 (2009)] [Lab. of Pharm. Anal. Chemistry]

Cross-Link Dimer Formation of the Acetaldehyde-Derived Cyclic 1,N^2-Propano-2'-deoxyguanosine Adduct Using Electrochemical Oxidation.

Hiroya MURAKAMI, Yukihiro ESAKA, and Bunji UNO*

The electrochemically oxidative lesion of the acetaldehyde-derived cyclic propano adduct 2 of 2_-deoxyguanosine 1 was identified as the cross-linked dimer 4 of adduct 2. Cross-link formation is explained by the nucleophilic preference of the exocyclic amino group in 2 to the carbocation 3 electrogenerated by 1-proton and 2-electron transfers. Dimer formation was also detected in duplex DNA during exposure to acetaldehyde followed by electrochemical oxidation. The dimer has been deduced to be an intrastrand cross-link generated specifically in the G–G sequence in duplex DNA, which is expected to contribute to acetaldehyde-mediated genotoxicity.

[*Euro. J. Pharm.Biopharm.,* **72**, 1–8 (2009)] [Lab. of Pharm. Engineering]

pH-Sensitive Nanospheres for Colon-Specific Drug Delivery in Experimentally-Induced Colitis Rat Model.

Abdallah MAKHLOF, Yuichi TOZUKA, and Hirofumi TAKEUCHI*

Novel pH-sensitive nanospheres designed for colon-specific delivery were prepared using polymeric mixtures of poly (lactic-co-glycolic) acid (PLGA) and a pH-sensitive methacrylate copolymer. The prepared nanospheres showed strongly pH-dependent drug release properties in acidic and neutral pH values followed by a sustained release phase at pH 7.4. Animal experiments revealed the superior therapeutic efficiency of BSD-loaded nanospheres in alleviating the conditions of TNBS-induced colitis model. In conclusion, the proposed nanosphere system combined the properties of pHsensitivity, controlled release, and particulate targeting that could be useful for colon-specific delivery in inflammatory bowel disease.

[*J. Control. Release,* **136,** 247–253 (2009)] [Labs. of Pharm. Engineering]

Design and Evaluation of a Liposomal Ocular Delivery System Targeting the Posterior Segment of the Eye.

Kohei HIRONAKA, Yuta INOKUCHI, Yuichi TOZUKA, Masamitsu SHIMAZAWA, Hideaki HARA, and Hirofumi TAKEUCHI*

The purpose of this study was to evaluate the potential of submicron-sized liposomes (ssLips) as a novel system for delivering ocular drugs to the eye's posterior segment. Fluorescence emission of coumarin-6 formulated into ssLip was obvious in that segment in mice after eyedrop administration of the liposomal suspension. The ssLip based on L-α-distearoyl phosphatidylcholine (DSPC ssLip) showed higher fluorescence emission in the retina than that based on egg phosphatidylcholine (EPC ssLip). Images of the entire eye showed that ssLip was delivered via the non-corneal pathway after administration. The liposomes tested in ocular cells showed little cytotoxicity. These results suggest that ssLip can be used to deliver drugs to the posterior segment of the eye.

[*Asian J. Pharm. Sci.*, **4**, 1–7 (2009)] [Labs. of Pharm. Engineering]

Antibiotic activity of teracycline released from a mucoadhesive complex with sucralfate against *Helicobacter pylori.*

Syouichi. HIGO, Hirofumi TAKEUCHI*, Hiromitsu YAMAMOTO, Tomoaki HINO, Machiko MIYATA, Hiroshi MORI, and Yoshiaki KAWASHIMA

In this study, we evaluated the actual antibiotic activity of the tetracycline released from the acidic complex, because tetracycline apparently binds to sucralfate by chelation. Activity against two bacteria was identified: *Staphylococcus aureus* as a general target and *H. pylori* as a specific target. The *H. pylori* was cultured in test tubes filled with CO_2 gas, which yielded satisfactory results without requiring the use of a CO_2 incubator. The sucralfate showed no antibiotic activity but the tetracycline released from the complex showed antibiotic activity even after acidic treatment in the preparation of the complex. Tetracycline showed far greater antibiotic activity against *H. pylori* than against *Staphylococcus aureus*.

[*J Pharm Sci.*, **98**, 1643–1656 (2009)] [Lab. of Pharm. Engineering]

Modified chitosans for oral drug delivery.

Martin WERLE, Hirofumi TAKEUCHI*, and Andreas BERNKOP-SCHNÜRCH

The cationic polysaccharide chitosan has been extensively studied for oral drug delivery. In recent years, chemically modified chitosans developed in order to improve the properties of chitosan for oral drug delivery have gained increasing attention. Representatives of these novel polymers are trimethyl-chitosans, thiolated chitosans, carboxymethyl chitosan and derivatives, hydrophobic chitosans, chitosan succinate and phthalate, PEGylated chitosans and chitosan-enzyme inhibitor conjugates. Besides their use for oral delivery of therapeutic peptides and proteins, they have recently been evaluated regarding their potential for the delivery of other substance classes, including genes and efflux pump substrates. Within the current review, various modified chitosan derivatives, their properties and synthesis are discussed.

[*Drug Dev Ind Pharm,*. **35**:209–215 (2009)] [Lab. of Pharm. Engineering]

Development and in vitro characterization of liposomes coated with thiolated poly(acrylic acid) for oral drug delivery.

Martin WERLE, Kohei HIRONAKA, and Hirofumi TAKEUCHI*

The aim of this study was to investigate the feasibility of preparing liposomes that are coated with the multifunctional polymer poly(acrylic acid)-cysteine (PAA-Cys). Cationic multilamellar vesicles (MLV) as well as cationic submicron-sized liposomes (ssLip) were prepared and coated with PAA-Cys. These effects were attributed to interactions between the markers and the poly(acrylates). Coating of liposomes with PAA-Cys and PAA did not influence the release profile of FD4 and CF, whereas the release profile was affected by the molecular mass of the marker and the liposome size. In conclusion, the feasibility of coating liposomes with PAA-Cys was demonstrated, and it could be shown that this novel carrier system fulfills the basic requirements for an intended use in oral drug delivery.

[*Int. J. Pharm.,* **370**(1-2): 26–32. (2009)] [Lab. of Pharm. Engineering]

Chitosan-aprotinin coated liposomes for oral peptide delivery: Development, characterisation and in vivo evaluation.

Martin WERLE, and Hirofumi TAKEUCHI*

In order to improve the systemic uptake of therapeutic peptides/proteins after oral administration, the polymer-protease inhibitor conjugate chitosan-aprotinin was synthesised and polyelectrolyte complexes between negatively charged multilamellar vesicles (MLV) and positively charged chitosan-aprotinin conjugate were prepared. It could be demonstrated that chitosan-aprotinin was capable of significantly inhibiting Trypsin in vitro in concentrations of 0.05% and 0.1%, whereas no inhibition was observed in the presence of 0.1% chitosan. The size range of the prepared MLV was between 3 and 4.5microm and the initially negative zeta potential (ca. -90mV) of the core liposomes switched to a positive value after polymer coating (ca. +40mV).

[*Recent Pat Drug Deliv Formul,* **3,** 94–104 (2009)] [Lab. of Pharm. Engineering]

Oral protein delivery: a patent review of academic and industrial approaches

Martin WERLE, and Hirofumi TAKEUCHI*

Protein therapeutics are used in the treatment of a broad variety of diseases, however, usually they are not available as peroral formulations. Oral delivery systems of proteins including insulin, glucagon like peptide, calcitonin or parathyroid hormone are highly demanded by patients suffering from chronic diseases such as diabetes or osteoporosis. The need for oral protein formulations has been recognized by researchers of various scientific disciplines and a number of patents have been filed that deal with technologies capable of facilitating oral protein delivery. Within the current review, patents based on approaches such as particulate delivery systems, multifunctional polymers, enzyme inhibitors, permeation enhancers and ligand-specific binding and uptake are discussed. In addition, the technology platforms of several innovative drug delivery companies are highlighted.

[*Int. J. Pharm.,* **376**, 169–175 (2009)] [Lab. of Pharm. Engineering]

Nanoparticle Formation from Probucol/PVP/Sodium Alkyl Sulfate Co-Ground Mixture.

Chalermphon WANAWONGTHAI, Adchara PONGPEERAPAT, Kenjirou HIGASHI, Yuichi TOZUKA*, Kunikazu MORIBE, and Keiji YAMAMOTO

Nanoparticles of a poorly water-soluble drug, probucol, have been obtained by co-grinding with PVP and SDS. The purpose of this studywas to investigate the effect of the alkyl chain length of sodium alkyl sulfates on probucol nanoparticle formation. The alkyl chain length of the sodium alkyl sulfate affected the probucol nanoparticle formation. The efficiency, based on the quantitative determination of nanoparticles,was in the order: C18S > C16S > C12S > C8S > C6S. ^{13}C solid-state NMR of co-ground mixtures showed a new peak originating from the probucol interaction with PVP together with the existence of probucol crystal peaks. Excess amounts of surfactantswere expected to play an important role for stabilizing the probucol nanoparticles in the suspension via the electrostatic repulsive effect.

[*Int. J. Pharm.*, **378**, 17–22 (2009)] [Lab. of Pharm. Engineering]

Molecular States of Prednisolone Dispersed in Folded Sheets Mesoporous Materials.

Akinori NISHIWAKI, Aya WATANABE, Kenjirou HIGASHI, Yuichi TOZUKA*, Kunikazu MORIBE, and Keiji YAMAMOTO

Molecular interaction modes between prednisolone and mesoporous materials by the technique of solid-state NMR have been investigated. Folded sheets mesoporous materials (FSM-16) was used as host material and prednisolone was used as guest molecule. The suspension of FSM-16 in prednisolone dichloromethane solution was evaporated to prepare the evaporated samples. ^{13}C-NMR spectroscopy was used as well as powder X-ray diffractometry and differential scanning calorimetry. NMR peak shifts and the broading could be attributed to the molecular interaction between A-ring of prednisolone and FSM-16. The results indicated that the thermal stability of the dispersion made from FSM-16 of large pore size was superior to that of small pore size. A double bond at the C-1 and C-2 positions of prednisolone might play an important role in the process of adsorption of prednisolone to FSM-16.

[*Int. J. Pharm.*, **380**, 201–205 (2009)] [Lab. of Pharm. Engineering]

Preparation of Prednisolone Multicomponent Nanoparticles using Aerosol Solvent Extraction System.

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

Prednisolone nanoparticles were prepared in the presence of a hydrophilic polymer and a surfactant by the aerosol solvent extraction system (ASES). A ternary mixture of prednisolone, polyethylene glycol (PEG), and sodium dodecyl sulfate (SDS) dissolved in methanol was sprayed through a nozzle into the reaction vessel filled with supercritical carbon dioxide. When a methanolic solution of prednisolone/PEG 4000/SDS at a weight ratio of 1:6:2 was sprayed under the optimized ASES conditions, the mean particle size of prednisolone obtained after dispersing the precipitates in water was observed to be ca. 230 nm. Prednisolone nanoparticles were not obtained by the binary ASES process for prednisolone, in the presence of either PEG or SDS. The ASES process that was applied to the ternary system appeared to be one of the most promising methods for the preparation of drug nanoparticles using the multicomponent system.

[*J. Drug Deliv. Sci. Tech.*, **19**, 401–404 (2009)] [Lab. of Pharm. Engineering]

Adsorption State of Naphthoic Acids onto Different Pore Sizes of Folded Sheets Mesoporous Materials (FSM).

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

Adsorption properties of 1- and 2-naphthoic acids (1- and 2-NPAs), model compounds of medicines that have naphthalene moiety such as naproxen, naphazoline, rifampicin etc., in the presence of folded sheets mesoporous materials (FSM-16) were investigated by solid-state fluorescence emission spectroscopy. The molecular states of NPAs adsorbed onto FSM-16 mesopores changed depending on the NPA concentration on the FSM-16 surface. The redshift of the fluorescence emission spectra was attributed to intermolecular overlapping of the naphthalene moieties of the NPA molecules. The amount of 2-NPA adsorbed onto FSM-16(Oc) was significantly higher than that of 1-NPA; this result shows that it is possible to selectively separate 1-NPA and 2-NPA by using FSM-16(Oc) as a mesoporous molecular sieve.

[*Int. J. Pharm*., **382**, 198–204 (2009)] [Lab. of Pharm. Engineering]

Improved cellular uptake of chitosan-modified PLGA nanospheres by A549 cells

Kohei TAHARA, Tsuyoshi SAKAI, Hiromitsu YAMAMOTO, Hirofumi TAKEUCHI*, and Yoshiaki KAWASHIMA

The purpose of this paper was to establish the surface modified poly(D,L-lactide-co-grycolide)(PLGA) nanosphere platform with chitosan(CS) for gene delivery by using the emulsion solvent diffusion (ESD) method. The advantages of this method are a simple process under mild conditions without sonication. This method requires essentially dissolving both polymer and drug in the organic solvent. Nucleic acid can easily form an ion-complex with cationic compound, which can be dissolved in the organic solvent. Thereafter, nucleic acid solubility for organic solution can increase by complexation with cationic compound. We used DOTAP as a cationic compound to increase the loading efficiency of nucleic acid. By coating the PLGA nanospheres with CS, the loading efficiency of nucleic acid in the modified nanospheres increased significantly.

[*Biol.Pharm.Bull*., **32**, 1266–1271, (2009)] [Lab. of Pharm. Engineering]

Mucoadhesive properties of chitosan-coated ophthalmic lipid emulsion containing indomethacinin tear fluid

Masazumi YAMAGUCHI, Kayoko UEDA, Akiharu ISOWAKI, Akira OHTORI, Hirofumi TAKEUCHI*, Nobuyuki OHGURO, and Kakuji TOJO

To evaluate the residence of chitosan-coated emulsion (CE) containing indomethacin in tear, the drug retention of CE in tear fluid was compared with the non-coated emulsion (NE) after instillation in rabbit eyes. EC showed the average concentration 3.6-fold and 3.8 fold higher than that of NE at 0.5 h and 0.75 h after instillation, respectively. Mean residence time and half-life of CE were prolonged to 1.5-flod and 1.8fold compared to NE, respectively. Volume of distribution of CE was also 1.6-fold greater than that of NE. These findings indicated that retention of the drug in tear was appreciably prolonged by chitosan-coated emulsion, and CE had higher distribution onto the ocular surface than NE. The drug levels in cornea, conjunctiva and aqueous humor at 1 hour after instillation were clearly higher than that of NE.

[*J. Photopolym. Sci. Technol.,* **22**, 477–480 (2009)] [Lab. of Pharm. Physical Chemistry]

Plasma-Assisted Fabrication of Self-Assembled Phospholipid Layer onto Polymer Surface and Its Characterization.

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

The self-assembled phospholipid layer was fabricated onto low density polyethylene film immobilizing hexamethylene diamine by the immersion into phospholipid suspension. The self-assembled phospholipid layer was thermally stable. Fatty acid (stearic acid) could be incorporated into the self-assembled phospholipid layer, and albumin was immolilized onto this film. The amount of immobilized albumin depended on the density of searic acid in the phospholipid layer. It was also shown that the self-assembled phospholipid layer possessed fluidity.

[*J. Photopolym. Sci. Technol.,* **22**, 503–506 (2009)] [Lab. of Pharm. Physical Chemistry]

Immobilization of Bioactive Molecule onto Polymer Surface Functionalized by Plasma Techniques and its Application to Cell Culture.

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

Polystyrene (PS) petri-dish immobilizing vinyl methyl ether-maleic acid (VEMAC), prepared by our method reported previously, was used as a substrate to immobilize covalently a cell adhesive peptide, Glycine-Arginine-Glycine-Aspartic acid-Serine (GRGDS) to control cell adhesion. The adhesion and proliferation of mouse embryonic fibroblast cell (NIH3T3) was significantly prompted on the GRGDS peptide-immobilized PS surface, indicating that the surface thus prepared acts as an artificial extra cellular matrix.

[*Curr. Drug. Discov. Technol.*, **6**, 135–150 (2009)] [Lab. of Pharm. Physical Chemistry]

Novel Application of Plasma Treatment for Pharmaceutical and Biomedical Engineering.

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

The nature of plasma-induced surface radicals formed on a variety of organic polymers has been studied by electron spin resonance (ESR), making it possible to provide a sound basis for future experimental design of polymer surface processing using plasma treatment. On the basis of the findings from such studies, several novel bio-applications in the field of drug-and biomedical-engineering have been developed. Applications for drug engineering include the preparation of reservoir-type drug delivery system (DDS) of sustaind-and delayed-release, and floating drug delivery system (FDDS) possessing gastric retention capabilities, followed by preparation of "Patient-Tailored DDS". In applications for biomedical engineering, the novel method to introduce the durable surface hydrophilicity and lubricity on hydrophobic biomedical polymers was developed by plasma-assisted method.

[*J Toxicol Sci.,* **34**(5): 449−458 (2009)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Protective Role of Metallothionein in Benzo[a]pyrene-induced DNA Damage.

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

Metallothionein (MT) is known to reduce chemical carcinogenesis. Carcinogenesis induced by benzo[a]pyrene (B[a]P) is related to DNA adduct formation and oxidative damage through metabolic activation. Ten-week-old male MT-I/II null mice and wild-type mice were given a single injection of B[a]P, and B[a]P-induced DNA damage was evaluated. The frequencies of micronucleated reticulocytes in MT-I/II null mice were significantly increased compared with that of wild-type mice. Comet scores were significantly increased in MT-I/II null mice but not in wild-type mice. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) was significantly increased in liver of MT-I/II null mice after B[a]P administration, although that of wild-type mice was only slightly changed. These results demonstrate that MT acts as an endogenous defensive factor against B[a]P-induced DNA damage.

[*Biol Pharm Bull.*, **32**, 1209–1214 (2009)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Anti-clastogenic Effect of Magnolol-containing Hange-koboku-to, Dai-joki-to, Goshaku-san, and Magnoliae Cortex on Benzo(a)pyrene-induced Clastogenicity in Mice.

Junichiro SAITO, Hiroko FUKUSHIMA, and Hisamitsu NAGASE*

In this study, we investigate the effects of the magnolol-containing *kampo* (traditional) medicines *Hange-koboku-to*, *Dai-joki-to*, and *Goshaku-san*, as well as *Magnoliae Cortex*, on the clastogenesis induced by benzo(*a*)pyrene (B(*a*)P) using the mouse micronucleus test. The mice were first treated with a single intraperitoneal injection of B(*a*)P, followed by a single oral dose of *Hange-koboku-to*, *Dai-joki-to*, *Goshaku-san*, or *Magnoliae Cortex*. The anti-clastogenic mechanisms employed by the *kampo* medicines and *Magnoliae Cortex* were also investigated by evaluating *in vivo* CYP1A1 activity using the zoxazolamine paralysis test. Results show that *Hange-koboku-to*, *Dai-joki-to*, and *Magnoliae Cortex*, which contain high levels of magnolol, significantly inhibited the clastogenesis and sufficiently inhibited *in vivo* CYP1A1 activity. These findings suggest that magnolol is a major contributor to the inhibition of B(*a*)P-induced clastogenesis, and that *kampo* medicines exert significant anti-clastogenic effects.

[*J. Health Sci.*, **55**(3), 396–404 (2009)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

17*β*-Estradiol Enhances Interleukin-18 mRNA Expression after Sensitization of Mice with Contact Hypersensitivity.

Fumitoshi SAKAZAKI, Masahiro FUJIYAMA, Hitoshi UENO, Hisamitsu NAGASE*, and Katsuhiko NAKAMURO

To clarify the mechanism underlying the enhancing effect of 17*β*-estradiol (E2) on contact hypersensitivity (CHS) and the expression of interferon (IFN)-*γ* in mice, the mRNA expression levels of interleukin (IL)-18 were evaluated. Female BALB/c mice aged 3 weeks were ovariectomized, administered E2, and sensitized by 3% 4-ethoxymethylene-2-phenyl-2-oxazolin-one (OXA). Seven days later, CHS was elicited by the application of 1% OXA on the ear auricles. The auricles, cervical lymph nodes and spleens were excised, and gene expression was evaluated by reverse transcription-polymerase chain reaction. E2 enhanced the expression of IL-18 mRNA in the spleen on the following day and in the ear auricles on days 4 and 7 after sensitization with OXA. E2 also enhanced the expression of IFN-*γ* and IL-18 mRNAs in splenocytes cultured with lipopolysaccharide (LPS). These results suggest that E2 enhances lymphocyte activation in the sensitization phase of CHS, and that IFN-*γ* mRNA expression is enhanced in the elicitation phase of CHS.

[*J. Health Sci.*, **55**, 560–566 (2009)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

p-Hydroxybenzoate Esters Enhance Mouse Contact Hypersensitivity.

Fumitoshi SAKAZAKI, Hitoshi UENO, Hisamitsu NAGASE*, and Katsuhiko NAKAMURO

In this paper, we evaluate the effects of *p*-hydroxybenzoate esters (parabens) on contact hypersensitivity. Female BALB/c mice were administered 1200 mg/kg of butylparaben and sensitized by painting 3% 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (OXA) on their backs. Seven days later, the mice were challenged by painting 1% OXA on the ear and the ear thickness was measured. Ear auricles were excised and the RNA expressions of interleukin (IL)-18 and interferon (IFN)-γ were evaluated by reverse transcription-polymerase chain reaction (RT-PCR). Butylparaben enhanced ear swelling at 6 hr after the elicitation of allergy. Butylparaben also enhanced the RNA expression of IL-18 before the challenge with OXA and the RNA expression of IFN-γ at 6 hr after the challenge. These results suggest that parabens could enhance IL-18 and IFN-γ expression and exacerbate mouse contact hypersensitivity to OXA.

[*Chem-Bio. Interact.,* **180**, 238–244 (2009)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Structure-Dependent Activation of Peroxisome Proliferator-Activated Receptor (PPAR) γ by Organotin Compounds.

Youhei HIROMORI, Jun-ichi NISHIKAWA, Ichiro YOSHIDA, Hisamitsu NAGASE, and Tsuyoshi NAKANISHI*

Previously, we reported that tributyltin (TBT) and triphenyltin (TPT) function as powerful agonists for peroxisome proliferator-activated receptor (PPAR) γ. Our current study investigates the structure-dependent binding of butyltin and phenyltin compounds to PPARγ and their ability to activate the receptor. Our observations indicate that trialkylated and triphenylated tin compounds are the most potent PPARγ agonists among the alkylated and phenylated tin compounds, and a phenyl substituent on a tin atom enhances the potency of organotin compounds as a PPARγ agonist much more than a butyl substituent.

[*Gen. Comp. Endocrinol.,* **163**, 285–291 (2009)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Placental Steroidogenesis in Rats is Independent of Signaling Pathways Induced by Retinoic Acids.

Kenji ITOH, Youhei HIROMORI, Naoko KATO, Ichiro YOSHIDA, Norio ITOH, Michihiko IKE, Hisamitsu NAGASE, Keiichi TANAKA, and Tsuyoshi NAKANISHI*

We investigated the effects of retinoic acids (RAs) on steroid hormone production and mRNA expression of steroidogenic enzymes in rat placenta *in vitro* and *in vivo*. In the rat trophoblast giant cell line Rcho-1, the retinoid X receptor (RXR) agonist 9-*cis* retinoic acid (9cRA) slightly promoted production of progesterone and androgen, whereas the natural retinoic acid receptor (RAR) agonist all-*trans* retinoic acid (atRA) did not. Furthermore, although administration of the RAs into the rat uterus at 13.5 days postcoitum robustly induced mRNA expression of cellular retinol binding protein II, the gene for which is targeted by RAR and/or RXR, in the placenta, neither RA affected the expression of placental steroidogenic enzymes, and both had little effect on progesterone and androgen levels in the placenta and embryo, suggesting that rat placental steroidogenesis is not regulated by RAs.

[*Environ. Sci. Technol.,* **43**, 6611–6616 (2009)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Identification of Retinoic Acid Receptor Agonists in Sewage Treatment Plants.

Huajun ZHEN, Xiaoqin WU, Jianying HU, Yang XIAO, Min YANG, Junji HIROTSUJI, Jun-ichi NISHIKAWA, Tsuyoshi NAKANISHI*, and Michihiko IKE

We identified the specific RAR agonists in sewage treatment plants (STPs) and receiving rivers using an RAR yeast two-hybrid bioassay. Water samples were extracted by solid-phase extract cartridges, which were successively eluted by hexane, ethyl acetate, and methanol for bioassay. Among the three fractions, the ethyl acetate fraction showed the highest RAR agonistic activities. Following a two-step fractionation using high-performance liquid chromatography and ultra-performance liquid chromatography (UPLC) directed by the bioassay, two bioactive fractions were obtained from Gaobeidian STP influent and all-*trans*-4-oxo-RA (4.7-10.4 ng/L in influents, < 0.2-0.9 ng/L in effluents) and 13-*cis*-4-oxo-RA (2.3-7.1 ng/L in influents, < 0.4-1.1 ng/L in effluents) were identified in these fractions with UPLC-MS/MS.

[*Biochem. Biophys. Res. Commun.,* **378**, 308–312 (2009)] [Lab. of Molecular Biology]

Pyrroloquinoline quinone attenuates iNOS gene expression in the injured spinal cord.

Akihiro HIRAKAWA, Katsuji SHIMIZU, Hidefumi FUKUMITSU, and Shoei FURUKAWA*

Pyrroloquinoline quinone (PQQ) is a naturally occurring redox cofactor that acts as an antioxidant, and redox modulator. PQQ has been demonstrated to oxidize the redox modulatory site of N-methyl-d-aspartic acid (NMDA) receptor and protect the neuronal cell death in experimental stroke models. Therefore, we examined the possible ameliorating effect of PQQ on spinal cord injury (SCI) in adult rats. Intraperitoneal administration of PQQ effectively promoted the functional recovery of SCI rats after hemi-transection, which was preceded by the attenuation of the expression of inducible nitric oxide (NO) synthase (iNOS) mRNA in the injury site. NO is involved in the secondary detrimental mechanisms and has been implicated in NMDA receptor-mediated neurotoxicity. In fact, administration of PQQ induced significantly decreased lesion size and increased axon density adjoining the lesion area, suggesting that PQQ protects against the secondary damage by reducing iNOS expression following primary physical injury to the spinal cord.

[*J. Neurosci. Res.,* **87**, 301–306 (2009)] [Lab. of Molecular Biology]

Neurotrophin-3 stimulates neurogenic proliferation via ERK signaling pathway.

Masanari OHTSUKA, Hidefumi FUKUMITSU, and Shoei FURUKAWA*

The effects of neurotrophin-3 (NT3) administered into the ventricular space of 13.5-day-old mouse embryos on neurogenesis in the developing cerebral cortex were examined. 5-Bromo-2'-deoxyuridine (BrdU) was injected into pregnant mice 3 hr after the NT3 administration to label the neural progenitor cells. NT3 increased the number of BrdU-positive cells without altering their distribution. The increment in BrdU-positive cells 24 hr after the BrdU injection was attributed to transient increse in the number of Pax6-/BrdU-positive cells (neural stem cells), which was followed by a significant elevation of the number of Tuj1-/BrdU-positive cells (neurons) 36 hr afterwards, suggesting that NT3 facilitated neurogenesis by acting in two sequential steps, i.e., causing proliferation of neural stem cells and generation of neurons from these progenitors, which were cancelled by an MEK inhibitor.

[*Evid. Based Complement Alternat. Med.,* Apr 17 (2009)] [Lab. of Molecular Biology]

Royal Jelly Facilitates Restoration of the Cognitive Ability in Trimethyltin-Intoxicated Mice.

Noriko HATTORI, Shozo OHTA, Tsutomu SAKAMOTO, Satoshi MISHIMA, and Shoei FURUKAWA*

Trimethyltin (TMT) is a toxic organotin compound that induces acute neuronal death selectively in the hippocampal dentate gyrus (DG) followed by cognition impairment; however the TMT-injured hippocampal DG itself is reported to regenerate the neuronal cell layer through rapid enhancement of neurogenesis from neural stem/progenitor cells. Therefore, we investigated whether royal jelly (RJ) stimulates the regenerating processes of the TMT-injured hippocampal DG, and found that orally administered RJ significantly increased the number of DG granule cells and simultaneously improved the cognitive impairment. These present resultssuggest that the orally administered RJ may be a promising avenue for ameliorating neuronal function by regenerating hippocampal granule cells that function in the cognition process.

[*Biomed. Res.,* **30** 121–128 (2009)] [Lab. of Molecular Biology]

Stem cell factor induces heterotopic accumulation of cells (heterotopia) in the mouse cerebral cortex.

Hitomi SOUMIYA, Hidefumi FUKUMITSU, and Shoei FURUKAWA*

The roles of the stem cell factor (SCF)-c-kit signals during the development of the cerebral cortex are pooly understood. We investigated the effects of SCF by directly administering it into the telencephalic ventricular space of 13.5-day-old mouse embryos. SCF produced the heterotopic accumulation of cortical cells in several distinct area of the cerebral cortex at the postnatal stage, including the subcortical periventricular area, marginal zone, and lateral ventricular space. Additional analysis revealed that the heterotopia included both neurons and astrocytes and that SCF initially increased the number of neural stem cells without affecting that of intermediate progenitors and also disturbed their organization. These results suggest that SCF alters the timing of the genesis and migration of neural stem/progenitor cells, which may lead to formation of the observed heterotopia.

[*Redox Rep.*, **14**, 34–40 (2009)] [Lab. of Clinical Pharmaceutics]

Expression of Extracellular-Superoxide Dismutase during Adipose Differentiation in 3T3-L1 Cells.

Tetsuo ADACHI*, Taisuke TOISHI, Haoshu WU, Tetsuro KAMIYA and Hirokazu HARA

In this report, the expression of extracellular-superoxide dismutase (EC-SOD) was compared to other adipocytokines in mice 3T3-L1 preadipocytes. EC-SOD expression levels were increased after the induction of differentiation and then declined, which was similar to adiponectin and transcription factors such as peroxisome proliferator-activated receptor-γ (PPARɣ) and CCAAT/enhancer-binding protein-α (C/EBPα). On the other hand, the expression levels of pro-inflammatory adipocytokines, such as tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1), increased markedly in the development stage of cells. It was observed that the expression of EC-SOD in differentiated 3T3-L1 cells co-cultured with LPS-stimulated J774 macrophages was up-regulated, while the addition of TNF-α down-regulated EC-SOD and adiponectin expression in adipocytes. It is possible that the expression of EC-SOD in adipocytes was stimulated to protect them from oxidative stress in the co-culture system.

[*Neurochem. Res.,* **34**, 1498–1506 (2009)] [Lab. of Clinical Pharmaceutics]

Zinc Induces Expression of the BH3-only Protein PUMA through p53 and ERK Pathways in SH-SY5Y Neuroblastoma Cells.

Hirokazu HARA*, Tetsuro KAMIYA and Tetsuo ADACHI

PUMA is known to promote apoptosis through a tumor suppressor p53-dependent and -independent mechanism. In this study, we examined the effect of Zn^{2+} on the induction of the PUMA gene in human neuroblastoma SH-SY5Y cells. The expression of PUMA was induced by Zn^{2+} in a dose- and time-dependent manner. A reporter assay revealed that Zn^{2+} activated the PUMA promoter. In addition, the mutation of the p53 binding site in the PUMA promoter region reduced promoter activation by Zn^{2+}. These findings suggest that p53 participates in Zn^{2+}-induced PUMA expression. Furthermore, we also demonstrated here that Zn^{2+} stimulates the phosphorylation of ERK and that the MEK-ERK pathway inhibitor, U0126, suppressed Zn^{2+}-induced PUMA expression. Taken together, these results indicate that Zn^{2+} regulates the induction of PUMA through p53 and ERK pathways.

[*Med. Biol.,* **153**, 611–618 (2009)] [Lab. of Clinical Pharmaceutics]

Improvement of the Assessment of Serum Oxidative Stress Index in Health Screening Examinees: A Test for Detecting the Wait State of Metabolic Syndrome Using GAP Ratio.

Eisuke MAEHATA, Yasuhiro TOYOKURA, Yoshinori TSURUSAKI, Ikukatsu SUZUKI, Matsuo TANIYAMA, Takahiro IMAZATO, Noriko ISHIDA, Teruo SHIBA, Masao YANO, Naoko IKOSHI, Akira TANAKA, Hiroji SHIMOMURA, Naoya KISHIKAWA, Naotaka KURODA, Tetsuo ADACHI*, Chieko KUDO, Kae SAKAI and Naoko TAKAHASHI

We have previously assessed oxidative stress in health screening examinees using FRAS4, but it was impossible to determine the antioxidant gap from the difference between these two potential levels. Instead, we examined the antioxidation (BAP) / oxidation (d-ROMS) ration (GAP ratio), and found 9 cases in the region of GAP < 6.0. The above results supported the possibility of a test for detecting the pre-stage of metabolic syndrome using GAP ratio and the clinical importance of such a test.

[*Ningen Dock*, **23**, 7–13 (2009)] [Lab. of Clinical Pharmaceutics]

Pathologic Background of Abnormal Serum Amyloid A and Interleukin-6 Levels Revealed by a Piecewise Linear Regression Model in the Population of Diabetic Patients.

Yojiro MAEHATA, Masaichi-Chang-il LEE, Eisuke MAEHATA, Minoru INOUE, Fukashi ISHIBASHI, Chieko KUDO, Minoru YAMAKADO, Teruo SHIBA, Hiroji SHIMOMURA, Tetsuo ADACHI*, Yoshinori TSURUSAKI, Ikukatsu SUZUKI, Kiyoshi HIROSAWA, Takahiro IMAZATO, Noriko ISHIDA, Naoya KISHIKAWA, Naotaka KURODA, Naoko IKOSHI, Yutaka MIDORIKAWA and Tatsuya ASHIKAWA

Significant associations were found between serum amyloid A (SAA) and interleukin-6 (IL-6) and between ferritin and lipoprotein lipase (LPL) mass. We attempted to detect pathologic tendencies related to SAA in the abnormal range using the piecewise linear regression method, and confirmed the close relationship between SAA and IL-6 related to the mechanism for cytokine induction.

[*J. Neurochem.*, **110**, 106–117 (2009)] [Lab. of Clinical Pharmaceutics]

Protein Kinase C Regulation of Neuronal Zinc Signaling Mediates Survival during Preconditioning.

Mandar A. ARAS, Hirokazu HARA*, Karen A. HARTNETT, Karl KANDLER, and Elias AIZENMAN

As intracellular Zn^{2+} liberation mediates neuronal death pathways, we tested whether a sub-lethal increase in free Zn^{2+} could also trigger neuroprotection. Neuronal free Zn^{2+} transiently increased following preconditioning, and was both necessary and sufficient for conferring excitotoxic tolerance. Lethal exposure to NMDA led to a delayed increase in Zn^{2+} that contributed significantly to excitotoxicity in non-preconditioned neurons, but not in tolerant neurons, unless preconditioning-induced free Zn^{2+} was chelated. Thus, preconditioning may trigger the expression of Zn^{2+}-regulating processes, which, in turn, prevent subsequent Zn^{2+}-mediated toxicity. Indeed, preconditioning increased Zn^{2+}-regulated gene expression in neurons. Examination of the molecular signaling mechanism leading to this early Zn^{2+} signal revealed a critical role for protein kinase C (PKC) activity, suggesting that PKC may act directly on the intracellular source of Zn^{2+}.

[*Jpn. J. Pharm. Health Care Sci.*, **35**, 195–201 (2009)] [Labs. of Clinical Pharmaceutics]

Conduct of a PBL Tutorial on "Appropriate Use of Medicines" for First-grade Students and Analysis of Product Presentation Evaluations.

Tetsuo ADACHI*, Hitomi TERAMACHI, Hirokazu HARA, Shigeyuki USUI, Tetsuro KAMIYA, Yumi KUZUYA, Teruo TSUCHIYA, Kazuyuki HIRANO, and Hiroichi NAGAI

Gifu Pharmaceutical University conducted a problem-based learning (PBL) tutorial on appropriate use of medicines as part of an introductory course in pharmacy for first-grade students in the first semester. This tutorial made use of scenarios with a simple background based on situations likely to be encountered in daily clinical practice. The presentation of their products developed by each group was evaluated by students in the presenter's group as well as the audience (instructors and students in other groups), and their evaluations were subjected to customer satisfaction (CS) analysis. The PBL tutorial in the introductory course to pharmacy appeared to be beneficial for students and the use of the new approach of PBL rather than a more conventional learning method had been effective.

[*YAKUGAKU ZASSHI*, **129**, 177–182 (2009)] [Lab. of Clinical Pharmaceutics]

An Effort to Improve Advanced Problem-Based Learning Tutorial

Tetsuo ADACHI*, Masumi SUZUI, Kuniko NAOI, Tetsuro KAMIYA, and Hirokazu HARA

To prepare for the introduction of the advanced problem-based learning (PBL) tutorial for higher-grade students under the six-year pharmacy curriculum, a trial of the tutorial was performed in a fourth-grade class under the former four-year curriculum in 2007. A questionnaire survey conducted to identify any problems in performing the tutorial revealed: 1) the number of students in each group was too large; 2) the contents of presentations seemed to overlap due to the limited number of task cases, which forced more than one group to address a particular case; and 3) the time-line from the day of product presentation to that of periodic examination was too short to hold a sufficient group discussion. In 2008, to resolve these problems: 1) the number of groups was increased to reduce the number of students in each group; 2) new task cases were added to decrease the number of groups addressing a particular case; and 3) an adequate time period was arranged between the days of product presentation and periodic examination.

[*Biol. Pharm. Bull.*, **32**, 1101–1104 (2009)] [Lab. of Pharmaceutics]

Overexpression of Thymosin b4 Increases Pseudopodia Formation in LNCaP Prostate Cancer Cells.

Mai ITO, Kazuhiro IGUCHI, Shigeyuki USUI, and Kazuyuki HIRANO*

Thymosin β4, a major G-actin-sequestering protein, is known to be involved in tumor metastasis. In the present study, we found that thymosin β4 expression promotes the formation of actin-based pseudopodia-like extensions, associated with cell migration, in human prostate cancer LNCaP cells. Treatment with the phosphatidylinositol 3-kinase (PI3K) inhibitor wortmannin and Cdc42/Rac1/RhoA inhibitor Clostridium difficile toxin B significantly reduced pseudopodia formation in thymosin β4-overexpressing LNCaP cells, suggesting that the pseudopodia formation by thymosin β4 is probably involved in PI3K and Rho family pathway. We recently reported that thymosin β4 expression is upregulated by androgen deprivation in prostate cancer cells. The increase in thymosin β4 may be one of the causes of prostate cancer progression after androgen ablation therapy.

[*Biol. Pharm. Bull.*, **32**, 1160–1165 (2009)] [Lab. of Pharmaceutics]

Regulation of Glyceraldehyde 3-Phosphate Dehydrogenase Expression by Metformin in HepG2 Cells.

Yuichi YOKOYAMA, Masafumi KUBOTA, Kazuhiro IGUCHI, Shigeyuki USUI, Tadashi KIHO, and Kazuyuki HIRANO*

We examined the signaling pathway and regulatory factors for the expression of the GAPD gene triggered by metformin in HepG2 cells. The mRNA and protein expression of GAPD decreased upon treatment of the cells with metformin. Metformin induced phosphorylation of AMP-activated protein kinase (AMPK). The expression of GAPD mRNA decreased on treatment with an activator for AMPK, 5-amino-imidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR). Inhibitors for signal transducers, Compound C, H-89, and MDL-12,330A, restored the level of GAPD mRNA. A mutant reporter plasmid with an altered cAMP-response element (CRE) counteracted the metformin-mediated repression of GAPD transcription. These results suggest that signal transducers, adenylate cyclase (AC), protein kinase A (PKA), and AMPK, are involved in the signaling pathway triggered by metformin and CRE-binding protein is one of the transcription factors for the GAPD gene down-regulated by metformin.

[*Toxicology,* **255**, 124–130 (2009)] [Lab. of Pharmaceutics]

Involvement of Interleukin 18 in Indomethacin-induced Lesions of the Gastric Mucosa in Adjuvant-induced Arthritis Rat.

Noriaki NAGAI, Takashi FUKUHATA, Yoshimasa ITO, Shigeyuki USUI, and Kazuyuki HIRANO*

We demonstrate whether interleukin 18 (IL-18) expression relate the aggravation of gastric lesion in adjuvant-induced arthritis (AA) rats following the oral administration of indomethacin. Arthritis was induced by injecting 50 μl of a suspension of 10mg/ml heat-killed butyricum into the plantar region of the right hind foot and tail of Dark Agouti rats. Two weeks after injection, the rats were administered indomethacin (40mg/kg) orally, and were killed under deep ether anesthesia 6h later. Oral administration of indomethacin caused hemorrhagic lesions in the gastric mucosa of AA rats. The expression of the IL-18 mRNA and mature IL-18 protein in the gastric mucosa of the rats administered indomethacin were also higher in comparison with normal rats receiving indomethacin. In addition, IFNγ and NO levels in the gastric mucosa of the rats were increased by the oral administration of indomethacin. It is possible that IL-18 expression in the rats is more sensitive to indomethacin, and the IL-18 may play a role in the aggravation of gastric lesions in AA rats treated with indomethacin.

[*Biol. Pharm. Bull*., **32**, 116–120 (2009)] [Lab. of Pharmaceutics]

Preventive Effect of Co-administration of Water Containing Magnesium Ion on Indomethacin Induced Lesions of Gastric Mucosa in Adjuvant-Induced Arthritis Rat.

Noriaki NAGAI, Takashi FUKUHATA, Yoshimasa ITO, Shigeyuki USUI, and Kazuyuki HIRANO*

We demonstrate the preventive effect of the co-administration of bittern water (BW, nigari-sui in Japanese), which enables the effective intake of Mg^{2+}, on the ulcerogenic response to indomethacin in adjuvant-induced arthritis (AA) rats. Oral administration of indomethacin (40 mg/kg) caused hemorrhagic lesions in the gastric mucosa of AA rats at 14 d after adjuvant injection. The expression of the mRNA for iNOS mRNA expression and the production of NO in the gastric mucosa of the rats were also increased by the administration of indomethacin. The co-administration of BWs decreased the ulcerogenic response to indomethacin in the rats. In addition, the administration of BW attenuated the increase in iNOS mRNA expression and NO production in AA rats receiving indomethacin. The oral administration of Mg2+ to AA rats had a potent preventive effect on the ulcerogenic response to indomethacin in AA rats, probably due to an inhibition in the rise in iNOS and NO levels in the gastric mucosa.

[*Atarashii Ganka (J. Eye),* **26**, 709–713 (2009)] [Lab. of Pharmaceutics]

Effect of Enhanced Nitric Oxide Production on Plasma Membrane Ca^{2+}-ATPase Expression in Human Lens Epithelial Cell Line SRQ 01/04 Treated with Combination of Interferon-γ and Lopopolysaccharide.

Noriaki NAGAI, Yoshimasa ITO, Shigeyuki USUI, and Kazuyuki HIRANO*

We investigated the changes in plasma membrane Ca^{2+}-ATPase (PMCA) mRNA expression in human lens epithelial cell line SRA 01/04 (HLE cell) following treatment with INF-γ and LPS, which induce iNOS expression. mRNA levels of PMCA1 and 4, which were expressed in the HLE cells, were increased with duration of incubation with INF-γ and LPS. Aminoguanidine, a selective inhibitor for iNOS, attenuated the increase in expression of PMCA1 and 4 mRNA. A close relationship was observed between PMCA1 and 4 mRNA expression and NO production. In conclusion, the present study demonstrated that excessive production of NO by iNOS may cause increased PMCA1 and 4 mRNA expression in the cels.

[*Oncol. Rep.,* **22**, 349–354 (2009)] [Lab. of Med. Ther. & Mol. Ther.]

Growth inhibitory activity of ethanol extracts of Chinese and Brazilian propolis in four human colon carcinoma cell lines.

Masashi ISHIHARA, Kuniko NAOI, Masanari HASHITA, and Masumi SUZUI*

The objective of this study was to examine whether the ethanol extracts of Chinese and Brazilian propolis may exert anticancer activities in four human colon carcinoma cell lines. The findings indicate that the ethanol extracts of propolis contain components that may have anticancer activity. Thus, propolis and related products may provide a novel approach to the chemoprevention and treatment of human colon carcinoma.

[*Mol. Med. Rep.,* **2**, 45–49 (2009)] [Lab. of Med. Ther. & Mol. Ther.]

Inhibitory effect of rice bran-derived crude glycosphingolipid on colon preneoplastic biomarker lesions induced by azoxymethane in male F344 rats.

Nao SUNAGAWA, Morihiko INAMINE, Takamitsu MORIOKA, Itaru CHIBA, Nanae MORITA, Yoichi AOKI, Masumi SUZUI*, and Naoki YOSHIMI

The aim of the present study was to examine whether crude glycosphingolipid (cGSL) has short-term chemopreventive effects on the preneoplastic biomarker lesions involved in carcinogen-induced rat colon carcinogenesis. The results suggest that dietary cGSL had a potent chemopreventive effect in the present short-term colon carcinogenesis bioassays, and that this effect may be associated with the inhibition of ACF and MDF and the induction of apoptosis.

[*Support Care Cancer,* published online (2009)] [Lab. of Med. Ther. & Mol. Ther.]

Pharmaceutical interventions facilitate premedication and prevent opioid-induced constipation and emesis in cancer patients.

Masashi ISHIHARA, Hirotoshi IIHARA, Shinji OKAYASU, Koji YASUDA, Katsuhiko MATSUURA, Masumi SUZUI*, and Yoshinori ITOH

BACKGROUND: Opioid analgesics possess a number of side effects, among which constipation and nausea/vomiting occur most frequently. RESULTS: Prophylactic treatment with laxatives and antiemetics were conducted in 57% and 52%, respectively. CONCLUSION: Intervention to promote prophylactic medication was highly effective in reducing the risk of opioid-induced constipation and nausea/vomiting.

[*Bioorg. Med. Chem.*, **17**, 1244–1250 (2009)] [Lab. of Biochemistry]

Correlation of binding constants and molecular modelling of inhibitors in the active sites of aldose reductase and aldehyde reductase.

Vincenzo CARBONE, Hai-Tao ZHAO, Roland CHUNG, Satoshi ENDO, Akira HARA*, and Ossama El-KABBANI

Aldose reductase (ALR2) is the first enzyme involved in the polyol pathway of glucose metabolism and has been linked to the pathologies associated with diabetes. Our studies revealed that the correlation between binding constants and molecular modeling of four inhibitors tolrestat, minalrestat, quercetin and 3,5-dichlorosalicylic acid (DCL) in ALR2 and aldehyde reductase (ALR1). Additionally, X-ray crystallography revealed that Arg312 in ALR1 (missing in ALR2) contributes favourably to the binding of DCL through an electrostatic interaction with the inhibitor's halide atom. In ALR2, Thr113 (Tyr116 in ALR1) forms electrostatic interactions with the fluorobenzyl moiety of minalrestat and the 3- and 4-hydroxy groups on the phenyl ring of quercetin. Our modeling studies suggest that a conformational change of the Tyr116 achieve the selectivity for ALR1 over ALR2 by minalrestat.

[*Acta Crystallogr. Sect. D-Biol. Crystallogr.*, **65**, 257–265 (2009)] [Lab. of Biochemistry]

Structure of the G225P/G226P mutant of mouse 3(17)α-hydroxysteroid dehydrogenase (AKR1C21) ternary complex: implications for the binding of inhibitor and substrate.

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

3(17)α-Hydroxysteroid dehydrogenase (AKR1C21) is a unique member of the aldo-keto reductase (AKR) superfamily owing to its ability to reduce 17-ketosteroids to 17α-hydroxysteroids, as opposed to other AKR members, which can only produce 17β-hydroxysteroids. In this paper, the crystal structure of a G225P/G226P mutant of AKR1C21 in complex with $NADP^+$ and an inhibitor refined at 2.1 Å resolution is presented. Kinetic analysis and molecular-modelling studies of 17α- and 17β-hydroxysteroid substrates in the active site of AKR1C21 suggested that Gly225 and Gly226 play an important role in determining the substrate stereospecificity of the enzyme. Additionally, the G225P/G226P mutation of the enzyme reduced the affinity for both 3α- and 17α-hydroxysteroid substrates by up to 160-fold, indicating that these residues are critical for the binding of substrates.

[*Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.*, **65**, 395–397 (2009)] [Lab. of Biochemistry]

Crystallization and preliminary X-ray analysis of a rat aldose reductase-like protein (AKR1B14).

Roland CHUNG, Satoshi ENDO, Akira HARA*, and Ossama El-KABBANI

Mouse vas deferens protein/aldo-keto reductase 1B7 (AKR1B7) is involved in the detoxification of isocaproaldehyde, a steroidogenesis byproduct, and of 4-hydroxynonenal formed by lipid peroxidation. The rat orthologue of AKR1B7 has recently been named AKR1B14 in the AKR superfamily. Recombinant AKR1B14 was expressed in a bacterial system and purified to homogeneity. The purified protein was crystallized from polyethylene glycol solutions using the hanging-drop vapour-diffusion method and an X-ray diffraction data set was collected to 1.86 Å resolution. The crystals belonged to space group $P2_1$, with unit-cell parameters a = 50.66, b = 69.14, c = 72.27 Å, β= 96.4°. This is the first crystallization report of a rodent AKR1B7 orthologue.

[*J. Biochem.*, **146**, 51–60 (2009)] [Lab. of Biochemistry]

Expression analysis of the aldo-keto reductases involved in the novel biosynthetic pathway of tetrahydrobiopterin in human and mouse tissues.

Haruka HIRAKAWA, Hiroshi SAWADA, Yumi YAMAHAMA, Shin-Ichiro TAKIKAWA, Haruo SHINTAKU, Akira HARA*, Keisuke MASE, Tomoyoshi KONDO and Teruhiko IINO

Tetrahydrobiopterin (BH4) is synthesized by sepiapterin reductase (SPR) from 6-pyruvoyl-tetrahydropterin (PPH4). A patient with SPR deficiency shows no hyperphenylalaninemia (HPA); however, an SPR knockout mouse exhibits HPA. We have reported on the SPR-unrelated novel pathway from PPH4 to BH4 in which 3α-hydroxysteroid dehydrogenase type 2 and aldose reductase work in concert. In this study, we performed the expression analysis of both proteins in humans and mice, which indicated that the pathway worked only in human liver but not human brain or mouse liver and brain. For this reason, a patient with SPR deficiency may show progressive neurological deterioration without HPA, and SPR knockout mice may exhibit HPA and abnormal locomotion activity.

[*Cell. Mol. Life Sci.*, **66**, 1570–1579 (2009)] [Lab. of Biochemistry]

Structure/function analysis of a critical disulfide bond in the active site of human L-xylulose reductase: Crystal structure of the holoenzyme displays both reduced and oxidized forms.

Hai-Tao ZHAO, Satoshi ENDO, Shuhei ISHIKURA, Roland CHUNG, Philip J. HOGG, Akira HARA*, and Ossama El-KABBANI

L-Xylulose reductase (XR) is involved in water re-absorption and cellular osmoregulation. The crystal structure of Cys138Ala human XR mutant indicated that the disulfide bond in the active site between Cys138 and Cys150 is unstable. The formation of the disulfide-bond was reversibily and resulted in three-fold decrease in catalytic efficiency. Furthermore, the addition of cysteine (>2 mM) inactivated human XR and was accompanied by a 10-fold decrease in catalytic efficiency. TOF-MS analysis of the inactivated enzyme showed the S-cysteinylation of Cys138 in the wild-type and Cys150 in the mutant enzymes. Thus, the action of human XR may be regulated by cellular redox conditions through reversible disulfide-bond formation and by S-cysteinylation.

[*J. Med. Chem.*, **52**, 3259–3264 (2009)] [Lab. of Biochemistry]

Structure-Guided Design, Synthesis and Evaluation of Salicylic Acid-Based Inhibitors Targeting a Selectivity Pocket in the Active Site of Human 20α-Hydroxysteroid Dehydrogenase (AKR1C1).

Ossama El-KABBANI, Peter J. SCAMMELLS, Joshua GOSLING, Urmi DHAGAT, Satoshi ENDO, Toshiyuki MATSUNAGA, Midori SODA, and Akira HARA*

The first design, synthesis, and evaluation of AKR1C1 inhibitors based on the recently published crystal structure of its ternary complex with inhibitor are reported. While the interactions observed in the crystal structure remain conserved with the newly designed inhibitors, the additional phenyl group of the most potent compound targets a nonconserved hydrophobic pocket in the active site of AKR1C1 resulting in 21-fold improved potency (Ki = 4 nM) over AKR1C2. The compound is hydrogen bonded to Tyr55, His117 and His222, and the phenyl ring forms additional van der Waals interactions with Leu308, Phe311, and the nonconserved Leu54. Moreover, the compound potently inhibited the metabolism of progesterone in AKR1C1-overexpressed cells.

[*Arch. Biochem. Biophys.*, **481**, 183–190 (2009)] [Lab. of Biochemistry]

Molecular determinants for the stereospecific reduction of 3-ketosteroids and reactivity towards all-trans-retinal of a short-chain dehydrogenase/reductase (DHRS4).

Satoshi ENDO*, Satoshi MAEDA, Toshiyuki MATSUNAGA, Urmi DHAGAT, Ossama El-KABBANI, Nobutada TANAKA, Kazuo T. NAKAMURA, Kazuo TAJIMA, and Akira HARA

DHRS4 reduces all-*trans*-retinal and xenobiotic carbonyl compounds. Human DHRS4 differs from other animal enzymes in kinetic constants for the substrates, particularly in its low reactivity to retinoids. Mutation (F158S/L161F) of substrate-binding residues predicted from the crystal structure of pig DHRS4 led to an effective switch of its substrate affinity and stereochemistry into those similar to human DHRS4. Additional mutation of T177N into the human S158F/F161L mutant resulted in almost complete kinetic conversion into a pig form, suggesting a role of N177 in forming the substrate-binding cavity through an intersubunit interaction in pig and other animal DHRS4s, and explaining why the human enzyme shows low reactivity towards retinoids.

[*Chem. Biol. Interact.*, **176**, 151–157 (2009)] [Lab. of Biochemistry]

Characterization of a rat NADPH-dependent aldo-keto reductase (AKR1B13) induced by oxidative stress.

Satoshi ENDO*, Toshiyuki MATSUNAGA, Hiroaki MAMIYA, Akira HARA, Yukio KITADE, Kazuo TAJIMA, and Ossama El-KABBANI

AKR1B13 was identified as a hepatoma-derived protein, exhibiting high sequence identity with mouse fibroblast growth factor (FGF)-induced reductase, AKR1B8. In this study, we elucidated that AKR1B13 exhibits NADPH-linked reductase activity towards reactive carbonyl compounds such as methylglyoxal, glyoxal, acrolein, 4-hydroxynonenal and 3-deoxyglucosone. Immunochemical and RT-PCR analyses revealed that the enzyme is expressed in many rat tissues, endothelial cells and fibroblasts. Gene expression in YPEN-1 and NRK cells was up-regulated by treatments with hydrogen peroxide and 1,4-naphthoquinone, but not with FGF-1, FGF-2, steroids. These results suggest that it functions as a defense system against oxidative stress in rat tissues.

[*Arch. Biochem. Biophys.*, **487**, 1–9 (2009)] [Lab. of Biochemistry]

Kinetic studies of AKR1B10, human aldose reductase-like protein: Endogenous substrates and inhibition by steroids.

Satoshi ENDO*, Toshiyuki MATSUNAGA, Hiroaki MAMIYA, Chisato OHTA, Midori SODA, Yukio KITADE, Kazuo TAJIMA, Hai-Tao ZHAO, Ossama El-KABBANI, and Akira HARA

AKR1B10 identified as a biomarker of lung cancer exhibits high sequence identity with human aldose reductase (AKR1B1). We compared their substrate specificity and inhibition and found the following unique features of AKR1B10. AKR1B10 efficiently reduced isoprenoid aldehyde such as farnesal and geranylgeranial. The enzyme oxidized aliphatic and aromatic alcohols including 20α-hydroxysteroids. In addition, AKR1B10 was inhibited by steroid hormones, bile acids and their metabolites, showing IC_{50} values of 0.03-25 μM. Thus, we propose a novel role of AKR1B10 in controlling isoprenoid homeostasis that is important in cholesterol synthesis and cell proliferation through salvaging isoprenoid alcohols, as well as its metabolic regulation by endogenous steroids.

[*Chem. Biol. Interact.*, **176**, 117–126 (2009)] [Lab. of Biochemistry]

Biochemical and Structural Characterization of a Short-chain Dehydrogenase/reductase of *Thermus thermophilus* HB8

A hyperthermostable aldose-1-dehydrogenase with broad substrate specificity.

Yukuhiko ASADA, Satoshi ENDO, Yukari INOUE, Hiroaki MAMIYA, Akira HARA, Naoki KUNISHIMA, and Toshiyuki MATSUNAGA*

Thermus thermophilus HB8 is a hyperthermophilic bacterium. An enzyme encoded in a gene (TTHA0369) among proteins of this bacterium showed NAD^+-dependent aldose 1-dehydrogenase activity against various aldoses. The enzyme was stable at pH 2–13 and up to 85 ˚C. We have determined the crystal structure of the enzyme–NAD^+ binary complex. Additionally, two unique structural features, aromatic–aromatic interactions among Phe141 and Phe249 in the subunit interface and hydrogen networks around the C-terminal Asp–Gly–Gly sequence at positions 242–244 contribute to the hyperthermostability of the enzyme.

[*Chem. Biol. Interact.*, **181**, 52–60 (2009)] [Lab. of Biochemistry]

Involvement of an aldo-keto reductase (AKR1C3) in redox cycling of 9,10-phenanthrenequinone leading to apoptosis in human endothelial cells.

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

Here, we show that 9,10-Phenanthrenequinone (9,10-PQ) evokes apoptosis in human aortic endothelial cells (HAECs) and its apoptotic signaling includes ROS generation and caspase activation. Comparison of mRNA expression levels and kinetic constants in the 9,10-PQ reduction among 10 human reductases suggests that aldo-keto reductase 1C3 (AKR1C3) is a 9,10-PQ reductase in HAECs. AKR1C3 overexpression in endothelial cells augmented the ROS generation and cytotoxicity by 9,10-PQ, and the ROS scavengers or AKR1C3 inhibitors, flufenamic acid and indomethacin, suppressed the 9,10-PQ-induced the toxic effects. These results suggest that AKR1C3 is a key enzyme in the initial step of 9,10-PQ-induced cytotoxicity in HAECs.

[*Biochem. Biophys. Res. Commun.,* **389**, 128–132 (2009)] [Lab. of Biochemistry]

Potent and selective inhibition of the tumor marker AKR1B10 by bisdemethoxycurcumin: Probing the active site of the enzyme with molecular modeling and site-directed mutagenesis.

Toshiyuki MATSUNAGA*, Satoshi ENDO, Midori SODA, Hai-Tao ZHAO, Ossama El-KABBANI, Kazuo TAJIMA, and Akira HARA

AKR1B10 shares high sequence identity with aldose reductase (AR), and was recently identified as a therapeutic target in the treatment of several types of cancer. We have compared the inhibitory effects of plant components on AKR1B10 and AR. Curcuminoids, magnolol, honokiol and resveratrol more potently inhibited AKR1B10 over AR. Among them, bisdemethoxycurcumin was the most potent competitive inhibitor (Ki=22 nM) with the highest selectivity (85-fold versus AR), and acted as an effective inhibitor in cellular level. Molecular docking studies and site-directed mutagenesis suggest that Gln114, Val301 and Gln303 are important for determining the inhibitory potency and selectivity of the curcuminoids.

[*Eur. J. Pharmacol.,* **605**, 153–157 (2009)] [Lab. of Pharmacology]

The Role of IgE and Repeated Challenge in the Induction of Persistent Increases in Scratching Behavior in a Mouse Model of Allergic Dermatitis.

Hirotaka YAMASHITA*, Daisuke TASAKI, Toshiaki MAKINO, Kunie MATSUOKA, Mitsuhiko NOSE, Naoki INAGAKI, and Hajime MIZUKAMI

The relationship between serum IgE level and induction of scratching behavior was confirmed to use BALB/c-nu/nu (nude) mice and trinitrophenol (TNP)-specific IgE-transgenic mice. Nude mice had been supplemented with IgE treated with 2,4,6-trinitrochlorobenzene (TNCB) 6 times exhibited scratching behavior with degranulation of mast cells but not significant ear swelling. On the other hand, the IgE-transgenic mice failed to exhibit scratching behavior after a single TNCB treatment because few mast cells degranulated. These results indicate that mast cell accumulation and continuous mast cell degranulation depended on IgE induce persistent increase in scratching behavior.

[*Am. J. Respir. Crit. Care. Med.,* **179**, 992–998 (2009)] [Lab. of Pharmacology]

Pulmonary Suppressor of Cytokine Signaling-1 Induced by IL-13 Regulates Allergic Asthma Phenotype

Satoru FUKUYAMA, Takako NAKANO, Takafumi MATSUMOTO, Brian G. OLIVER, Janette K. BURGESS, Atsushi MORIKAWA, Kentaro TANAKA, Masato KUBO, Tomoaki HOSHINO, Hiroyuki TANAKA*, Andrew N. J. McKENZIE, Koichiro MATSUMOTO, Hisamichi AIZAWA, Yoichi NAKANISHI, Akihiko YOSHIMURA, Judith L. BLACK, and Hiromasa INOUE

Suppressor of cytokine signaling (SOCS) proteins are known to play a critical role in helper T cell differentiation in allergic disease; however, their local function in airway structural cells is less well defined. Since SOCS1 induction in the airways negatively controlled allergic responses in the mouse models of IL-13- and ovalbumin-induced airway inflammation, and airway smooth muscle cells cultured from patients of asthma, aberrant action of SOCS1 may contribute to the pathophysiology of asthma.

[*Pharmacology,* **84**, 249–256 (2009)] [Lab. of Pharmacology]

The Effects of Inhaled KP-496, a Novel Dual Antagonist for Cysteinyl Leukotriene Receptor and Thromboxane A_2 Receptor, on Allergic Asthmatic Responses in Guinea Pigs.

Masahiro SUDA, Toshiaki OKUDA, Masakazu ISHIMURA, Shigeo KUROKAWA, Shota TOKUOKA, Tsutomu NAKAMURA, Yoshimasa TAKAHASHI, Hiroyuki TANAKA*, and Hiroichi NAGAI

Guinea pigs were exposed to ovalbumin (OA) to evaluate the effects of inhaled KP-496 (0.01 and 0.1%), a dual antagonist for cysteinyl leukotriene (cysLT) receptor 1 and thromboxane A_2 (TXA_2) receptor, on asthmatic responses, such as immediate and late asthmatic response (IAR and LAR), and airway hyperresponsiveness (AHR) by exposure of acetylcholine. KP-496 significantly inhibited both LAR and AHR with suppression of eosinophils infiltration around airway smooth muscle, epithelial hypertrophy, and increasing mucus production in the airway. Since these broad ameliorative effects of KP-496 on asthmatic pathology are thought to result from the inhibition of cysLT and TXA_2, KP-496 will be a potent agent in the treatment of bronchial asthma.

[*Eur. J. Pharm. Biopharm.,* **73**, 361–365 (2009)] [Lab. of Pharmacology]

Preparation of a Fast Dissolving Oral Thin Film Containing Dexamethasone: A Possible Application to Antiemesis During Caner Cemotherapy.

Hiroyoshi SHIMODA, Kazumi TANIGUCHI, Misao NISHIMURA, Katsuhiko MATSUURA, Tadao TSUKIOKA, Hirotaka YAMASHITA, Naoki INAGAKI*, Kazuyuki HIRANO, Mayumi YAMAMOTO, Yasutomi KINOSADA, and Yoshinori ITOH

Oral thin film containing dexamethasone with base materials, including microcrystalline cellulose, polyethylene glycol, low-substituted hydroxypropyl cellulose, and so on, showed excellent stability, when stored at 40◦C and 75% in humidity. The film was disintegrated within 15 sec after immersion into distilled water, and approximately 90% of dexamethasone was dissolved within 5 min.Pharmacokinetic parameter, involved in Cmax, Tmax, AUC, and half-life in the film were equal to those of oral administration. These findings suggest that dexamethasone film is likely to become one of choices for antiemetic during cancer chemotherapy.

[*J. Biol. Chem.,* **284**, 31463–31472 (2009)] [Lab. of Pharmacology]

Evidence That Integrin alpha IIb beta 3-dependent Interaction of Mast Cells with Fibrinogen Exacerbates Chronic Inflammation.

Toshihiko OKI, Koji ETO, Kumi IZAWA, Yoshinori YAMANISHI, Naoki INAGAKI*, Jon FRAMPTON, Toshio KITAMURA, and Jiro KITAURA.

Integrin alpha IIb beta 3 on bone marrow-derived mast cells (BMMCs) was required for that enhancement of proliferation, degranulation, cytokine production, and interaction with fibrinogen (FB). Integrin alpha IIb deficiency in mast cell strongly suppressed chronic inflammation related with the administration of FB, although it did not affect acute allergic responses or mast cell numbers in tissues in steady states. Additionally, soluble FB promoted cytokine production of BMMCs in response to *Staphylococcus aureus* with FB-binding capacity through integrin alpha IIb beta 3-dependent recognition of the pathogen. In fact, integrin alpha IIb beta 3 in mast cells plays an important part in FB-associated chronic inflammation and innate immune responses.

[*PLoS ONE*, **4(10)**: e7461 (2009)] [Lab. of Molecular Pharmacology]

Generation and characterization of conditional heparin-binding EGF-like growth factor knockout mice.

Atsushi OYAGI, Yasuhisa OIDA, Kenichi KAKEFUDA, Masamitsu SHIMAZAWA, Norifumi SHIODA, Shigeki MORIGUCHI, Kiyoyuki KITAICHI, Daisuke NANBA, Kazumasa YAMAGUCHI, Yasuhide FURUTA, Kohji FUKUNAGA, Shigeki HIGASHIYAMA, and Hideaki HARA*

We generated mice in which heparin-binding epidermal growth factor-like growth factor (HB-EGF) activity is disrupted specifically in the ventral forebrain. These knockout mice showed behavioral abnormalities similar to those described in psychiatric disorders, which were ameliorated by typical or atypical antipsychotics. These results suggest the alterations affecting HB-EGF signaling could comprise a contributing factor in psychiatric disorder.

[*Neurosci. Lett.*, **467**, 11–14 (2009)] [Lab. of Molecular Pharmacology]

Metallothionein-3 deficient mice exhibit abnormalities of psychological behaviors.

Akihiro KOUMURA, Kenichi KAKEFUDA, Akiko HONDA, Yasushi ITO, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Yoko UCHIDA, Isao HOZUMI, Masahiko SATOH, Takashi INUZUKA, and Hideaki HARA*

We carried out behavioral tests on Metallothionein-3 (MT-3) knock-out (KO) mice. The duration of the MT-3 KO mice's social interactions were significantly shorter than that of the wild-type (WT) mice. The acoustic startle response of the MT-3 KO mice showed diminished prepulse inhibition (PPI) at all prepulse intensities. However, the locomotor activity tests of the MT-3 KO mice displayed normal circadian rhythm, activity, and habituation to a novel environment. In the novel object recognition test, the MT-3 KO mice exhibited normal memory. These findings indicate that abnormalities of psychological behavior were observed in the MT-3 KO mice. Further experiments will be needed to clarify the involvement of MT-3 in higher brain function.

[*Neurobiol. Dis.*, **36**, 470–476 (2009)] [Lab. of Molecular Pharmacology]

Involvement of CHOP, an ER-stress apoptotic mediator, in both human sporadic ALS and ALS model mice.

Yasushi ITO, Mitsunori YAMADA, Hirotaka TANAKA, Kazunari AIDA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Isao HOZUMI, Takashi INUZUKA, Hitoshi TAKAHASHI, and Hideaki HARA*

Endoplasmic reticulum (ER) stress-induced neuronal death may play a critical role in the pathogenesis of amyotrophic lateral sclerosis (ALS). However, whether CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP), an ER-stress apoptotic mediator, is involved in the pathogenesis of ALS is controversial. In the spinal cords of sporadic ALS patients, CHOP was markedly up-regulated but typically expressed at low levels in those of the control. Furthermore, localizations of CHOP were merged in motor neurons and glial cells, such as oligodendrocytes, astrocytes, and microglia. These results indicate that the up-regulation of CHOP in motor neurons and glial cells may play pivotal roles in the pathogenesis of ALS.

[*Neuroscience*, **159**, 760–769 (2009)] [Lab. of Molecular Pharmacology]

Thalidomide protects against ischemic neuronal damage induced by focal cerebral ischemia in mice.

Kana HYAKKOKU, Yoshimi NAKAJIMA, Hiroshi IZUTA, Masamitsu SHIMAZAWA, Takeshi YAMAMOTO, Norio SHIBATA, and Hideaki HARA*

We aimed to examine whether thalidomide might inhibit the neuronal damage resulting from focal cerebral ischemia, and if so to explore the neuroprotective mechanism. Thalidomide significantly reduced (a) the infarct area and volume at 24 and 72 h after middle cerebral artery occlusion (MCAO) and (b) the neurological score at 72 h after MCAO. Thalidomide reduced both the number of terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)-positive cells and the oxidative damage. Thalidomide inhibited both the lipid peroxidation and the production of H_2O_2 and O_2^- (but not HO^-) radicals. These findings indicate that thalidomide has neuroprotective effects against ischemic neuronal damage in mice, and that an inhibitory action of thalidomide against oxidative stress may be partly responsible for these neuroprotective effects.

[*NeuroReport*, **20**, 139–144 (2009)] [Lab. of Molecular Pharmacology]

Molecular imaging reveals unique degenerative changes in experimental glaucoma.

Kazuyuki IMAMURA, Hirotaka ONOE, Masamitsu SHIMAZAWA, Satoshi NOZAKI, Yasuhiro WADA, Koichi KATO, Hideki NAKAJIMA, Hiroshi MIZUMA, Kayo ONOE, Takazumi TANIGUCHI, Masaaki SASAOKA, Hideaki HARA*, Shigeru TANAKA, Makoto ARAIE, and Yasuyoshi WATANABE

Experimentally induced changes in the central visual pathway were studied by using positron emission tomography in monkeys with unilateral hypertension glaucoma. In 2-[^{18}F]fluoro-2-deoxy-glucose studies, monocular visual stimulation of the affected eye yielded significantly reduced neural responses in the occipital visuocortical areas. In addition, in [^{11}C]PK11195 positron emission tomography and immunohistochemical studies, selective accumulation of activated microglia, a sign of neural degeneration, was found bilaterally in lateral geniculate nuclei. The present findings establish the usefulness of noninvasive molecular imaging for early diagnosis of glaucoma by providing a sharper surrogate end point for an early phase of glaucoma.

[*J. Pharmacol. Exp. Ther.*, **330**, 13–22 (2009)] [Lab. of Molecular Pharmacology]

Combination treatment with normobaric hyperoxia and cilostazol protects mice against focal cerebral ischemia-induced neuronal damage better than each treatment alone.

Yuko NONAKA, Akihiro KOUMURA, Kana HYAKKOKU, Masamitsu SHIMAZAWA, Shinichi YOSHIMURA, Toru IWAMA, and Hideaki HARA*

We evaluated the potential neuroprotective effects of combination treatment with normobaric hyperoxia (NBO; 95% O_2) and cilostazol against acute and subacute brain injuries after simulated stroke. Mean acute and subacute lesion volumes were significantly reduced in the combination group but not in the two monotherapy groups. The combination therapy increased endothelial nitric-oxide synthase (eNOS) activity in the lesion area after ischemia versus vehicle. Combination therapy with NBO plus cilostazol protected mice subjected to focal cerebral ischemia by improvement of rCBF after reperfusion, in part in association with eNOS activity.

[*Neurosci. Lett.*, **452**, 156–161 (2009)] [Lab. of Molecular Pharmacology]

Cilostazol protects against hemorrhagic transformation in mice transient focal cerebral ischemia-induced brain damage.

Yuko NONAKA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Shinichi YOSHIMURA, Toru IWAMA, and Hideaki HARA*

We evaluated the protection afforded by cilostazol against ischemic brain injury and hemorrhagic transformation. Mice subjected to a 2-h filamental middle cerebral artery (MCA) occlusion were treated with cilostazol (10mg/kg, intraperitoneally just after the occlusion) or with vehicle. Histological outcomes (infarct volume and hemorrhagic transformation) and Evans blue extravasation were assessed after reperfusion. Mean infarct volume, hemorrhagic transformation, and Evans blue extravasation were all significantly reduced in the cilostazol-treated group. Thus, cilostazol protected against ischemic brain injury and hemorrhagic transformation in mice subjected to transient focal cerebral ischemia.

[*Brain Res.*, **1292**, 148–154 (2009)] [Lab. of Molecular Pharmacology]

Metallothionein-III knockout mice aggravates the neuronal damage after transient focal cerebral ischemia.

Akihiro KOUMURA, Junya HAMANAKA, Masamitsu SHIMAZAWA, Akiko HONDA, Kazuhiro TSURUMA, Yoko UCHIDA, Isao HOZUMI, Masahiko SATOH, Takashi INUZUKA, and Hideaki HARA*

We examined neuronal damage after a middle cerebral artery occlusion (MCAO) in metallothionein-III (MT-III) knockout (KO) mice to elucidate the relationship between MT-III and cerebral infarction. After 2-h MCAO and 22-h reperfusion, cerebral infarction in the MT-III KO mice was aggravated compared with the wild-type mice. Furthermore, fatal rate of MT-III KO mice increased from 3 days after MCAO, and neurological deficits at 5 and 7 days after MCAO of MT-III KO mice were worse than those of wild-type. These findings indicate that neuronal damage was aggravated by reperfusion injury in the MT-III KO mice compared with the wild-type mice, suggesting that MT-III plays anti-oxidative and neuroprotective roles in transient cerebral ischemia.

[*Exp. Eye Res.*, **89**, 246–255 (2009)] [Lab. of Molecular Pharmacology]

Morphological changes in the visual pathway induced by experimental glaucoma in Japanese monkeys.

Yasushi ITO, Masamitsu SHIMAZAWA, Yi-Ning CHEN, Kazuhiro TSURUMA, Tetsumori YAMASHITA, Makoto ARAIE, and Hideaki HARA*

We investigated time-dependent alterations in the ONH, the optic nerve (ON), and the LGN after intraocular pressure (IOP) elevation in Japanese monkeys. Nine Japanese monkeys, each with an experimental glaucomatous left eye, and two naive monkeys were studied. Loss of axons and a decrease in the area of ON were first observed at 4 and 28 weeks, respectively. Neuronal loss was first observed at 2 weeks in layers 1 and 2 of LGN [magnocellular (M)-layer] and at 12 weeks in layers 3-6 of LGN [parvocellular (P)-layer]. Neuronal shrinkage was first observed at 2 weeks in all layers in LGN. These findings indicate that in Japanese monkeys, damage to neurons in LGN can be detected in the early phase (first few weeks) after an IOP elevation, as can damage to ONH.

[*Mol. Vision*, **15**, 662–669 (2009)] [Lab. of Molecular Pharmacology]

Involvement of brain-derived neurotrophic factor in time-dependent neurodegeneration in the murine superior colliculus after intravitreal injection of *N*-methyl-D-aspartate.

Hirotaka TANAKA, Yasushi ITO, Shinsuke NAKAMURA, Masamitsu SHIMAZAWA, and Hideaki HARA*

We examined the morphological alterations present in the superior colliculus (SC) after *N*-methyl-D-aspartate (NMDA)-induced retinal damage in mice. The number of neuronal nuclear specific protein (NeuN)-immunostained neurons showed a significant decrease in the contralateral SC at both 90 and 180 days after intravitreal NMDA injection. An increase in glial fibrillary acid protein (GFAP) immunoreactivity was observed in the contralateral SC at 7, 30, and 90 days after NMDA injection and in the ipsilateral SC at 7 days, while brain-derived neurotrophic factor (BDNF) expression was increased in the contralateral SC at 30 and 90 days..Hence, these findings may provide useful information concerning the pathological mechanisms of several disorders accompanied by retinal degeneration.

[*Biochem. Biophys. Res. Commun.*, **387**, 784–788 (2009)] [Lab. of Molecular Pharmacology]

Sirtuin 1 overexpression mice show a reference memory deficit, but not neuroprotection.

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Sirtuin 1 (SIRT1) is the closest mammalian homologue of yeast silent information regulator 2 (Sir2) and has a role in lifespan modulation. Reportedly, SIRT1 is also linked to neurodegenerative diseases. In the present study, we generated neuron-specific enolase (NSE) SIRT1 Tg mice that overexpress human SIRT1 in neurons. We examined possible neuroprotective effects of SIRT1 overexpression and compared their higher brain functions with those of wild-type (WT) mice. Overexpression of SIRT1 did not have any neuroprotective effects against the neuronal damage induced by ischemia or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). However, SIRT1 Tg mice exhibited a reference memory deficit. These findings suggest that an excessive expression of SIRT1 might induce the memory deficit in mice, but not neuroprotective effects.

[*Invest. Ophthalmol. Vis. Sci.*, **50**, 334–344 (2009)] [Lab. of Molecular Pharmacology]

Effect of an inducer of BiP, a molecular chaperone, on endoplasmic reticulum (ER) stress-induced retinal cell death.

Yuta INOKUCHI, Yoshimi NAKAJIMA, Masamitsu SHIMAZAWA, Takanori KURITA, Mikiko KUBO, Atsushi SAITO, Hironao SAJIKI., Takashi KUDO, Makoto AIHARA., Kazunori IMAIZUMI, Makoto ARAIE, and Hideaki HARA*

The effect of a preferential inducer of immunoglobulin heavy-chain binding protein (BiP; BiP inducer X, BIX) against tunicamycin-induced cell death in RGC-5, and also against tunicamycin- or *N*-methyl-D-aspartate (NMDA)-induced retinal damage in mice was evaluated. BIX significantly reduced tunicamycin-induced cell death in RGC-5. Co-administration of BIX (5 nmol) significantly reduced both the retinal cell death in GCL induced by tunicamycin or NMDA. These findings suggest that this BiP inducer may have the potential to be a therapeutic agent for endoplasmic reticulum (ER) stress-induced retinal diseases.

[*J. Pharmacol. Exp. Ther.*, **329**, 687–698 (2009)] [Lab. of Molecular Pharmacology]

Edaravone, a free radical scavenger, protects against retinal damage *in vitro* and *in vivo*.

Yuta INOKUCHI, Shunsuke IMAI, Yoshimi NAKAJIMA, Masamitsu SHIMAZAWA, Makoto AIHARA, Makoto ARAIE, and Hideaki HARA*

Edaravone, a free radical scavenger, is used for the treatment of acute cerebral infarction. In this study, we investigated whether edaravone is neuroprotective against retinal damage. Edaravone significantly decreased radical generation and reduced the RGC-5 cell death induced by OGD stress. Edaravone at 5 and 50 nmol intravitreous injection or at 1 and 3 mg/kg i.v. significantly protected against NMDA-induced retinal cell death. At 50 nmol intravitreous injection, it 1) decreased the retinal expressions of TUNEL-positive cells, 4-HNE, and 8-OHdG and 2) reduced the retinal expressions of NMDA-induced phosphorylated JNK and phosphorylated p38 but not that of phosphorylated ERK. These findings suggest that oxidative stress plays a pivotal role in retinal damage and that edaravone may be a candidate for the effective treatment of retinal diseases.

[*J. Neurosci. Res.*, **87**, 906–917 (2009)] [Lab. of Molecular Pharmacology]

A Na^+/Ca^{2+} exchanger isoform, NCX1, is involved in retinal cell death after *N*-methyl-D-aspartate injection and ischemia-reperfusion.

Yuta INOKUCHI, Masamitsu SHIMAZAWA, Yoshimi NAKAJIMA, Issei KOMURO, Toshio MATSUDA, Akemichi BABA, Makoto ARAIE, Satomi KITA, Takahiro IWAMOTO, and Hideaki HARA*

We investigated the expression of Na^+/Ca^{2+} exchanger (NCX) and the functional role of NCX in retinal damage by using NCX1-heterozygous deficient mice ($NCX1^{+/-}$) and SEA0400, a selective NCX inhibitor in vivo. The expression of NCX1 was confirmed and entirely localized in retina by immunoblotting and immunohistochemistry, respectively. $NCX1^{+/-}$ mice possessed significant protection against retinal damage induced by intravitreal injection of *N*-methyl-D-aspartate (NMDA). SEA0400 at 3 and 10 mg/kg significantly reduced NMDA- or high intraocular pressure-induced retinal cell damage in mice. In conclusion, these results suggest that NCX1 may play a role in retinal cell death induced by NMDA and ischemia-reperfusion.

[*Mol. Vision*, **15**, 2663–2672 (2009)] [Lab. of Molecular Pharmacology]

Extracellular SOD and VEGF are increased in vitreous bodies from proliferative diabetic retinopathy patients.

Hiroshi IZUTA, Yuichi CHIKARAISHI, Tetsuo ADACHI, Masamitsu SHIMAZAWA, Tetsuya SUGIYAMA, Tsunehiko IKEDA, and Hideaki HARA*

We evaluated the relationship between vascular endothelial growth factor (VEGF) and extracellular superoxide dismutase (EC-SOD) in vitreous body in patients with proliferative diabetic retinopathy (PDR). Intravitreal concentrations of EC-SOD were significantly higher ($p<0.01$) in PDR (58.0+/-23.8 ng/ml, mean+/-SD) than in MH (29.3+/-6.6 ng/ml). Intravitreal concentrations of VEGF were dramatically higher ($p<0.01$) in PDR (798.2+/-882.7 pg/ml) than in MH (17.7+/-15.5 pg/ml). The vitreous concentrations of VEGF correlated with those of EC-SOD in all patients ($rs=0.61$, $p<0.001$). EC-SOD was increased together with VEGF in the vitreous body from PDR patients, suggesting that EC-SOD may play a pivotal role in the pathogenesis of angiogenesis.

[*Curr. Neurovasc. Res.*, **6**, 140–147 (2009)] [Lab. of Molecular Pharmacology]

CB-12181, a new azasugar-based matrix metalloproteinase/tumor necrosis factor-α converting enzyme inhibitor, inhibits vascular endothelial growth factor-induced angiogenesis *in vitro* and retinal neovascularization *in vivo*.

Yuichi CHIKARAISHI, Masamitsu SHIMAZAWA, Koichi YOKOTA, Koichiro YOSHINO, and Hideaki HARA*

To evaluate the anti-angiogenic efficacy of CB-12181 [an azasugar derivative that has inhibitory actions against matrix metalloproteinases (MMPs) and TNF-alpha converting enzyme], we investigated the suppressing ability on in vitro and in vivo models of angiogenesis. In the in vitro angiogenesis model, CB-12181 significantly suppressed VEGF-induced HUVEC tube formation. Furthermore, in the in vivo angiogenesis model, administration of CB-12181 significantly suppressed retinal neovascularization. These results suggest that CB-12181 might be useful in the treatment of various diseases that depend on pathologic angiogenesis, and especially valuable for the treatment of diabetic retinopathy and retinopathy of prematurity.

[*Curr. Eye Res.*, **34**, 311–318 (2009)] [Lab. of Molecular Pharmacology]

Zeaxanthin, a retinal carotenoid, protects retinal cells against oxidative stress.

Yoshimi NAKAJIMA, Masamitsu SHIMAZAWA, Kazumasa OTSUBO, Takashi ISHIBASHI, and Hideaki HARA*

We investigated whether zeaxanthin, the predominant carotenoid pigment of the macular pigments in human retina, provides neuroprotection against retinal cell damage. When added to RGC-5 cell cultures, 0.1, 10, and 1 microM zeaxanthin scavenged the free radicals induced by H_2O_2, O_2^-, and HO^-, respectively. In addition, pretreatment with 1 microM zeaxanthin permitted scavenging of staurosporine-induced intracellular radicals. Zeaxanthin also inhibited the neurotoxicity induced by H_2O_2 or serum deprivation and scavenged the intracellular radicals induced by H_2O_2 or serum deprivation. Our results suggest that zeaxanthin provides effective protection against oxidative stress-induced retinal cell damage.

[*Brain Res.*, **1251**, 269–275 (2009)] [Lab. of Molecular Pharmacology]

Docosahexaenoic acid (DHA) has neuroprotective effects against oxidative stress in retinal ganglion cells.

Masamitsu SHIMAZAWA, Yoshimi NAKAJIMA, Yukihiko MASHIMA, and Hideaki HARA*

We examined the radical-scavenging activity of docosahexaenoic acid (DHA) and its effects on the neuronal cell death induced by oxidative or hypoxic stress in cultured retinal ganglion cells (RGC-5). DHA concentration-dependently scavenged the intracellular radical productions induced by H_2O_2 radical, O_2^-, and ·OH. Treatment with DHA at 0.1 and 1 microM significantly inhibited the decrease in cell viability induced by H_2O_2. Treatment with DHA at 0.1, 1, or 10 microM significantly inhibited the decrease in cell viability induced by OGD/reoxygenation exposure. However, DHA (0.1 to 10 microM) had no effect on the decrease in cell viability induced by tunicamycin. These results indicate that DHA may be protective against oxidative or hypoxic stress-induced cell damage in retinal ganglion cells.

[*Mol. Nutr. Food Res.*, **53**, 869–877 (2009)] [Lab. of Molecular Pharmacology]

Bilberry and its main constituents have neuroprotective effects against retinal neuronal damage *in vitro* and *in vivo*.

Nozomu MATSUNAGA, Shunsuke IMAI, Yuta INOKUCHI, Masamitsu SHIMAZAWA, Shigeru YOKOTA, Yoko ARAKI, and Hideaki HARA*

Our aim was to determine whether a Vaccinium myrtillus (bilberry) anthocyanoside (VMA) and/or its main anthocyanidin constituents (cyanidin, delphinidin, and malvidin) can protect retinal ganglion cells (RGCs) against retinal damage in vitro and in vivo. VMA and all three anthocyanidins significantly inhibited SIN-1 (a peroxynitrite donor)-induced neurotoxicity and radical activation in RGC-5. Intravitreously injected VMA significantly inhibited the NMDA-induced morphological retinal damage in the ganglion cell layer. Thus, VMA and its anthocyanidins have neuroprotective effects (exerted at least in part via an anti-oxidation mechanism) in these in vitro and in vivo models of retinal diseases.

[*eCAM*, **6**, 489–494 (2009)] [Lab. of Molecular Pharmacology]

10-Hydroxy-2-decenoic acid, a major fatty acid from Royal jelly, inhibits VEGF-induced angiogenesis in human umbilical vein endothelial cells.

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

Royal jelly (RJ) is a honeybee product containing various proteins, sugars, lipids, vitamins and free amino acids. 10-Hydroxy-2-decenoic acid (10HDA), a major fatty acid component of RJ, is known to have various pharmacological effects; its antitumor activity being especially noteworthy. However, the mechanism underlying this effect is unclear. We examined the effect of 10HDA on vascular endothelial growth factor (VEGF)-induced proliferation, migration and tube formation in human umbilical vein endothelial cells (HUVECs). Our findings showed that, 10HDA at 20 microM or more significantly inhibited such proliferation, migration and tube formation. These findings indicate that 10HDA exerts an inhibitory effect on VEGF-induced angiogenesis, partly by inhibiting both cell proliferation and migration.

[*Biol. Pharm. Bull.*, **32**, 1947–1951 (2009)] [Lab. of Molecular Pharmacology]

1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity of bee products and their constituents determined by ESR.

Hiroshi IZUTA, Yukimi NARAHARA, Masamitsu SHIMAZAWA, Satoshi MISHIMA, Shin-ichi KONDO, and Hideaki HARA*.

The aim of this work was to investigate the antioxidant property of honeybee products and their constituents using an ESR method. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical radical scavenging activities, in descending order, were: ethanol extract of Chinese red propolis>ethanol extract of Brazilian green propolis>water extract of Brazilian green propolis>ethanol extract of bee pollen. Caffeoylquinic acid derivatives exhibited DPPH radical scavenging activity as strong as that of ascorbic acid and trolox. Both Brazilian and Chinese propolis and their constituents (caffeoylquinic acid derivatives and caffeic acid phenethyl ester) therefore appear to be powerful scavengers of DPPH radical, and the effects may be partly dependent on the nature of their caffeoyl groups.

[*Phytother. Res.*, **23**, 1431–1438 (2009)] [Lab. of Molecular Pharmacology]

Neuroprotective effects of Brazilian green propolis and its main constituents against oxygen-glucose deprivation stress, with a gene-expression analysis.

Yoshimi NAKAJIMA, Masamitsu SHIMAZAWA, Satoshi MISHIMA, and Hideaki HARA*

We investigated the neuroprotective effects (and the underlying mechanism) exerted by water extract of Brazilian green propolis (WEP) and its main constituents against the neuronal damage induced by oxygen-glucose deprivation (OGD)/reoxygenation in retinal ganglion cells (RGC-5). WEP and some of its main constituents attenuated the cell damage. Expression of casein kinase 2 (CK2) was down-regulated and that of Bcl-2-related ovarian killer protein (Bok) was up-regulated following OGD stress. These effects were normalized by WEP. Our findings indicate that WEP has neuroprotective effects against OGD/reoxygenation-induced cell damage. Furthermore, the protective mechanism may involve normalization of the expressions of antioxidant- and apoptosis-related genes (such as CK2 and Bok, respectively).

[*BMC Complement. Altern. Med.*, **9**: 4 (2009)] [Lab. of Molecular Pharmacology]

Comparison of bee products based on assays of antioxidant capacities.

Yoshimi NAKAJIMA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Satoshi MISHIMA, and Hideaki HARA*

We compared antioxidant effects among water and ethanol extracts of Brazilian green propolis (WEP or EEP), its main constituents, water-soluble royal jelly (RJ), and an ethanol extract of bee pollen. The rank order of antioxidant potencies was as follows: WEP > EEP > pollen, but neither RJ nor 10-hydroxy-2-decenoic acid (10-HDA) had any effects. Concerning the main constituents of WEP, the rank order of antioxidant effects was: caffeic acid > artepillin C > drupanin, but neither baccharin nor coumaric acid had any effects. The scavenging effects of caffeic acid were as powerful as those of trolox, but stronger than those of *N*-acetyl cysteine (NAC) or vitamin C. On the basis of the present assays, propolis is the most powerful antioxidant of all the bee product examined, and its effect may be partly due to the various caffeic acids it contains. Pollen, too, exhibited strong antioxidant effects.

[*BMC Complement. Altern. Med.*, **9**: 45 (2009)] [Lab. of Molecular Pharmacology]

Bee products prevent VEGF-induced angiogenesis in human umbilical vein endothelial cells.

Hiroshi IZUTA, Masamitsu SHIMAZAWA, Kazuhiro TSURUMA, Yoko ARAKI, Satoshi MISHIMA, and Hideaki HARA*

The aim of this study was to investigate the anti-angiogenic effects of bee products using human umbilical vein endothelial cells (HUVECs). Royal jelly (RJ), bee pollen, Chinese red propolis, and caffeic acid phenethyl ester (CAPE) significantly suppressed vascular endothelial growth factor (VEGF)-induced in vitro tube formation in the descending order: CAPE > Chinese red propolis >> bee pollen > RJ. RJ and Chinese red propolis suppressed both VEGF-induced HUVEC proliferation and migration. In contrast, bee pollen and CAPE suppressed only the proliferation. Among the bee products, Chinese red propolis and CAPE in particular showed strong suppressive effects against VEGF-induced angiogenesis. These findings indicate that Chinese red propolis and CAPE may have potential as preventive and therapeutic agents against angiogenesis-related human diseases.

[*Biol. Pharm. Bull.*, **32**, 1244–1250 (2009)] [Lab. of Molecular Pharmacology]

Antihypertensive effects of flavonoids isolated from Brazilian green propolis in spontaneously hypertensive rats.

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

The present study was performed to investigate whether Brazilian green propolis exerts antihypertensive effects in spontaneously hypertensive rats (SHR) and which constituents are involved in its effects. The ethanol-eluted fractions at 10 mg/kg were administered orally to SHR for 14 d. Significant decreases in blood pressure were observed in fractions 6 and 7. The active constituents were purified and identified to be four flavonoids. Isosakuranetin, dihydrokaempferide and betuletol produced significant decrease in blood pressure, especially marked were the effects observed in the group that received isosakuranetin. Therefore, these finding suggest that the vasodilating action may be partly involved in the mechanism of antihypertensive effect. Hence, the ethanol extract of Brazilian green propolis and its main constituents may be useful for prevention of hypertension.

[*Bioorg. Med. Chem. Lett.*, **19**, 3973–3976 (2009)] [Lab. of Molecular Pharmacology]

Synthesis, configurational stability and stereochemical biological evaluations of (*S*)- and (*R*)-5-hydroxythalidomides.

Takeshi YAMAMOTO, Norio SHIBATA, Daisuke SUKEGUCHI, Masayuki TAKASHIMA, Shuichi NAKAMURA, Takeshi TORU, Nozomu MATSUNAGA, Hideaki HARA*, Motohiro TANAKA, Tohru OBATA, and Takuma SASAKI

The first asymmetric synthesis of (S)- and (R)-5-hydroxythalidomides, one of thalidomide's major metabolites, was achieved using HMDS/ZnBr(2)-induced imidation as a key reaction. 5-Hydroxythalidomide was found to be configurationally more stable than thalidomide at physiological pH. Stereochemical biological effects of thalidomide and 5-hydroxythalidomide on anti-angiogenesis and antitumor activities were also investigated using racemic and pure enantiomers.

[*J. Pharm. Comun*, **7**, 37–48 (2009)] [Lab. of Clinical Pharmacy]

Evaluation for Communication Skill Training Curriculum in Pre-education of Pharmacy Practical Training.

Hitomi TERAMACHI*, Yumi KUZUYA, Tetsuo ADACHI, and Teruo TSUCHIYA

A communication skill training curriculum as a part of the practice of pharmaceutics was implemented for the 4th- year students (n = 67). We staffed graduate students as teaching assistants (TA, n = 17), and studied students performance using video. A questionnaire survey was conducted among the students who had participated in this program. The results suggested that students learned activity, mastered the needed skills, and that video learning is promising for acquirement of the skills. Furthermore, the results suggested that both students felt that the introduction of the TA system was promising. In communication assessment by objective evaluation, all students were judged to "be good" more than 70%. We infer that this communication skill training curriculum with assistance of TAs and using video, is a most effective curriculum step for improvement of communication skill.

[*J. Jpn. Soc. Hosp. Pharm.,* **45**, 961–964 (2009)] [Lab. of Clinical Pharmacy]

Improvement of Procedures for Preparation of Aseptic Injectable Anticancer Drugs by means of the Quality Control Technique.

Masahiro YASUDA, Michi UMEDA, Yumi KUZUYA*, Yuka TANAKA, Atsushi ICHIHASHI, Makoto SAHASHI, Katsutoshi GOTO, Masamitsu SAKAIDA and Kazuo SATOMI.

We examined improvement of safety and promotion of efficiency of procedures in a preparation of aseptic injectable anticancer drugs by the quality control (QC) technique in the union of Japanese scientists and engineers. As a problem factor, the complexity of the procedures and the insufficiency of the check system of the attention item were given. We constructed the system that automatically makes the preparation slip which necessary information such as ‘characteristics of the drug’, ‘kinds of solvent’, and ‘the dosage time’ was mentioned in. We could promote improvement of the safety and efficiency of procedures by utilizing this system.

[*Int. J. Pharm.,* **368**, 98–102 (2009)] [Labs.of Pharmacy Practice and Social Science]

In vitro and *in vivo* characteristics of prochlorperazine oral disintegrating film.

Misao NISHIMURA, Katuhiko MATSUURA, Tadao TSUKIOKA, Hirotaka YAMASHITA, Naoki INAGAKI, Tadashi SUGIYAMA*, and Yoshinori ITOH

Oral disintegrating film containing prochlorperazine, a dopamine D_2 receptor antagonist with anti-emetic property, was newly developed using microcrystalline cellulose, polyethlene glycol and hydroxypropylmethyl cellulose as the base materials. The uniformity of dosage units of the preparation was acceptable according to the criteria of JP15 or USP27. The film showed an excellent stability at least for 8 weeks when stored at 40 degrees C and 75% in humidity. None of the parameters, including T(max), C(max), area under curves, clearance and steady-state distribution volume at rats was significantly different between oral disintegrating film and oral solution. These findings suggest that the present prochlorperazine-containing oral film is potentially useful to control emesis induced by anti-cancer agents or opioid analgesics in patients who limit the oral intake.

[*Chemotherapy,* **55**, 234–240 (2009)] [Labs.of Pharmacy Practice and Social Science]

Development of computer-assisted biohazard safety cabinet for preparation and verification of injectable anticancer agents

Shinji OKAYASU, Mitsuhiro NAKAMURA, Tadashi SUGIYAMA*, Koichi CHIGUSA, Kiyoshi SAKURAI, Katuhiko MATSUURA, Mayumi YAMAMOTO, Yoshiomi KINOSADA and Yoshinori ITOH.

A computer-assisted biohazard safety cabinet was newly developed for verification and preparation of anticancer agents. Using a barcode reader, information on prescription orders was transmitted from an electronic medical record to the computer system installed in the safety cabinet. The names of anticancer agents were verified using a personal digital assistant and the volume of injection taken, which was automatically converted to weight on the basis of the specific gravity of anticancer solution, was recorded on the computer through a digital scale.

[*Jpn. J. Drug Inform.,* **10**, 304–308 (2009)] [Lab. of Drug Informatics]

Survey of the over-the-counter drug package inserts for cold medicine and analgesic antipyretic drug.

Mitsuhro NAKAMURA*, Tadashi HORIUCHI, Hitomi TERAMACHI, Masafumi KUBOTA, Eiji TAKASHIMA, Yumi KUZUYA, and Teruo TSUCHIYA

In this study, we surveyed the descriptions on the information content of over-the-counter (OTC) drug package inserts for cold medicine and analgesic antipyretic drug. We investigated the description format of OTC package inserts, components of drugs, the presence or absence of described contraindication information and contents of description. OTC package inserts for cold medicine and analgesic antipyretic drug (593 and 216 respectively) were surveyed. The package inserts of non-steroidal anti-inflammatory drugs (NSAIDs) were carefully evaluated. On most package inserts of NSAIDs, contraindication information of acetylsalicylic acid was not written. Since there are many OTC drugs containing NSAIDs showing no warning indication, it is necessary to describe appropriate and clear information on OTC package inserts.

[*Biomed. Chromatogr.*, **23**, 357–364(2009)] [Lab. of Drug Informatics]

Simultaneous determination of benzodiazepines and their metabolites in human serum by liquid chromatography-tandem mass spectrometry using a high resolution octadecyl silica column compatible with aqueous compounds.

Mitsuhiro NAKAMURA*, Tomofumi OHMORI, Yoshinori ITOH, Masato TERASHITA, and Kazuyuki HIRANO

Our new LC-MS/MS method enabled us to determine multiple benzodiazepines, including flurazepam, bromazepam, chlordiazepoxide, nitrazepam, clonazepam, flunitrazepam, estazolam, clobazam, lorazepam, alprazolam, triazolam, brotizolam, fludiazepam, diazepam, quazepam, prazepam and their metabolites such as 7-aminonitrazepam, 7-aminoclonazepam, 7-acetamidonitrazepam, N-desmethylclobazam and N-desmethyldiazepam. The limit of detection ranged from 0.3 to 11.4 ng/mL. Linearity was satisfactory for all compounds. These data suggest that the present method can be applicable to routine assay for benzodiazepines in the clinical setting.

[*World J. Pediatr*, **5,** 316–318 (2009)] [Lab. of Drug Informatics]

Theophylline-associated status epilepticus in an infant: pharmacokinetics and the risk of suppository use.

Zenichiro KATO, Atsushi YAMAGISHI, Mitsuhiro NAKAMURA*, and Naomi KONDO

Theophylline has been widely used to treat asthma, but recent studies have revealed that the possible risks for seizure may result in the revision of the therapeutic guidelines. METHODS: An 8-month-old boy who had been treated with oral sustained-release theophylline and additional aminophylline suppository was hospitalized. A combination of diazepam, lidocaine and thiopental was required to stop his convulsion. RESULTS: The pharmacokinetic study indicated that the usage of a sustained-release formula should not usually be over 15 mg/ml, but the additional use of an aminophylline suppository elevated the concentration to over 20 mg/ml and resulted in the severe adverse effects. CONCLUSION: The parents of children and also physicians should be educated to ensure the proper use of the suppository formula.

[*Jpn. J. Ther. Drug Monit.,* **26**, 125–131 (2009)] [Lab. of Drug Informatics]

Development and evaluation of a TDM management system linked to an electronic medical ordering system.

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We newly developed a therapeutic drug monitoring (TDM) management system linked to an electronic medical ordering system. This system offered the following advantages: 1) the patient's profile, drug profile, laboratory data could be automaticaly downloaded from the electrical medical records. 2) TDM analysis software could be accessed through this system. 3) This system could save TDM reports containing pharmacists' comments and manage the report history. In order to evaluate the efficacy of this system, we compared the time required for TDM analysis with this TDM management to that without this system use. The required time was reduced from 46±6 (Mean±SD) to 16±3 minutes ($p<0.0001$). Using this TDM management system, TDM services have been performed efficiently, and this system can contribute to clinical support.

[*Pediatric Reports,* **1**, 30–31 (2009)] [Lab. of Drug Informatics]

Pediatric thioridazine poisoning as a result of a pharmacy compounding error.

Zenichiro KATO, Mitsuhiro NAKAMURA*, Yuka YAMAGISHI, Takahide TERAMOTO, and Naomi KONDO

A three-year-old boy with bronchitis was prescribed erythromycin by a local clinic, but he started to complain of severe drowsiness and became unconscious. It was decided that this was a result of a compounding error of thioridazine instead of erythromycin owing to their similar commercial name. The thioridazine concentration in the child's serum on admission was two to three times higher than the Cmax for adults with the same dosage. The concentration of the lavage saline on admission was only 0.3% of the ingested amount, indicating that the lavege was not effective in our case. Pharmacokinetic analysis revealed the parameters as Tmax, 1.5hr; Cmax, 1700ng/mL; Ka, 2.01L/hr; Vd, 3.6L/kg; and T1/2, 6.8hr. Further investigations on clinical cases with a pharmacokinetic analysis should be done to confirm the pharmacokinetic evidence obtained here and to give specific therapeutic guidelines for overdose management especially in children.

[*J. Biol. Chem.*, **284**, 10422–10432 (2009)] [Labs. of Drug infomatics]

Sphingosine kinase isoforms regulate oxaliplatin sensitivity of human colon cancer cells through ceramide accumulation and AKT activtion.

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Among nine colon cancer cell lines, sphingosine kinase (SPHK) 1 and 2 activity and protein expression was highest in RKO cells and lowest in HCT116 cells. A viability assay revealed that HCT116 cells were sensitive to the effects of oxaliplatin (l-OHP), whereas RKO cells were resistant to those of l-OHP. The increase in ceramide and caspase activation induced by l-OHP in HCT116 cells was abolished by a sphingomyelinase inhibitor, and in contrast, in RKO cells, an SPHK inhibitor or SPHK silencing suppressed the alterations after l-OHP treatment. In addition, SPHK silencing in RKO cells suppressed Akt phosphorylation induced by l-OHP, suggesting that SPHK and sphingomyelinase regulate chemosensitivity by controlling ceramide formation and Akt pathway.

[*J. Jpn. Health Med. Associ.,* **17**, 10–15 (2009)] [Lab. of Anatomy]

Effects of the Outdoor Activity Experience on the Mood in the University Students.

Haruo SUGIURA*, Hiroko SUGIURA, Maki UEYA, and Etsuo UEYA

The purpose of the present study was to investigate the effects of the outdoor activity experience on the mood in the university students. We assessed the pre- and post-outdoor activity experience mood with the Profile of Mood States (POMS). The vigor score in the post-outdoor activity was higher than that in the pre-outdoor activity. The negative mood score in the pre- outdoor activity was significantly low in the post-outdoor activity. Total Mood Disturbance sore in the post-outdoor activity was significantly lower than that in the pre-outdoor activity. These resuts suggest that outdoor activity experience effectively decreased negative mood score and increased positive mood score in the university students.

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