[*Angew. Chem. Int. Ed. Engl.*, **47**, 6411–6413 (2008)] [Lab. of Pharm. Med. Chemistry]

Synthesis of 2-Arylbenzoxazoles by Copper-Catalyzed Intramolecular Oxidative C-O Coupling of Benzanilides.

Satoshi UEDA,* and Hideko NAGASAWA

A wide variety of functionalized 2-arylbenzoxazoles can be prepared with high functional-group tolerance and regioselectivity by a copper-catalyzed intramolecular oxidative CO coupling of benzanilides. The catalytic cycle is completed by the regeneration of the copper catalyst using molecular oxygen as a terminal oxidant without the need for additives.

[*Cancer Lett.*, **272**, 325–335 (2008)] [Lab. of Pharm. Med. Chemistry]

The Novel Hypoxic Cell Radiosensitizer, TX-1877 Has Antitumor Activity through Suppression of Angiogenesis and Inhibits Liver Metastasis on Xenograft Model of Pancreatic Cancer.

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

In the present study, we investigated the antitumor effect of hypoxic cell radiosensitizer, TX-1877 in inhibiting angiogenesis and liver metastasis on pancreatic cancer xenograft model. In vitro, TX-1877 inhibited the proliferation and potentiated the radiosensitivity of various pancreatic cancer cell lines. In an orthotopic model, tumors from nude mice injected with pancreatic cancer cells and treated with TX-1877 and irradiation showed significant reductions in volume. Quantitative real-time reverse transcription-PCR and immunohistochemical analysis revealed that treatment with TX-1877 alone or with TX-1877 and irradiation inhibited expression of the angiogenic molecules, vascular endothelial growth factor. These treatments also induced apoptosis in cancer cells. These data show that treatment of TX-1877 and irradiation decreased growth of human pancreatic cancer, suppressed angiogenesis and inhibited liver metastasis, leading to prolonged survival.

[*Bioorg. Med. Chem.*, **16**, 8661–8669 (2008)] [Lab. of Pharm. Med. Chemistry]

Synthesis and Biological Activity of 1-Methyl-tryptophan-tirapazamine Hybrids as Hypoxia-targeting Indoleamine 2,3-Dioxygenase Inhibitors.

Hitomi NAKASHIMA, Yoshihiro UTO, Eiji NAKATA, Hideko NAGASAWA,* Kazuhiro IKKYU, Noriko HIRAOKA, Kouichiro NAKASHIMA, Yuki SASAKI, Hiroshi SUGIMOTO, Yoshitsugu SHIRO, Toshihiro HASHIMOTO, Yasuko OKAMOTO, Yoshinori ASAKAWA, and Hitoshi HORI

We have designed and synthesized new hypoxic-neoplastic cells-targeted indoleamine 2,3-dioxygenase (IDO) inhibitors. 1-Methyl-tryptophan (1MT)-tirapazamine (TPZ, 3-amino-1,2,4-benzotriazine 1,4-dioxide) hybrid inhibitors including 1 (TX-2236), 2 (TX-2235), 3 (TX-2228), and 4 (TX-2234) were prepared. All of these compounds were uncompetitive IDO inhibitors. TPZ-monoxide hybrids 1 and 3 showed higher IDO inhibitory activities than TPZ hybrids 2 and 4. These data suggest that TPZ hybrids 2 and 4 may act through their dual biological functions: first, they function as hypoxic cytotoxins in hypoxic cells, and then are metabolized to their TPZ-monoxide (3-amino-1,2,4-benzotriazine 1-oxide) hybrids, which function as IDO inhibitors.

[*Bioorg. Med. Chem*, **16**, 6042–6053 (2008)] [Lab. of Pharm. Med. Chemistry]

Design of Antiangiogenic Hypoxic Cell Radiosensitizers: 2-Nitroimidazoles Containing a 2-Aminomethylene-4-cyclopentene-1,3-dione Moiety.

Yoshihiro UTO, Hideko NAGASAWA,* Cheng-Zhe JIN, Shinichi NAKAYAMA, Ayako TANAKA, Saori KIYOI, Hitomi NAKASHIMA, Mariko SHIMAMURA, Seiichi INAYAMA, Tomoya FUJIWARA, Yoshio TAKEUCHI, Yoshimasa UEHARA, Kenneth L. KIRK, Eiji NAKATA, and Hitoshi HORI

We designed chiral 2-nitroimidazole derivatives containing a 2-aminomethylene-4-cyclopentene-1,3-dione moiety as antiangiogenic hypoxic cell radiosensitizers. We evaluated the antiangiogenic and radiosensitizing effects of the new compounds, along with other biological properties including their activities as hypoxic cytotoxicities and protein tyrosine kinase (PTK) inhibitory activities. Our results show that these chiral 2-nitroimidazole derivatives that contain the 2-aminomethylene-4-cyclopentene-1,3-dione moiety as a potent antiangiogenic pharmacophoric descriptor are promising lead candidates for the development of antiangiogenic hypoxic cell radiosensitizers.

[*Bioorg. Med. Chem.*, **16**, 7705–7714 (2008)] [Lab. of Pharm. Med. Chemistry]

TX-2152: a Conformationally Rigid and Electron-rich Diyne Analogue of FTY720 with *in vivo* Antiangiogenic Activity.

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We designed FTY720 analogues with conformationally rigid and electron-rich acetylenic chains as antiangiogenic agents (the monoyne 1: TX-2148, the diyne 2: TX-2152, the triyne 3: TX-2256). The construction of the acetylenic chain was carried out by an iterative strategy using a Sonogashira cross-coupling reaction and desilylative bromination in two steps. The in vivo antiangiogenic activities of these acetylenic analogues and FTY720 were evaluated by the chick embryo chorioallantoic membrane (CAM) assay and compared to the activities of the known antiangiogenic agent TNP-470. The diyne 2 showed more potent antiangiogenic activity (90% inhibition) than FTY720 (77% inhibition) and other acetylenic analogues (the monoyne 1: 42% inhibition, the triyne 3: 60% inhibition), and TNP-470 (82% inhibition) at a dose of 10 microg/CAM, without showing toxicity. These results indicate that the flexibility of C8 alkyl chain of FTY720 is not required for its antiangiogenic activity. We suggest that the diyne 2 (TX-2152) may be a promising candidate as an antiangiogenic agent for antineoplastic drug discovery.

[*Eur. J. Pharmacol.*, **587**, 296–301 (2008)] [Lab. of Pharm. Med. Chemistry]

Anti-inflammatory Effects of a Bioavailable Compound, Artepillin C, in Brazilian Propolis.

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Artepillin C is the major compound in the Brazilian green propolis from Baccharis dracunculifolia. Our aim in this study was to investigate the anti-inflammatory effects, absorption, and bioavailability of Artepillin C in mice. The animals used were male Swiss mice subjected to: paw oedema by carrageenan (300 microg/paw), carrageenan-induced peritonitis, and prostaglandin E(2) determination. We also measured in vitro nitric oxide production by RAW 264.7 cells and NF-kappaB activity in HEK 293 cells. Finally, we measured the absorption and bioavailability of Artepillin C in plasma from mice by means of GC-MS after a single oral dose (10 mg/kg). Collectively, Artepillin C showed anti-inflammatory effects mediated, at least in part, by prostaglandin E(2) and nitric oxide inhibition through NF-kappaB modulation, and exhibited bioavailability by oral administration.

[*Bioorg. Med. Chem.*, **16**, 675–682 (2008)] [Lab. of Pharm. Med. Chemistry]

Design, Synthesis, and Radiosensitizing Activities of Sugar-hybrid Hypoxic Cell Radiosensitizers

Takashi NAKAE, Yoshihiro UTO, Motoko TANAKA, Haruna SHIBATA, Eiji NAKATA, Masahide TOMINAGA, Hiroshi MAEZAWA, Toshihiro HASHIMOTO, Kenneth L. KIRK, Hideko NAGASAWA,* and Hitoshi HORI

We have designed sugar-hybrid TX-1877 derivatives conjugated with sugar moieties including beta-glucose (beta-Glc), beta-galactose (beta-Gal), alpha-mannose (alpha-Man) and N-acetyl-beta-galactosamine (beta-GalNAc). Compound 1 (TX-1877) was glycosylated with appropriate peracetylated sugars using BF(3)-OEt(2) to give acetylated sugar-hybrids, 5 (TX-2244), 6 (TX-2245), 7 (TX-2246), and 10 (TX-2243). Removal of the acetyl groups afforded the sugar-hybrids having free hydroxyl groups, 11 (TX-2141), 12 (TX-2218), 13 (TX-2217) and 14 (TX-2068). Among these, 5 (TX-2244) is the most active radiosensitizer (ER=2.30). In the present study, we have succeeded in producing sugar-hybrid hypoxic cell radiosensitizers that have an increased radiosensitizing activity that does not depend on increased hydrophobicity.

[*Chem. Biol.*, **15**, 493–500 (2008)] [Lab. of Pharm. Med. Chemistry]

An Uncharged Amine in the Transition State of the Ribosomal Peptidyl Transfer Reaction.

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The ribosome has an active site comprised of RNA that catalyzes peptide bond formation. To understand how RNA promotes this reaction requires a detailed understanding of the chemical transition state. Here, we report the Bronsted coefficient of the alpha-amino nucleophile with a series of puromycin derivatives. Both 50S subunit- and 70S ribosome-catalyzed reactions displayed linear free-energy relationships with slopes close to zero under conditions where chemistry is rate limiting. These results indicate that, at the transition state, the nucleophile is neutral in the ribosome-catalyzed reaction, in contrast to the substantial positive charge reported for typical uncatalyzed aminolysis reactions. This suggests that the ribosomal transition state involves deprotonation to a degree commensurate with nitrogen-carbon bond formation. Such a transition state is significantly different from that of uncatalyzed aminolysis reactions in solution.

[*Bioorg. Med. Chem. Lett.*, **18**, 4124–4127 (2008)] [Lab. of Pharm. Med. Chemistry]

Identification of Novel Non-peptide CXCR4 Antagonists by Ligand-based Design Approach.

Satoshi UEDA,* Manabu KATO, Shinsuke INUKI, Hiroaki OHNO, Barry EVANS, Zi-xuan WANG, Stephen C. PEIPER, Kazuki IZUMI, Eiichi KODAMA, Masao MATSUOKA, Hideko NAGASAWA, Shinya OISHI, and Nobutaka FUJII

The design and synthesis of novel non-peptide CXCR4 antagonists is described. The peptide backbone of highly potent cyclic peptide-based CXCR4 antagonists was entirely replaced by an indole framework, which was expected to reproduce the disposition of the key pharmacophores consistent with those of potential bioactive conformations of the original peptides. A structure-activity relationship study on a series of modified indoles identified novel small-molecule antagonists having three pharmacophore functional groups through the appropriate linkers.

[*Chem. Bio. Chem.*, **9**, 1154–1158 (2008)] [Lab. of Pharm. Med. Chemistry]

Synthesis and Application of Fluorescein- and Botin-labeled Molecular Probes for Chemokine Receptor CXCR4.

Shinya OISHI, Ryo MASUDA, Barry EVANS, Satoshi UEDA,* Yukiko GOTO, Hiroaki OHNO, Akira HIRASAWA, Gozo TSUJIMOTO, Zixuan WANG, Stephen C. PEIPER, Eiichi KODAMA, Masao MATSUOKA, and Nobutaka FUJII

The design, synthesis, and bioevaluation of fluorescence- and biotin-labeled CXCR4 antagonists are described. The modification of D-Lys8 at an epsilon-amino group in the peptide antagonist Ac-TZ14011 derived from polyphemusin II had no significant influence on the potent binding of the peptide to the CXCR4 receptor. The application of the labeled peptides in flow cytometry and confocal microscopy studies demonstrated the selectivity of their binding to the CXCR4 receptor, but not to CXCR7, which was recently reported to be another receptor for stromal cell-derived factor 1 (SDF-1)/CXCL12.

[*Angew. Chem. Int. Ed.*, **47**, 5394–5397 (2008)] [Lab. of Organic Chemistry]

Mild and Efficient H/D Exchange of Alkanes Based on C–H Activation Catalyzed by Rhodium on Charcoal.

Tomohiro MAEGAWA, Yuta FUJIWARA, Yuya INAGAKI, Hiroyoshi ESAKI, Yasunari MONGUCHI, and Hironao SAJIKI*

In the presence of Rh/C in D_2O under H_2 at 160 °C the H/D exchange reaction of unfunctionalized alkanes can easily occur. Inexpensive reagents and mild reaction conditions are used, and fullydeuterated products can be obtained after a simple work up procedure.

[*Chem. Eur. J.*, **14**, 664–673 (2008)] [Lab. of Organic Chemistry]

Efficient and Convenient Heterogeneous Palladium-Catalyzed Regioselective Deuteration at the Benzylic Position.

Takanori KURITA, Kazuyuki HATTORI, Saori SEKI, Takuto MIZUMOTO, Fumiyo AOKI, Yuki YAMADA, Kanoko IKAWA, Tomohiro MAEGAWA, Yasunari MONGUCHI, and Hironao SAJIKI*

The Pd/C-catalyzed efficient and regioselective hydrogen-deuterium (H-D) exchange reaction on the benzylic site proceeded in D_2O in the presence of a small amount of H_2 gas. The use of the Pd/C-ethylenediamine complex [Pd/C(en)] as a catalyst instead of Pd/C led to the efficient deuterium incorporation into the benzylic site of *O*-benzyl protective groups without hydrogenolysis. These H-D exchange reactions provide a post synthetic and D_2-gas-free deuterium-labeling method on a wide variety of benzylic sites using D_2O as the deuterium source and heterogeneous Pd/C or Pd/C(en) as a reusable heterogeneous palladium catalyst under mild and neutral conditions.

[*Adv. Synth. Catal.*, **350**, 406–410 (2008)] [Lab. of Organic Chemistry]

Novel Palladium-on-Carbon/Diphenyl Sulfide Complex for Chemoselective Hydrogenation: Preparation, Characterization, and Application.

Akinori MORI, Tomoteru MIZUSAKI, Masami KAWASE, Tomohiro MAEGAWA, Yasunari MONGUCHI, Shinobu TAKAO, Yukio TAKAGI, and Hironao SAJIKI*

A diphenyl sulfide immobilized on palladium-on-carbon system, Pd/C[Ph_2S], was developed to achieve the highly chemoselective hydrogenation of alkenes, acetylenes, azides, and nitro groups in the presence of aromatic ketones, halides, benzyl esters, and N-Cbz protective groups. Instrumental analyses of the heterogeneous catalyst demonstrated that diphenyl sulfide was embedded on Pd/C *via* coordination of its sulfur atom to palladium metal or physical interaction with graphite layers of the activated carbon. The catalyst could be recovered and reused at least five times without any significant loss of the reactivity.

[*Chem. Eur. J.*, **14**, 3371–3379 (2008)] [Lab. of Organic Chemistry]

Facile and Convenient Method of Deuterium Gas Generation Using a Pd/C-Catalyzed H_2-D_2 Exchange Reaction and Its Application to Synthesis of Deuterium-Labeled Compounds.

Takanori KURITA, Fumiyo AOKI, Takuto MIZUMOTO, Toshihide MAEJIMA, Hiroyoshi ESAKI, Tomohiro MAEGAWA, Yasunari MONGUCHI, and Hironao SAJIKI*

The Pd/C-catalyzed H_2-D_2 exchange reaction using a H_2-D_2O combination provided a general, efficient and environmentally friendly route for the preparation of deuterium gas (D_2). H_2 sealed in a reaction flask was converted into nearly pure D_2, which could be used for the Pd/C-catalyzed one-pot reductive deuteration of various reducible functionalities and the chemoselective one-pot deuterogenation of olefin and acetylene. Additionally, we established the capturing method of the generated D_2 in a balloon, which was successfully applied to the Pd/C-catalyzed reductive mono-*N*-alkylation of a primary amine using nitrile as the alkylating reagent.

[*Chem. Eur. J.*, **14**, 5109–5111 (2008)] [Lab. of Organic Chemistry]

Partial Hydrogenation of Alkynes to *cis*-Olefins by Using a Novel Pd^0-Polyethyleneimine Catalyst.

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

Various mono and disubstituted alkynes were partially hydrogenated to give cis-olefins with high selectivity using a 5% Pd(0)-polyethyleneimine complex as a catalyst without additives

[*Synthesis*, 1467–1478 (2008)] [Lab. of Organic Chemistry]

H-D Exchange Reaction Taking Advantage of the Synergistic Effect of Heterogeneous Palladium and Platinum Mixed Catalyst.

Nobuhiro ITO, Tsutomu WATAHIKI, Tsuneaki MAESAWA, Tomohiro MAEGAWA, and Hironao SAJIKI*

An effective and applicable deuteration method for alkyl-substituted aromatic compounds using a heterogeneous Pd/C and Pt/C mixed catalyst in deuterium oxide in the presence of a small amount of hydrogen gas was developed. Mixing a heterogeneous palladium and platinum catalyst provides an interesting synergistic effect in the H-D exchange reaction and leads to full H-D exchange results even on sterically hindered sites, which indicated only low-deuterium efficiencies when either Pd/C or Pt/C were used independently as a catalyst. We investigated the synergistic effect using a variety of substrates and proved the broad generality of the heterogeneous Pd-Pt-D_2O-H_2 system in the H-D exchange reaction. Furthermore, this system could be applied to a multigram scale synthesis of useful deuterium-labeled compounds, such as deuterium-labeled bis-aniline derivatives as raw materials for polyimides, aryl iodides as synthetic building blocks, and biologically active compounds.

[*Appl. Catal. B: Environmental.*, **81**, 274–282 (2008)] [Lab. of Organic Chemistry]

Pd/C-Catalyzed Practical Degradation of PCBs at Room Temperature.

Akira KUME, Yasunari MONGUCHI, Kazuyuki HATTORI, Hisamitsu NAGASE, and Hironao SAJIKI*

The catalytic degradation method of polychlorinated biphenyls (PCBs) using the palladium on activated carbon–triethylamine (Pd/C–Et_3N) system under ambient hydrogen pressure and temperature was developed. Aroclor® 1254, Aroclor® 1248, 10% Aroclor® 1254 in paraffin oil and PCBs from capacitor could be completely dechlorinated to afford biphenyl and Et_3N·HCl. Fifteen pure PCB congeners, including the highly toxic co-planar PCBs, were smoothly dechlorinated to biphenyl within 1 or 2 h using 10% Pd/C (10% of substrate weight) and Et_3N (1.2 equiv. vs. Cl numbers). However, the dechlorination of the fully *ortho*-substituted PCB congeners was delayed and chlorine atoms on the *ortho*-positions still remained under the hydrogenation conditions, but these PCB congeners are only slightly present in the commercial PCB mixture. The Pd/C–Et_3N–H_2 system offers a simple, safe, and inexpensive degradation method of PCBs under mild reaction conditions.

[*Bull. Chem. Soc Jpn.*, **81**, 278–286 (2008)] [Lab. of Organic Chemistry]

Efficient and Selective Pt/C-Catalyzed H–D Exchange Reaction of Aromatic Rings.

Nobuhiro ITO, Hiroyoshi ESAKI, Tsuneaki MAESAWA, Eikoh IMAMIYA,
Tomohiro MAEGAWA, and Hironao SAJIKI*

An effective and applicable deuteration method for aromatic rings using Pt/C–D_2O–H_2 system was established. Especially, phenol was fully deuterated even at room temperature, and other electron-rich aromatic nuclei were efficiently deuterated under mild conditions. The scope and limitations of the presence method and its application to the synthesis of deuterium-labeled biologically active compounds and deuterium-labeled building blocks for practical multi-gram scale syntheses are reported.

[*Chem. Eur. J.*, **14**, 6994–6999 (2008)] [Lab. of Organic Chemistry]

Ligand-Free Sonogashira Coupling Reactions with Heterogeneous Pd/C as the Catalyst.

Shigeki MORI, Takayoshi YANASE, Satoka AOYAGI, Yasunari MONGUCHI,
Tomohiro MAEGAWA, and Hironao SAJIKI*

A variety of aryl iodides were coupled with aromatic and aliphatic terminal alkynes to give the corresponding 1,2-disubstituted aromatic alkynes in good yields by using only 0.4 mol % of the heterogeneous 10% Pd/C as the catalyst without a ligand, copper salt, or amine in an aqueous medium.

[*Synlett*, 2291–2294 (2008)] [Lab. of Organic Chemistry]

Pd/C-Catalyzed Direct α-Oxygenation of 1,3-Dicarbonyl Compounds Using Molecular Oxygen.

Yasunari MONGUCHI, Tohru TAKAHASHI, Yusuke IIDA, Yuta FUJIWARA, Yuya INAGAKI, Tomohiro MAEGAWA, and Hironao SAJIKI*

A hydroxyl group was readily and directly introduced into the α-position of a variety of β-dicarbonyl compounds by heterogeneous Pd/C-catalyzed oxygenation using molecular oxygen.

[*Adv. Synth. Catal.*, **350**, 2215–2218 (2008)] [Lab. of Organic Chemistry]

A Convenient and Effective Method for the Regioselective Deuteration of Alcohols.

Tomohiro MAEGAWA, Yuta FUJIWARA, Yuya INAGAKI, Yasunari MONGUCHI, and Hironao SAJIKI*

The convenient and regioselective deuteration of hydroxy groups on vicinal carbons was achieved by the combination of 5% ruthenium on carbon (Ru/C), hydrogen gas and deuterium oxide (D_2O).

[*Adv. Synth. Catal.*, **350**, 2767–2777 (2008)] [Lab. of Organic Chemistry]

Evaluation of Aromatic Amination Catalyzed by Palladium on Carbon: A Practical Synthesis of Triarylamines.

Yasunari MONGUCHI, Katsunori KITAMOTO, Takashi IKAWA, Tomohiro MAEGAWA, and Hironao SAJIKI*

A heterogeneous palladium on carbon (Pd/C)-catalyzed coupling between amines and aromatic halides including aromatic chlorides has been achieved using sodium *tert*-butoxide (NaO-*t*-Bu) and 1,1'-bis(diphenylphosphino)ferrocene (dppf) as a ligand in cyclopentyl methyl ether (CPME). The use of potassium *tert*-butoxide (KO-*t*-Bu) in place of NaO-*t*-Bu brought about the benzyne-mediated aromatic amination even without Pd/C and dppf, giving a mixture of regioisomers when 4-substituted bromobenzenes were employed as the substrate. The combination of Pd/C, dppf, NaO-*t*-Bu could be utilized for the syntheses of a broad range of triarylamines by replacing CPME with mesitylene which can provide a higher reaction temperature. The Pd/C could be quantitatively recovered and reused until at least the fourth cycle without any loss in catalytic activity. The quite low leaching of palladium (<1.1%) was demonstrated by an inductively coupled plasma-atomic emission spectrometric analysis.

[*Synlett*, 2811–2814 (2008)] [Lab. of Organic Chemistry]

Alternative I-D Exchange Reaction on Pyrimidine and Purine Nuclei Mediated by Tributyltin Hydride Using THF-d_8 as a Deuterium Source.

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

A method for the regioselective deuteration of pyrimidine and purine rings mediated by Bu_3SnH using THF-d_8 as a deuterium source on the basis of a radical reaction was developed.

[*Synlett*, 675–678 (2008)] [Lab. of Synthetic Chemistry]

Aerobic Photooxidation of Benzylamide in the Presence of Catalytic Iodine

Hiroki Nakayama, and Akichika Itoh*

Benzylamides were found to be oxidized to the corresponding imides in the presence of catalytic iodine under photoirradiation. This oxidation is a facile and convenient method in the view point of synthetic organic chemistry.

[*Tetrahedron Lett.*, **49**, 2792–2794 (2008)] [Lab. of Synthetic Chemistry]

Aerobic Photo-decarboxylation of α-Hydroxy Carboxylic Acid Derivatives under Visible Light Irradiation in the Presence of Catalytic Iodine

Hiroki Nakayama, and Akichika Itoh*

A catalytic amount of iodine enables us to carry out aerobic photo-decarboxylation of α-hydroxy carboxylic acid derivatives to the corresponding carboxylic acids or ketones selectively in high yields under irradiation of VIS. This new oxidation is interesting in keeping with the notion of Green Chemistry due to the non-use of heavy metals and halogenated solvents, waste reduction, and use of molecular oxygen.

[*Chem. Pharm. Bull.*, **56**, 921–925 (2008)] [Lab. of Pharm. Physical Chemistry]

Plasma-Assisted Immobilization of Heparin onto Low-Density Polyethylene Surface.

Shin-ichi KONDO,* Yasuyo FUKUNAGA, Michinori OIKAWA, Yasushi SASAI, and Masayuki KUZUYA.

In this study heparin was covalently immobilized onto LDPE-VEMAC sheet fabricated by the introduction of carboxyl groups to the surface of low-density polyethylene (LDPE) using a plasma technique. When heparin was directly immobilized on the LDPE-VEMAC sheet, the density of the immobilized heparin depended on that of the carboxyl groups. Heparin was also immobilized with a spacer, hexamethylene diamine, and the density of such heparin was about 1.6 times that of the directly immobilized heparin. This result suggests that the introduction of a spacer may be an effective way to increase the density of immobilized heparin.

[*Surf. Coat. Technol.*, **202**, 5724–5727 (2008)] [Lab. of Pharm. Physical Chemistry]

Introduction of Carboxyl Group onto Polystyrene Surface Using Plasma Techniques.

Yasushi SASAI,* Natsuko MATSUZAKI, Shin-ichi KONDO, and Masayuki KUZUYA

We report the methods to introduce a large amount of carboxyl group onto polystyrene (PS) surface using plasma techniques. The method involves the immobilization of vinylmethylether-maleic anhydride copolymer (VEMA) on PS by plasma-induced crosslink reaction of PS, followed by hydrolysis of the maleic anhydride moiety to generate carboxyl group. The resultant PS immobilizing vinylmethylether-maleic acid copolymer (VEMAC) was characterized by water-contact angle measurement, X-ray photoelectron spectroscopy (XPS). The surface wettability was significantly improved as compared with that for non-treated PS surface, and remained nearly unchanged at a low level, close to the initially acquired value.

[*J. Photopolym. Sci. Technol.*, **21**, 277–280 (2008)] [Lab. of Pharm. Physical Chemistry]

Surface Engineering of Polystyrene Dish for Improvement of Cell Adhesion Using Plasma Techniques.

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

We have previously reported the preparation of polystyrene (PS) dish immobilizing vinylmethylether-maleic acid copolymer (VEMAC) (PS/ VEMAC) using plasma techniques to introduce carboxyl groups on the hydrophobic PS. In this study, we exmanined the effect of plasma conditions on the surface density of carboxyl group on PS/VEMAC and the application of PS/VEMAC to a cell culture substrate. The density of carboxyl group on PS/VEMAC was well-controlled by plasma conditions. The cell adhesion and proliferation were significantly enhanced on PS/VEMAC with the highest density of carboxyl group, prepared under the present plasma conditions, as compared with that on the non-treated PS. These results suggested a good cell-compatibility of VEMAC immobilized on PS.

[*Carbohydrate Polymers*, **71**, 324–329 (2008)] [Lab. of Pharm. Engineering]

Atomic Force Microscopy Imaging of Novel Self-assembling Pectin-liposome Nanocomplexes.

Pornsak SRIAMORNSAK, Nartaya THIRAWONG, Jurairat NUNTHANID,
Satit PUTTIPIPATKHACHORN, Jringjai THONGBORISUTE, and Hirofumi TAKEUCHI*

Self-assembling pectin liposome nanocomplexes (PLNs) were prepared by a simple mixing of cationic liposomes with pectin solution. Nanostructures of liposomes, pectin, and PLNs were observed by atomic force microscopy (AFM). The AFM images of pectin show a chain-like structure with a small number of branches while those of liposomes show a spherical form. The AFM images also provided a direct evidence for association of cationic liposomes on the pectin chain. This was confirmed by the FTIR analysis.

[*J. Controlled Release*, **125**, 236–245 (2008)] [Lab. of Pharm. Engineering]

Improved Intestinal Absorption of Calcitonin by Mucoadhesive Delivery of Novel Pectin-liposome Nanocomplexes.

Nartaya THIRAWONG, Jringjai THONGBORISUTE, Hirofumi TAKEUCHI,* and Pornsak SRIAMORNSAK

Self-assembling pectin-liposome nanocomplexes (PLNs) were prepared by a simple mixing of cationic liposomes with pectin solution, in order to improve intestinal absorption of calcitonin (eCT). Both in-vitro and in-vivo evaluations for PLNs were evaluated. The results showed that average particle size of PLNs was significantly larger than that of initial cationic liposomes. The eCT-loaded PLNs demonstrated a strong pharmacological action over the eCT solution and eCT-loaded liposomes, in which an enhanced and prolonged reduction in plasma calcium concentration of rats was observed. This was attributed to the ability of pectin to adhere to the mucus layer and prolong retention in the intestinal mucosa.

[*Adv. Drug Deliv. Rev.*, **60**, 388–398 (2008)] [Lab. of Pharm. Engineering]

Particle Design of Poorly Water-soluble Drug Substances Using Supercritical Fluid Technologies.

Takehiko YASUJI, Hirofumi TAKEUCHI,* and Yoshiaki KAWASHIMA

In order to improve the dissolution properties of poorly water soluble drugs, various drugs were either subjected to micronization or prepared as composite particles using supercritical fluid (SCF) technology with carbon dioxide (CO_2). Solubility in CO_2 is key for using this method. Solubility affects the supersaturation of the materials in the solvent, as well as the mass transfer of that solvent, which are both critical to the micronization of the materials and the formation of composite particles. The problems posed by the characteristics of the drug itself can be addressed by combining SC-CO_2 with other technologies, such as the formation of coacervates or emulsions, and other equipment types, such as milling or ultrasound fields. Another advantage of SCF technology is that it is considered to be green chemistry. SC-CO_2 can improve the solubility of poorly water-soluble drug substances using few or no organic solvents, and with little or no heating.

[*Int. J. Pharmaceutics*, **354**, 204–209 (2008)] [Lab. of Pharm. Engineering]

Evaluation of Mucoadhesiveness of Polymers by BIACORE Method and Mucin-particle Method.

Jringjai THONGBORISUTE, and Hirofumi TAKEUCHI*

To evaluate the reliability of the BIACORE method as a useful method for measuring the mucoadhesive interaction between chitosan and mucin, the mucin-particle method was used for comparison. In this study, the adhesivities of different-molecular-weight chitosans (chitosan Mw. 150,000, CS; low-molecular-weight chitosan, LCS) and hydrophobically modified chitosans (dodecylated CS, d-CS; dodecylated LCS, d-LCS) to mucin were determined. The BIACORE method showed that CS, LCS and d-CS could interact with mucin based on the increased RU response after mucin was passed over the chitosans-immobilized sensor chip surface. The results from both BIACORE and the mucin-particle method implied that hydrophobic modification of chitosan reduced its adhesivity to mucin. The results from these two methods corresponded well. Therefore, the BIACORE method has promise as an alternative method for evaluating the adhesivity of adhesive polymers to mucin.

[*Int. J. Pharmaceutics,* **354**,174–179 (2008)] [Lab. of Pharm. Engineering]

Effect of Surface Properties of Liposomes Coated with a Modified Polyvinyl Alcohol (PVA-R) on the Interaction with Macrophage Cells.

Koji NAKANO, Yuichi TOZUKA, and Hirofumi TAKEUCHI*

The purpose of this study was to investigate the effect of a polymer coating using modified polyvinyl alcohol (PVA-R) on the interaction between liposomes and macrophage cells (J774 cells). The PVA-R-coated liposomes, which were labeled with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocianin perchlorate (DiI) as a fluorescence reagent, were prepared with the conventional hydration method followed by extrusion and surface modification with PVA-R. When liposomes with or without PVA-R coating were incubated with J744 cells, the fluorescence emission intensity of DiI from J774 cells was significantly smaller than in the case of non-coated liposomes. These *in vitro* tests explained the differences in blood circulation of polymer-coated liposomes having different lipid formulations in rats.

[*Int. J. Pharm.,* **355**, 203–209 (2008)] [Lab. of Pharm. Engineering]

A Novel Method for Measuring Elasticity of Submicron-size Liposomes with Atomic Force Microscopy.

Koji NAKANO, Yuichi TOZUKA, Hirofumi TAKEUCHI,* Hiromitsu YAMAMOTO, and Yoshiaki KAWASHIMA

There are many useful colloidal drug delivery systems that use liposomes. The rigidity of the carrier particle is one of the most important properties affecting drug delivery effectiveness, assessed by particle stability, release profile of encapsulated drug, and blood circulation time. However, it is difficult to evaluate the rigidity of such fine particles; so far, no useful methods have been reported.We demonstrate a unique method to evaluate the rigidity of liposomes using atomic force microscopy (AFM) and dynamic light scattering (DLS) in this report. We showed that the combination of two types of particle-size measurements, tapping mode AFM in buffer solution with another conventional method such as DLS, is useful for evaluating the rigidity of submicron-size particles such as liposomes.

[*Int. J. Pharm.*, **357**, 280–285 (2008)] [Lab. of Pharm. Engineering]

Cyclodextrins as Stabilizers for the Preparation of Drug Nanoparticles by the Emulsion Solvent Diffusion Method.

Abdallah MAKHLOF, Yuta MIYAZAKI, Yuichi TOZUKA, and Hirofumi TAKEUCHI*

Cyclodextrins (CyDs) were employed as protective stabilizers for the preparation of surfactant-free nanocrystals of indomethacin (IMC) by using the emulsion solvent diffusion method. The effect of changing the type and concentration of CyDs on the formation of IMC nanocrystals was investigated. Dispersions were freeze-dried to characterize the size, shape, nanoparticle yield, crystallinity, and dissolution behavior of the obtained particles. Submicron-sized particles of IMC with average diameters in the range of 300–500 nm were obtained by incorporating α-, β-,or γ-CyD in the outer phase of the primary emulsions. Quantitative determination demonstrated that more than 80% of IMC was recovered as fine particles smaller than 0.8 μm. A significant enhancement in the dissolution rate of IMC nanocrystals was observed when compared to the commercial powder.

[*Drug Devel. Ind. Pharm.*, **17**, 1–7 (2008)] [Lab. of Pharm. Engineering]

Development and *in vitro* Characterisation of Liposomes Coated with Thiolated Poly(acrylic acid) for Oral Drug Delivery.

Martin WERLE, Herbert HOYER , Kohei HIRONAKA, and Hirofumi TAKEUCHI*

Mucoadhesive drug delivery systems offer promising opportunities for oral drug delivery. 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. Size, zeta potential, amount of free thiol groups, aggregation behavior, drug-loading, and drug release of these novel carriers were evaluated. A switch of the initial positive zeta potential to a negative value after coating indicated the successful coating procedure. 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.

[*Exp. Rev. Clin. Pharmacol.*, **1**, 429–440 (2008)] [Lab. of Pharm. Engineering]

New Generation Efflux Pump Inhibitors.

Martin WERLE, Hirofumi TAKEUCHI,* and Bernkop-Schnürch ANDREWS

The development of novel efflux pump inhibitors is an emerging and challenging research field. Besides the use of such excipients in cancer therapy, they are gaining increasing interest in drug delivery. In particular, inhibition of efflux pumps located in the intestine and the blood brain barrier offers promising prospects. Nowadays, third generation inhibitors such as elacridar, zosuquidar, laniquidar, OC144-093 and tariquidar have been evaluated in clinical trials. Apart from these small molecular inhibitors which will be discussed within the current review, a focus has been set on polymeric- and polymer based inhibitors including poly(ethylene glycols) and derivatives, poloxamers and thiomers.

[*Chem. Pharm. Bull.*, **56**, 1412–1416 (2008)] [Lab. of Pharm. Engineering]

Slow Release of Tetracycline from a Mucoadhesive Complex with Surcralfate for Eradication of *Helicobacter pylori*

Syoichi HIGO, Hirofumi TAKEUCHI,* Hiromitsu YAMAMOTO, Tomoaki HINO, and Yoshiai KAWASHIMA

Retention of antibiotics on the gastric mucosa has shown to be effective for the eradication of *Helicobacter pylori* (*H. pylori*), which resides on the surface of the gastric mucosa. Treatment composed of a gastric mucoadhesive antibiotic with slow release drug delivery has proven to be an effective therapy. Change in the zeta potential of the acidic complex particles seems useful for clarifying the release mechanisms of tetracycline. The data indicated that immediate release of tetracycline in early stage of the test was indispensable to the following paste formation for its slow release. If administrated orally, the acidic complex rapidly adheres to the gastric mucosa with long-term sustained release of the tetracycline to the gastric lumen or mucus layer. This antibiotic delivery mechanism, which requires only a minimum dosage, may be effective for efficient eradication of *H. pylori*.

[*J. Drug Del. Sci. Tech.*, **18**, 375–386 (2008)] [Lab. of Pharm. Engineering]

Mucoadhesive Drug Carriers and Polymers for Effective Drug Delivery

Abdallah MAKHLOF, Martin WERLE, and Hirofumi TAKEUCHI*

The consept of mucoadhesion for the design of non-invasive drug delivery systems has gained increasing attention in recent years.Within the current review, various mucoadhesive polymers, including poly(acrylates), chitosan, thiolated polymers and others, as well as their use in mucosal drug delivery are discussed. An emphasis has been put on the development of mucoadhesive formulations, and in particular on recently developed micro-and nanoparticulate systems. Moreover, the applications of the mucoadhesive carrier systems for different administration routes such as the oral, nasal, pulmonary and ocular route are discussed.

[*Adv. Drug Deliv. Rev.*, **60**, 328–338 (2008)] [Lab. of Pharm. Engineering]

Supercritical Carbon Dioxide Processing of Active Pharmaceutical Ingredients for Polymorphic Control and for Complex Formation.

Kunikazu MORIBE, Yuichi TOZUKA,* and Keiji YAMAMOTO

Supercritical fluid technique have been exploited in extraction, separation and crystallization processes. In the field of pharmaceutics, supercritical carbon dioxide (scCO2) has been used for the purpose of micronization, polymorphic control, and preparation of solid dispersion and complexes. Particle design of active pharmaceutical ingredients is important to make the solid dosage forms with suitable physicochemical properties. Control of the characteristic properties of particles, such as size, shape, crystal structure and morphology is required to optimize the formulation. For solubility enhancement of poorly water-soluble drugs, preparation of the solid dispersion or the complexation with proper drugs or excipients should be a promising approach. This review focuses on aspects of polymorphic control and complexation behavior of active pharmaceutical ingredients by scCO2 processing.

[*Int. J. Pharm.*, **352**, 309–316 (2008)] [Lab. of Pharm. Engineering]

Formation Mechanism of Colloidal Nanoparticles Obtained from Probucol/PVP/SDS Ternary Ground Mixture.

Adchara PONGPEERAPAT, Chalermphon WANAWONGTAI, Yuichi TOZUKA,*
Kunikazu MORIBE, and Keiji YAMAMOTO

The purpose of this study was to investigate the formation mechanism of colloidal nanoparticles after dispersion of probucol/polyvinylpyrrolidone (PVP)/sodium dodecyl sulphate (SDS) ternary ground mixture (GM) into water. SEM images confirmed the presence of 20 nm size primary particles in the GMpowder of probucol/PVP K17/SDS. Spherical nanoparticles with a size of around 100 nm, formed after dispersion of the GM into water, suggested an agglomeration of the primary particles. ^{13}C NMR results suggested that intermolecular interactions between PVP K12 and SDS did not reach the same level as the interactions between PVP K17 and SDS.

[*Drug Dev. Ind. Pharm.*, **34**, 314–322 (2008)] [Lab. of Pharm. Engineering]

Formation, Physical Stability and in vitro Antimalarialactivity of Hihydroartemisinin Nanosuspensions Obtained by Co-grinding Method.

Jiraporn CHINGUNPITAK, Satit PUTTIPIPATKACHORN, Porntip Chavalitshewinkoon-PETMITR,
Yuichi TOZUKA,* Kunikazu MORIBE, and Keiji YAMAMOTO

The formation of drug nanoparticles from binary and ternary mixtures, consisting of dihydroartemisinin (DHA), a poorly water-soluble antimalarial drug, with water-soluble polymer and/or surfactant was investigated. Nanosuspension was successfully formed after dispersing ternary ground mixtures or DHA/NaDC ground mixtures in water. Atomic force microscopy and transmission electron microscopy with selected area diffraction indicated that DHA in nanosuspension was existed as nanocrystals. The obtained nanosuspensions had higher in vitro antimalarial acitivity against Plasmodium falciparum than microsuspensions.

[*Drug Dev. Ind. Pharm.*, **34**, 609–617 (2008)] [Lab. of Pharm. Engineering]

Micronization of Dihydroartemisinin by Rapid Expansion of Supercritical Solutions.

Jiraporn CHINGUNPITAK, Satit PUTTIPIPATKACHORN, Yuichi TOZUKA,*
Kunikazu MORIBE, and Keiji YAMAMOTO

The aim of this study was to prepare fine particles of antimalarial drug dihydroartemisinin (DHA) by rapid expansion of supercritical solutions (RESS) using carbon dioxide as supercritical fluid. In the RESS process, drug particles were prepared by varying processing conditions, including extraction condition, pre-expansion condition, nozzle diameter, nozzle temperature, and collecting distance. The particle size of drug was related to the solubility of drug in supercritical CO_2 at each processing condition. The fine particles of DHA (about 1 μm) with narrow size distribution could be obtained at extraction pressure of 18 MPa and extraction temperature of 32°C, which was closed to the critical temperature of supercritical CO_2 whereas broad size distribution was obtained at extraction temperature of 60°C. The results revealed that RESS process is applicable for micronization of DHA.

[*Pharm. Dev. Tech.*, **13**, 541–547 (2008)] [Lab. of Pharm. Engineering]

Physicochemical, Morphological and Therapeutic Evaluation of Agarose Hydrogel Particles as a Reservoir for Basic Fibroblast Growth Factor

Kunikazu MORIBE, Natsuko NOMIZU, Shunsuke IZUKURA, Yuichi TOZUKA,* Manabu SAKURAI,
Atsushi ISHIDA, Hirofumi NISHIDA, Masaru MIYAZAKI, and Keiji YAMAMOTO

Micron-sized agarose hydrogel particles were prepared using an emulsification/gelation method as a controlled release reservoir for basic fibroblast growth factor (bFGF). Mean particle size of agarose hydrogel particles decreased with an increase in stirring speed and/or temperature of the oil phase before the cooling. Porous polymer matrix structure was observed in the hydrogel particles by cryo-SEM. More than 99% of bFGF was encapsulated and the release from the agarose hydrogel particles was less than 3% during the incubation in phosphate buffered saline. bFGF molecules were not only adsorbed on the particle surface but also permeated and retained within the matrix. The therapeutic efficacy of bFGF retained in agarose hydrogel particles was significantly higher than that dissolved in saline. Agarose hydrogel particle seems to be a potential candidate for a bFGF reservoir.

[*J. Sep. Sci.*, **31**, 735–740 (2008)] [Lab. of Pharm. Anal. Chem.]

Human Liver Dihydrodiol Dehydrogenase 1-catalyzed Reaction Generating 9α,11β-prostaglandin F_2 from Prostagrandin D_2 Followed by Micellar Electrokinetic Chromatography.

Shinsuke INAGAKI, Yukihiro ESAKA,* Yoshihiro DEYASHIKI, Bunji UNO, Akira HARA, and Toshimasa TOYO'OKA

An Enzyme reaction converting PGD2 to 9α, 11β-PGF_2 by a human liver-originated recombinant DD1 has been studied using CE. Four PGs: PGD_2, 9α,1β-PGF_2, PGE_2, and PGF_2α were completely separated by using SDS as a buffer additive. The pH dependence and the dependence of reaction temperature on the enzyme activity have been studied. The present method enabled us to detect all of the participants of the enzyme reaction: PGD_2, 9α,11β-PGF_2, NADPH and $NADP^+$. Thus, direct, comprehensive and reliable analysis of the enzyme reaction is possible, while the enzyme activity has been estimated indirectly with decrease of fluorescence derived from NADPH as an index of progress of the enzyme reactions in batch methods employed in conventional studies. In addition, the small sample consumption as a nature of CE should be a significant advantage of the present method in characterization of less commonly available enzymes such as the recombinant one in this work.

[*Bunseki Kagaku*, **57**, 961–968 (2008)] [Lab. of Pharm. Anal. Chem.]

Separation Analysis of Reactive Chemical Species by Non-Aqueous Capillary Electrophoresis.

Yukihiro ESAKA,* Noriko OKUMURA, and Bunji UNO

We have developed methods for separation analysis of highly reactive chemicals such as organic radicals by non-aqueous capillary zone electrophoresis（CZE）. Five anion radicals and a dianion of quinoide compounds, generated by electrolysis in an acetonitrile solutions under anaerobic conditions, was successfully electrophoresed to be detected as single components using the acetonitrile solutions as a separation matrix. We studied effects of solvated O_2 and water contents in running solutions on detection of the reactive species to optimize analysis conditions. We also investigated manners to transfer the reactive chemicals from the generation cell to the separation capillary not to be decomposed during the transfers. Finally, we developed an on-line generator which enables us fast completion of electrolysis and following quick transfer of the generated species to CE separation systems without any contact with outside compounds such as air. Employing the present methods, we also studied hydrogen-bonding interactions between the radical anions and hydrogen-donating reagents occurring in the separation systems.

[*Seibutsu-butsuri-kagaku*, **52**, 161–166 (2008)] [Lab. of Pharm. Anal. Chem.]

Gradient Micellar Electrokinetic Chromatography.

Yukihiro ESAKA,* and Bunji UNO

In the present method, electroosmotic flow was suppressed almost completely and thus, micelles in a running solution of the inlet vial were introduced in turn into the capillary during operations. We represented MEKC separations of unsubstituted benzoate and nine substituted benzoates as model organic anions using mixed systems of CTAC as a cationic surfactant and Tween 20 or Brij 35 as non-ionic surfactants possessing polyoxyethylene chains (POE-NSs). In a pure CTAC system, the synergistic influences of attractive electrostatic and hydrophobic interactions gave rise to quite large retention factors of many of the benzoate anions, resulting in their co-elution. Addition of an adequate amount of the POE-NSs to the pure CTAC system decreased the electrostatic interaction significantly to give remarkably improved separation of the analytes, but long analysis time was required. Surfactant gradient methods decreasing the concentration of POE-NSs in the mixed systems were useful to decrease analysis time and to improve separation simultaneously.

[*J. Androl.*, **29**, 207–212 (2008)] [Lab. of Pharmaceutics]

Downregulation of Thymosin β4 Expression by Androgen in Prostate Cancer LNCaP Cells.

Kazuhiro IGUCHI, Mai ITO, Shigeyuki USUI, Atsushi MIZOKAMI, Mikio NAMIKI, and Kazuyuki HIRANO*

Androgen-ablation therapy is an effective treatment for advanced prostate cancer, but the tumor often progresses toward a more aggressive phenotype. We determined the changes in genes associated with the malignant progression, and found increased thymosin β4, involved in tumor metastasis, in androgen-sensitive LNCaP cells grown in the medium with androgen-deficient charcoal-stripped FCS. Androgen receptor antagonist bicalutamide inhibited thymosin β4 expression in a dose-dependent manner in LNCaP cells. In androgen receptor-negative PC-3 cells, no significant effects on thymosin β4 gene expression were observed. The regulation of thymosin β4 mRNA expression by androgen is due to the transcriptional activation, and deletion analysis revealed that the region between -83 bp and -46 bp of thymosin β4 gene is responsible for the regulation of the transcriptional activity by androgen. From these results, thymosin β4 expression is negatively controlled at the transcriptional level by androgen.

[*Eur. J. Pharmacol.*, **588**, 26–32 (2008)] [Lab. of Pharmaceutics]

Regulation of Aquaporin 3 Expression by Magnesium Ion.

Masashi OKAHIRA, Masafumi KUBOTA, Kazuhiro IGUCHI, Shigeyuki USUI, and Kazuyuki HIRANO*

For understanding the actions of magnesium formulations, magnesium oxide and magnesium sulfate as a constituent of antacid, in the gastrointestinal tract, the effect of magnesium ion on the water channel aquaporin 3 (AQP3) known to be permeable mainly to water and glycerol was investigated in Caco-2 cells. The mRNA and protein of aquaporin 3 were found to increase significantly after treatment with magnesium acetate. Inhibitors for signal transducers, MDL-12330A, H-89, U0126, and Ro 31-8220, were shown to repress the increase in expression of the mRNA. siRNA for the cAMP response element binding protein (CREB) sequence located between bp -404 and –190 counteracted the magnesium ion-mediated activation of aquaporin 3 transcription. These results suggest that signal transducers, adenylyl cyclase , PKA, MEK1/2, and MSK1, were involved in the signaling pathway for regulating transcription of the aquaporin 3 gene and CREB is one of the transcriptional regulators for aquaporin 3 gene expression mediated by magnesium ion.

[*Biochem. Biophyis. Res. Commun.*, **373**, 613–617 (2008)] [Lab. of Pharmaceutics]

Membrane Trafficking of Aquaporin 3 Induced by Epinephrine.

Hideyuki YASUI, Masafumi KUBOTA, Kazuhiro IGUCHI, Shigeyuk USUI, Tadashi KIHO, and Kazuyuki HIRANO*.

We investigated the membrane trafficking of AQP3 induced by epinephrine in Caco-2 cells to clarify the digestive absorption of glycerol permeated by AQP3. Epinephrine was found to promote within 60 min the translocation of AQP3 from the cytoplasmic fraction to the plasma membrane. This increased trafficking of AQP3 was suppressed by phospholipase C and protein kinase C (PKC) inhibitors and a phorbol ester accelerated the trafficking of AQP3 to the membrane fraction. In contrast, adenylyl cyclase (AC) and protein kinase A (PKA) inhibitors did not have any effect on the increased in trafficking of AQP3 by epinephrine and an AC activator did not affect the trafficking of AQP3. Phosphorylation of a threonine (514) residue in PKC was detected upon the treatment with epinephrine and the temporal transitional pattern of this phosphorylation paralleled that of the increased trafficking of AQP3. These results suggest that PKC modulates the trafficking of AQP3.

[*Biol. Pharm. Bull.*, **31**, 1990–1995 (2008)] [Lab. of Pharmaceutics]

Comparison of the Mechanisms of Cataract Development Involving Differences in Ca^{2+} Regulation in Lenses among Three Hereditary Cataract Model Rats.

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

We compare the mechanisms for dysfunction in Ca^{2+} regulation in UPL rat (UPLR), Shumiya cataract rat (SCR), and Ihara cataract rat (ICR). Decreases in the activity of Ca^{2+}-ATPase were found in the lenses of SCR and ICR concurrent with cataract development. In contrast, the Ca^{2+}-ATPase activity in UPLR with opaque lenses was higher than in those with transparent lenses. On the other hand, ATP levels were markedly decreased in UPLR with opaque lenses. The expression of cytochrome c oxidase (CCO)-1 mRNA and CCO activity in UPLR lenses was found to decrease during cataract development. The nitric oxide (NO) and lipid peroxide levels were also increased in the lenses of UPLR, SCR and ICR with opaque lenses. The decrease in Ca^{2+}-ATPase activity may cause the elevation in the level of lens Ca^{2+}, thus leading to lens opacification. Our findings show that the Ca^{2+} contents in the cataractous lenses of all three model rats are increased, the mechanisms for this Ca^{2+} enhancement is different in each rat model.

[*Toxicology*, **243**, 75–83 (2008)] [Lab. of Hygienics]

Enhancing Effect of Chlorinated Organic Solvents on Histamine Release and Inflammatory Mediator Production.

Makoto SEO, Koji IKEDA, Tetsunori OKAMURA, Kumiko KIDA, Masahiko SATOH, Naoki INAGAKI, Hiroichi NAGAI, and Hisamitsu NAGASE*

Trichloroethylene (TCE) and tetrachloroethylene (PCE) enhanced histamine release in a dose-dependent manner from antigen-stimulated non-purified rat peritoneal mast cells and rat basophilic leukemia (RBL-2H3) cells sensitized with anti-dinitrophenol (DNP) monoclonal IgE antibody and stimulated with DNP-conjugated bovine serum albumin. In addition., TCE and PCE increased IL-4 and TNF-α production from antigen-stimulated RBL-2H3. In an in vivo study, we investigated the effect of TCE and PCE on passive cutaneous anaphylaxis (PCA) reaction. TCE and PCE enhanced PCA reaction markedly.

[*Immunobiology*, **213**, 663–669 (2008)] [Lab. of Hygienics]

A Small Amount of Tetrachloroethylene Ingestion from Drinking Water Accelerates Antigen-stimulated Allergic Responses.

Makoto SEO, Takeo YAMAGIWA, Ryo KOBAYASHI, Koji IKEDA, Masahiko SATOH, Naoki INAGAKI, Hiroichi NAGAI, and Hisamitsu NAGASE*

After exposure of Wistar rats to tetrachloroethylene (PCE) in drinking water for 2 or 4 weeks, we performed a passive cutaneous anaphylaxis (PCA) reaction. PCE exposure for 4 weeks enhanced PCA reaction in a dose-dependent manner. Non-purified mast cells (NPMCs) from rats treated with 1 mg/L PCE in drinking water for 2 weeks increased antigen-stimulated histamine release. Furthermore, the leukocytes of rats treated with PCE in drinking water for 4 weeks showed increased interleukin (IL)-4 expression. The mechanism of enhancing the PCA reaction is assumed to be that PCE increases IL-4 production and PCE causes T helper (Th) 1/Th2-type helper T-cell imbalance and increases histamine release from excessively accumulated mast cells.

[*Regul. Toxicol. Pharmacol.*, **52**, 140–146 (2008)] [Lab. of Hygienics]

Augmentation of Antigen-stimulated Allergic Responses by a Small Amount of Trichloroethylene Ingestion from Drinking Water.

Makoto SEO, Takeo YAMAGIWA, Ryo KOBAYASHI, Koji IKEDA, Masahiko SATOH, Naoki INAGAKI, Hiroichi NAGAI, and Hisamitsu NAGASE*

After exposure of Wistar rats to trichloroethylene (TCE) ingestion for 2 or 4 weeks, we performed a passive cutaneous anaphylaxis (PCA) reaction. TCE ingestion for 2 and 4 weeks enhanced PCA reaction in a dose-dependent manner. On histological examninatin, TCE ingestion for 2 weeks exacerbated inflammation characterized by infiltration of lymphocytes and accumulation of mast cells around the vessel in the skin. After TCE ingestion for 4 weeks, the mesenteric lymph nodes (MLNs) showed increase of the size and wet weight, and germinal centers changed distinctly. The interleukin-4 (IL-4) mRNA levels on spleen, MLNs and leukocytes were increased. Moreover, serum total IgE levels of TCE ingestion increased in a time-dependent manner.

[*Food Chem. Toxicol*., **46**, 694–700 (2008)] [Lab. of Hygienics]

Anti-clastogenic Effect of Magnolol on Benzo(a)pyrene-induced Clastogenicity in Mice.

Junichiro SAITO, Kiyoshi SHIBUYA, and Hisamitsu NAGASE*

In this study, the in vivo anti-clastogenic effect of magnolol against clastogenicity induced by B(a)P was evaluated using the micronucleus test in mice. Animals were treated with an oral administration of magnolol (1, 10, and 100 mg/kg) at -24, 0, 24, 48, 72, and 96 h before a single i.p. injection of B(a)P. In order to elucidate the mechanism behind this effect, the authors measured the activity of the detoxifying enzymes [UDP-glucuronosyltransferase (UGT) and glutathione-S-transferase (GST)] and antioxidative enzymes [superoxide dismutase (SOD) and catalase] in the liver when treated with an oral administration of magnolol at various administration times. Magnolol had an anti-clastogenic effect on B(a)P in the micronucleus test as well as an anti-mutagenic effect on indirect mutagens in the Ames test. The anti-clastogenic effect of magnolol was also suggested by the increases in UGT and SOD enzyme activity, and by the attenuation of oxidative damage induced by x-ray irradation.

[*Asian Pac. J. Cancer Prev.*, **9**, 279–282 (2008)] [Lab. of Hygienics]

Aspirin Intake Suppresses MMC-induced Genotoxicity in Mice.

Miki NIIKAWA, Tetsunori OKAMURA, Katsunori SUGIURA, and Hisamitsu NAGASE*

The genotoxicity induced by mitomycin C (MMC) was found to be decreased by aspirin on alkaline single cell gel electrophoresis (SCG) assay in multiple organs of mice. Aspirin at doses of 0.5, 5 and 50 mg/kg and MMC at 2 mg/kg were administered and then liver, lung, kidney, spleen, colon and bone marrow were sampled after 3 h. Significant protective effects of aspirin against MMC-induced genotoxicity was observed in all but the bone marrow, where no change was evident. The results suggest that the radical scavenging ability of aspirin prevents danage by MMC-induced reactive oxygen species (ROS) in multiple organs.

[*Endocrinology*, **149**, 73–83 (2008)] [Lab. of Hygienics]

Ascorbic Acid Transported by Sodium-Dependent Vitamin C Transporter 2 Stimulates Steroidogenesis in Human Choriocarcinoma Cells.

Ximei WU, Takuma IGUCHI, Norio ITOH, Kousuke OKAMOTO, Tatsuya TAKAGI, Keiichi TANAKA, and Tsuyoshi NAKANISHI*

Ascorbic acid (AA) which is taken up to into cells by sodium-dependent vitamin C transporter (SVCT) 1 and 2 is believed to be important for hormone synthesis, but its role in generating placental steroids needed to maintain pregnancy and fetal development is not clear. To determine the steroidogenic effect of AA and the role of SVCT2 in AA-induced steroidogenesis, we tested the effects of AA treatment and SVCT2 knockdown on steroidogenesis in human choriocarcinoma cell lines. AA treatment of JEG-3, BeWo, and JAR cells dose dependently increased progesterone and estradiol levels. Additionally, stabel knockdown of SVCT2 in JEG-3 cells by retrovirally mediated RNA interference significantly suppressed the AA-induced progesterone and estradiol production.

[*Biochem J.*, **415**, 477–482 (2008)] [Lab. of Hygienics]

Chromium(VI) Inhibits Mouse Metallothionein-I Gene Transcription by Preventing the Zinc-dependent Formation of an MTF-1-p300 Complex.

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

Mouse MT-I (metallothionein-I) transcription is regulated by MTF-1 (metal-response-element-binding transcription factor-1) which is recruited to the promoter in response to zinc. Here we showed that Cr(VI) [chromium(VI)] inhibits the ability of MTF-1 to transactivate this gene in response to zinc. In addition, we demonstrated that Cr(VI) pretreatment blocks the zinc-induced formation of this co-activator complex. Thus Cr(VI) inhibits mouse MT-I gene expression in response to zinc by interfering with the ability of MTF-1 to form a co-activator complex containing and the histone acetyltransferase p300, which plays an essential role in the activation of MT-I transcription, and recruiting RNA polymerase II to the promoter.

[*J. Nat. Med.*, **62**, 228–231 (2008)] [Lab. of Herbal Garden]

A New *Erythrina* alkaloide from *Erythrina herbacea.*

Hitoshi TANAKA, Hisanori HATTORI, Toshihiro TANAKA, Eiji SAKAI,* Nobuyuki TANAKA,
Aditya KULKARNI, and Hideo ETOH

A new *Erythrina* alkaloid, 10-hydroxy-11-oxoerysotrine (1), has been isolated from the flowers of *Erythrina herbacea* together with five known compounds: erythabine (2), 10,11-dioxoerysotrine (3), erythrartine (4), crysotramidine (5) and erysotrine-*N*-oxide (6). The structure of the new compound was elucidated on the baseis of its spectraldata, including 2-D-NMR and mass (MS) spectra. The new compound is a rare C-10 oxygenated *Erythrina* alkaloide. The antioxidant activities of the isolated compounds 1-6 were evaluated by scavenging with peroxynitrite.

[*J. Nat. Med.*, **62**, 354–355 (2008)] [Lab. of Herbal Garden]

Main Phenolic Compounds from the Flower of *Trachelospermum asiaticum* var. *intermedium* (Apocynaceae).

Shinzo HOSOI, Eri SHIMIZU, Toshihiro TANAKA, Eiji SAKAI,*
Mitsuko YAMADA, and Akiyo SAKUSHIMA

Six phenolic compounds were isolated from the flowers of *Trachelospermum asiaticum* var. *intermedium* (Apocynaceae). These structures were determined on the basis of spectral data.

[*生薬学雑誌*, **62**, 15–18 (2008)] [Lab. of Herbal Garden]

Characteristics of CALCULUS BOVIS SATIVUS First Introduced in the Chinese Pharmacopoeia 2005 Edition.

Shigeharu YAMAGUCHI, Kasumi IWAI, Koji OHBA, Youichi HISATA, Eiji SAKAI,* and Toshihiro TANAKA

CALCULUS BOVIS SATIVUS (CBS) was first introduced in the Chinese Pharmacopoeia 2005 edition. In China, CBS hs been used as an alternative to BEZOAR BOVIS (BB). CBS consists of several ingerdients derived from BB and is made ex vivo. This experiment has been carried out to enable the discrimination between CBS and BB. Comparison with BB revealed the follwing characteristics of CBS: the thick ring layers in the transverse plane forms a concentric circle; the crack entered from the surface to the center, rather than alonf the boundaries of the layers with BB; the surface color presented a more vivid yellow than that of BB. Furthermor, each sample was extracted with ethanol to examine the time course of the changf in extract solution color. The maximum optical absobance of both extract solutions originally showed 415nm in wavelength. However, 24 hours after the preparation, only the peak absorbance of BB was stable. This indicates that the fading speed of ethanol extract solution for CBS was faster. As a result, it was concluded that CBS can be distinguished from BB by the above characteristics.

[生薬学雑誌, **62**, 72–78 (2008)] [Lab. of Herbal Garden]

Comparative Study on Testing Methods and Specification Values for Crude Drugs in Pharmacopoeias among Four Western Pasific Regional Countries (Japan, China, Korea and Vietnam) (IV) Comparative Study on TLC Identification for Crude Drugs Considering Harmonization and Clean Analysis

Nobuo KAWAHARA, Yoshie IDO, Ikumi NAKAJIMA, Takeshi KAWASAKI, Eiji SAKAI,* and Yukihiro GODA

Recently, from the viewpoint of prevention of environmental pollution and the health protection of researcher, clean analysis removing harmful chemical reagents such as benzene and chloroform is recommended worldwide. At the fourth Western Pacific Regional Forum for the Harmonization of Herbal Medicines (FHH) Standing Committee in Tokyo 2006, we proposed the collaborative study of the developing solvent for TLC identification in Pharmacopoeia, considering clean analysis. The proposed collaboration study is as follows: each member state tests the TLC analysis using non-toxic solvent systems which are described in other members' Pharmacopoeia with regard to the designgted crude drugs in the comparative table. In this paper, we show the results of the task work. From our comparative study, it is suggested that for almost all TLC identification of crude drugs using chloroform (or benzene) as a developing solvent, non-toxic solvent systems will be able to replace the toxic solvents.

[*Eur. J. Pharmacol.*, **578**, 87–96 (2008)] [Lab. of Pharmacology]

Repeated Instillations of *Dermatophagoides farinae* into the Airways can Induce Th2-Dependent Airway Hyperresponsiveness, Eosinophilia and Remodeling in Mice. Effect of Intratracheal Treatment of Fluticasone Propionate.

Keiko WAKAHARA, Hiroyuki TANAKA,* Go TAKAHASHI, Mayumi TAMARI, Reishi NASU, Tatsuyuki TOYOHARA, Hirohisa TAKANO, Saburo SAITO, Naoki INAGAKI, Kaoru SHIMOKATA, and Hiroichi NAGAI

Dermatophagoides farinae are a common environmental allergen causing allergic asthma; however, little is known about their pathophysiological effect. We established a mouse model of asthma induced by repeated instillations of *D. farinae* without additional adjuvants. This model demonstrated the local Th2-dominant inflammation and airway remodeling feature. This model is a useful tool for the investigation of mechanisms involved in the development of atopic asthma.

[*Int. Immunopharmacol.*, **8**, 453–457 (2008)] [Lab. of Pharmacology]

Inhibitory Effects of *Piper Betle* on Production of Allergic Mediators by Bone Marrow-derived Mast Cells and Lung Epithelial Cells.

Mali WIROTESANGTHONG, Naoki INAGAKI,* Hiroyuki TANAKA, Witchuda THANAKIJCHAROENPATH, and Hiroichi NAGAI

The leaves of the *Piper betle* Linn. are used in traditional medicine in Thailand. In this study, the effects of *P. betle* ethanolic extract (PE) on the production of histamine and granulocyte macrophage-colony-stimulating factor (GM-CSF) by murine bone marrow mast cells (BMMCs) and on the secretion of eotaxin and IL-8 by the human lung epithelial 0cell line, BEAS-2B, were investigated *in vitro*. PE significantly decreased histamine and GM-CSF produced by an IgE-mediated hypersensitive reaction, and inhibited eotaxin and IL-8 secretion in a TNF-alpha and IL-4-induced allergic reaction. The results suggest that *P. betle* may offer a new therapeutic approach for the control of allergic diseases through inhibition of production of allergic mediators.

[*Int. Arch. Allergy Immunol.*, **147**, 6–16 (2008)] [Lab. of Pharmacology]

Immunomodulatory Effects of CpG Oligodeoxynucleotides on House Dust Mite-Induced Airway Inflammation in Mice.

Izumi HIROSE, Hiroyuki TANAKA,* Go TAKAHASHI, Keiko WAKAHARA, Mayumi TAMARI, Tatsuo SAKAMOTO, Seiji KOJIMA, Naoki INAGAKI, and Hiroichi NAGAI

CpG oligodeoxynucleotides (CpG) are reported to protect against airway eosinophilia and hyperresponsiveness in animal models of asthma. In the present study, we evaluated the immunomodulatory effects of CpG on the development of house dust mite-induced airway inflammation and remodeling in mice. Mice were instilled with *Dermatophagoides farinae* and CpG into the trachea without additional adjuvants. CpG showed inhibition of airway eosinophilia, hyperresponsiveness, levels of Th2 cytokines in the bronchoalveolar lavage fluid, goblet cell hyperplasia, the thickness of the epithelium and subepithelial fibrosis dose-dependently. Our results demonstrated CpG can be a therapeutic approach for the house dust mite-induced asthma.

[*J. Immunol.*, **180**, 6262–6269 (2008)] [Lab. of Pharmacology]

Identification of Pendrin as a Common Mediator for Mucus Production in Bronchial Asthma and Chronic Obstructive Pulmonary Disease.

Isao NAKAO, Sachiko KANAJI, Shoichiro OHTA, Hidetomo MATSUSHITA, Kazuhiko ARIMA, Noriko YUYAMA, Mutsuo YAMAYA, Katsutoshi NAKAYAMA, Hiroshi KUBO, Mika WATANABE, Hironori SAGARA, Kumiya SUGIYAMA, Hiroyuki TANAKA,* Shuji TODA, Hiroaki HAYASHI, Hiromasa INOUE, Tomoaki HOSHINO, Aya SHIRAKI, Makoto INOUE, Koichi SUZUKI, Hisamichi AIZAWA, Satoshi OKINAMI, Hiroichi NAGAI, Mamoru HASEGAWA, Takeshi FUKUDA, Eric D. GREEN, and Kenji IZUHARA

Excessive production of airway mucus is a cardinal feature of bronchial asthma and COPD. We identified pendrin related to a molecule responsible for airway mucus production. The expression of pendrin in airway epithelial cells rapidly induced mucus overproduction with neutrophilic infiltration in mice. Pendrin may be a therapeutic target candidate for bronchial asthma and COPD.

[*J. Pharmacol. Sci.*, **108**, 355–363 (2008)] [Lab. of Pharmacology]

Nafamostat Mesilate, a Potent Serine Protease Inhibitor, Inhibits Airway Eosinophilic Inflammation and Airway Epithelial Remodeling in a Murine Model of Allergic Asthma.

Masayuki ISHIZAKI, Hiroyuki TANAKA,* Daisuke KAJIWARA, Tatsuyuki TOYOHARA, Keiko WAKAHARA, Naoki INAGAKI, and Hiroichi NAGAI

To clarify the involvement of serine proteases in the development of allergic airway inflammation, we investigated the effect of nafamostat mesilate in a murine model of allergic asthma. In sensitized mice, repeated allergen challenge induced an increase in tryptase proteolytic activity, marked increases in the numbers of inflammatory cells, levels of T helper type 2 (Th2) cytokines and eotaxin in bronchoalveolar lavage fluid and numbers of goblet cells in the epithelium. Naphamostat mesilate clearly inhibited these parameters dose dependently. These findings suggest that increased serine protease activity in the airways is involved in the development of antigen-induced allergic eosinophilic inflammation and epithelial remodeling in bronchial asthma.

[*Biol. Pharm. Bull.* **31**, 2108–2113 (2008)] [Lab. of Pharmacology]

Effect of Bakumijiogan, an Herbal Formula in Traditional Chinese Medicine, on Atopic Dermatitis-Like Skin Lesions Induced by Mite Antigen in NC/Jic Mice.

Toshiaki MAKINO, Minako HAMANAKA, Hirotaka YAMASHITA,* and Hajime MIZUKAMI

We evaluated the effectiveness of bakumijiogan (BJG), an herbal formula in traditional Chinese medicine used to treat atopic dermatitis (AD), using a NC/Jic mouse model of AD. AD symptoms were induced by repeated injections of *Dermatophagoides farinae* antigen (Df-antigen) into the ear auricle. Ear thickness dramatically increased up to 16 day after the first injection of Df –antigen and significantly reduced by daily oral administration of BJG. Augmented serum concentrationsof total IgE and Df-antigen-specific IgG1 were slightly suppressed by BJG administration. Serum IFN-gamma and lesional IFN-gamma mRNA levels were significantly higher, whereas lesional IL-1alfa and tumor necrosis factor-alfa mRNA levels were lower in BJG-treated mice than those in control mice. These results suggest that BJG suppressed AD-like symptoms by correcting the Th1/Th2 imbalance.

[*Free Radic. Biol. Med.*, **44**, 1191–1202 (2008)] [Lab. of Biochemistry]

L-Xylulose Reductase Is Involved in 9,10-Phenanthrenequinone-Induced Apoptosis in Human T Lymphoma Cells.

Toshiyuki MATSUNAGA,* Tetsuro KAMIYA, Daigo SUMI, Yoshito KUMAGAI, B. KALYANARAMAN, and Akira HARA

Treatment of human acute T-lymphoblastic leukemia MOLT-4 cells with 9,10-phenanthrenequinone (9,10-PQ) elicited not only apoptotic signaling, but also intracellular reactive oxygen species (ROS) generation and consequent glutathione depletion. The ROS generation and cytotoxicity by 9,10-PQ were augmented in an L-xylulose reductase (XR)-transformed cell line, and prurified XR indeed reduced 9,10-PQ and produced superoxide anion through redox cycling. In addition, the 9,10-PQ-induced apoptosis was partially inhibited by the pretreatment with XR-specific inhibitors. Moreover, the expression levels of XR and its mRNA in the cells were markedly enhanced by ROS, suggesting that initially produced ROS induce XR, which accelerates the generation of ROS.

[*Arch. Biochem. Biophys.*, **477**, 339–347 (2008)] [Lab. of Biochemistry]

Characterization of Human DHRS4: an Inducible Short-Chain Dehydrogenase/reductase Enzyme with 3β-Hydroxysteroid Dehydrogenase Activity

Toshiyuki MATSUNAGA,* Satoshi ENDO, Satoshi MAEDA, Syuhei ISHIKURA, Kazuo TAJIMA, Nobutada TANAKA, Kazuo T. NAKAMURA, Yorishige IMAMURA, and Akira HARA

We show that human DHRS4 is inactivated at low temperature without dissociation into subunits. The cold inactivation was prevented by a mutation of Thr177 with the corresponding residue, Asn, in cold-stable pig DHRS4. While its activity towards all-*trans*-retinal was low, human DHRS4 efficiently reduced 3-keto-C_{19}/C_{21}-steroids into 3β-hydroxysteroids. The mRNA for the enzyme was ubiquitously expressed in human tissues and several cancer cells, and the enzyme in HepG2 cells was induced by PPARα ligands. The results suggest a novel mechanism of cold inactivation and role of the inducible human DHRS4 in 3β-hydroxysteroid synthesis and xenobiotic carbonyl metabolism.

[*Biochem. Biophys. Res. Commun.*, **377**, 1326–1330 (2008)] [Lab. of Biochemistry]

Human Carbonyl Reductase 4 Is a Mitochondrial NADPH-dependent Quinone Reductase.

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

A protein encoded in the gene *Cbr4* on human chromosome 4q32.3 belongs to the short-chain dehydrogenase/reductase family. Contrary to the functional annotation as carbonyl reductase 4 (CBR4), we show that the recombinant tetrameric protein exhibits NADPH-dependent reductase activity for o- and p-quinones, but not for other aldehydes and ketones. The enzyme was insensitive to dicumarol and quercetin, potent inhibitors of cytosolic quinone reductases. The 25-kDa CBR4 was detected in human liver, kidney and cell lines. The overexpression of CBR4 in bovine endothelial cells reveals that the enzyme has a non-cleavable mitochondrial targeting signal. We further demonstrate that the in vitro quinone reduction by CBR4 generates superoxide through the redox cycling, and suggest that the enzyme may be involved in the induction of apoptosis by cytotoxic 9,10-phenanthrenequinone.

[*Proteins*, **70**, 176–187 (2008)] [Lab. of Biochemistry]

Structures of Dimeric Dihydrodiol Dehydrogenase Apoenzyme and Inhibitor Complex: Probing the Subunit Interface with Site-directed Mutagenesis.

Vincenzo CARBONE, Satoshi ENDO, Rie SUMII, Roland P.-T. CHUNG, Toshiyuki MATSUNAGA, Akira HARA,* and Ossama El-KABBANI

Dimeric dihydrodiol dehydrogenase (DD) catalyses $NADP^+$-dependent oxidation of trans-dihydrodiols of aromatic hydrocarbons to their corresponding catechols. This is the first report of the crystal structure of the enzyme. The active-site of DD is located in the C-terminal domain of the protein. The dimer interface is stabilized by a lot of intermolecular contacts, which includes an intricate hydrogen bonding network. Site-directed mutagenesis has demonstrated that the dimer is not essential for the activity. The similarity between the quaternary structures of mammalian DD and glucose-fructose oxidoreductase in the prokaryotic organism suggests that both enzymes are members of a unique family of oligomeric proteins and may share a common ancestral gene.

[*Bioorg. Med. Chem.*, **16**, 3245–3254 (2008)] [Lab. of Biochemistry]

Inhibition of 3(17)α-Hydroxysteroid Dehydrogenase (AKR1C21) by Aldose Reductase Inhibitors.

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

Mouse 3(17)α-hydroxysteroid dehydrogenase (AKR1C21) is a member of the aldo-keto reductase superfamily that catalyses the oxido-reduction of steroid hormones such as estrogens, androgens and neurosteroids. Inhibitors of aldose reductase, a member of the same superfamily, were evaluated against AKR1C21. Models of the enzyme-inhibitor complexes suggest that Tyr118 and Phe311 are important residues for inhibitor recognition and orientation in the active site of AKR1C21.

[*J. Med. Chem.*, **51**, 4844–4848 (2008)] [Lab. of Biochemistry]

Selectivity Determinants of Inhibitor Binding to Human 20α-Hydroxysteroid Dehydrogenase: Crystal Structure of the Enzyme in Ternary Complex with Coenzyme and the Potent Inhibitor 3,5-Dichlorosalicylic Acid.

Urmi DHAGAT, Satoshi ENDO, Rie SUMII, Akira HARA,* and Ossama EL-KABBANI

The crystal structure of human 20α-hydroxysteroid dehydrogenase (AKR1C1) in ternary complex with the coenzyme $NADP^+$ and the potent inhibitor 3,5-dichlorosalicylic acid was determined at a resolution of 1.8 Å. The inhibitor is held in place by a network of hydrogen bonding interactions with the active site residues, Tyr55, His117, and His222, of AKR1C1. The important role of the nonconserved residues Leu54, His222, Leu306, and Leu308 in inhibitor binding and selectivity was determined by site-directed mutagenesis.

[*J. Neurochem.*, **104**, 1372–1386 (2008)] [Lab. of Biochemistry]

Depolarization-induced Differentiation of PC12 Cells Is Mediated by Phospholipase D_2 through the Transcription Factor CREB Pathway.

Yoshiko BANNO, Satoshi NEMOTO, Masashi MURAKAMI, Masashi KIMURA, Yoshihito UENO, Kenji OHGUCHI, Akira HARA,* Yukio OKANO, Yukio KITADE, Minoru ONOZUKA, Takashi MURATE, and Yoshinori NOZAWA

Depolarization of rat pheochromocytoma (PC12) cells with 50 mM KCl increased neurite outgrowth and elevated expression of growth-associated protein-43 (GAP-43) and synapsin I. The Results on Western blotting using antibodies against a variety of signaling molecules suggest that the signaling pathway of depolarization-induced PLD_2 activation was downstream of Ca^{2+}-calmodulin-dependent protein kinase II delta and Src, and upstream of proline-rich protein tyrosine kinase 2 and extracellular signal-regulated kinase / cAMP response element-binding protein, but independent of the protein kinase A.

[*Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.*, **64**, 228–230 (2008)] [Lab. of Biochemistry]

Crystallization and Preliminary X-ray Crystallographic Analysis of Rabbit L-Gulonate 3-Dehydrogenase.

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

Rabbit L-gulonate 3-dehydrogenase was crystallized using the oil-microbatch method at 295 K. X-ray diffraction data were collected to 1.70 Å resolution from a crystal at 100 K using synchrotron radiation. The crystal belongs to the C-centred monoclinic space group C2, with unit-cell parameters a = 71.81, b = 69.08, c = 65.64 Å, β= 102.7 degrees. Assuming the presence of a monomeric protomer in the asymmetric unit gives a V_M value of 2.21 $Å^3$ Da^{-1} and a solvent content of 44.4%. A cocrystal with NADH, which was isomorphous to the apo form, was also prepared and diffraction data were collected to 1.85 Å resolution using Cu Kα radiation at 100 K.

[*Structure*, **16**, 388–397 (2008)] [Lab. of Biochemistry]

Molecular Basis for Peroxisomal Localization of Tetrameric Carbonyl Reductase

Nobutada TANAKA, Ken-ichi AOKI, Shuhei ISHIKURA, Makoto NAGANO, Yorishige IMAMURA, Akira HARA,* and Kazuo T. NAKAMURA

Pig heart peroxisomal carbonyl reductase (PerCR) belongs to the SDR family, and its sequence comprises a C-terminal SRL tripeptide, which is a variant of the type 1 peroxisomal targeting signal (PTS1) Ser-Lys-Leu. PerCR is imported into peroxisomes of HeLa cells when the cells are transfected with vectors expressing the enzyme. To understand the structural basis for peroxisomal localization of PerCR, we determined the crystal structure of PerCR. Our data revealed that the C-terminal PTS1 of each subunit of PerCR was involved in intersubunit interactions and was buried in the interior of the tetrameric molecule. These findings indicate that the PTS1 receptor Pex5p in the cytosol recognizes the monomeric form of PerCR whose C-terminal PTS1 is exposed, and that this PerCR is targeted into the peroxisome, thereby forming a tetramer.

[*Acta Crystallogr. D Biol. Crystallogr.*, **64**, 532–542 (2008)] [Lab. of Biochemistry]

Structure of Monkey Dimeric Dihydrodiol Dehydrogenase in Complex with Isoascorbic Acid.

Vincenzo CARBONE, Rie SUMII, Shuhei ISHIKURA, Yukuhiko ASADA, Akira HARA,* and Ossama El-KABBANI

Mammalian dimeric dihydrodiol dehydrogenase (DD) is identical to $NADP^+$-dependent D-xylose dehydrogenase. The crystal structure of monkey dimeric DD complexed with the inhibitor isoascorbic acid has been determined at 2.59 Å resolution. Several key residues contribute to the high affinity for coenzyme, Arg37, Arg41, His76 and His79, have been identified. The interaction of Arg37 and Arg41 with the coenzyme has been established from the large increases (29-fold to 438-fold) in the K_d values for NADP(H) for the R37D and R41D mutant enzymes. The mutation of several residues lining the inhibitor-binding site of DD suggested the involvement of Trp125, Phe154, Trp254 and Phe279 in determining the broad substrate specificity and inhibitor potency of the enzyme. In addition, mutants of Lys97, which is present near the catalytic residue Tyr180, greatly reduced the k_{cat} value without changing the K_d values for coenzyme, suggesting the importance of Lys97 in the catalytic mechanism of DD.

[*Arch. Biochem. Biophys.*, **477**, 339–347 (2008)] [Lab. of Biochemistry]

Structure of Aldehyde Reductase Holoenzyme in Complex with the Potent 20α-Hydroxysteroid Dehydrogenase Inhibitor 3,5-Dichlorosalicylic Acid: Implications for Inhibitor Selectivity.

Vincenzo CARBONE, Roland CHUNG, Satoshi ENDO, Akira HARA,* and Ossama EL-KABBANI

The structure of aldehyde reductase (ALR1) in ternary complex with the NADPH and 3,5-dichlorosalicylic acid (DCL), a potent inhibitor of human 20α-hydroxysteroid dehydrogenase (AKR1C1), was determined at a resolution of 2.41 Å. The inhibitor formed a network of hydrogen bonds with the several residues in the active sites. Molecular modelling calculations together with inhibitory activity measurements indicated that DCL was a less potent inhibitor of ALR1 (256-fold) when compared to AKR1C1. In AKR1C1, the inhibitor formed a 10-fold stronger binding interaction with the catalytic residue (Tyr55), non-conserved hydrogen bonding interaction with His222, and additional van der Waals contacts with the non-conserved C-terminal residues Leu306, Leu308 and Phe311 that contribute to the inhibitor's selectivity advantage for AKR1C1 over ALR1.

[*Jpn. J. Pharm. Health Care Sci*., **34**, 361–365 (2008)] [Lab. of Clinical Pharmacy]

A Retrospective Study on the Use of G-CSF in Paclitaxel plus Carboplatin Combination Chemotherapy for the Treatment of Advanced Lung Cancer.

Katsumi TANIZAWA, Yoshiko KIRIYAMA, Toshikazu SHINTANI, Kunio HAYASE, Ochimi KATO, Hitomi TERAMACHI, and Teruo TSUCHIYA*

A logistic regression analysis showed that the risk factors that affect the prolongation of G-CSF use were found to be monthly dosage regimen [odds ratio: 3.571], dose of PTX > 140mg/m2 [7.387] and multiple (>2) courses of the chemotherapy [3.261]. However, the duration of G-CSF use was not influenced by gender, age, performance status, serum albumin or radiation therapy. In addition, none of these factors had any influence on the timing of the start of G-CSF use. From these findings, it is suggested that pharmacists should carefully monitor the serological data on myeloid function in patients who undertook PTX plus CBDCA combination chemotherapy for advanced lung cancer, particularly in those with risk factors for prolongation of G-CSF use.

[*Jpn. J. Pharm. Health Care Sc*i., **34**, 374–380 (2008)] [Lab. of Clinical Pharmacy]

Questionnaire Survey to Evaluate Improvement in Satisfaction Levels among Fourth Year Students in Practical Training at a Hospital Pharmacy.

Hitomi TERAMACHI,* Mitsuhiro NAKAMURA, Eiji TAKASHIMA, Masafumi KUBOTA, Tetsuo ADACHI, and Teruo TSUCHIYA

We conducted a questionnaire survey of fourth year student at Gifu Pharmaceutical University to investigate satisfaction levels after practical training in a pharmacy. The response rate was 97.5%. Levels of satisfaction with the training overall were higher among students whose training had lasted for four weeks and involved numerous sickbeds than among those who did not receive such extensive training. We consider there is a need for hospitals to establish their own curriculums and based on this, provide an environment that will create the desire to learn in students, that the actual experience of training will have a great effect in improving satisfaction levels.

[*Jpn. J. Pharm. Health Care Sci*., **34**, 755–763 (2008)] [Lab. of Clinical Pharmacy]

Trial Introduction of Advanced Problem-Based Learning and Analysis of its Evaluation by Students.

Hitomi TERAMACHI,* Yumi KUZUYA, and Teruo TSUCHIYA

We conducted a PBL trial in the clinical pharmacy course for graduate students (n=16). The results of a questionnaire survey concerning the PBL lectures indicated a high level of satisfaction for many students and in the “general evaluation” of the self-assessment by students, they gave high scores to both Typical Type PBL and Question Type PBL. When applied to the self assessment, CS analysis uncovered problems that would have been overlooked by conventional statistical analysis such as those in mean assessment values. The use of Customer Satisfaction analysis in this way enabled us to infer that the combined use of Typical Type PBL and Question Type PBL was effective in self learning by students.

[*J. Jpn. Soc. Hosp. Pharm*., **44**, 219–222 (2008)] [Lab. of Clinical Pharmacy]

A Study on the Guidebooks for Appropriate Use of Injection Anticancer Agents Provided by the Manufacturer.

Katsumi TANIZAWA, Keiko TAGUCHI, Tomoyuki HIRASHITA, Kazuyo MORISHITA, Toshikazu SHINTANI, Hitomi TERAMACHI, and Teruo TSUCHIYA*

We consider that a reasonable guidebooks can administer them, and it should be stated the grounds of the condition clearly. In addition, we consider that it is desirable to mention them in an attached document to be able to publicize important information to medical staff enough.

[*Jpn. J. Pharm. Health Care Sci*., **34**, 1137–1146 (2008)] [Lab. of Clinical Pharmacy]

Evaluation of a Support System and Textbook Developed for Practical Training -Self Learning by Students Effective in Improving Pharmacy Practical Training-.

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

Ten graduate students used the system and the textbook in a pharmacy training trial based on the model core curriculum conducted at the pharmacy of Gifu Pharmaceutical University which lasted two months. Many of the students commented that the system and the textbook increased their motivation in the practical training (respective mean score 3.8, 4.0, on a 5.0 grade scale) and made it more meaningful (4.2). In addition, many of them were highly satisfied with the training. The execution rate for all the specific behavioral objectives(SBOs) was significantly higher for the optimal stage graduate students (n=5) than it was for the suboptimal stage graduate students((n=5). Further, a self-evaluation by students produced mean scores for all SBOs that were significantly higher for the former (3.6) than they were for the latter (2.6). We conclude that the use of the support system and textbook helped motivate students to learn on their own and enhanced the effectiveness of the practical training.

[*J. Pharm. Comun*., **6**, 13–22 (2008)] [Lab. of Clinical Pharmacy]

Introduction and Evaluation of a PBL Method for Communication Education in the Medical Psychology Course.

Hitomi TERAMACHI,* Tetsuo ADACHI, and Teruo TSUCHIYA

As a component of the medical psychology curriculum in 2006, we lectured the 2nd-year students (n = 42) with a PBL format, and staffed the clinical pharmacy course for graduate students (teaching assistant: TA, n = 8) as facilitators. The self-assessment of the students to the PBL method showed high satisfaction, active learning, and increased motivation (respective mean score on a 5-grade scale: 3.82, 3.90, 3.87). Furthermore, it was demonstrated that the facilitation by TAs had helped students learning (3.95 for students, 3.13for TAs). Besides, students and TAs felt that the introduction of the TA system was promising (3.97 for students, 4.13for TAs). We infer that the PBL method with cases and staffed TAs as facilitators, is the most effective curriculum step for improvement of communication in the age-mixing of students and TAs in medical psychology.

[*J. Gifu Byoyaku*, **47**, 12–18 (2008)] [Lab. of Clinical Pharmacy]

A Questionnaire Survey and Analysis on the Issue of "Crushing of Tablets and Opening of Capsules" and "Tube Administration".

Hitomi TERAMACHI,* Naomi FUJITA, Ayako BAN, Teruo TSUCHIYA, and Hirofumi TAKEUCHI

We investigated the issue of "grinding of tablets and opening of capsules" and "tube administration" by means of a questionnaire sent to pharmacists, working at 94 hospitals in Gifu prefecture (58.5%). Most pharmacists (96.4%) reported a marked problem for grinding of tablets and opening of capsules. For tube administration, the use of grinding technique was 84.6%, while a simple suspension method was 9.6%. The results suggested the grinding technique was dominant in this clinical treatment. Regardless of the method used, many inconveniences in the tube administration were reported. For instance, as the particle size of grinding of Panaldine®tablet decreases by grinding time, the recovery of Panaldine® becomes difficult due to increasing adhesion to dispensing appliance. We confirmed the problem in grinding of Panaldine® by conducting model experiments in our laboratory. Based on these results, we suggest strong requirement to develop a suitable dosage form for medical needs such as tube administration.

[*Int. J. Hematol.*, **87**, 266–275 (2008)] [Lab. of Drug Informatics]

Implications of Sphingosine Kinase 1 Expression Level for the Cellular Sphingolipid Rheostat: Relevance as a Marker for Daunorubicin Sensitivity of Leukemia Cells.

Sayaka SOBUE, Satoshi NEMOTO, Masashi MURAKAMI, Hiromi ITO, Ami KIMURA, Sigiang GAO, Ayako FURUHATA, Akira TAKAGI, Tetsuhito KOJIMA, Mitsuhiro NAKAMURA,* Yoshinori ITO, Motoshi SUZUKI, Yoshiko BANNO, Yoshinori NOZAWA, Takashi MURATE

We reported increased sphingosine kinase 1 (SPHK1) and decreased neutral sphingomyelinase 2 (NSMase2) gene expression in myelodysplastic syndromes and acute leukemia. This alteration is supposed to change the cellular sphingolipid metabolites. Positive correlations were observed between daunorubicin (DA)-IC_{50} and the SPHK1 message, when 16 different leukemia cell lines were used to analyze the relationship between gene expressions and chemosensitivity against DA. SPHK1 is both a good marker to predict the DA sensitivity of leukemia cells and a potential therapeutic target for leukemia with high SPHK1 expression, and suggest that the sphingolipid rheostat plays a significant role in DA-induced cytotoxicity.

[*Biomed. Chromatogr.*, **22**, 387–393 (2008)] [Lab. of Drug Informatics]

A Simple and Rapid Determination of Valproic Acid in Human Plasma Using a Non-porous Silica Column and Liquid Chromatography with Tandem Mass Spectrometric Detection.

Katsuhiko MATSUURA, Tomofumi OHMORI, Mitsuhiro NAKAMURA,* Yoshinori ITOH, and Kazuyuki HIRANO

An LC-MS/MS assay was developed and validated to determine valproic acid in human plasma. The method involved a solid-phase extraction of valproic acid and betamethasone valerate, an internal standard, from plasma and detection using an LC-MS/MS system with electrospray ionization source in negative ion mode. Separation was achieved within 3 min on a non-porous silica column with mobile phase containing ammonium acetate and methanol. Multiple reaction monitoring was utilized for detection monitoring at 142.89-142.89 for valproic acid and 457.21-457.21 for the internal standard. The calibration curve for valproic acid was linear over the range of 0.5-150 microg/mL. The limit of detection was 0.17 microg/mL and the lower limit of quantification was 0.5 microg/mL, when 0.2 mL plasma was used for extraction. The percentage coefficient of validation for accuracy and precision (inter- and intra-day) for this method was less than 9.5% with recovery ranging from 82.3 to 86.9% for valproic acid.

[*J. Chromatogr. B*, **861**, 95–100 (2008)] [Lab. of Drug Informatics]

A Highly Sensitive Assay for Ritodrine in Human Serum by Hydrophilic Interaction Chromatography-Tandem Mass Spectrometry.

Tomofumi OHMORI, Mitsuhiro NAKAMURA,* Shin TADA, Tadashi SUGIYAMA, Yoshinori ITOH, Yasuhiro UDAGAWA, and Kazuyuki HIRANO

We developed a sensitive assay for ritodrine (RTD) in human serum. This method was based upon the selective technique by MS/MS using a hydrophilic interaction chromatography (HILIC) technique. This method involved a mixed-mode cation-exchange solid-phase extraction of RTD and isoxsuprine (IS). The detection was made with electrospray ionization source in positive ion mode. The separation of the analytes was achieved with a mobile phase of ammonium acetate and acetonitrile (10:90, v/v). MRM was utilized by monitoring 288.2→121.1 for RTD, 302.2→107.0 for IS. The lower limit of quantification was 0.39 ng/mL (97.5 fg on-column). The percent coefficient of validation for accuracy and precision (inter- and intra-day) was less than 9.8% and the recovery was ranged from 83.5 to 94.7% for RTD. This method enabled us to successfully determine RTD in maternal and fetal sera.

[*Helv. Chim. Acta*, **91**, 1989–1998 (2008)] [Lab.of Pharmacognosy]

Oligostilbenoids from Dipterocarpaceaeous Plants: a New Resveratrol Tetramer from *Vateria indica* and the Revised Structure of Isohopeaphenol.

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

The investigation of phenolic constituents in *Vateria indica* afforded five resveratrol tetramers, vateriaphenols B and C, isohopeaphenol, hopeaphenol, and shoreaketone. Their structures and configurations were established by spectroscopic methods. The structure assigned to vateriaphenol C was found to be identical with the structure reported in the literature for the resveratrol tetramer isohopeaphenol. The structure of isohopeaphenol was revised and confirmed by spectroscopic evidences.

[*Biol. Pharm. Bull.*, **31**, 1973–1976 (2008)] [Lab.of Pharmacognosy]

Histamine Release Inhibitory Activity of *Piper nigrum* Leaf.

Noriko HIRATA, Shunsuke NARUTO, Kazunori INABA, Kimihisa ITOH, Masashi TOKUNAGA, Munekazu IINUMA,* and Hideaki MATSUDA

Oral administration of an extract of *Piper nigrum* leaf showed a potent dose-dependent inhibition of dinitrofluorobenzene (DNFB)-induced cutaneous reaction at 1 h [immediate phase response (IPR)] after and 24 h [late phase response (LPR)] after DNFB challenge in mice which were passively sensitized with anti-dinitrophenyl IgE antibody. Ear swelling inhibitory effect of PN-extract on very late phase response (vLPR) in the model mice was significant but weaker than that on IPR. Oral administration of PN-extract inhibited picryl chloride-induced ear swelling in PC sensitized mice. PN-extract exhibited in vitro inhibitory effect on compound 48/80-induced histamine release. Two lignans of PN-extract, (-)-cubebin and (-)-3,4-dimethoxy-3,4-desmethylenedioxycubebin, were identified as major active principles having histamine release inhibitory activity.

[*Bioorg. Med. Chem.*, **16**, 7592–7598 (2008)] [Lab.of Pharmacognosy]

Inhibitory Effects of Polymethoxy Flavones Isolated from *Citrus reticulate* on Degranulation in Rat Basophilic Leukemia RBL-2H3: Enhanced Inhibition by Their Combination.

Tomohiro ITOH, Kenji OHGUCHI, Munekazu IINUMA,* Yoshinori NOZAWA, and Yukihiro AKAO

To clarify the inhibitory mechanism of degranulation by polymethoxy flavones (PMFs), we examined the activation of intracellular signaling molecules such as Lyn, Syk, and PLCgamma's. All the PMFs significantly suppressed the activation of Syk and PLCgamma's. In Ag-mediated activation of FceRI on mast cells, three major subfamilies of mitogen-activated protein kinases, especially ERK44/42, were activated. These PMFs reduced the level of phospho-ERKs. It was suggested that the suppression of Ag-stimulated degranulation by these PMFs mainly is due to the Syk/PLCgamma s/PKC pathway and Ca^{2+} influx.

[*Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.*, **47B**, 952–956 (2008)] [Lab.of Pharmacognosy]

Chemical Constituents of *Dendrobium gratiosissimum* and Their Cytotoxic Activities.

Chao-Feng ZHANG, Min WANG, Lei WANG, Munekazu IINUMA,* Mian ZHANG, Luo-Shan XU, and Zheng-Tao WANG

Two new bibenzyl derivatives, named dengraols A1 and B2, are isolated from stems of *Dendrobium gratiosissimum* Rchb. (Orchidaceae), together with 7 known compounds: 3,5,4'-trihydroxybibenzyl; 3,4'-dihydroxy-5-methoxybibenzyl; 3,4-dihydroxy-5,4'-dimethoxybibenzyl; 3,4'-dihydroxy-4,5,3'-trimethoxybibenzyl; 5,4'-dihydroxy-3,3'-dimethoxybibenzyl; 5,3',4'-trihydroxy-3-methoxy-bibenzyl and 5,3'-dihydroxy-3-methoxybibenzyl. Among the isolated compounds, dengraols A1 and B2, moscatilin and gigantol showed inhibitory activity of proliferation on HL-60 cells with IC_{50} values at 2.1, 6.4, 0.082 and 10.6 micro M, respectivery.

[*Nat. Prod. Commun.*, **3**, 809–814 (2008)] [Lab.of Pharmacognosy]

Membrane Activity-guided Isolation of Antiproliferative and Antiplatelet Constituent from *Evodiopanax innovans*.

Hironori TSUCHIYA, Toshiyuki TANAKA, Motohiko NAGAYAMA, Masayoshi OYAMA, and Munekazu IINUMA*

Bark of *Evodiopanax innovans* (Araliaceae) was subjected to membrane activity-guided fractionation. The potency to interact with lipid membranes and change their fluidity was determined by measuring fluorescence polarization of liposomal and cell membranes. Further purification led to the isolation of maltol 3-*O*-beta-glucopyranoside, which inhibited tumor cell growth and platelet aggregation, together with rigidifying the cell membranes as well as the membrane-active antitumor compound (-)-epigallocatechin gallate and doxorubicin. *E. innovans* is considered as a medicinal plant contg. a potent bioactive constituent that exerts antiproliferative and antiplatelet effects through interaction with cell membranes to modify their fluidity.

[*Bioorg. Med. Chem.*, **16**, 4500–4508 (2008)] [Lab.of Pharmacognosy]

Inhibitory Effect of Xanthones Isolated from the Pericarp of *Garcinia mangostana* L. on Rat Basophilic Leukemia RBL-2H3 Cell Degranulation.

Tomohiro ITOH, Kenji OHGUCHI, Munekazu IINUMA,* Yoshinori NOZAWA, and Yukihiro AKAO

In this study, we examined the effect of xanthones on cell degranulation in rat basophilic leukemia RBL-2H3 cells. These xanthones suppressed the release of histamine from IgE-sensitized RBL-2H3 cells. We examined the activation of intracellular signaling molecules such as Lyn, Syk, and PLCgamma's. All the xanthones tested significantly suppressed the signaling involving Syk and PLCgamma's. In Ag-mediated activation of FceRI on mast cells, three major subfamilies of mitogen-activated protein kinases were activated. The xanthones decreased the level of phospho-ERKs. Furthermore, the levels of phospho-ERKs were obsd. to be regulated by Syk/LAT/Ras/ERK pathway rather than PKC/Raf/ERK pathway, suggesting that the inhibitory mechanism of xanthones was mainly due to suppression of the Syk/PLCgamma s/PKC pathway.

[*Bioorg. Med. Chem.*, **16**, 2803–2810 (2008)] [Lab.of Pharmacognosy]

Interactive Effects of Polymethoxy Flavones from *Citrus* on Cell Growth Inhibition in Human Neuroblastoma SH-SY5Y Cells.

Yukihiro AKAO, Tomohiro ITOH, Kenji OHGUCHI, Munekazu IINUMA,* and Yoshinori NOZAWA

In the present study, we found that tangeretin, nobiletin, and 5-demethyl nobiletin exhibited a cancelling, synergistic, or additive effect when combinations of two of these three compounds were tested. As to the structure-activity relationship, the Me group at C-5 in nobiletin was shown to contribute to the anti-proliferative effect. By the combined treatment with tangeretin and 5-demethyl nobiletin, the apoptotic cell population and the activity of caspase-3 were synergistically elevated. The finding that tangeretin and 5-demethyl nobiletin induced apoptosis by reducing the mitochondrial membrane potential suggested that an intrinsic pathway of apoptosis was synergistically activated by the combination treatment with tangeretin and 5-demethyl nobiletin. These results indicate the relevance of the combination of phytochemicals for the enhancement of the anticancer effect.

[*Biochem. Biophys. Res. Commun.*, **368**, 948–954 (2008)] [Lab.of Pharmacognosy]

Preconditioning by Sesquiterpene Lactone Enhances H_2O_2-induced Nrf2/ARE Activation.

Ken UMEMURA, Tomohiro ITOH, Nanako HAMADA, Yasunori FUJITA, Yukihiro AKAO, Yoshinori NOZAWA, Nobuyasu MATSUURA, Munekazu IINUMA,* and Masafumi ITO

The Nrf2/ARE pathway plays a pivotal role in chemoprevention and neuroprotection. Here, we report that sesquiterpene lactones extractd. from *Calea urticifolia* and fever few increased enhancer activity of the ARE. The results suggest a possibility that preconditioning by sesquiterpene lactone may enhance activation of the Nrf2/ARE pathway and induction of phase II detoxification/antioxidant enzymes upon oxidative stress, thereby resulting in increased resistance to oxidative damage.

[*Bioorg. Med. Chem.*, **16**, 721–731 (2008)] [Lab.of Pharmacognosy]

Eupalinin A Isolated from *Eupatorium chinense* L. Induces Autophagocytosis in Human Leukemia HL60 Cells.

Tomohiro ITOH, Yuko ITO, Kenji OHGUCHI, Masayoshi OHYAMA, Munekazu IINUMA,* Yoshinori OTSUKI, Yoshinori NOZAWA, and Yukihiro AKAO

Eupalinin A, a natural phytoalexin included in *Eupatorium chinense* L., exhibited a marked inhibitory effect on cell growth in HL60 cells. We conclude that eupalinin A-induced cell death was mainly due to autophagy, which was initiated by increased ROS, resulting in the perturbation of mitochondrial membrane potential. Since the class III PI3K inhibitor such as 3-MA or LY294002 did not inhibit the eupalinin A-induced type II programmed cell death (PCD II), it was suggested that the PCD II was executed by Beclin-1 independent pathway of damage-induced mitochondrial autophagy (mitophagy).

[*J. Neurosci. Res.*, **86**, 1789–1800 (2008)] [Lab. of Mol. Biology]

Adenovirus-mediated Retrograde Transfer of Neurotrophin-3 Gene Enhances Survival of Anterior Horn Neurons of *twy/twy* Mice with Chronic Mechanical Compression of the Spinal Cord.

Kenzo UCHIDA, Hideaki NAKAJIMA, Tomoo INUKAI, Takaharu TAKAMURA, Shigeru KOBAYASHI, Shoei FURUKAWA,* and Hisatoshi BABA

Chronic mechanical compression of the spinal cord causes neural tissue damage. We investigated the efficacy of retrograde gene delivery of adenoviral vector (AdV) carrying neurotrophin-3 (NT-3) gene into twy (*twy/twy*) mouse spinal cord anterior horn neurons with chronic and progressive mechanical compression at C1-C2 level. Immunoreactivity to NT-3 was significantly enhanced in the AdV-NT-3-injected twy mice compared with the AdV-LacZ-injected mice. Retrograde NT-3 gene transfer to twy mouse anterior horn neurons increased neurite axonal length and arborization of WGA-HRP-labeled neurons. Our results suggest that targeted retrograde NT-3 gene delivery is feasible in the intact animal and that it enhances neuronal survival even under chronic mechanical compression of the spinal cord.

[*Spine.*, **33**, 2596–2604 (2008)] [Lab. of Mol. Biology]

Gene Expression Profiles of Neurotrophic Factors in Rat Cultured Spinal Cord Cells under Cyclic Tensile Stress.

Kenzo UCHIDA, Hideaki NAKAJIMA, Takaharu TAKAMURA, Shoei FURUKAWA,* Shigeru KOBAYASHI, Takafumi YAYAMA, and Hisatoshi BABA

OBJECTIVE: We evaluated *in vitro* expression of neurotrophic factors and receptors in cultured rat spinal cord cells under cyclic tensile stress. METHODS: Spinal cord cells were isolated for culture from 15-day Sprague-Dawley rat embryos. We used the FX3000 Flexercell Strain Unit to induce mechanical stress. RESULTS: Tensile stress for 6 hours resulted in reduction of spinal cord cells and loss of neurites. Cells that survived 24-hours stress showed swollen irregular-shaped soma, bleb formation, and fragmented neurites. The cell survival rate decreased, whereas lactate dehydrogenase release increased significantly at 6 hours. There were significant increases in mRNA expression levels of nerve growth factor, brain-derived neurotrophic factor, trkB, p75 neurotrophin receptor, glial cell line-derived neurotrophic factor, and caspase-9 during the early period after application of tensile stress.

[*Biochem. Biophys. Res. Commun.*, **369**, 1144–1149 (2008)] [Lab. of Mol. Biology]

PACAP Decides Neuronal Laminar Fate *via* PKA Signaling in the Developing Cerebral Cortex.

Masanari OHTSUKA, Hidefumi FUKUMITSU, and Shoei FURUKAWA*

Laminar formation in the developing cerebral cortex requires the precisely regulated generation of phenotype-specified neurons. To test the possible involvement of pituitary adenylate cyclase-activating polypeptide (PACAP) in this formation, we investigated the effects of PACAP administered into the telencephalic ventricular space of 13.5-day-old mouse embryos. PACAP partially inhibited the proliferation of cortical progenitors and altered the position and gene-expression profiles of newly generated neurons otherwise expected for layer IV to those of neurons for the deeper layers, V and VI, of the cerebral cortex. The former and latter effects were seen only when the parent progenitor cells were exposed to PACAP in the later and in earlier G1 phase, respectively; and these effects were suppressed by co-treatment with a protein kinase A (PKA) inhibitor. These observations suggest that PACAP participates in the processes forming the neuronal laminas in the developing cortex via the intracellular PKA pathway.

[*Biomed. Res.*, **29**, 163–170 (2008)] [Lab. of Mol. Biology]

Effects of Estrogens on Proliferation and Differentiation of Neural Stem/Progenitor Cells.

Makiko OKADA, Koichi MURASE, Akihisa MAKINO, Mitsunari NAKAJIMA, Teppei KAKU, Shoei FURUKAWA,* and Yoshiko FURUKAWA

We investigated the effect of the female hormone 17β-estradiol (E2) and the hormone mimic bisphenol A (BPA) on the proliferation and differentiation of rat neural stem/progenitors cells (NS/PCs) cultured from the telencephalon of embryonic day-15 rats. Basic fibroblast growth factor (FGF-2) is a potent mitogen of early generated NS/PCs, and is used for the proliferation of NS/PCs *in vitro*. Administration of E2 or BPA alone to the NS/PCs stimulated their proliferation in the absence but not in the presence of FGF-2. E2- or BPA-treatment increased the ratio of the oligodendrocytes generated from the NS/PCs to total cells; however, this ratio did not change when the cells were stimulated with platelet-derived growth factor (PDGF), a mitogen for oligodendrocyte precursors, or with neurotrophin-3, an oligogenic factor for glial progenitor cells. These results suggest that estrogens would influence the fate of NS/PCs when the cells are poorly supplied with mitogens or differentiation factors during the early stages of neurogenesis.

[*J. Neurosci. Res.*, **86**, 720–725 (2008)] [Lab. of Mol. Biology]

Ebselen, a Redox Regulator Containing a Selenium Atom, Induces Neurofilament M Expression in Cultured Rat Pheochromocytoma PC12 Cells *via* Activation of Mitogen-activated Protein Kinase.

Atsuyoshi NISHINA, Akihiro SEKIGUCHI, Yuxi HE, Mamoru KOKETSU, and Shoei FURUKAWA*

We found that ebselen [2-phenyl-1,2-benzisoselenazol-3(2H)-one] caused phosphorylation of mitogen-activated protein kinase (MAPK), followed by expression of neurofilament-M in cultured PC12 cells. The ebselen-induced MAPK activation was suppressed by U0126, an inhibitor of MAPK kinase (MEK1/2), but not by K252a, a inhibitor of Trk tyrosine kinases; AG1478, an antagonist of epidermal growth factor receptor (EGFR); pertussis toxin, an inhibitor of Gi/o; or GP antagonist-2A, an inhibitor of Gq. Furthermore, we observed that N-acetyl-L-cysteine, an inhibitor of tyrosine kinases, suppressed ebselen-induced MAPK activation and buthionine sulfoximine, an activator of protein tyrosine phosphatases, enhanced the effect, indicating that ebselen activated MEK1/2 through one or more tyrosine kinases. We propose that ebselen stimulated tyrosine kinase activity, thus activating a MAPK cascade (tyrosine kinase-MEK1/2-ERK1/2) in PC12 cells and that this activation resulted in their neuronal differentiation.

[*Free Radic. Res.*, **42**, 949–956 (2008)] [Lab. of Clinical Pharmaceutics]

Cobalt Chloride Decreases EC-SOD Expression through Intracellular ROS Generation and p38-MAPK Pathways in COS7 Cells.

Tetsuro KAMIYA,* Hirokazu HARA, Harutaka YAMADA, Hirokazu IMAI, Naoki INAGAKI, and Tetsuo ADACHI

The purpose of this study was to elucidate the regulation of extracellular-superoxide dismutase (EC-SOD) expression in cells under hypoxia. Our results show that the expressions of EC-SOD mRNA and protein in cobalt chloride ($CoCl_2$)-treated COS7 cells decreased in a dose- and time-dependent manner, whereas the expressions of other SOD isoforms, (Cu/Zn-SOD and Mn-SOD), were not changed. The down-regulation of EC-SOD mRNA was suppressed by pretreatment with the antioxidant trolox and the p38 mitogen-activated protein kinase (p38-MAPK) inhibitor SB203580. We concluded that the expression of EC-SOD is decreased through ROS and p38-MAPK signaling cascades and that the down-regulation of EC-SOD leads to a decrease in the resistance to oxidative stress of COS7 cells under hypoxia induced by $CoCl_2$.

[*Glia*, **56**, 89–96 (2008)] [Lab. of Clinical Pharmaceutics]

Microglia Induce Neurotoxicity via Intraneuronal Zn^{2+} Release and a K^+ Current Surge.

Megan E. KNOCH, Karen A. HARTNETT, Hirokazu HARA,* Karl KANDLER, and Elias AIZENMAN

We report here that reactive species released from activated microglia induce the liberation of Zn^{2+} from intracellular stores in cultured cortical neurons, with a subsequent enhancement in neuronal voltage-gated K^+ currents, two events that have been intimately linked to apoptosis. Both the intraneuronal Zn^{2+} release and the K^+ current surge could be prevented by the NADPH oxidase inhibitor apocynin and the free radical scavenging mixture of superoxide dismutase and catalase. The enhancement of K^+ currents was prevented by neuronal overexpression of metallothionein III or by expression of a dominant negative (DN) vector for the upstream mitogen-activated protein kinase apoptosis signal regulating kinase-1 (ASK-1). These results establish a direct link between microglial-generated oxygen and nitrogen reactive products and neuronal cell death mediated by intracellular Zn^{2+} release and a surge in K^+ currents.

[*Am. J. Respir. Crit. Care Med.*, **177**, 219–226 (2008)] [Lab. of Clinical pharmaceutics]

Gene Transfer of Extracellular Superoxide Dismutase Ameliorates Pulmonary Hypertension in Rats.

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Pulmonary hypertension (PH) is a life-threatening disease, characterized by vascular remodeling and vasoconstriction. Evidence suggests that oxidative stress may contribute to the pathogenesis and/or development of PH. In the present study, we examined whether intratracheal gene transfer of human extracellular superoxide dismutase (EC-SOD) could ameliorate monocrotaline (MCT)-induced PH in rats. EC-SOD overexpression to the lung ameliorated MCT-induced PH in rats. We suggest that EC-SOD may act as an anti-oxidant in PH and that increased oxidative stress may be important in the pathogenesis of MCT-induced PH.

[*Int. J. Cardiol., Mar 24 EPub* (2008)] [Lab. of Clinical pharmaceutics]

Serum Soluble Lectin-like Oxidized Low-density Lipoprotein Receptor-1 Correlates with Oxidative Stress Markers in Stable Coronary Artery Disease.

Fumihiko KAMEZAKI, Kazuhito YAMASHITA, Hiromi TASAKI, Noriaki KUME, Hirokazu MITSUOKA, Toru KITA, Tetsuo ADACHI,* and Yutaka OTSUJI

Although serum soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) is reported to be associated with acute coronary syndrome (ACS), its correlation with oxidative stress markers has not been elucidated. We therefore investigated the association of serum sLOX-1 with the severity of CAD, and serum biomarkers for oxidative stress and inflammation, as well as extracellular superoxide dismutase (EC-SOD), which is protective against oxidative stress in the vascular wall. Serum sLOX-1 levels were positively correlated with urinary 8-isoprostane levels and inversely correlated with EC-SOD levels. These results thus suggest that increased serum sLOX-1 levels may reflect enhanced oxidative stress in vascular walls.

[*Jpn. J. Pharm. Health Care Sci.*, **34**, 311–319 (2008)] [Lab. of Clinical Pharmaceutics]

Analysis of Advanced PBL Tutorial Trial and Assessment of Product Presentation.

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

Fourth-year students in the conventional 4-year pharmaceutical education curriculum took part in a trial on advanced problem-based learning (PBL) in preparation for its introduction at higher levels of the 6-year curriculum of pharmaceutical education, which was stated in April, 2006. Students were divided into 15 groups and the products prepared by each one were evaluated using the same evaluation scale by the audience (instructors and students of other groups) as well as students of the group that made the presentation. Data obtained were subjects to Customer Satisfaction (CS) analysis, which is generally used for market survey. A case of acute promyelocytic leukemia, typhoid fever, acute myocardial infarction, anaphylactic shock or sigmoid colon cancer was randomly assigned to each group.

[*Nutri. Res.*, **28**, 137–143 (2008)] [Lab. of Clinical Pharmaceutics]

Supplementation of Hydrogen-rich Water Improves Lipid and Glucose Metabolism in Patients with Type 2 Diabetes or Impaired Glucose Tolerance.

Shizuo KAJIYAMA, Goji HASEGAWA, Mai ASANO, Hiroko HOSODA, Michiaki FUKUI, Naoto NAKAMURA, Jo KITAWAKI, Saeko IMAI, Koji NAKANO, Mitsuhiro OHTA, Tetsuo ADACHI,* Hiroshi OBAYASHI and Toshikazu YOSHIKAWA

Oxidative stress is recognized widely as being associated with various disorders including diabetes, hypertension, and atherosclerosis. It is well established that hydrogen has a reducing action. We therefore investigated the effects of hydrogen-rich water intake on lipid and glucose metabolism in patients with either type 2 diabetes mellitus or impaired glucose tolerance. Hydrogen-rich water intake was also associated with a trend of decreased serum concentrations of oxidized LDL and free fatty acids, and increased plasma levels of adiponectin and extracellular-superoxide dismutase. In conclusion, these results suggest that supplementation with hydrogen-rich water may have a beneficial role in prevention of type 2 diabetes mellitus and insulin resistance.

[*Ningen Dock*, **22**, 35–41 (2008)] [Lab. of Clinical Pharmaceutics]

Evaluation of Immunoglobulin G Antibody Titer Measurement in the Simplified Test for Multiple Bacterial Infection in Periodontal Disease Based on Self-Sampling of Fingertip Capillary Blood. –Focusing of *Porphyromonas gingivalis* Antigen–

Eisuke MAEHATA, Yojiro MAEHATA, Masaichi-Cong-il LEE, Chieko KUDO, Shogo TAKASHIBA, Hiroji SHIMOMURA, Minoru YAMAKADO, Masao YANO, Teruo SHIBA, Ikuo HATAKEYAMA, Minoru INOUE, Kunio KOUKA, Tetsuo ADACHI,* Naoya KISHIKAWA, Naotaka KURODA, Shinya SUGIMOTO, Hiromi WATANABE, Kazumasa KOGA, Naoko IKOSHI, and Katsuhisa SHIMIZU

Periodontal disease is a multiple infection caused by bacteria represented by *Porphyromonas gingivalis*. The prevalence of periodontal disease is high, as it predominantly affects the people in the same age range as diabetes mellitus, but it is often overlooked because of the lack of subjective symptoms. The ability to detect periodontal disease in the setting of "Human Dry Dock" screening using the sampling and test methods proposed by us would be an important means to improve services.

[*Yakugaku Zasshi*, **128**, 1227–1233 (2008)] [Lab. of Clinical Pharmaceutics]

Evaluation of the Cardio Pulmonary Resuscitation / Automated External Defibrillator Class and Disability Experiences Class Provided as Part of the Early Exposure Program.

Eiji SAKAI, Hitomi TERAMACHI, Hiroyuki NISHIDA, and Tetsuo ADACHI*

Many students had already participated in a previous CPR class (79%) and/or disability experiences class (55%), and some students had even experienced a real-life situation requiring these techniques (9% and 14%, respectively). Those with previous training experience performed more effectively in this year's early exposure program than those without such an experience. Due to the active participation of most students during the training, a significantly higher level of satisfaction with the program than that previously expected was achieved (mean score on a 5-grade scale: 3.98 vs. 3.31 for the CPR/AED class; 4.35 vs. 3.69 for the disability experiences class, respectively). Furthermore, many students commented that their experience in this year's program increased their motivation for future training and confidence to cope with a real-life situation in the future.

[*J. Cereb. Blood Flow. Metabol.*, **28**, 354–366 (2008)] [Lab. of Molecular Pharmacology]

Propofol Exerts Greater Neuroprotection with Disodium Edentate than without It.

Yoshinori KOTANI, Yoshimi NAKAJIMA, Tatsuya HASEGAWA, Masahiko SATOH, Hisamitsu NAGASE, Masamitsu SHIMAZAWA, Shinichi YOSHIMURA, Toru IWAMA, and Hideaki HARA*

Propofol provided neuroprotection against both *in vivo* and *in vitro* ischemic damage, and its effects were enhanced when EDTA is added, EDTA itself protected against ischemic neuronal damage, possibly part by due to its zinc-chelating action.

[*Exp. Eye Res.*, **86**, 131–137 (2008)] [Lab. of Molecular Pharmacology]

Rifampicin Inhibits the Retinal Neovascularization *in vitro* and *in vivo*.

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

We examined the anti-angiogenic effect on tube formation and proliferation by human umbilical vein endothelial cells (HUVECs) *in vitro* and on retinal neovascularization in a murine oxygen-induced retinopathy model *in vivo*. Rifampicin significantly suppressed retinal neovascularization (versus vehicle treatment), but revascularization of the capillary-free area did not differ between vehicle and rifampicin treatment. Rifampicin had anti-angiogenic effects *in vitro* and *in vivo*, and may be useful as an anti-angiogenic agent in the treatment of retinal neovascularization diseases.

[*Neuroscience*, **151**, 111–119 (2008)] [Lab. of Molecular Pharmacology]

Involvement of Endoplasmic Reticulum Stress in The Neuronal Death Induced by Transient Forebrain Ischemia in Gerbil.

Yasuhisa OIDA, Masamitsu SHIMAZAWA, Kazunori IMAIZUMI, and Hideaki HARA*

Endoplasmic reticulum (ER)-stress, which is caused by an accumulation of unfolded proteins in the ER lumen, is associated with stroke and with neurodegenerative diseases such as Parkinson's and Alzheimer diseases. We assessed the expression patterns of immunoglobulin heavy chain binding protein (BiP)/ glucose-regulated protein (GRP) 78 (an ER-resident molecular chaperone whose expression serves as a good marker of ER-stress), activating transcription factor (ATF)-4, and C/EBP homology protein (CHOP) by immunohistochemistry and/or Western blotting after transient forebrain ischemia in gerbils. In conclusion, we suggest that ER-stress is involved in the CA1-selective neuronal cell death we observed in a gerbil transient forebrain ischemia model.

[*Cell Death Differ.*, **15**, 364–375 (2008)] [Lab. of Molecular Pharmacology]

A Molecular Chaperone Inducer Protects Neurons from ER Stress.

Takashi KUDO, Soshi KANEMOTO, Hideaki HARA,* Nobutaka MORIMOTO, Takashi MORIHARA, Ryo KIMURA, Takeshi TABIRA, Kazunori IMAIZUMI, and Masatoshi TAKEDA

Pretreatment of neuroblastoma cells with Bip inducer X (BIX) reduced cell death induced by ER stress. Intracerebroventricular pretreatment with BIX reduced the area of infarction due to focal cerebral ischemia in mice. In the penumbra of BIX-treated mice, ER stress-induced apoptosis was suppressed, leading to a reduction in the number of apoptotic cells. Considering these results together, it appears that BIX induces BiP to prevent neuronal death by ER stress, suggesting that it may be a potential therapeutic agent for cerebral diseases caused by ER stress.

[*J. Pharmacol. Sci.*, **106**, 128–135 (2008)] [Lab. of Molecular Pharmacology]

Phosphatidylinositol Inhibits Vascular Endothelial Growth Factor-A-induced Migration of Human Umbilical Vein Endothelial Cells.

Nozomu MATSUNAGA, Masamitsu SHIMAZAWA, Kazumasa OTSUBO, and Hideaki HARA*

Phosphatidylinositol (PI), a phospholipid in component of cell membranes, is widely distributed in animals, plants, and microorganisms. We examined *in vitro* whether PI inhibits the angiogenesis induced by vascular endothelial growth factor-A (VEGF-A). The data in the present study indicate that PI inhibits VEGF-induced angiogenesis by inhibiting HUVECs migration, and that inhibition of phosphorylated-Akt and -p38MAPK may be involved in the mechanism. Therefore, PI may be expected to prevent some diseases caused by angiogenesis.

[*Biosci. Biotech. Biochem.*, **72**, 335–345 (2008)] [Lab. of Molecular Pharmacology]

Laxative Effect of Agarwood Leaves and Its Mechanism.

Hideaki HARA,* Yasuaki ISE, Nobutaka MORIMOTO, Masamitsu SHIMAZAWA, Koji ICHIHASHI, Masayoshi OHYAMA, and Munekazu IINUMA

We investigated the laxative activity of an extract of agarwood leaves from *Aquilaria sinensis*. Laxative activity was measured in mice by counting stool frequency and stool weight. The drugs were orally administered. Acetone extract of agarwood leaves and senna increased stool frequency and weight, but methanol extract did not. The laxative effect of acetone extract was milder than that of the anthraquinoid laxative senna, and the former did not induce diarrhea as a severe side effect. We identified the main constituent contributing to the laxative effect of acetone extract as genkwanin 5-*O*-β-primeveroside (Compound 4). Compound 4 strengthened spontaneous motility and induced constraction in the ileum. The ileal contraction induced by Compound 4 was inhibited by atropine, but not by azasetron, suggesting that the effect of Compound 4 was mediated by acetylcholine receptors, not by serotonin. The laxative mechanism of Compound 4 may in part involve stimulation of intestinal motility via acetylcholine receptors.

[*Brain Res.*, **1214**, 169–176 (2008)] [Lab. of Molecular Pharmacology]

Protective Effects of SUN N8075, a Novel Agent with Antioxidant Properties, in *in vitro* and *in vivo* Models of Parkinson's Disease.

Atsushi OYAGI, Yasuhisa OIDA, Hirokazu HARA, Hiroshi IZUTA, Masamitsu SHIMAZAWA, Nozomu MATSUNAGA, Tetsuo ADACHI, and Hideaki HARA*

SUN N8075 is a novel antioxidant with neuroprotective properties. This study was designed to elucidate its neuroprotective effects against 6-hydroxy dopamine (6-OHDA)-induced cell death and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity (known as *in vitro* and *in vivo* models of Parkinson's disease, respectively). The present results indicate that SUN N8075 exerts protective effects, at least in part via an anti-oxidation mechanism, in these *in vitro* and *in vivo* models of Parkinson's disease.

[*Brain Res.*, **1212**, 89–101 (2008)] [Lab. of Molecular Pharmacology]

Degenerative Alterations in the Visual Pathway after NMDA-induced Retinal Damage in Mice.

Yasushi ITO, Masamitsu SHIMAZAWA, Yuta INOKUCHI, Hidefumi FUKUMITSU, Syoei FURUKAWA, Makoto ARAIE, and Hideaki HARA*

We demonstrated time- and region-dependent alterations in lateral geniculate nucleus (LGN) following *N*-methyl-D-aspartate (NMDA)-induced retinal damage in mice. Furthermore, BDNF may be a beneficial factor protecting LGN neurons after NMDA-induced retinal damage. Indeed, in our previous report, BDNF has been reported to exhibit a protective effect against shrinkage of retinal ganglion cells at in the early phase after optic nerve axotomy in rats. Although the mechanisms underlying neuronal degeneration and protection are not yet fully understood, further studies using transgenic mice may be an effective way of elucidating these mechanisms, to judge from the findings made in the present study.

[*Org. Biomol. Chem.*, **6**, 1540–1543 (2008)] [Lab. of Molecular Pharmacology]

Enzymatic Resolution and Evaluation of Enantiomers of *cis*-5'-Hydroxythalidomide.

Takeshi YAMAMOTO, Norio SHIBATA, Masayuki TAKASHIMA, Shuichi NAKAMURA, Takeshi TORU, Nozomu MATSUNAGA, and Hideaki HARA*

The straightforward synthesis of both enantiomers of *cis*-5'-hydroxythalidomide, a major metabolite of thalidomide, has been accomplished by enzymatic kinetic resolution of a racemic substrate catalyzed by *Pseudomonas stutzeri* lipase TL. *Cis*-5'-hydroxythalidomide shows resistance to racemization (and epimerization) at physiological pH. A tube formation assay to assess the ability to inhibit angiogenesis revealed that *cis*-5'-hydroxythalidomides are inactive.

[*Brain Res.*, **1208**, 217–224 (2008)] [Lab. of Molecular Pharmacology]

Induction of BiP, an ER-resident Protein, Prevents the Neuronal Death Induced by Transient Forebrain Ischemia in Gerbil.

Yasuhisa OIDA, Hiroshi IZUTA, Atsushi OYAGI, Masamitsu SHIMAZAWA, Takashi KUDO, Kazunori IMAIZUMI, and Hideaki HARA*

We evaluated the effect of a selective inducer of immunoglobulin heavy chain binding protein (BiP) (BiP inducer X; BIX) against both tunicamycin-induced cell death (in SH-SY5Y cells) and the effects of global transient forebrain ischemia. BIX significantly induced BiP expression both *in vitro* and *in vivo*. Pretreatment with BIX reduced the cell death induced by tunicamycin in SH-SY5Y cells. In gerbils subjected to forebrain ischemia, prior treatment with BIX (intracerebroventricular injection) protected against cell death and decreased TUNEL-positive cells in the hippocampal CA1 subfield. These findings indicate that this selective inducer of BiP could be used to prevent the neuronal damage both *in vitro* and *in vivo*.

[*Exp. Eye Res.*, **86**, 770–782 (2008)] [Lab. of Molecular Pharmacology]

Changes in Visual Fields and Lateral Geniculate Nucleus in Monkey Laser-induced High Intraocular Pressure Model.

Masaaki SASAOKA, Katsuki NAKAMURA, Masamitsu SHIMAZAWA, Yasushi ITO, Makoto ARAIE, and Hideaki HARA*

We examined visual field changes, retinal ganglion cell (RGC) and lateral geniculate nucleus (LGN) numbers, and glial fibrous acidic protein (GFAP) immunohistochemistry in the LGN in each of two monkeys. Visual field sensitivity, RGC number, and neuronal density of LGN were all decreased by high intraocular pressure (IOP). The relationship between loss of RGC and decrease in visual field sensitivity depended on the eccentricity from the fovea. Moreover, LGN immunohistochemistry revealed greater increases in GFAP expression in the layers receiving a neuronal input from the high IOP eye than in those receiving a neuronal input from the contralateral untreated eye.

[*Ophthalmology*, **115**, 1916–1922 (2008)] [Lab. of Molecular Pharmacology]

Role of Soluble Vascular Endothelial Growth Factor receptor-1 in the Vitreous in Proliferative Diabetic Retinopathy.

Nozomu MATSUNAGA, Yuichi CHIKARAISHI, Hiroshi IZUTA, Naoko OGATA, Masamitsu SHIMAZAWA, Miyo MATSUMURA, and Hideaki HARA*

The purpose was to determine the vitreous level of soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) in patients with proliferative diabetic retinopathy (PDR) or idiopathic macular hole (MH) and to investigate the relationships among sVEGFR-1, vascular endothelial growth factor (VEGF), and pigment epithelium-derived factor (PEDF). In the vitreous fluids of PDR patients, the sVEGFR-1 level was increased (vs. that in MH patients), and sVEGFR-1 correlated significantly with VEGF. *In vitro*, sVEGFR-1 reduced VEGF-induced angiogenesis. Hence, sVEGFR-1 may play a pivotal antiangiogenic role in PDR.

[*J. Agr. Food Chem.*, **56**, 8944–8953 (2008)] [Lab. of Molecular Pharmacology]

Protective Effects of Chinese Propolis and Its Component, Chrysin against Neuronal Cell Death *via* Inhibition of Mitochondrial Apoptosis Pathway in SH-SY5Y Cells.

Hiroshi IZUTA, Masamitsu SHIMAZAWA, Shigemi TAZAWA, Yoko ARAKI, Satoshi MISHIMA, and Hideaki HARA*

The purpose of this study was to evaluate the effects of Chinese propolis and its constituents against tunicamycin-induced neuronal cell death in SH-SY5Y cells. Both Chinese propolis and chrysin concentration-dependently inhibited such cell death, the tunicamycin-induced activation of caspase-3, and the effects of tunicamycin on mitochondria. Chinese propolis and chrysin each inhibited the staurosporine-induced cell death. These findings indicate that the inhibitory effects of Chinese propolis against the neuronal cell death induced by ER stress or staurosporine may be exerted primarily by chrysin. Moreover, the mechanism underlying the protective effects may, at least partly, involve inhibitions of caspase-3 activity and the mitochondrial apoptotic pathway.

[*CNS Neurosci. Ther.,* **14**, 192–202 (2008)] [Lab. of Molecular Pharmacology]

Memantine Protects against Secondary Neuronal Degeneration in Lateral Geniculate Nucleus and Superior Colliculus after Retinal Damage in Mice.

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

The purpose of this study, on mice, was to determine whether memantine, a glutamate-receptor antagonist of the *N*-methyl-D-aspartate (NMDA) subtype, protects against neuronal degeneration in the dorsal lateral geniculate nucleus (dLGN) and superior colliculus (SC) after the induction of retinal damage by intravitreal injection of NMDA. Memantine protected against the secondary neuronal degeneration in brain regions in the visual pathway that occurs after retinal damage in mice.

[*Neurosci. Lett.*, **441**, 224–228 (2008)] [Lab. of Molecular Pharmacology]

Combination Effects of Normobaric Hyperoxia and Edaravone on Focal Cerebral Ischemia-induced Neuronal Damage in Mice.

Yuko NONAKA, 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 occlusion were treated with cilostazol 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. Cilostazol protected against ischemic brain injury and hemorrhagic transformation in mice subjected to transient focal cerebral ischemia.

[*Brain Res.,* **1226**, 226–233 (2008)] [Lab. of Molecular Pharmacology]

Coenzyme Q_{10} Protects Retinal Cells against Oxidative Stress *in vitro* and *in vivo*.

Yoshimi NAKAJIMA, Yuta INOKUCHI, Masahiro NISHI, Masamitsu SHIMAZAWA, Kazumasa OTSUBO, and Hideaki HARA*

The purpose was to investigate the neuroprotective effects of coenzyme Q_{10} and/or a vitamin E analogue on retinal damage both *in vitro* and *in vivo*. In a rat ganglion cell-line transformed using E1A virus, a combination of coenzyme Q_{10} and trolox, a water-soluble vitamin E analogue (a derivative of α-tocopherol), prevented cell damage more effectively than either agent alone. Coenzyme Q_{10} and α-tocopherol (separately or together) reduced the retinal damage, number of TUNEL-positive cells in the ganglion cell layer, and 4-hydroxyl-2-nonenal expression induced by NMDA in mice i*n vivo*. Coenzyme Q_{10} and/or these vitamin E analogues exerted neuroprotective effects against retinal damage both *in vitro* and *in vivo*.

[*J. Neurochem.,* **107**, 279–290 (2008)] [Lab. of Molecular Pharmacology]

Reduced Retinal Function in Amyloid Precursor Protein-over-expressing Transgenic Mice *via* Attenuating Glutamate-*N*-Methyl-D-Aspartate Receptor Signaling.

Masamitsu SHIMAZAWA, Yuta INOKUCHI, Takashi OKUNO, Yoshihiro NAKAJIMA, Gaku SAKAGUCHI, Akira KATO, Hidehiro OKU, Tetsuya SUGIYAMA, Takashi KUDO, Tsunehiko IKEDA, Masatoshi TAKEDA, and Hideaki HARA*

We examined whether amyloid-β (Aβ) protein participates in cell death and retinal function using three types of transgenic (Tg) mice *in vivo* [human mutant amyloid precursor protein (APP) Tg (Tg 2576) mice, mutant presenilin-1 (PS-1) knock-in mice, and APP/PS-1 double Tg mice]. We found evidence suggesting that exposure to Aβ may reduce activation of NMDA-receptor signaling pathways and lead to retinal dysfunction. Our findings may indicate a potential new target for therapeutic interventions against retinal diseases.

[*J. Pharmacy Pharmacol.*, **60**, 1365–1374 (2008)] [Lab. of Molecular Pharmacology]

Astaxanthin, a Dietary Carotenoid, Protects Retinal Cells against Oxidative Stress *in vitro* and *in vivo*.

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

We investigated whether astaxanthin exerts neuroprotective effects in retinal ganglion cells *in vitro* and/or *in vivo*. Astaxanthin has neuroprotective effects against retinal damage both *in vitro* and *in vivo*, and that its protective effects may be partly mediated *via* its antioxidant effects.

[*J. Compl. Integr. Med.*, **5**, 1, 20 (2008)] [Lab. of Molecular Pharmacology]

Antiangiogenic Effects of Chinese Medicines (Hachimijiogan and Kogikujiogan) on Vascular Endothelial Growth Factor-A-induced Tube Formation in Human Umbilical Vein Endothelial Cells Co-cultured with Fibroblasts.

Rumi UCHIBAYASHI, Yuri YOKOYAMA, Yoshimi NAKAJIMA, Nozomu MATSUNAGA, Masamitsu SHIMAZAWA, and Hideaki HARA*

The aim of this study was to examine the antiangiogenic properties and antioxidant activities of two Chinese medicines (Hachimijiogan and Kogikujiogan). Each of these medicines concentration-dependently inhibited vascular endothelial growth factor-A (VEGF-A)-induced tube formation in a co-culture of human umbilical vein endothelial cells (HUVECs) and fibroblasts. In addition, they each at 100 μg/ml inhibited the VEGF-A-induced cell proliferation of HUVECs. Hachimijiogan at 10 and 100 μg/ml and Kogikujiogan at 100 μg/ml also inhibited the VEGF-A-induced cell migration of HUVECs, and Hachimijiogan at 50 μg/ml and Kogikujiogan at 5, 50, and 100 μg/ml inhibited lipid peroxidation in mouse forebrain homogenates. These findings indicate that Hachimijiogan and Kogikujiogan have antiangiogenic and antioxidant effects, and that their antioxidant effects may be partly responsible for their antiangiogenic effects.

[*Neurosci.*, **157**, 309–318 (2008)] [Lab. of Molecular Pharmacology]

A Novel Calpain Inhibitor, ((1S)-1((((1S)-1-Benzyl-3-cyclopropylamino-2,3-di-oxopropyl)amino)carbonyl)-3-methylbutyl) Carbamic Acid 5-Methoxy-3-oxapentyl Ester, Protects Neuronal Cells from Cerebral Ischemia-induced Damage in Mice.

Akihiro KOUMURA, Yuko NONAKA, Kana HYAKKOKU, Takayuki OKA, Masamitsu SHIMAZAWA, Isao HOZUMI, Takashi INUZUKA, and Hideaki HARA*

We evaluated the effect of SNJ-1945, ((*1S*)-1((((*1S*)-1-Benzyl-3-cyclopropylamino-2,3-di-oxopropyl)amino)carbonyl)-3-methylbutyl) carbamic acid 5-methoxy-3-oxapentyl ester, on the focal brain ischemia induced by middle cerebral artery occlusion (MCAO) in mice. SNJ-1945 is a novel calpain inhibitor that has good membrane permeability and water solubility. SNJ-1945 inhibited the activation of calpain, and offers neuroprotection against the effects of acute cerebral ischemia in mice even when given up to 6 h after MCAO. SNJ-1945 may therefore be a potential drug for stroke.

[*Jpn. J. Pharm. Health. Care Sci.*, **34**, 103–111 (2008)] [Lab.of Pharmacy Practice and Social Science]

Development of the System for Supporting Pharmaceutical Practices Based on the Electronic Medical Record System.

Takashi NIWA, Tadashi SUGIYAMA,* Shinji OKAYASU, Eriko YAMAUCHI, Minako NISHIGAKI, Katuhiko MATSUURA, Chitoshi GOTO, and Yoshinori ITOH

We have developed the support system for pharmaceutical care using the commercial pharmaceutical care services system that was modified based on the electronic medical record system. The system exploited data from the pharmacy information system and those from electronic medical record system, which enabled us to easily transmit and receive clinical data between the pharmacy information system and the electronic medical record system. It was evident that the application of this system enabled us to spare time for pharmaceutical care services. Thus, we concluded that our system was potentially useful for the support of work of clinical pharmacists.

[*J. Jpn. Health Med. Associ.*, **16**, 21–27 (2008)] [Lab. of Anatomy]

The Influence of Recreational Activities on One's Emotional State.

Toshiro OKAZAKI, Hiroko SUGIURA, Masato INOUE and Haruo SUGIURA*.

The purpose of this study is to examine, by Profile of Mood States (POMS), Total Mood Disturbance (TMD) and Effective Recreation Activity Points (ERAP), how different levels of leadership ability affect people's emotional states in a Janken-pon activity. The sample population, four males and twenty-five females was voluntarily recruited. In an experimental setting, a beginner instructor and an advanced instructor separately took a lead in the same Janken-pon activity. The results of this experimental study evidently show that regardless of different levels of leadership ability, the Janken-pon activity decreases negative moods and enhance positive moods in participants.

[*Heterocycles*, **76**, 833–843 (2008)] [Lab. of General Chemistry]

Synthesis of Chiral (Sulfinyl)furyl Oxazoline Ligands and Its Application to Enantioselective Palladium-catalyzed Allylic Alkylation.

Yusuke BUNYA, Takaaki SENGOKU, Yoko IMAMURA, and Yoshitsugu ARAI*

Chiral ligands containing an oxazoline substituent and a sulfur functionality at the furan ring have been synthesized and examined for enantioselective palladium catalyzed allylic substitution reaction. The enantioselectivity observed was found to be dependent upon the nature of the sulfur center and oxazoline group, up to 91% ee was given with chiral (3-sulfinyl)furan-2-yl ligand.

[*Vaccine*, **26**, 469–476 (2008)] [Lab. of Microbiology]

Protection of Mice from Shiga Toxin-2 Toxemia by Mucosal Vaccine of Shiga Toxin 2B-His with *Escherichia coli* Enterotoxin.

Takao TSUJI, Takeshi SHIMIZU, Keiko SASAKI, Yoshiyasu SHIMIZU, Kentaro TSUKAMOTO, Hideyuki ARIMITSU, Sadayuki OCHI, Satoshi SUGIYAMA, Koki TANIGUCHI, Paola NERI, and Hiroshi MORI*

Shiga toxin (Stx) 1 and Stx-2 produced by enterohemorrhagic *E. coli* (EHEC) strains cause severe diseases. The nontoxic B subunits of Stx-2 may be useful as antigen, despite the low immunogenicity. In the present study, we examined if His-tagged recombinant Stx-2B (Stx-2B-His) could be used to induce neutralizing antibodies. Mice were immunized intranasally with Stx-2B-His alone or along with a mutant of *E. coli* heat-labile enterotoxin (mLT) as adjuvant. Mice immunized with Stx-2B-His plus mLT showed higher titers of specific antibodies in the serum and lung fluid lavage than those immunized with Stx-2B-His alone. Serum from mice immunized with Stx-2B-His plus mLT neutralized the cytotoxicity of Stx-2 in vitro and in vivo.

[*Vaccine*, **26**, 2092–2099 (2008)] [Lab. of Microbiology]

A Nasal Vaccine Comprising B-subunit Derivative of Shiga Toxin 2 for Cross-protection against Shiga Toxin Types 1 and 2.

Takao TSUJI, Takeshi SHIMIZU, Keiko SASAKI, Kentaro TSUKAMOTO, Hideyuki ARIMITSU, Sadayuki OCHI, Toshiyasu SHIMIZU, Koki TANIGUCHI, Masatoshi NODA, Paola NERI, and Hiroshi MORI*

It is reported that Shiga toxin (Stx) 1 and Stx-2 are antigenically different and antibodies to Stx-1 did not neutralize Stx-2 and viceversa. In this study, we assessed if immunization with His-tagged recombinant Stx-1B (Stx-1B-His) or Stx-2B-His could induce antibodies neutralizing both toxins. Mice were immunized intranasally with Stx-1B-His or Stx-2B-His along with a mutant of *E. coli* heat-labile enterotoxin (mLT) as adjuvant. Antibodies in serum and bronchial alveolar fluid lavage (BALF) from mice immunized with Stx-1B-His reacted with Stx-1 and Stx-2, but they neutralized only Stx-1 in vitro. In contrast, antibodies in serum and BALF from mice immunized with Stx-2B-His reacted with Stx-1 and Stx-2, and neutralized both toxins in vitro and in vivo.

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