[*Chem. Pharm. Bull.* **60**, 402-407 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Development of a Hypoxia-selective Near-infrared Fluorescent Probe for Non-invasive Tumor Imaging.

Bahaa G. M. YOUSSIF, Kensuke OKUDA*, Tetsuya KADONOSONO, Ola I. A. R. SALEM, Alaa A. M. HAYALLAH, Mostafa A. HUSSEIN, Shinae KIZAKA-KONDOH and Hideko NAGASAWA*

A near-infrared fluorochrome, GPU-311, was designed, synthesized and evaluated for its application in non-invasive imaging of tumor hypoxia. Efficient synthesis was achieved by nucleophilic substitution and click chemistry using the bifunctional glycol linker containing thiol and azide groups for the conjugation of the propargylated nitroimidazole and the heptamethine cyanine dye. GPU-311 exhibited long excitation and emission wavelength (Ex/Em=785/802 nm) and a decent quantum yield (0.05). After *in vitro* treatment of SUIT-2/HRE-Luc cells with GPU-311, a higher fluorescence was observed selectively in hypoxia than in normoxia. However, *in vivo* imaging revealed inadequate accumulation of GPU-311 in tumors due to its rapid elimination through the liver.

[*Bioconjugate Chem.* **23**, 324-329 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

2-Nitroimidazole-tricarbocyanine Conjugate as a Near-infrared Fluorescent Probe for in Vivo Imaging of Tumor Hypoxia.

Kensuke OKUDA*, Yasuyuki OKABE, Tetsuya KADONOSONO, Takahiro UENO, Bahaa G. M. YOUSSIF, Shinae KIZAKA-KONDOH and Hideko NAGASAWA*

We developed a novel near-infrared (NIR) fluorescent probe, GPU-167, for in vivo imaging of tumor hypoxia. GPU-167 comprises a tricarbocyanine dye as an NIR fluorophore and two 2-nitroimidazole moieties as exogenous hypoxia markers that undergo bioreductive activation and then selective entrapment in hypoxic cells. After treatment with GPU-167, tumor cells contained significantly higher levels of fluorescence in hypoxia than in normoxia. *In vivo* fluorescence imaging specifically detected GPU-167 in tumors 24 h after administration. *Ex vivo* analysis revealed that fluorescence showed a strong correlation with hypoxia inducible factor (HIF)-1 active region. These data suggest that GPU-167 is a promising in vivo optical imaging probe for tumor hypoxia.

[*J. Med. Chem.* **55**, 2970-2980 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Discovery of a Novel Class of Potent Human Deoxyuridine Triphosphatase Inhibitors Remarkably Enhancing the Antitumor Activity of Thymidylate Synthase Inhibitors.

Seiji MIYAHARA, Hitoshi MIYAKOSHI, Tatsushi YOKOGAWA, Khoon Tee CHONG, Junko TAGUCHI, Toshiharu MUTO, Kanji ENDOH, Wakako YANO, Takeshi WAKASA, Hiroyuki UENO, Yayoi TAKAO, Akio FUJIOKA, Akihiro HASHIMOTO, Kenjirou ITOU, Keisuke YAMAMURA, Makoto NOMURA, Hideko NAGASAWA*, Satoshi SHUTO and Masayoshi FUKUOKA

A novel class of dUTPase inhibitors were developped based on the SAR studies of uracil derivatives. We developed compound **26**, which is the most potent human dUTPase inhibitor (IC_{50} = 0.021 μM) reported to date. Not only does compound **26** significantly enhance the growth inhibition activity of FdUrd against HeLa S3 cells (EC_{50} = 0.075 μM) but also shows robust antitumor activity against MX-1 breast cancer xenograft model in mice when administered orally with a continuous infusion of 5-FU.

[*J. Med. Chem.* **55**, 2960-2969 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Synthesis and Discovery of *N*-Carbonylpyrrolidine- or *N*-Sulfonylpyrrolidine-containing Uracil Derivatives as Potent Human Deoxyuridine Triphosphatase Inhibitors.

Hitoshi MIYAKOSHI, Seiji MIYAHARA, Tatsushi YOKOGAWA, Khoon Tee CHONG, Junko TAGUCHI, Kanji ENDOH, Wakako YANO, Takeshi WAKASA, Hiroyuki UENO, Yayoi TAKAO, Makoto NOMURA, Satoshi SHUTO, Hideko NAGASAWA* and Masayoshi FUKUOKA

We have developed potent drug-like dUTPase inhibitors based on SAR studies of uracil derivatives. *N*-Carbonylpyrrolidine- and N-sulfonylpyrrolidine-containing uracils were found to be promising scaffolds that led us to human dUTPase inhibitors (**12k**) having excellent potencies (IC_{50} = 0.15 μM). The X-ray structure of a complex of **16a** and human dUTPase revealed a unique binding mode, and are stacked on each other. Compounds **12a** and **16a** markedly enhanced the inhibition activity of FdUrd against HeLa S3 cells (EC_{50} = 0.27-0.30 μM), suggesting that our dUTPase inhibitors were effective in combination with TS inhibitors.

[*Exp. Cell. Res.* **318**, 1554-1563 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

The Novel Hypoxic Cytotoxin, TX-2098 Has Antitumor Effect in Pancreatic Cancer; Possible Mechanism Through Inhibiting VEGF and Hypoxia Inducible Factor-1α Targeted Gene Expression.

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

In the present study, we investigated the antitumor effect of a novel hypoxic cytotoxin, TX-2098 in inhibiting the expression of HIF-1α, and consequently VEGF expression in pancreatic cancer. *In vitro*, TX-2098 inhibited the proliferation of various pancreatic cancer cell lines. In s.c model, tumors from nude mice injected with pancreatic cancer cells and treated with TX-2098 showed significant reductions in volume ($P<0.01$ versus control). Quantitative real-time RT-PCR analysis revealed that TX-2098 significantly inhibited mRNA expression of the HIF-1 associated molecules, VEGF, glucose transporter 1 and Aldolase A ($P<0.01$ versus control). These treatments also prolong the survival in orthotopic models.

[*World J. Oncol.* **3**, 103-112 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Evaluating the Usefulness of a Novel ^{10}B-carrier Conjugated With Cyclic RGD Peptide in Boron Neutron Capture Therapy.

Shin-ichiro MASUNAGA, Sadaaki KIMURA, Tomohiro HARADA, Kensuke OKUDA*, Yoshinori SAKURAI, Hiroki TANAKA, Minoru SUZUKI, Natsuko KONDO, Akira MARUHASHI, Hideko NAGASAWA* and Koji ONO

To evaluate the usefulness of a novel ^{10}B-carrier conjugated with an integrin-binding cyclic RGD peptide (GPU-201) in boron neutron capture therapy (BNCT). The ^{10}B from BSH was washed away rapidly in all these tissues and the retention of ^{10}B from BSH-CD and GPU-201 was similar except in blood where the ^{10}B concentration from GPU-201 was higher for longer. GPU-201 showed a significantly stronger radio-sensitizing effect under neutron beam irradiation on both total and Q cell populations than any other ^{10}B-carrier. Consequently, GPU-201 that sensitized tumor cells more markedly than conventional ^{10}B-carriers may be a promising candidate for use in BNCT. However, its toxicity needs to be tested further.

[*J. Med. Chem.* **55**, 5483-5496 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Discovery of Highly Potent Human Deoxyuridine Triphosphatase Inhibitors Based on the Conformation Restriction Strategy.

Seiji MIYAHARA, Hitoshi MIYAKOSHI, Tatsushi YOKOGAWA, Khoon T. CHONG, Junko TAGUCHI, Toshiharu MUTO, Kanji ENDOH, Wakako YANO, Takeshi WAKASA, Hiroyuki UENO, Yayoi TAKAO, Akio FUJIOKA, Akihiro HASHIMOTO, Kenjirou ITOU, Keisuke YAMAMURA, Makoto NOMURA, Hideko NAGASAWA*, Satoshi SHUTO and Masayoshi FUKUOKA

In this study, we describe the discovery of a novel class of human dUTPase inhibitors based on the conformation restriction strategy. On the basis of the X-ray cocrystal structure of dUTPase and its inhibitor, we designed and synthesized two conformation restricted analogues. Further SAR studies identified a compound **43** with the highest *in vitro* potency (IC_{50} = 39 nM, EC_{50} = 66 nM). Furthermore, compound **43** had a favorable oral PK profile and exhibited potent antitumor activity in combination with 5-FU.

[*J. Med. Chem.* **55**, 6427-6437 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

1,2,3-Triazole-containing Uracil Derivatives with Excellent Pharmacokinetics as a Novel Class of Potent Human Deoxyuridine Triphosphatase Inhibitors.

Hitoshi MIYAKOSHI, Seiji MIYAHARA, Tatsushi YOKOGAWA, Kanji ENDOH, Toshiharu MUTO, Wakako YANO, Takeshi WAKASA, Hiroyuki UENO, Khoon T. CHONG, Junko TAGUCHI, Makoto NOMURA, Yayoi TAKAO, Akio FUJIOKA, Akihiro HASHIMOTO, Kenjirou ITOU, Keisuke YAMAMURA, Satoshi SHUTO, Hideko NAGASAWA* and Masayoshi FUKUOKA

We describe the design and synthesis of a novel class of human dUTPase inhibitors, 1,2,3-triazole-containing uracil derivatives. Compound **45a**, which possesses 1,5-disubstituted 1,2,3-triazole moiety locked in a *cis* conformation showed potent inhibitory activity, and its SAR studies led us to the discovery of highly potent inhibitors **48c** and **50c** (IC_{50} = ~0.029 μM). Compound **50c** is a promising candidate for combination cancer chemotherapies with TS inhibitors.

[*Synth. Commun.* **42**, 865–871 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 74: Rearrangement Reaction of 5-Amino-1,2-dihydrofuro[2,3-*c*]isoquinolines with α,ω-Dibromoalkanes and Evaluation of Product Bronchodilator Activity.

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

Reaction of 5-amino-1,2-dihydrofuro[2,3-c]isoquinolines with 1,2-dibromoethane and 1,3-dibromopropane in the presence of base afforded 2',3'-dihydrospiro[cyclopropane-1,6'(5'*H*)-imidazo[2,1-*a*]isoquinolin]-5'-ones and 3',4'-dihydro-2'*H*-spiro[cyclopropane-1,7'(6'*H*)-pyrimido[2,1-*a*]isoquinolin]-6'-ones, respectively. Certain of the products showed significant bronchodilator activity.

[*J. Heterocycl. Chem.* **49**, 281–287 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 69: Synthesis of 5-Amino-1,2-dihydro[1]benzofuro[3,2-*d*]furo[2,3-*b*]pyridines and Their Transformations to Related Compounds.

Kensuke OKUDA*, Jun-ichi TAKANO, Takashi HIROTA and Kenji SASAKI

Reaction of 3-(3-cyanopropoxy)[1]benzofuran-2-carbonitriles with potassium tert-butoxide gave 5-amino-1,2-dihydro[1]benzofuro[3,2-*d*]furo[2,3-*b*]pyridines and 5-amino-2,3-dihydro[1]benzofuro[3,2-*b*]oxepin-4-carbonitriles as new ring systems. Reactions of the 5-chloro derivative, obtained from 5-amino-1,2-dihydro[1]benzofuro[3,2-*d*]furo[2,3-*b*]pyridine, produced a dihydrofuran ring-opened compound as well as 5-substituted compounds.

[*J. Heterocycl. Chem.* **49**, 742–747 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 71: Synthesis and Bronchodilator Evaluation of 5-Substituted 1,2-Dihydrofuro[3,2-*f*][1,7]naphthyridines and Related Compounds.

Kensuke OKUDA*, Tetsuo MATSUSHITA, Takashi HIROTA and Kenji SASAKI

Several 5-substituted 1,2-dihydrofuro[3,2-*f*][1,7]naphthyridines were synthesized as part of our research to develop new effective bronchodilators. Amines, sulfanyls, and alcohols were used as substituents at the 5-position. Tetracyclic compounds were also obtained. Evaluation of the effects of the newly synthesized compounds on carbamoylcholine chloride-induced contractions of trachea revealed one promising bronchodilator candidate with potency comparable to that of 3-isobutyl-1-methylxanthine.

[*J. Heterocycl. Chem.* **49**, 755–762 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 72: Reaction of *N*-([1]Benzofuro (or Benzothieno)[3,2-*d*]pyrimidin-4-yl)formamidine and *N*-(Pyrido[2,3-*d*]pyrimidin-4-yl)formamidine Derivatives with Hydroxylamine Hydrochloride.

Kensuke OKUDA*, Kiyoko TSUCHIE and Takashi HIROTA

The reactions of *N*-([1]benzofuro[3,2-*d*]pyrimidin-4-yl)formamidines with hydroxylamine hydrochloride gave rearranged cyclization products via ring cleavage of the pyrimidine component accompanied by a ring closure of the 1,2,4-oxadiazole to give *N*-[2-([1,2,4]oxadiazol-5-yl)[1]benzofuran-3-yl)formamide oximes. *N*-([1]Benzothieno[3,2-*d*]pyrimidin-4-yl)formamidines and *N*-(pyrido[2,3-*d*]pyrimidin-4-yl)formamidines with hydroxylamine hydrochloride gave similar results.

[*Acta Cryst*. E **68**, o2819 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

3-(2-Oxo-2,3,4,5-tetrahydrofuran-3-yl)[1]benzofuran-2-carbonitrile.

Kensuke OKUDA*, Takashi HIROTA, Yuta NISHINA and Hiroyuki ISHIDA

The asymmetric unit of the title compound, $C_{13}H_9NO_3$, consists of two crystallographically independent molecules. In each molecule, the tetrahydrofuran (THF) ring adopts an envelope conformation with one of the methylene C atoms positioned at the flap. The dihedral angles between the mean plane of the THFand the benzofuran ring system are 70.85 (5) and 89.59 (6)º. In the crystal, molecules are stacked in a column along the a-axis direction through C—H···O hydrogen bonds, with columns further linked by C—H···N and C—H···O interactions.

[*Acta Cryst*. E **68**, o3252 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

10'-Chloro-3',4'-dihydro-2'*H*-spiro[cyclopropane-1,7'(6'*H*)-pyrimido[2,1-*a*]isoquinolin]-6'-one.

Kensuke OKUDA*, Takashi HIROTA, Yuta NISHINA and Hiroyuki ISHIDA

In the title compound, $C_{14}H_{13}ClN_2O$, the fused hydropyrimidine ring adopts an envelope conformation with one of the methylene C atoms at the flap. The three-membered ring is approximately perpendicular to the attached isoquinoline ring system, with a dihedral angle of 89.44 (11)º. In the crystal, molecules are linked by a weak C—H···π interaction, forming a helical chain along the *c* axis.

[*Bioorg. Med. Chem. Lett.* **22**, 7410-7413 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Development of a Dual Functional Luminescent Sensor for Zinc Ion Based on a Peptidic Architecture.

Tasuku HIRAYAMA*, Masayasu TAKI, Kazushi AKAOKA and Yukio YAMAMOTO

A synthetic peptide bearing a lanthanide complex, TbOTZ exhibits a decrease of chromophore fluorescence and a concomitant luminescence enhancement due to sensitized Tb^{3+} upon Zn^{2+} binding. Thus, TbOTZ can be a valuable tool for ratiometric sensing of Zn^{2+} as well as for time-resolved fluorescence detection with a single molecule.

[*Proc. Natl. Acad. Sci. USA.* **109**, 2228-2233 (2012)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Near-infrared Fluorescent Sensor for *in vivo* Copper Imaging in a Murine Wilson Disease Model.

Tasuku HIRAYAMA*, Genevieve C. Van de BITTNER, Lawrence W. GRAY, Svetlana LUTSENKO and Christopher J. CHANG

Copper is an essential metal nutrient that is tightly regulated in the body because loss of its homeostasis is connected to severe diseases. The complex relationships between copper status and various stages of health and disease remain challenging to elucidate, in part due to a lack of methods for monitoring dynamic changes in copper pools in whole living organisms. Here we present Coppersensor 790, a first-generation fluorescent sensor for visualizing labile copper pools in living animals. This probe is capable of monitoring fluctuations in exchangeable copper stores in living cells and mice under basal conditions, as well as in situations of copper overload or deficiency. Moreover, we demonstrate the utility of this unique chemical tool to detect aberrant increases in labile copper levels in a murine model of Wilson disease, a genetic disorder that is characterized by accumulation of excess copper.

[*Chem. Eur. J.* **18**, 16608-16611(2012)] [Lab. of Organic Chemistry]

Iron-catalyzed Chemoselective Azidation of Benzylic Silyl Ethers.

Yoshinari SAWAMA, Saori NAGATA, Yuki YABE, Kosuke MORITA, Yasunari MONGUCHI and Hironao SAJIKI *

Siloxy groups derived from secondary and tertiary benzyl alcohols can be transformed into azide groups at room temperature using $TMSN_3$ in the presence of an iron catalyst (see scheme; TMS=trimethylsilyl). Secondary and tertiary benzylic silyl ethers can be transformed in the presence of primary silyl ethers, and other reactive functional groups, such as alkyl chlorides, α,β-unsaturated esters, and aldehydes, are stable under the reaction conditions.

[*Chem. Eur. J.* **18**, 16436-16442 (2012)] [Lab. of Organic Chemistry]

Stereo- and Regioselective Direct Multi-deuterium-labeling Methods for Sugars.

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

Deuterium-labeled sugars can be utilized as powerful tools for the architectural analyses of high-sugar-containing molecules represented by the nucleic acids and glycoproteins, and chiral building blocks for the syntheses of new drug candidates (heavy drugs) due to their potential characteristics, such as simplifying the ^1H NMR spectra and the stability of C-D bonds compared with C-H bonds. We have established a direct and efficient synthetic method of deuterated sugars from non-labeled sugars, such as pyranosides and furanosides represented by ribose and deoxyribose, by using the heterogeneous Ru/C-catalyzed H–D exchange reaction in D_2O under a hydrogen atmosphere with perfect chemo- and stereoselectivities.

[*Adv. Synth. Catal*. **354**, 2561-2567 (2012)] [Lab. of Organic Chemistry]

Palladium on Carbon-catalyzed Cross-coupling Using Triarylbismuths.

Yasunari MONGUCHI, Tomohiro HATTORI, Yasuhiro MIYAMOTO, Takayoshi YANASE, Yoshinari SAWAMA and Hironao Sajiki*

Simple and efficient protocols for the 10% palladium on carbon (Pd/C)-catalyzed cross-coupling reactions between triarylbismuths and aryl halides have been developed. A variety of iodo- and bromobenzenes possessing an electron-withdrawing group on the aromatic nucleus were smoothly cross-coupled in the presence of 10% Pd/C, $Na_3PO_4 \cdot 12\,H_2O$ and DABCO in heated *N*-methyl-2-pyrrolidone (NMP) as the solvent. For the arylations of iodobenzenes, the reactions effectively proceeded under the combined use of CsF and 2,2′-biquinoline. Furthermore, a ligand-free 10% Pd/C-catalyzed cross-coupling reaction between the aryl iodides and triarylbismuths was also established by the addition of tetra-*n*-buthylammonium fluoride trihydrate ($TBAF \cdot 3\,H_2O$) in which the palladium metals were hardly leached from the catalyst into the reaction media.

[*Tetrahedron* **68**, 8293-8299 (2012)] [Lab. of Organic Chemistry]

Chemoselective Hydrogenation Using Molecular Sieves-supported Pd Catalysts: Pd/MS3A and Pd/MS5A.

Tohru TAKAHASHI, Masatoshi YOSHIMURA, Hiroyasu SUZUKA, Tomohiro MAEGAWA, Yoshinari SAWAMA, Yasunari MONGUCHI and Hironao SAJIKI*

Palladium catalysts embedded on molecular sieves (MS3A and MS5A) were prepared by the adsorption of $Pd(OAc)_2$ onto molecular sieves with its in situ reduction to Pd^0 by MeOH as a reducing agent and solvent. 0.5% Pd/MS3A and 0.5% Pd/MS5A catalyzed the hydrogenation of alkynes, alkenes, and azides with a variety of coexisting reducible functionalities, such as nitro group, intact. It is noteworthy that terminal alkenes of styrene derivatives possessing electron-donating functionalities on the benzene nucleus were never hydrogenated under 0.5% Pd/MS5A-catalyzed conditions, while internal alkenes of 1-propenylbenzene derivatives were readily reduced to the corresponding alkanes.

[*J. Hazard. Mater.* **229-230**,15-19 (2012)] [Lab. of Organic Chemistry]

Pd/C-catalyzed Dechlorination of Polychlorinated Biphenyls under Hydrogen Gas-free Conditions.

Shinji ISHIHARA, Akiko IDO, Yasunari MONGUCHI, Hisamitsu NAGASE and Hironao SAJIKI*

The simultaneous use of catalytic amount of palladium on carbon (Pd/C) and Mg metal (1.5-2.0 equiv vs. Cl numbers of the substrates) in MeOH achieved the complete dechlorination of a variety of aryl chlorides at room temperature under a nitrogen atmosphere in the absence of hydrogen gas. The present method could be successfully used for the detoxification of PCBs based on the dechlorinaton reaction. Both virgin PCBs, such as Aroclors 1242, 1248 and 1254, and used PCBs as a high-tension capacitor oil, were smoothly dechlorinated into harmless biphenyl without any byproducts within 2 h at rt. The distinctive features of this method are convenience and safety due to no needs for the pretreatment of catalyst and Mg and complete degradation of PCBs under mild conditions without hydrogen gas.

[*Molecules* **17**, 6519-6546 (2012)] [Lab. of Organic Chemistry]

Development of Diversified Methods for Chemical Modification of the 5,6-Double Bond of Uracil Derivatives Depending on Active Methylene Compounds.

Hironao SAJIKI*, Yusuke IIDA, Kanoko IKAWA, Yoshinari SAWAMA, Yasunari MONGUCHI, Yukio KITADE, Yoshifumi MAKI, Hideo INOUE and Kosaku HIROTA

The reaction of 5-halogenouracil and uridine derivatives **1** and **7** with active methylene compounds under basic conditions produced diverse and selective C-C bond formation products by virtue of the nature of the carbanions. Three different types of reactions such as the regioselective C-C bond formation at the 5- and 6-positions of uracil and uridine derivatives (products **2**, **5**, **8**, **17**, **20** and **21**), and the formation of fused heterocycle derivatives 2,4-diazabicyclo[4.1.0]heptane (**15**) and 2,4-diazabicyclo-[4.1.0]nonane (**16**) via dual C-C bond formations at both the 5- and 6-positions were due to the different active methylene compounds used as reagents.

[*Adv. Synth. Catal.* **354**, 1264-1268 (2012)] [Lab. of Organic Chemistry]

Development of a Palladium on Boron Nitride Catalyst and its Application to the Semihydrogenation of Alkynes.

Yuki YABE, Tsuyoshi YAMADA, Saori NAGATA, Yoshinari SAWAMA, Yasunari MONGUCHI and Hironao SAJIKI*

The simple preparative method for a novel palladium supported on boron nitride catalyst (Pd/BN) was accomplished. Pd/BN is widely applicable for the semihydrogenation of mono- as well as disubstituted alkynes to furnish the corresponding alkenes in the presence of diethylenetriamine (DETA), which exhibits both an unprecedented acceleration effect toward the semihydrogenation and a suppression effect with regard to the overhydrogenation to alkanes.

[*ChemCatChem.* **4**, 546-558 (2012)] [Lab. of Organic Chemistry]

Carbon-carbon Bond Formation by Ligand-free Cross-coupling Reaction Using Palladium Catalyst Supported on Synthetic Adsorbent.

Yasunari MONGUCHI, Keita SAKAI, Koichi ENDO, Yuki FUJITA, Masaru NIINURA, Masatoshi YOSHIMURA, Tomoteru MIZUSAKI, Yoshinari SAWAMA and Hironao SAJIKI*

A palladium catalyst supported on a commercial synthetic adsorbent, DIAION HP20, could be employed for the cross-coupling reactions of aryl halides with organoboronic acids, alkenes, and alkynes in a ligand-free manner. 10 % Pd/HP20 was highly active as the catalyst at approximately the same level as 10 % palladium on carbon (10 % Pd/C) for many of the reactions, and was especially effective for the Suzuki–Miyaura reaction using bromoaniline derivatives without the *N*-protection and Sonogashira reaction of the heteroaryl iodides. As a certain quality of HP20 are available as an industrial product, 10 % Pd/HP20 would be in practical use for a variety of cross-coupling reactions and hydrogenation as the substitute for 10 % Pd/C.

[*Adv. Synth. Catal*. **354**, 777-782 (2012)] [Lab. of Organic Chemistry]

Platinum on Carbon-catalyzed Hydrodefluorination of Fluoroarenes using Isopropyl Alcohol-water-sodium Carbonate Combination.

Yoshinari SAWAMA, Yuki YABE, Masahiro SIGETSURA, Tsuyoshi YAMADA, Saori NAGATA, Yuta FUJIWARA, Tomohiro MAEGAWA, Yasunari MONGUCHI and Hironao SAJIKI*

We have developed a platinum on carbon-isopropyl alcohol-catalyzed and widely applicable defluorination method for fluoroarenes, and the addition of water and sodium carbonate efficiently accelerated the reaction. The defluorination readily occurred under the reaction conditions in comparison with the dehalogenation of other aromatic halides (fluorine>chlorine>bromine≫iodine).

[*Org. Biomol. Chem.* **10**, 293-304 (2012)] [Lab. of Organic Chemistry]

Selective N-Alkylation of Amines Using Nitriles under Hydrogenation Conditions: Facile Synthesis of Secondary and Tertiary Amines.

Takashi IKAWA, Yuki FUJITA,Tomoteru MIZUSAKI, Sae BETSUIN, Haruki TAKAMATSU, Tomohiro MAEGAWA, Yasunari MONGUCHI and Hironao SAJIKI *

Nitriles were found to be highly effective alkylating reagents for the selective *N*-alkylation of amines under catalytic hydrogenation conditions. While aliphatic amines were effectively converted to the corresponding tertiary amines under Pd/C-catalyzed conditions, Rh/C effectively catalyzed the *N*-monoalkylation of aliphatic primary amines to the tertiary amines. The combination of the Rh/C-catalyzed *N*-monoalkylation of the aliphatic primary amines and additional Pd/C-catalyzed alkylation of the resulting secondary aliphatic amines afforded aliphatic tertiary amines possessing three different alkyl groups. Mechanistic studies suggested that nitriles were reduced to aldimines before the nucleophilic attack of the amine during the first step of the reaction.

[*RSC Adv.* **2**, 590-594 (2012)] [Lab. of Organic Chemistry]

Ligand-free Hiyama Cross-coupling Reaction Catalyzed by Palladium on Carbon.

Takayoshi YANASE, Yasunari MONGUCHI and Hironao SAJIKI *

A ligand-free Pd/C-catalyzed Hiyama cross-coupling reaction has been developed. A variety of aryl bromides were efficiently cross-coupled with aryltriethoxysilanes with only 0.5 mol% of 5% Pd/C. The protocol would be practical for use as an economical synthetic method for the construction of biphenyl derivatives.

[*Tetrahedron* **68**, 1712-1722 (2012)] [Lab. of Organic Chemistry]

One-pot Aromatic Amination Based on Carbon-nitrogen Coupling Reaction between Aryl Halides and Azidocompounds.

Toshihide MAEJIMA,Yutaka SHIMODA, Kei NOZAKI, Shigeki MORI, Yoshinari SAWAMA, Yasunari MONGUCHI and Hironao SAJIKI*

An efficient copper-mediated C-N coupling reaction between various aryl halides and azido compounds to produce the corresponding aromatic primary amines was established. The present amination is apparently involved in both the reduction of an azido functionality to the corresponding primary amino group and its cross-coupling reaction with aryl halides in a one-pot manner. The present amination could be applied to the synthesis of procaine, a local anesthetic drug. A mechanistic study indicated that 2-aminoethanol could work as a major hydrogen donor and the reaction would proceed without the formation of the intermediary aryl azide.

[*Heterocycles* **84**, 419-429 (2012)] [Lab. of Organic Chemistry]

Deuterium-labeled Benzyladenine: Synthesis and Application as a Surrogate.

Nkaelang MODUTLWA, Hiroyuki TADA, Yoshiki SUGAHARA, Koichi SHIRAKI, Nobuyuki HARA, Yoshihiro DEYASHIKI, Tomohiro MAEGAWA, Yasunari MONGUCHI and Hironao SAJIKI*

N^6-Benzyladenine (benzyladenine), a plant growth regulator, was efficiently deuterated by the hydrogen–deuterium (H–D) exchange reaction catalyzed by palladium on carbon-ethylenediamine complex [Pd/C(en)], while use of palladium on carbon (Pd/C) as a catalyst led to a low deuterium incorporation at room temperature or complete removal of the N^6-benzyl group at 110 °C or higher temperature. The obtained benzyladenine-d_5 was used as an internal standard (surrogate) for the quantification of residual benzyladenine in fruits, vegetables, cereals, and beans using LC/MS/MS. Satisfactory recovery of benzyladenine between 94.2 and 105.7% (100.4% on the average) was obtained. The agrochemical could be detected within the concentration range of 0.25–0.50 ng/g in agricultural products using the present quantification method.

[*Adv. Synth. Catal.* **354**, 771-776 (2012)] [Lab. of Organic Chemistry]

Platinum on Carbon-catalyzed Precise Reduction Control of Trichloromethyl to Geminal-dichloromethyl Groups.

Takahiro IMANISHI, Yuta FUJIWARA, Yoshinari SAWAMA, Yasunari MONGUCHI and Hironao SAJIKI*

The *Geminal*-dichloromethyl derivatives could be efficiently synthesized by the highly chemoselective platinum on carbon-catalyzed mono-dechlorination of trichloromethyl substrates in dimethylacetamide (DMA) as a specific solvent at 25 °C under a hydrogen atmosphere.

[*Chem. Eur. J.* **18**, 13861-13870 (2012)] [Lab. of Organic Chemistry]

Asymmetric Total Synthesis of (-)-Stenine and 9a-epi-Stenine.

Hiromichi FUJIOKA, Kenji NAKAHARA, Naoyuki KOTOKU, Yusuke OHBA, Yasushi NAGATOMI, Tsung-Lung WANG, Yoshinari SAWAMA*, Kenichi MURAI, Kie HIRANO, Tomohiro OKI, Shintaro WAKAMATSU and Yasuyuki KITA

A route for the asymmetric synthesis of (−)-stenine, a member of the *Stemona* alkaloid family used as folk medicine in Asian countries, is described. The key features of the sequence employed include stereoselective transformations on a cyclohexane ring controlled by a chiral auxiliary unit and an intramolecular Mitsunobu reaction to construct the perhydroindole ring system. By using an intermediate in the route to (−)-stenine, an asymmetric synthesis of 9a-*epi*-stenine was also executed. The C(9a) stereocenter in 9a-*epi*-stenine was installed by using a Staudinger/aza-Wittig reaction of a keto–azide precursor followed by reduction of the resulting imine.

[*Chem. Asian. J.* **7**, 367-373 (2012)] [Lab. of Organic Chemistry]

Reaction of Acetals with Various Carbon Nucleophiles under Non-acidic Conditions: C-C Bond Formation via aPyridinium-type Salt.

Hiromichi FUJIOKA, Kenzo YAHATA, Tomohito HAMADA, Ozora KUBO, Takashi OKITSU, Yoshinari SAWAMA*, Takuya OHNAKA, Tomohiro MAEGAWA and Yasuyuki KITA

Siloxy Mild substitution reactions of acetals with carbon nucleophiles via the pyridinium-type salts generated by the treatment ofacetals with TESOTf and 2,4,6-collidine or 2,2'-bipyridyl have been developed. Various carbon nucleophiles, such as organocuprates, silyl enol ethers, enamines, etc., reacted with the pyridinium-type salts to give the corresponding substituted products in good yields. The reactions proceeded under very mild conditions (non-acidic conditions) and thus acid-sensitive functional groups can be tolerated during the reaction. In addn., only an acetal can form the pyridiniumtype salt and react with nucleophiles in the presence of a ketal. This unusual selectivity is in contrast to general methods conducted under acidic conditions.

[*Chem. Eur. J.* **18**, 11423-11432 (2012)] [Lab. of Organic Chemistry]

Effects of Phosphorus Substituents on Reactions of α-Alkoxyphosphonium Salts with Nucleophiles.

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

The effects of phosphorus substituents on the reactivity of α-alkoxyphosphonium salts with nucleophiles has been explored. Reactions of α-alkoxyphosphonium salts, prepared from various acetals and tris(*o*-tolyl)phosphine, with a variety of nucleophiles proceeded efficiently. These processes represent the first examples of high-yielding nucleophilic substitution reactions of α-alkoxyphosphonium salts. In addition, a novel reaction of α-alkoxyphosphonium salts derived from Ph_3P with Grignard reagents was observed to take place in the presence of O_2 to afford alcohols in good yields. A radical mechanism is proposed for this process that has gained support from isotope-labeling and radical-inhibition experiments.

[*Photochem. Photobiol. Sci.* **11**, 616-619 (2012)] [Lab. of Pharmaceutical Synthetic Chemistry]

Direct Aerobic Photo-oxidative Syntheses of Aromatic Methyl Esters from Methyl Aromatics Using Anthraquinone-2,3-dicarboxylic Acid as Organophotocatalyst.

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

This paper reports a useful method for facile direct syntheses of aromatic methyl esters from methyl aromatics by aerobic photo-oxidation using anthraquinone-2,3-dicarboxylic acid as an organophotocatalyst. The electron-rich and neutral methyl aromatics were good substrates for oxidative esterification to afford the corresponding methyl carboxylates in high yields; however, the electron-deficient methyl aromatics with the cyano group were poor substrates. Interestingly, methyl 4-methylbenzoate was obtained from *p*-xylene in moderate yields in the optimized condition. Furthermore, ethyl, propyl, and *iso*-propyl carboxylates were also obtained in good to moderate yields, respectively. These reactions are the first examples of the direct synthesis of ethyl, propyl, and *iso*-propyl esters from methyl aromatics.

[*Org. Biomol. Chem.* **10**, 2209-2213 (2012)] [Lab. of Pharmaceutical Synthetic Chemistry]

Highly Efficient Asymmetric Aldol Reaction in Brine Using a Fluorous Sulfonamide Organocatalyst.

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

The novel fluorous organocatalyst can be easily prepared from L-tyrosine. Fluorous organocatalyst, which is a simple β-aminosulfonamide with only one chiral center, efficiently catalyzes the direct aldol reactions of various aromatic aldehydes with ketones in brine to afford the corresponding *anti*-aldol products with high enantioselectivity. Fluorous organocatalyst is an excellent catalyst and can efficiently catalyze aldol reactions even under mild reaction conditions, at only low catalyst loading (0.05 equiv) and with reasonable amount of cyclohexanone (5 equiv). The excellent performance is probably due to the ability of the fluorous tag ($–C_8F_{17}$) to function as a preferable hydrophobic reaction field in brine. Fluorous organocatalyst was readily recovered by simple solid phase extraction using fluorous silica gel and was immediately reusable without purification.

[*Synlett* **23**, 2059-2062 (2012)] [Lab. of Pharmaceutical Synthetic Chemistry]

Aerobic Photooxidative Cleavage of Vicinal Diols to Carboxylic Acids Using 2-Chloroanthraquinone.

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

We developed the aerobic photooxidative cleavage of vicinal diols to carboxylic acids using 2-chloroanthraquinone in presence of photoirradiation with a high-pressure mercury lamp. Generally, the corresponding carboxylic acids are obtained in high yields regardless of the electron-donating or electron-withdrawing group on the benzene ring. On the other hand, the 2-naphthyl derivative was the poor substrate, and resulted in low yield with many minor by-products. Aliphatic vicinal diols are also converted to the corresponding carboxylic acids in moderate to good yields. Cyclic vicinal diol yielded the corresponding dicarboxylic acid. In these aliphatic substrates, further prolonging of reaction time resulted in lower yields. Moreover, tetrasubstituted vicinal diol was converted to the corresponding ketone in good yields. This is the first metal-free reaction using molecular oxygen as the terminal oxidant.

[*Tetrahedron Lett.* **53**, 5306-5308 (2012)] [Lab. of Pharmaceutical Synthetic Chemistry]

Aerobic Oxidative Esterification of Benzyl Alcohols with Catalytic Tetrabromomethane under Visible Light Irradiation.

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

We report a useful method for the facile synthesis of aromatic esters from benzyl alcohols with molecular oxygen and catalytic tetrabromomethane in alcohol under visible light irradiation with a fluorescent lamp. In general, the corresponding aromatic esters were obtained in good to high yields regardless of the electron-donating or electron-withdrawing group in the benzene ring. Both 1-naphthalenemethanol and 2-naphthalenemethanol were suitable substrates for this reaction affording the corresponding esters **i**n high yields, respectively. Interestingly, ethanol and propanol can be used under these oxidative esterification conditions giving the corresponding aromatic esters, respectively. This is the first metal-free reaction using molecular oxygen as the terminal oxidant.

[*Synlett* **23**, 2385-2388 (2012)] [Lab. of Pharmaceutical Synthetic Chemistry]

Highly Efficient Asymmetric Conjugate Additions of Aldehydes with Vinyl Sulfones Using a Sulfonamide Organocatalyst.

Tsuyoshi MIURA*, Hiroki YUASA, Miho MURAHASHI, Mariko INA, Kosuke NAKASHIMA, Norihiro TADA and Akichika ITOH

The novel organocatalyst can easily be prepared from L-valine. Organocatalyst, which is a simple β-aminosulfonamide with only one stereogenic center, efficiently catalyzes conjugate additions of various branched aldehydes to vinyl sulfone with a short reaction time at room temperature to afford the corresponding addition products with high enantioselectivities. Organocatalyst is an excellent catalyst and is effective for conjugate additions of vinyl sulfone with branched aldehydes to give compounds possessing all-carbon quaternary stereocenters. The excellent performance is probably due to the carbon skeleton of valine and the electron-withdrawal effect of the perfluorobutyl group.

[*Green Chem.* **14**, 3007-3009 (2012)] [Lab. of Pharmaceutical Synthetic Chemistry]

Facile Aerobic Photooxidative Oxylactonization of Oxocarboxylic Acids in Fluorous Solvents.

Norihiro TADA, Lei CUI, Takafumi ISHIGAMI, Kazunori BAN, Tsuyoshi MIURA, Bunji UNO and Akichika ITOH*

We developed efficient aerobic photooxidative oxylactonizations of oxocarboxylic acids promoted by trifluoroacetic anhydride with molecular oxygen as the terminal oxidant in the fluorous solvent FC-72. Electron-deficient and electron-rich substrates gave the corresponding oxolactones in good yields. In contrast, the methoxy group, a stronger electron-donating group, retarded the reaction. Sterically hindered substrates possessing two methyl groups on the aromatic ring gave the corresponding oxolactones in good yields. Furthermore, 4-(2-Naphthoyl)butyric acid gave the corresponding oxolactone in moderate yield. Unfortunately, 4-acetylbutyric acid, 3-benzoylpropionic acid, and 5-benzoylpentanoic acid were poor substrates. This method represents the first successful oxidation in a fluorous solvent and direct oxylactonization via an enol lactone intermediate under visible light irradiation.

[*Tetrahedron* **68**, 2421-2428 (2012)] [Lab. of Pharmacognosy]

Novel Quinolinone Alkaloids Bearing a Lignoid Moiety and Related Constituents in the Leaves of *Melicope denhamii*.

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

Six novel quinolinone alkaloids bearing a phenylpropanoid or a coumarin moiety, named melicodenines C-H, two new cinnamyl alcohol derivatives, named melicodins A and B, and a new coumarinolignan, named melicodin C, were isolated from the leaves of *Melicope denhamii* (Seem.) T. G. Hartley. Their structures were established by spectroscopic analyses including extensive 2D-NMR expriments. Ten quinolinone alkaloids and a coumarinolignan were tested for anti-proliferative activity against DLD-1 human colon cancer cells. Melicodenine G showed the most potent inhibitory activity, causing the induction of apoptosis.

[*Chem. Biodiversity* **9**, 2195-2202 (2012)] [Lab. of Pharmacognosy]

Novel Zierane- and Guaiane-Type Sesquiterpenes from the Root of *Melicope denhamii*.

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

Two novel zierane-type sesquiterpenes, named melicodenones A and B, and three new guaiane-type sesquiterpenes, named melicodenones C-E, were isolated from the root of *Melicope denhamii* (Seem.) T. G. Hartley together with zierone. Their structures were established by extensive NMR-spectroscopic analyses. The isolates were tested for cytotoxicity using human colon cancer DLD-1 cells, and melicodenone A was found to exhibit moderate activity.

[*Tetrahedron* **68**, 2950-2960 (2012)] [Lab. of Pharmacognosy]

Absolute Structure of Shoreaketone: a Rotational Isomeric Resveratrol Tetramer in Dipterocarpaceaeous Plants.

Tetsuro ITO, Masayoshi OYAMA, Hironao SAJIKI, Ryuichi SAWA, Yoshikazu TAKAHASHI and Munekazu IINUMA*

A rotational isomeric shoreaketone, identified as a skeletal member of resveratrol tetramers, was isolated from three species of Dipterocarpaceaeous plants: *Shorea uliginosa*, *Shorea hemsleyana*, and *Vateria indica*. The absolute structure was elucidated by spectroscopic analysis including NMR and CD data. Shoreaketone has 10 asummetric carbons and a framework of fused heptacyclic ring system including a spiro ring that has not been reported in any other natural product. The conformations of rotational isomeric properties were studied by variable-temparature NMR, ROESY, a skeletal conversion.

[*Phytochem. Lett.* **5**, 267-270 (2012)] [Lab. of Pharmacognosy]

Novel Isolation of Stilbenoids with Enantiomeric and Meso Forms from a *Cyperus* Rhizome.

Tetsuro ITO, Hidetatsu ENDO, Masayoshi OYAMA and Munekazu IINUMA*

Three stereoisomeric stilbene trimers bearing an (*E*)-2,3,5,6-tetraphenyl-4-styryl-2,3,5,6-tetrahydrobenzo[1,2-*b*:5,4-*b'*]difuran skeleton, (+)- and (−)-(*E*)-cyperusphenol A and (*E*)-mesocyperusphenol A, were isolated from a cyperus rhizome. Moreover, the geometrical isomers were identified as the artifacts of their (*E*)-forms. The isolated products are the first instance of the co-occurrence of racemates and a meso isomer, which resembles the *C2* symmetrical structure of an oligostilbenoid. These structures were characterized by NMR and CD spectroscopy. This is the first report that shows the occurrence of a racemate of a stilbenoid in the same plant material and the achievement of the enantiometric separation of stilbene oligomers.

[*J. Nutr. Sci. Vitaminol.* **58**, 136-142 (2012)] [Lab. of Pharmacognosy]

Quantification of Polyphenols and Pharmacological Analysis of Water and Ethanol-based Extracts of Cultivated Agarwood Leaves.

Tetsuro ITO, Mamoru KAKINO, Shigemi TAZAWA, Tatsuya WATARAI, Masayoshi OYAMA, Hiroe MARUYAMA, Yoko ARAKI, Hideaki HARA and Munekazu IINUMA*

Mangiferin and genkwanin 5-*O*-β-primeveroside are the two major bioactive polyphenols with laxative property present in the extracts of agarwood (*Aquilaria sinensis*) leaves. HPLC method to determine these bioactive components and four other major polyphenols in the extracts and evaluation of the pharmacological equivalence of organic and water extracts were evalusted. The laxative properties of 60% ethanol and four water extracts of *A. crassna* were evaluated by the frequency and weight of stools in loperamide-induced constipation model mice. The pharmacological equivalence of some extracts was identified in mice.

[*Food Sci. Technol. Res.* **18**, 259-262 (2012)] [Lab. of Pharmacognosy]

Identification of Phenolic Compounds in *Aquilaria crassna* Leaves via Liquid Chromatography-electrospray Ionization Mass Spectroscopy.

Tetsuro ITO, Mamoru KAKINO, Shigemi TAZAWA, Masayoshi OYAMA, Hiroe MARUYAMA, Yoko ARAKI, Hideaki HARA and Munekazu IINUMA*

HPLC-DAD-ESI-MS was performed to identify the phenolic constituents of *Aquilaria crassna* and iriflophenone 3,5-*C*-β-diglucoside, iriflophenone 3-*C*-β-glucoside, mangiferin, iriflophenone 2-*O*-α-rhamnoside, genkwanin 5-*O*-β-primeveroside, genkwanin 5-*O*-β-glucoside, genkwanin 4'-Me ether 5-*O*-β -primeveroside, and genkwanin were identified by the comparison with authentic samples. The MS/MS spectra of these polyphenols were detected using hybrid ion trap time-of-flight (IT-TOF) mass spectrometry. The results of the present study demonstrated the specific quality control of extracts of *A. crassna*.

[*Phytochem. Lett.* **5**, 325-328 (2012)] [Lab. of Pharmacognosy]

Novel Isolation of Acetophenone Derivatives with Spiroketal-hexosefuranoside in *Upuna borneensis*.

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

Our investigations of the chem. constituents in the leaves of *Upuna borneensis* Sym. (Dipterocarpaceae) resulted in the isolation of two novel diastereomeric acetophenone derivatives, upuborneols A (1) and B (2), along with four known derivatives. Their structures were determined by spectroscopic analysis including two-dimensional NMR and the speculation of biogenesis. Compounds 1 and 2 had a C6 unit derived from sugar unit and are the first known representatives of natural acetophenone derivatives bearing a spiroketal moiety.

[*Fitoterapia* **83**, 1420-1429 (2012)] [Lab. of Pharmacognosy]

Occurrence of Stilbene Oligomers in *Cyperus* Rhizomes.

Tetsuro ITO, Hidetatsu ENDO, Haruka SHINOHARA, Masayoshi OYAMA, Yukihiro AKAO and Munekazu IINUMA*

Investigation of the chem. constituents of Rhizoma Cyperi (*Cyperus rotundus* Linneus) resulted in the isolation of novel enantiomeric and meso-stilbene trimers [i.e., (+)- and (−)-(*E*)-cyperusphenol A (1, 2 respectively) and (*E*)-mesocyperusphenol A], a trimer bearing a novel hexacyclic ring system [cyperusphenol B], as well as known stilbenoids (cyperusphenols C and D, scirpusins A and B, and piceid) and luteolin. HPLC was used for the chiral separation of 1 and 2 as well as for the identification of cooccurrence of enantiomers of scirpsin A. The isolates were evaluated in terms of their antiproliferative activity employing the Jurkat cell line. The suppressive effect of cell growth by 6 was due to the induction of apoptosis.

[*Phytochem. Lett.* **5**, 743-746 (2012)] [Lab. of Pharmacognosy]

Occurrence of Bergenin Phenylpropanoates in *Vatica bantamensis*.

Tetsuro ITO, Yasumasa HARA, Masayoshi OYAMA, Toshiyuki TANAKA, Jin MURATA, Dedy DARNAEDI and Munekazu IINUMA*

We isolated five bergenin phenylpropanoates, i.e., 11-*O*-(*E*)-sinapate, 11-*O*-(*E*)-ferulate, 11-*O*-(*Z*)-ferulate, 11-*O*-(*E*)-coumalate, and 11-*O*-(*Z*)-coumalate, and three bergenin hydroxybenzoates, i.e., 11-*O*-syringate, 11-*O*-vanillate, and 11-*O*-*p*-hydroxybenzoate, along with bergenin, from the leaves of *Vatica bantamensis*. Moreover, we identified the geometrical isomerization between bergenin-11-*O*-(*E*)-ferulate and bergenin-11-*O*-(*Z*)-ferulate. These structures were characterized by NMR. This is the first report that shows the occurrence of bergenin phenolic acid esters in dipterocarpaceaeous plants.

[*J.Nat.Prod.* **75**, 694-698 (2012)] [Lab. of Pharmacognosy]

Terpenoids from *Chloranthus serratus* and Their Anti-inflammatory Activities.

Mi ZHANG, Munekazu IINUMA*, Jun-Song WANG, Masayoshi OYAMA, Tetsuro ITO and Ling-Yi KONG

Seven new terpenoids, including two sesquiterpene dimers (1, 2), two norditerpenoids (3, 4), and three sesquiterpenes (5-7), along with six known sesquiterpene dimers and four known sesquiterpenes were isolated from the whole plant of *Chloranthus serratus*. Their structures and relative configurations were elucidated on the basis of spectroscopic data analysis. The absolute configuration of 1 was determined by the CD exciton chirality method. These isolates were evaluated for their inhibitory effects on lipopolysaccharide-induced nitric oxide production in RAW264.7 cells. Compound 2 and two known compounds, shizukaols B and D, showed significant anti-inflammatory activities, with IC_{50} values of 0.22, 0.15, and 7.22 μM, respectively.

[*Fitoterapia* **83**, 1604-1609 (2012)] [Lab. of Pharmacognosy]

Sesquiterpenes from the Aerial Part of *Chloranthus japonicus* and Their Cytotoxicities.

Mi ZHANG, Jun-Song WANG, Peng-Ran WANG, Masayoshi OYAMA, Jun LUO, Tetsuro ITO, Munekazu IINUMA* and Ling-Yi KONG

Four new sesquiterpenes, chlorajapolides F-I, along with nine known terpenoids were isolated from the aerial part of *Chloranthus japonicus*. Their structures were elucidated on the basis of spectroscopic analysis, and a lindenane sesquiterpene, named 9-hydroxy-heterogorgiolide, previously isolated from the *C. japonicus*, was revised as its 8-epimer. Moreover, methanol extract, EtOAc fraction, water fraction, and all isolates were evaluated for their cytotoxicity using two human cancer cell lines.

[*J. Asian Nat. Prod. Res.* **14**, 708-712 (2012)] [Lab. of Pharmacognosy]

Anti-inflammatory Sesquiterpenes and Sesquiterpene Dimers from *Chloranthus fortunei*.

Mi ZHANG, Jun-Song WANG, Masayoshi OYAMA, Jun LUO, Chao GUO, Tetsuro ITO, Munekazu IINUMA* and Ling-Yi KONG

A novel lindenane sesquiterpene with an unprecedented 18-membered triester ring, named chlorafortulide, along with one known lindenane sesquiterpene and nine known lindenane sesquiterpene dimers, was isolated from the whole plant of *Chloranthus fortunei*. Their structures and relative configurations were elucidated on the basis of spectroscopic analysis. All the isolates were evaluated for their inhibitory effects on lipopolysaccharide-induced nitric oxide production in RAW264.7 cells. Henriol D, shizukaols E, G, M, and O showed significant anti-inflammatory activities with IC_{50} values of 1.90, 3.68, 1.95, 7.01, and 1.95 μM, respectively.

[*Chin. Med.* **7**, 19 (2012)] [Lab. of Pharmacognosy]

Effects of Alpha-Mangostin on the Expression of Anti-inflammatory Genes in U937 Cells.

Szu-Hsiu LIU, Lain-Tze LEE, Nai-Yun HU, Kuo-Kuei HUANG, Ying-Chu SHIH, Munekazu IINUMA*, Jen-Ming LI, Ting-Yu CHOU, Wei-Hsin WANG and Ting-Shou CHEN

This study aims to investigate the anti-inflammatory molecular action of alpha-mangostin on gene expression profiles. U937 and EL4 cells were treated with different concentrations of alpha-mangostin in the presence of LPS for 4 h. The anti-inflammatory effects were measured by the levels of TNF-alpha and IL-4 in cell culture media, which were determined with enzyme-linked immunosorbent assay kits. The gene expression profiles of all samples were analyzed with a whole human genome microarray. The protein levels were determined by Western blotting analyses. This study demonstrates that alpha-MG attenuates LPS-mediated activation of MAPK, STAT1, c-Fos, c-Jun and EIK-1, inhibiting TNF-alpha and IL-4 production in U937 cells.

[*J. Nat. Prod.* **75**, 563-566 (2012)] [Lab. of Pharmacognosy]

Hesperetin Upregulates ABCA1 Expression and Promotes Cholesterol Efflux from THP-1 Macrophages.

Akio IIO, Kenji OHGUCHI, Munekazu IINUMA*, Yoshinori NOZAWA and Masafumi ITO

Firefly luciferase reporter assays were developed for human ABCA1 promoters and LXR enhancers, and an in-house phytochemical library was screened. It was found that a citrus flavonoid, hesperetin, increased ABCA1 promoter and LXR enhancer activities in THP-1 macrophages. It was also found that this flavonoid promoted PPAR-enhancing activity. In accordance with these findings, 1 increased mRNA and protein expression of ABCA1 and consequently upregulated ApoA-I-mediated cholesterol efflux. These results provide evidence that 1 promotes ApoA-I-mediated cholesterol efflux from macrophages by increasing ABCA1 expression through the activation of LXRalpha and PPARgamma.

[*Arch. Virol.* **157**, 1489-1498 (2012)] [Lab. of Pharmacognosy]

In vitro and *in vivo* Evaluation of a Novel Antiherpetic Flavonoid, 4'-Phenylflavone, and its Synergistic Actions with Acyclovir.

Kyoko HAYASHI, Munekazu IINUMA*, Kohei SASAKI and Toshimitsu HAYASHI

We have evaluated the antiviral activities of a series of natural and synthetic flavonoids and found that a synthetic flavonoid, 4'-phenylflavone, showed the highest activity against acyclovir (ACV)-sensitive and ACV-resistant strains of HSV-1, as well as HSV-2, with a selectivity index of 213, 35 and 55, respectively. 4'-Phenylflavone plus ACV synergistically inhibited the replication of HSV-1. This flavonoid also showed efficacy *in vivo* and potentiated the antiherpetic effect of ACV in a mouse model of genital herpes. Our results suggest that 4'-phenylflavone might be useful as a candidate for the development of novel antiherpetic therapeutics.

[*PLoS One* **7**, e47950 (2012)] [Lab. of Pharmacognosy]

ATF6alpha Promotes Astroglial Activation and Neuronal Survival in a Chronic Mouse Model of Parkinson's Disease.

Koji HASHIDA, Yasuko KITAO, Hirofumi SUDO, Yoshitaka AWA, Shinichiro MAEDA, Kazutoshi MORI, Ryosuke TAKAHASHI, Munekazu IINUMA* and Osamu HORI

Accumulating evidence suggests a crucial role for the unfolded protein response (UPR) in Parkinson's disease. In this study, we investigated the relevance of the UPR in a mouse model of chronic MPTP/P injection, which causes severe and persistent degeneration of dopaminergic neurons. Enhanced activation of the UPR branches, including ATF6α and PERK/eIF2α/ATF4, was observed after MPTP/P injections into mice. The experimentl results suggest that the UPR is activated in a mouse model of chronic MPTP/P injection, and contributes to the survival of nigrostriatal dopaminergic neurons, in part, through activated astrocytes.

[*J. Biomed. Biotechnol.* **2012**, 672428-672429 (2012)] [Lab. of Pharmacognosy]

Alterations in Cell Cycle and Induction of Apoptotic Cell Death in Breast Cancer Cells Treated with Alpha-mangostin Extracted from Mangosteen Pericarp.

Hitomi KUROSE, Masa-Aki SHIBATA, Munekazu IINUMA* and Yoshinori OTSUKI

alpha-Mangostin has been shown to induce apoptosis in various cancer cell lines and to exhibit antitumor activity in a mouse mammary cancer model. In this study, we investigated the influence of alpha-mangostin on apoptosis and cell cycle in the human breast cancer cell line MDA-MB231 in order to elucidate its anticancer mechanisms. In alpha-mangostin-treated cells, induction of mitochondria-mediated apoptosis was observed. On cell-cycle analysis, G1-phase arrest, increased p21(cip1) expression and decreases in cyclins, cdc(s), CDKs and PCNA were observed. In conclusion, alpha-mangostin may be useful as a therapeutic agent for breast cancer carrying a p53 mutation and having HER2- and hormone receptor-negative subtypes.

[*Anal. Sci*., **28**, 257–265 (2012)] [Lab. of Pharm. Anal. Chemistry]

Mechanistic Study on the Electron-transfer Reaction of 9,10-Anthraquinone in the Presence of Hydrogen-bond and Proton Donating Additives .

Jiro KATSUMI, Tatsushi NAKAYAMA, Yukihiro ESAKA and Bunji UNO*

The electrochemical reduction of 9,10-anthraquinone (AQ) was investigated in CH_3CN in both the absence and presence of the hydrogen-bond and proton donating additives, CH_3OH, $CH(CF_3)_2OH$, phenol, 4-methoxyphenol, 4-cyanophenol, 2,4,6-trichlorophenol, and benzoic acid (BA). Three clearly different types of electrochemical behavior were observed with increasing concentrations of the additives, and were simulated to analyze the reaction mechanisms. Types I, II, and III were characterized by positive shifts of the two well-separated waves, a reversible or quasireversible two-electron reduction wave, and the 2-electorn cathodic and the broad anodic waves, respectively. The effects of hydrogen-bonding and protonation on the electrochemistry of AQ have been systematically demonstrated in terms of the potentials and reaction pathways.

[*J. Chromatogr. A*, **1236**, 202–206 (2012)] [Lab. of Pharm. Anal. Chemistry]

Micellar Electrokinetic Chromatography of Aromatic Anions and Non-ionic Aromatic Compounds with Stepwise Changes of the Concentration of Cetyltrimethylammonium Chloride.

Yukihiro ESAKA,* Miki KOBAYASHI, Hiroya MURAKAMI and Bunji UNO

Micellar electrokinetic chromatography in which the concentration of cetyltrimetylammmonium chloride (CTAC) was sequentially changed in the separation system was investigated. In isocratic elutions without EOF, the model analytes could be separated better with lower concentrations of CTAC but migration times of the analytes possessing relatively higher polarities increased markedly, and thus, long analysis times were required. Therefore, we attempted to increase the concentration of CTAC during a single measurement to reduce the analysis time without hindering the resultant separation of analytes obtained with lower concentrations. By the present stepwise method, both the 10 anionic analytes and the 11 non-ionic analytes were well separated within reasonable periods.

[*Eur. J. Pharm. Sci.* **46**, 374-380 (2012)] [Lab. of Pharm. Engineering]

Design and Evaluation of Poly(dl-lactic-co-glycolic acid) Nanocomposite Particles Containing Salmon Calcitonin for Inhalation.

Mingshi YANG, Hiromitsu YAMAMOTO, Homare KURASHIMA, Hirofumi TAKEUCHI, Toyokazu YOKOYAMA, Hiroyuki TSUJIMOTO and Yoshiaki KAWASHIMA*

Salmon calcitonin, for the treatment of calcium homeostasis and bone remodeling, was used as a model peptide drug and adsorbed on the surface of biodegradable polymeric poly(dl-lactic-co-glycolic acid) (PLGA) nanospheres. Subsequently, the nanospheres were treated using lyophilizer and loaded onto inhalable carrier using Mechanofusion™ to obtain nanocomposite particles suitable for inhalation. The physicochemical properties and in vitro inhalation properties of the nanocomposite particles were investigated. It suggested that the Mechanofusion™ technique can impart improved inhalation properties to the lyophilized nanospheres for pulmonary delivery of therapeutic peptide drugs.

[*Eur. J. Pharm. Sci.* **47**, 235-243 (2012)] [Lab. of Pharm. Engineering]

Design and Evaluation of Inhalable Chitosan-modified Poly (dl-lactic-co-glycolic acid) Nanocomposite Particles.

Mingshi YANG, Hiromitsu YAMAMOTO, Homare KURASHIMA, Hirofumi TAKEUCHI, Toyokazu YOKOYAMA, Hiroyuki TSUJIMOTO and Yoshiaki KAWASHIMA*

The aim of this study was to investigate two types of chitosan-modified poly (dl-lactic-co-glycolic acid) (PLGA) nanocomposite particles containing salmon calcitonin for pulmonary delivery, which were obtained using spray drying fluidized bed granulation (Agglomaster™) and dry powder coating techniques (Mechanofusion™), respectively. The physicochemical properties, pulmonary distribution, pulmonary clearance rate as well as in vivo hypocalcemia actions of the two types of nanocomposite particles were investigated. This can be attributed to the avoidance of aggregation of chitosan-modified PLGA nanocomposite particles when using Agglomaster™ rather than Mechanofusion™.

[*Int. J. Pharm.* **428**, 183-186 (2012)] [Lab. of Pharm. Engineering]

Nanocomposite Formation between Alpha-glucosyl Stevia and Surfactant Improves the Dissolution Profile of Poorly Water-soluble Drug.

Hiromasa. UCHIYAMA, Yuichi. TOZUKA, Masahiro NISHIKAWA and Hirofumi TAKEUCHI*

The formation of a hybrid-nanocomposite using alpha-glucosyl stevia (Stevia-G) and surfactant was explored to improve the dissolution of flurbiprofen (FP). As reported previously, the dissolution amount of FP was enhanced in the presence of Stevia-G, induced by the formation of an FP and Stevia-G-associated nanostructure. When a small amount of sodium dodecyl sulfate (SDS) was present with Stevia-G, the amount of dissolved FP was extremely enhanced. This dissolution-enhancement effect was also observed with the cationic surfactant of dodecyl trimethyl ammonium bromide, but not with the non-ionic surfactant of n-octyl-beta-D-maltopyranoside. To investigate the dissolution-enhancement effect of Stevia-G/SDS mixture, the pyrene I(1)/I(3) ratio was plotted versus the Stevia-G concentration.

[*Eur. J. Pharm. Biopharm.* **82**, 120-126 (2012)] [Lab. of Pharm. Engineering]

Transglycosylated Rutin-specific Non-surface-active Nanostructure Affects Absorption Enhancement of Flurbiprofen.

Yuichi. TOZUKA, Kenjirou. HIGASHI, Takeshi MORITA, Masahiro NISHIKAWA, Hiromasa UCHIYAMA, Junying ZHANG, Kunikazu MORIBE, Keiko NISHIKAWA, Hirofumi TAKEUCHI and Keiji YAMAMOTO*

Transglycosylated rutin (Rutin-G), a newly developed transglycosylated food additive, was used as a novel excipient for improving the dissolution and absorption properties of flurbiprofen. No surface activity was found up to 100 mg/mL of Rutin-G concentration. No cytotoxicity to Caco-2 cells was observed even at a high level of 100 mg/mL Rutin-G solution. 1H NMR study with concentration variation revealed that Rutin-G formed small aggregates in water, with the aggregation number of Rutin-G above the critical aggregation concentration of about 5.0 mg/mL being 4. Structural analyses by small-angle X-ray scattering determined the aggregate to be several nanometers in maximum length.

[*J. Liposome Res.* **22**, 72-79 (2012)] [Lab. of Pharm. Engineering]

Effectiveness of Submicronized Chitosan-coated Liposomes in Oral Absorption of Indomethacin.

Hikaru SUGIHARA, Hiromitsu YAMAMOTO, Yoshiaki KAWASHIMA and Hirofumi TAKEUCHI*

The plasma profile of indomethacin (IMC) after oral administration of IMC-loaded submicronized chitosan-coated liposomes (ssCS-Lip) was evaluated to reveal the effectiveness of the mucoadhesive function for improving the absorption of this poorly absorbable drug. The stomach and small intestine were removed from rats after 1, 2, and 4 hours of oral administration of submicron-sized liposomes (ssLip) or ssCS-Lip containing fluorescent dye, and the retentive properties were confirmed by measuring the amount of dye in each part of the gastrointestinal (GI) tract. Results showed that ssCS-Lip tended to be better retained in the upper part of the GI tract, compared with ssLip, at 1, 2, and 4 hours after administration, and was significantly better retained in the small intestine at 4 hours. The plasma profile and bioavailability of IMC after oral administration of both types of liposomes were improved, compared with oral administration of IMC solution.

[*Chem. Pharm. Bull. (Tokyo)* **60**, 1320-1323 (2012)] [Lab. of Pharm. Engineering]

Effects of Food Intake on the Mucoadhesive and Gastroretentive Properties of Submicron-sized Chitosan-coated Liposomes.

Hikaru SUGIHARA, Hiromitsu YAMAMOTO, Yoshiaki KAWASHIMA and Hirofumi TAKEUCHI*

The gastrointestinal transition of mucoadhesive drug carriers may be affected by food intake, since food changes the physiological conditions of the gastrointestinal tract, and the food content itself is a physical obstruction for the drug carriers. Here we investigated the effects of food intake on the gastrointestinal transition and mucoadhesive function of submicron-sized chitosan-coated liposomes (ssCS-Lip). The stomach and small intestine were removed after oral administration of ssCS-Lip and non-coated liposomes (ssLip) containing fluorescent dye to fasted or fed rats, and retentive properties were quantitatively confirmed by measuring the amount of dye in each part of the gastrointestinal tract. Both types of liposome were retained in the stomach at approx. 40% in the fed rats at 1 h after oral administration.

[*Eur. J. Pharm. Biopharm.* **80**, 340-346 (2012)] [Lab. of Pharm. Engineering]

Pulmonary Delivery of Elcatonin Using Surface-modified Liposomes to Improve Systemic Absorption: Polyvinyl Alcohol with a Hydrophobic Anchor and Chitosan Oligosaccharide as Effective Surface Modifiers.

Mitsutaka. MURATA, Koji. NAKANO, Kohei. TAHARA, Yuichi. TOZUKA and Hirofumi TAKEUCHI*?

The aim of this study was to investigate the feasibility of surface-modified liposomes for pulmonary delivery of a peptide. Chitosan oligosaccharide (oligoCS) and polyvinyl alcohol with a hydrophobic anchor (PVA-R) were used as surface modifiers. The effect of liposomal surface modification on the behavior of the liposomes on pulmonary administration and potential toxicity were evaluated in vitro and in vivo. PVA-R modification reduced interaction with A549 cells, whereas oligoCS modification electrostatically enhanced cellular interaction. The therapeutic efficacy of elcatonin (eCT) after pulmonary administration to rats was significantly enhanced and prolonged for 48 h after separate administration with oligoCS- or PVA-R-modified liposomes.

[*Drug. Dev. Ind. Pharm.* 1-7 (2012)] [Lab. of Pharm. Engineering]

Solventless Dry Powder Coating for Sustained Drug Release Using Mechanochemical Treatment Based on the Tri-component System of Acetaminophen, Carnauba Wax and Glidant.

Yohei HOASHI, Yuichi TOZUKA and Hirofumi TAKEUCHI*

Solventless dry powder coating methods have many advantages compared to solvent-based methods: they are more economical, simpler, safer, more environmentally friendly and easier to scale up. The purpose of this study was to investigate a highly effective dry powder coating method using the mechanofusion system, a mechanochemical treatment equipped with high compressive and shearing force. Effective CW coating onto the AAP surface was successfully achieved by strictly controlling the processing conditions and the composition of core particles, coating material and glidant. Our mechanochemical dry powder coating method using the mechanofusion system is a simple and promising means of solventless pharmaceutical coating.

[*Int. J. Pharm.* **436**, 564-567 (2012)] [Lab. of Pharm. Engineering]

Liposomal Diclofenac Eye Drop Formulations Targeting the Retina: Formulation Stability Improvement Using Surface Modification of Liposomes.

Takuya. FUJISAWA, Hiroko MIYAI, Kohei HIRONAKA, Toshimasa TSUKAMOTO, Kohei TAHARA, Yuichi TOZUKA, Masaki ITO and Hirofumi TAKEUCHI*

An efficient liposomal formulation for targeting the retina was produced as an optimal means of distributing therapeutic agents to the retina. Diclofenac was used as a model compound for liposome encapsulation, and the release rate and distribution to the retina were investigated. The calcium acetate gradient method was found to be the optimal method for encapsulating diclofenac into liposomes. Entrapment efficiency using this method was greater than 97%, whereas conventional hydration method achieved 51.3%. The resultant formulation obtained with the gradient method caused aggregation and/or fusion of liposomes. To avoid inhibition of retinal delivery due to the aggregation of the carrier, surface modification was performed simultaneously with the gradient method.

[*Eur. J. Pharm. Biopharm.* **82**, 250-261 (2012)] [Lab. of Pharm. Engineering]

Novel Pectin-based Nanoparticles Prepared from Nanoemulsion Templates for Improving in vitro Dissolution and in vivo Absorption of Poorly Water-soluble Drug.

Kanokporn BURAPAPADH, Hirofumi TAKEUCHI and Pornsak SRIAMORNSAK*

The purpose of this study was to improve in vitro dissolution and in vivo absorption of itraconazole (ITZ), a poorly water-soluble drug, by means of novel pectin-based nanoparticles prepared from nanoemulsion templates. Nanoemulsion templates were prepared by a high-pressure homogenization using pectin (i.e., 0.5-3.0% w/w low-methoxyl pectin (LMP), amidated low-methoxyl pectin (ALMP), or high-methoxyl pectin (HMP)) as an emulsifier and chloroform as an oil phase. HMP provided good oil-in-water emulsions with ITZ loaded in the oil phase. The chloroform in nanoemulsions was then removed to produce the suspensions of nanoparticles dispersed in water phase. After lyophilization, the dried core-shell nanoparticles with good properties in terms of redispersibility, dissolution, and stability were obtained.

[*Biomaterials* **33**, 343-351 (2012)] [Lab. of Pharm. Engineering]

The Suppression of IgE-mediated Histamine Release from Mast Cells Following Exocytic Exclusion of Biodegradable Polymeric Nanoparticles.

Kohei. TAHARA, Satoshi. TADOKORO, Hiromitsu. YAMAMOTO, Yoshiaki KAWASHIMA and Naohide HIRASHIMA*

The objective of this study is to evaluate the effect of polymeric nanoparticles (NPs) on the allergic response of mast cells that release inflammatory mediators such as histamine through exocytosis. Submicron-sized biodegradable poly(dl-lactide-co-glycolide) (PLGA) NPs were prepared by the emulsion solvent diffusion method. Here, we examined the interactions of the mast cells with two types of PLGA NPs, unmodified NPs and NPs modified with chitosan (CS), a biodegradable cationic polymer. NPs were taken up by mast cells through an endocytic pathway (endocytic phase) and then the cellular uptake was saturated and maintained plateau level by the exclusion of NPs through exocytosis (exocytic phase).

[*Langmuir* **28**, 7114-7118 (2012)] [Lab. of Pharm. Engineering]

Endocytosis-like Uptake of Surface-modified Drug Nanocarriers into Giant Unilamellar Vesicles.

Kohei TAHARA, Satoshi TADOKORO, Yoshiaki KAWASHIMA and Naohide HIRASHIMA*

We had previously developed surface-modified poly (d,l-lactide-co- glycolide) (PLGA) nanoparticles (NPs) for use as a cellular drug delivery system. The cellular uptake of PLGA-NPs was mediated predominantly by endocytosis, and this uptake was increased by surface modifications with polymers, such as chitosan (CS) and polysorbate 80 (P80). In the present study, we prepared a cell-sized giant unilamellar vesicle (GUV) that mimics a cell membrane to investigate the interaction between cell membranes and NPs. Endocytosis-like uptake of NPs into a GUV was observed when the NPs were modified with nonionic surfactant P80 probably due to change in viscoelasticity and enhanced fusion activity of the membrane induced by P80. In contrast, unmodified NPs and those modified with CS were not internalized into a GUV. These results suggest that surface properties of PLGA-NPs are an important formulation parameter for their interaction with lipid membranes.

[*J. Photopolym. Sci. Thechnol.* **25**, 501–506 (2012)] [Lab. of Pharmaceutical Physical Chemistry]

Evaluation on Fluidity of the Self-assembled Phospholipid Layer Fabricated by Plasma-assisted Method and its Application.

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

We carried out the measurement of thickness of self-assembled phospholipid layer (PC-SA) fabricated by plasma-assisted method. The thickness of PC-SA increased with the increase of surface density of phospholipid up to about 25 nm in this experimental condition. It was suggested that the PC-SA fabricated would be a multilayered structure. The fluidity of PC-SA containing more stearic acid was higher than the others, so that the fluidity might be controlled by the content of stearic acid. The antibody immobilized onto PC-SA possessed higher activity than that onto the polymer surface with carboxylic acids prepared by plasma-assisted method. It was suggested that PC-SA was very effective as a bio-interface for the immobilization of biomolecules.

[*J. Photopolym. Sci. Thechnol*, **25**, 551–554 (2012)] [Lab. of Pharmaceutical Physical Chemistry]

Fabrication of Hydrophilic Polymer Brushes on Polystyrene Substrate by Plasma-based Surface Functionalization.

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

We developed the method for fabrication of poly (sodium acrylate) (pSA) brushes by atom transfer radical polymerization on plasma-irradiated polystyrene (PS) substrate. The results from XPS and water contact angle measurement indicated that the well-defined pSA brushes were successfully synthesized on PS substrate. The surface thus prepared was superhydrophilic, so that the nonspecific physisorption of serum proteins onto the surface was minimized and the cell adhesion was effectively suppressed. We used the pSA grafted PS as a platform for immobilizing a specific peptide ligand to evaluate the interaction with cell surface. The cell culture experimants indicated that the peptide immobilized on pSA-grafted PS was specifically recognized by cell surface

[*J. Toxicol. Sci.* **37**, 381-387 (2012)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

Trichloroethylene Enhances TCR-CD3-induced Proliferation of $CD8^+$ Rather Than $CD4^+$ T Cells.

Ryo KOBAYASHI, Tsuyoshi NAKANISHI and Hisamitsu NAGASE*

We investigated whether trichloroethylene (TCE) modulates T cell receptor (TCR)-induced T cell activation and proliferation *in vitro*. TCE enhanced T cell proliferation primed by anti-CD3 antibody, but not concanavalin A. In addition, TCE enhanced anti-CD3-primed proliferation of $CD8^+$ rather than $CD4^+$ T cells. Consistent with this result, TCE markedly enhanced the Lck phosphorylation mediated by anti-CD3 antibody in $CD8^+$ but not $CD4^+$ T cells. Furthermore, in OVA-immunized mice, TCE exposure at the high concentration via drinking water for 2 weeks significantly expanded $CD3^+$, $CD8^+$ and $CD4^+$ splenocyte populations, however the effect at the lower concentration was significant only in the $CD8^+$ populations, whereas TCE had no effect on these cells population in non-immunized mice. These findings suggest that TCE enhances TCR-CD3-induced proliferation of $CD8^+$ rather than $CD4^+$ cells and disrupts various activities of peripheral T cells.

[*J. Toxicol. Sci.* **37**, 439-445 (2012)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

Enhancing Effects of Trichloroethylene and Tetrachloroethylene on Type I Allergic Responses in Mice.

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

In the current study, we investigated the effects of trichloroethylene (TCE) and tetrachloroethylene (perchloroethylene; PCE) on the passive cutaneous anaphylaxis (PCA) reaction *in vivo* using ICR mice. TCE and PCE significantly enhanced the PCA reaction in a dose-dependent manner. In addition, we examined the enhancing effects of ingesting small amount of TCE and PCE in drinking water on antigen-stimulated allergic responses. After the ICR mice had ingested TCE or PCE in their drinking water for 2 or 4 weeks, we performed the PCA reaction. Both TCE and PCE ingestion enhanced the PCA reaction in a dose-dependent manner for 4 weeks. These results suggest that exposure to TCE and PCE leads to the augmentation of type I allergic responses in many species.

[*Toxicol. Lett.* **211**, 257-265 (2012)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

Possible Aryl Hydrocarbon Receptor-independent Pathway of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin -induced Antiproliferative Response in Human Breast Cancer Cells.

Hiroki YOSHIOKA, Youhei HIROMORI, Akira AOKI, Tomoki KIMURA, Yoshiaki FUJII-KURIYAMA, Hisamitsu NAGASE and Tsuyoshi NAKANISHI*

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a ligand with high affinity for the aryl hydrocarbon receptor (AhR). Here, we investigated the possibility of TCDD-induced antiproliferative responses in human breast cancer cells through AhR-independent pathways. DNA synthesis was significantly suppressed in human breast cancer cells treated with TCDD at a very low concentration (0.01 nM), whereas that in human cancer cells in ovary, cervix uteri and placenta was unaffected, even by exposure to 10 nM TCDD. Furthermore, knockdown of the AhR by RNA interference had no effect on TCDD-induced antiproliferation in breast cancer cells. These findings suggest that the principal pathways of TCDD-induced antiproliferation in breast cancer cells are not AhR dependent.

[*Toxicol. Lett.* **212**, 91-96 (2012)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

Role of Megalin and the Soluble Form of its Ligand RAP in Cd-metallothionein Endocytosis and Cd-metallothionein-induced Nephrotoxicity *in vivo*.

Akira ONODERA, Miyuki TANI, Toshimi MICHIGAMI, Masayo YAMAGATA, Kyong-Son MIN, Keiichi TANAKA, Tsuyoshi NAKANISHI*, Tomoki KIMURA and Norio ITOH

In the current study, we investigated the effect of megalin, a multiligand endocytic receptor (also known as low-density lipoprotein receptor-related protein 2 or Lrp2), on Cd-metallothionein (MT) distribution and Cd-MT-induced nephrotoxicity in an animal model to inhibit megalin function using the soluble form of 39-kDa receptor-associated protein (sRAP; also known as Lrpap1), a ligand of megalin. Administration of sRAP to mice caused acute loss of megalin function by removing megalin in the brush border membrane. The pre-injection of sRAP decreased renal Cd content and decreased Cd-MT-induced kidney damage. Our results demonstrate that sRAP reduces Cd-MT-induced kidney toxicity *in vivo*.

[*Biochim. Biophys. Acta.* **1821**, 561-572 (2011)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

Retinoic Acid Receptor Agonists Regulate Expression of ATP-binding Cassette Transporter G1 in Macrophages.

Makoto AYAORI, Emi YAKUSHIJI, Masatsune OGURA, Kazuhiro NAKAYA, Tetsuya HISADA, Harumi UTO-KONDO, Shunichi TAKIGUCHI, Yoshio TERAO, Makoto SASAKI, Tomohiro KOMATSU, Maki IIZUKA, Makiko YOGO, Yoshinari UEHARA, Hiroyuki KAGECHIKA, Tsuyoshi NAKANISHI* and Katsunori IKEWAKI

In the current study, we investigated whether, and how, retinoic acid receptors (RARs) regulate the expression of ABC transporter G1 (ABCG1) plays a which play a pivotal role in HDL-mediated cholesterol efflux and atherogenesis, in macrophages. Our data indicates that RAR ligands increase ABCA1/G1 expression and apoA-I/HDL-mediated cholesterol efflux from macrophages, and modulate ABCG1 promoter activity via a liver X receptor-responsive element -dependent mechanisms.

[*Environ. Toxicol. Chem.* **31**, 307-315 (2012)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

Detection of Retinoic Acid Receptor Agonistic Activity and Identification of Causative Compounds in Municipal Wastewater Treatment Plants in Japan.

Kazuko SAWADA, Daisuke INOUE, Yuichiro WADA, Kazunari SEI, Tsuyoshi NAKANISHI* and Michihiko IKE

In the current study, to determine the occurrence of retinoic acid (RA) receptor (RAR) agonists, which are potential teratogenic toxicants for vertebrates, in municipal wastewater treatment plants (WWTPs), we examined the RARα agonistic activities of influent and effluent samples from several municipal WWTPs in Osaka, Japan, using a yeast two-hybrid assay. Significant RARα agonistic activity was detected in all the influent samples investigated, suggesting that municipal wastewater consistently contains RAR agonists. The RAR agonists, all-*trans* RA (atRA), 13-*cis* RA (13cRA), 4-oxo-atRA, and 4-oxo-13cRA were identified in WWTPs. However our data also suggests the occurrence of unidentified RAR agonists during the activated sludge treatment.

[*Pharmazie* **67**, 86-90 (2012)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

Efflux Transporter mRNA Expression Profiles in Differentiating JEG-3 Human Choriocarcinoma Cells as a Placental Transport Model.

Kenji IKEDA, Kyohei YAMASAKI, Manami HOMEMOTO, Satoko YAMAUE, Mitsue OGAWA, Erina NAKAO, Yumi FUKUNAGA, Tsuyoshi NAKANISHI*, Naoki UTOGUCHI, Michiaki MYOTOKU and Yoshihiko HIROTANI

In the current study, to clarify the usefulness of the differentiating JEG-3 cell placental drug transport model which was previously established by us, we investigated the mRNA expression profiles of the efflux transporters MRPs, MDR1, and BCRP in JEG-3 cells and compared them with those of BeWo cells and their known placental expression. We demonstrated the efflux transporters' expression profiles, as well as those of the BeWo cells, was demonstrated in the DJEG placental drug transport evaluating model as well as the BeWo cells, in the DJEG placental drug transport evaluation model. Based on these findings, we hope that the DJEG model will be adequate for use in evaluating placental drug transport in relation to the transporter proteins.

[*Int. J. Mol.* **13**, 13484-13500 (2012)] [Lab. of Molecular Biology]

Injury-Induced Accumulation of Glial Cell Line-derived Neurotrophic Factor in the Rostral Part of the Injured Rat Spinal Cord.

Takuya HARA, Hidefumi FUKUMITSU, Hitomi SOUMIYA, Yoshiko FURUKAWA and Shoei FURUKAWA*

The spinal cord of a 7-week-old female Wistar rat was hemi-transected at thoracic position 10 with a razor blade, and changes in glial cell line-derived neurotrophic factor (GDNF) protein and mRNA expression levels in the spinal cord were examined. Although GDNF is distributed in the healthy spinal cord from 150 to 400 pg/g tissue in a regionally dependent manner, hemi-transection (left side) of the spinal cord caused a rapid increase in GDNF content in the ipsilateral rostral but not in the caudal part of the spinal cord. On the other hand, injury-induced GDNF mRNA was distributed limitedly in both rostral and caudal stumps. These observations suggest the possibility that increased GDNF in the rostral part is responsible for the accumulation of GDNF that may be constitutively transported from the rostral to caudal side within the spinal cord.

[*Int. J. Mol. Sci.* **13**, 4968-4981 (2012)] [Lab. of Molecular Biology]

2-Decenoic Acid Ethyl Ester, a Compound That Elicits Neurotrophin-like Intracellular Signals, Facilitating Functional Recovery from Cerebral Infarction in Mice.

Yoshitaka TANAKA, Hidefumi FUKUMITSU, Hitomi SOUMIYA, Shinichi YOSHIMURA, Toru IWATA and Shoei FURUKAWA*

In our previous study, we found that trans-2-decenoic acid ethyl ester (DAEE), a derivative of a medium-chain fatty acid, elicits neurotrophin-like signals including the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) in cultured mouse cortical neurons. Here, we examined the efficacy of intraperitoneal administration of DAEE on the treatment of a mouse model of the cerebral infarction caused by unilateral permanent middlecerebral artery occlusion (PMCAO). DAEE-treatment (100 □g/kg body weight injected at 0.5, 24, 48, 72 h after PMCAO) significantly restored the mice from PMCAO-induced neurological deficits including motor paralysis when evaluated 48, 72, and 96 h after the PMCAO.

[*Biochem. Biophys. Res. Commun.* **425**, 848-853 (2012)] [Lab. of Molecular Biology]

Knockdown of Pre-mRNA Cleavage Factor Im 25kDa Promotes Neurite Outgrowth.

Hidefumi FUKUMITSU*, Hitomi SOUMIYA and Shoei FURUKAWA

Mammalian precursor mRNA (pre-mRNA) cleavage factor I (CFIm) plays important roles in the selection of poly(A) sites in a 3'-untranslated region (3'-UTR), producing mRNAs with variable 3' ends. Because 3'-UTRs often contain cis elements that impact stability or localization of mRNA or translation, alternative polyadenylation diversifies utilization of primary transcripts in mammalian cells. By using RNA interference tequnique, we revealed that knockdown of CFIm25 increased the number of primary dendrites of hippocampal neurons and promoted nerve growth factor (NGF)-induced neurite extension from rat pheochromocytoma PC12 cells. On the other hand, CFIm25 knockdown did not influence constitutively active or dominantly negative RhoA suppression or promotion of NGF-induced neurite extension from PC12 cells, respectively. Taken together, our results indicate that endogenous CFIm may promote neuritogenesis by coordinating events upstream of NGF-induced RhoA inactivation.

[*Neurosci. Lett.* **526**, 79-84 (2012)] [Lab. of Molecular Biology]

Absence of SHATI/Nat8l Reduces Social Interaction in Mice.

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

We previously identified a novel molecule "Shati/Nat8l" from the nucleus accumbens of mice. However, the physiological roles of the SHATI protein are not clear. To investigate the effect of SHATI on the central nervous system and behavior, we studied knockout mice of this protein. Shati-knockout mice did not differ from wild type mice in learning and memory. On the other hand, Shati-knockout mice showed increases in rearing and grooming time in the open field test, and exploration time of novel objects. These results suggested that knockout of the Shati gene may increase exploration in specific circumstances. Interestingly, the Shati-knockout mice avoided social interaction with unfamiliar mice out of their home cage, although there was no difference in social interaction in their home cage compared with wild type mice.

[*Evid. Based Complement Alternat. Med.* **2012**, 139140 (2012)] [Lab. of Molecular Biology]

Antidepressant-like Activity of 10-hydroxy-trans-2-decenoic Acid, a Unique Unsaturated Fatty Acid of Royal Jelly, in Stress-inducible Depression-like Mouse Model.

Satoru ITO, Yuji NITTA, Hidefumi FUKUMITSU, Hitomi SOUMIYA, Kumiko IKENO, Tadashi NAKAMURA and Shoei FURUKAWA*

Symptoms of depression and anxiety appeared in mice after they had been subjected to a combination of forced swimming for 15 min followed by being kept in cages that were sequentially subjected to leaning, drenching, and rotation within 1-2 days for a total of 3 weeks. Using several behavioral tests, we found that 10-hydroxy-trans-2-decenoic acid (HDEA), an unsaturated fatty acid unique to royal jelly (RJ), protected against the depression and anxiety when intraperitoneally administered once a day for 3 weeks simultaneously with the stress loading. Our present results demonstrate that HDEA and RJ, a natural source of it, were effective in ameliorating the stress-inducible symptoms of depression and anxiety.

[*Arch. Biochem. Biophys.* **520**, 30-35 (2012)] [Lab. of Clinical Pharmaceutics]

Contribution of p38 MAPK, NF-κB and Glucocorticoid Signaling Pathways to ER Stress-induced Increase in Retinal Endothelial Permeability.

Tetsuo ADACHI*, Mayumi TERAMACHI, Hiroyuki YASUDA, Tetsuro KAMIYA and Hirokazu HARA

Endoplasmic reticulum (ER) stress is known to play a pathogenic role in vascular impairment in diabetic retinopathy (DR). The present study demonstrated that the treatment of human retinal endothelial cells with ER stress inducers such as thapsigargin (Tg) and tunicamycin (Tm) significantly increased the permeability of exogenously added FITC-dextran, accompanied by a decrease of transendothelial electrical resistance (TEER). The expression of claudin-5 among tight junction proteins was significantly decreased by the treatment with Tg or Tm. A p38 MAPK inhibitor, SB203580, and an NF-□B inhibitor, dexamethasone, significantly suppressed the Tg-induced down-regulation of claudin-5, decrease of TEER and leakage of added FITC-dextran. The effects of dexamethasone are thought to be due to the transrepression of the above signaling and direct regulation of claudin-5 gene.

[*Free Radic. Res.* **46**, 637-644 (2012)] [Lab. of Clinical Pharmaceutics]

TPA Induces the Expression of EC-SOD in Human Monocytic THP-1Cells: Involvement of PKC, MEK/ERK and NOX-derived ROS.

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

Extracellular-superoxide dismutase (EC-SOD) is a major SOD isozyme mainly present in the vascular wall. EC-SOD is also observed in monocytes/macrophages, and its high expression contributes to the suppression of atherosclerosis by scavenging superoxide. In this study, we investigated the expression of EC-SOD during 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced monocytic differentiation of THP-1 cells. We confirmed the significant induction of EC-SOD in a TPA time-dependent manner, and that induction was completely blocked by pre-treatment with GF109203X, an inhibitor of protein kinase C, U0126 and PD98059, inhibitors of mitogen-activated protein kinase kinase/extracellular-signal regulated kinase. Moreover, we determined the involvement of NADPH oxidase-derived reactive oxygen species in that induction.

[*Biol. Pharm. Bull.* **35**, 1126-1131 (2012)] [Lab. of Clinical Pharmaceutics]

Propolis Suppresses $CdCl_2$-induced Cytotoxicity of COS7 Cells through the Prevention of Intracellular Reactive Oxygen Species Accumulation.

Tetsuro KAMIYA*, Misato IZUMI, Hirokazu HARA and Tetsuo ADACHI

Propolis is a natural product made by honeybees and contains various compounds. These compounds are considered to have antiviral, antibacterial and antioxidative properties. We previously reported that exposure to cadmium chloride ($CdCl_2$) induced cell death though intracellular reactive oxygen species (ROS) generation in kidney tubule epithelial COS7 cells. Pretreatment with propolis extracts suppressed $CdCl_2$-induced cytotoxicity and intracellular ROS generation. Propolis extracts not only showed antioxidative activities, but also increased the expression of heme oxygenase-1 (HO-1), an antioxidative enzyme. Moreover, we determined the involvement of hypoxia inducible factor-1α in propolis extract-derived HO-1 induction. We demonstrate the utility of propolis for Cd-related COS7 cytotoxicity, and these findings are considered to contribute to the control of ROS-derived disorders.

[*J. Agric. Food Chem.* **60**, 11065-11070 (2012)] [Lab. of Clinical Pharmaceutics]

Ethanol Extract of Brazilian Red Propolis Induces Apoptosis in Human Breast Cancer MCF-7 Cells through Endoplasmic Reticulum Stress.

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

Propolis, a natural product collected from plants by honey bees, is commonly used in folk medicines. Endoplasmic reticulum (ER) stress is known to induce apoptosis through the induction of CCAAT/enhancer-binding protein homologous protein (CHOP). Here, we investigated whether ethanol extracts of propolis and caffeic acid phenethyl ester (CAPE) induce apoptosis, mitochondrial dysfunction, and ER stress in human breast cancer MCF-7 cells and human fibroblasts. Brazilian red propolis (BRP) significantly reduced MCF-7 cell viability through the induction of mitochondrial dysfunction, caspase-3 activity, and DNA fragmentation but did not affect those of fibroblasts. Moreover, treatment with BRP significantly induced CHOP expression in MCF-7 cells compared to fibroblasts. Further, pretreatment with a chemical chaperone, 4-phenylbutyric acid, suppressed BRP-triggered MCF-7 cell death.

[*J. Jpn. Soc. Hosp. Pharm.* **48,** 627-631 (2012)] [Lab. of Clinical Pharmaceutics]

The Influence of Dexamethasone on Control of Blood Glucose Levels in Cancer Patients with Concurrent Diabetes.

Tomokazu FUJII, Hiroshi SUZUKI, Naoki SAWAYANAGI, Haruhiko NAKAMURA, Hisayuki TSUKAMOTO, Koutarou SHIBATA, Tetsuo ADACHI* and Tsuneyuki KAMIYA

For the safety management of cancer chemotherapy, pharmacists are expected to play an important role in prescription checking. We investigated the influence of dexamethasone on blood sugar level and of hemoglobin A_{1c} (HbA_{1c}) value in cancer patients with concurrent diabetes. A significant positive correlation was observed between the accumulation of dexamethasone and change of blood sugar level or HbA_{1c} within 12 months. It was suggested that severe control of pathophysiological conditions of diabetes is necessary when the accumulated dosage of dexamethasone exceeds 150 mg.

[*Ther. Res.* **33**, 905-909 (2012)] [Lab. of Clinical Pharmaceutics]

Benefit of Amlodipine/Atorvastatin Combination Tablet in Patients with Hypertension, Dyslipidemia and Stable Coronary Artery Disease.

Kiyoshi OZUMI, Hiromi TASAKI, Seiya TANAKA, Takashi HARADA, Tetsuo ADACHI* and Yutaka OTSUJI

The joint effects of systolic blood pressure and total cholesterol on cardiovascular disease seen consistent across various Asian populations. The incidence of ischemic stroke has declined significantly over the past 40 years, probably owing to better management of hypertension in the Japanese population. However, there is a need for greater primary prevention efforts in the treatment of hypertension and metabolic disorders. In this study, we observed the benefit of amlodipine/atorvastatin combination tablet in patients with hypertension, dislipidemia and stable coronary artery disease.

[*J. Anal. Bio-Sci.* **35**, 225-233 (2012)] [Lab. of Clinical Pharmaceutics]

The Degree of Oxidative Stress in Patients with Rheumatoid Arthritis (RA): The Introduction of the ''GAP Ratio'' Yardstick and Its Applications – A ''Sampled Studies'' Approach –.

Takahiro IMAZATO, Yukitaka UEKI, Naoyuki HIRAKATA, Naotaka KURODA, Naoya KISHIKAWA, Masao YANO, Teruo SHIBA, Noriko OHNISHI, Naoko IKOSHI, Hiroji SHIMOMURA, Tetsuo ADACHI*, Naoki SUZUKI, Matsuo TANIYAMA and Eisuke MAEHATA

The mean GAP ratio in a group of random selected RA patients was 7.6 for males and 6.8 for females. A GAP ratio of 6.0 or less was considered to indicate a state of oxidative stress. The GAP ratio showed significant negative correlations with ESR and CRP, as well as with DAS28-ESR and DAS28-CRP. Furthermore, the separation at the GAP ratio of 6.0 was consistent with significant classification according to RA markers ESR, CRP, and MMP-3. The GAP ratio is effective as an RA marker, particularly in examining subclinical conditions, and therefore considered highly useful as a vital sign.

[*Oncol. Rep.* **28**, 2009-2015 (2012)] [Lab. of Pharmaceutics]

Characterization of the Low pH/Low Nutrient-resistant LNCaP Cell Subline LNCaP-F10.

Kazuhiro IGUCHI, Yuri HAYAKAWA, Kenichiro ISHII, Kaoru MATSUMOTO, Shigeyuki USUI, Yoshiki SUGIMURA and Kazuyuki HIRANO*

In the present study, the subline LNCaP-F10, of the prostate cancer cell line LNCaP, was isolated and its low pH/low nutrient-resistant properties were examined. LNCaP-F10 cells were grown under low-pH/low-nutrient conditions, which caused cell death of the LNCaP cells. The cell death was associated with oligonucleosomal DNA fragmentation and poly (ADP-ribose) polymerase (PARP) cleavage, indicating that low-pH/low-nutrient induced apoptosis in these cells. Tumor growth caused by implantation of LNCaP-F10 cells into the renal subcapsular space of nude mice in the absence or presence of prostate stromal cell stimulation was greater than that caused by implantation of LNCaP cells. LNCaP-F10 cells were resistant to apoptosis induced by an environment of low-pH/low-nutrient in vitro, and displayed malignant potential in vivo.

[*J. Androl.* **33**, 1208-1215 (2012)] [Lab. of Pharmaceutics]

Antiandrogenic Activity of Resveratrol Analogs in Prostate Cancer LNCaP Cells.

Kazuhiro IGUCHI, Tomoaki TOYAMA, Tetsuro ITO, Toshinobu SHAKUI, Shigeyuki USUI, Masayoshi OYAMA, Munekazu IINUMA and Kazuyuki HIRANO*

The suppression of androgen signaling is a therapeutic target for the treatment of prostate cancer. Resveratrol (3,4',5-trihydroxystilbene) is known to inhibit the function of the androgen receptor (AR). In the present study, we investigated the antiandrogenic activities of resveratrol analogs in order to identify a potent antiandrogen compound. Among the resveratrol analogs tested, 4'-*O*-methylresveratrol (3,5-dihydroxy-4'-methoxystilbene) was the most effective inhibitor of AR transcriptional activity. Introduction of a methoxy group to the C-4' of resveratrol and its analogs increased their antiandrogenic activity compared with the unmodified counterparts. Conversely, modification of the 3- and/or 5-hydroxyl groups reduced the antiandrogenic activity. The hydroxyl groups in resveratrol play a key role in their antiandrogenic effect by modulating AR transcriptional activity.

[*J. Androl.* **33**, 660-666 (2012)] [Lab. of Pharmaceutics]

Low Androgen Sensitivity Is Associated With Low Levels of Akt Phosphorylation in LNCaP-E9 Cells

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

We previously characterized LNCaP-E9, a low-androgen-sensitive LNCaP cell subline. To understand the mechanism underlying low androgen sensitivity of LNCaP-E9 cells, we examined the activities of the Akt, p44/42, and p38 mitogen-activated protein kinase signaling pathways, all of which are known to be linked to androgen receptor signaling. We found that the phosphorylation of Akt at Ser473 was markedly lower in LNCaP-E9 cells than in LNCaP cells. Inhibition of Akt phosphorylation by the phosphatidylinositol 3-kinase inhibitor LY294002 resulted in reduction of PSA expression in LNCaP cells. Conversely, activation of Akt by serum starvation led to the induction of PSA expression in LNCaP-E9 cells. These results suggest that the impaired Akt phosphorylation in LNCaP-E9 cells is associated with low androgen sensitivity.

[*Biol. Trace Elem. Res.* **151**, 9-13 (2012)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Decreased Bioelements Content in the Hair of Patients with Fahr's Disease (Idiopathic Bilateral Calcification in the Brain).

Mari TAKAGI, Kazuhiro OZAWA, Hiroshi YASUDA, Mitsuko DOUKE, Kazunori HASHIMOTO, Yuichi HAYASHI, Takashi INUZUKA and Isao HOZUMI*

To determine the pattern of some biological metals in the hair of patients with Fahr's disease, we investigated the levels of 24 bioelements in the hair of 28 patients. We found decreases in the levels of several bioelements in the hair of patients. The significant tendencies of several bioelements in the hair of patients in this study suggest metabolic disorders of bioelements, especially biometals, on the background.

[*J. Neurol.* **259**, 1448-1452 (2012)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Is There Delayed Gastric Emptying in Patients with Multiple System Atrophy? An Analysis Using the ^{13}C-acetate Breath Test.

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

We investigated gastric emptying in 25 patients with MSA, 20 patients with sporadic adult-onset ataxia of unknown etiology, and 20 healthy volunteers using the ^{13}C-acetate breath test. Gastric emptying was significantly delayed in patients with MSA, and the delay already appeared in the early stage of the disease. Delayed gastric emptying is one of the autonomic failures and may be a clinical marker of MSA.

[*Curr. Neurovasc. Res.* **9**, 296-301 (2012)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Anti-endothelial Cell Antibodies in Patients with Cerebral Small Vessel Disease.

Akio KIMURA, Takeo SAKURAI, Megumi YAMADA, Akihiro KOUMURA, Yuichi HAYASHI, Yuji TANAKA, Isao HOZUMI*, Hirofumi OHTAKI, Mitsuhiro CHOUSA, Masao TAKEMURA, Mitsuru SEISHIMA and Takashi INUZUKA

We examined anti-endothelial cell antibodies (AECAs) in sera from 12 elderly subjects with cerebral small vessel disease (CSVD), 12 elderly subjects without CSVD, and 18 healthy volunteers by 2-dimensional immunoblotting using primary cultured human brain microvascular endothelial cells as the antigen source. The anti-TPM4 antibody level was significantly higher in the subjects with than without CSVD. An autoimmune, inflammatory process with high levels of anti-TPM4 antibody may contribute to the development of CSVD in the elderly.

[*J. Alzheimers Dis.* **29**, 373-377 (2012)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Antibodies against the Tom40 Subunit of the Translocase of the Outer Mitochondrial Membrane Complex and Cognitive Impairment in Alzheimer's Disease.

Akio KIMURA, Takeo SAKURAI, Megumi YAMADA, Akihiro KOUMURA, Yuichi HAYASHI, Yuji TANAKA, Isao HOZUMI*, Hirofumi OHTAKI, Mitsuhiro CHOUSA, Masao TAKEMURA, Mitsuru SEISHIM and Takashi INUZUKA

The purpose of this study was to examine the presence of antibodies against cerebral microvascular endothelial cells specific for Alzheimer's disease, and to evaluate the association of these antibodies with cognitive impairment. The anti-Tom40 antibody is significantly associated with cognitive impairment in patients with Alzheimer's disease.

[*Internal Med.* **51**, 579-584 (2012)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Late-onset Patients with Sporadic Amyotrophic Lateral Sclerosis in Japan Have a Higher Progression Rate of ALSFRS-R at Time of Diagnosis.

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

Forty-five patients with sporadic ALS were divided into 2 groups: 23 patients with early-onset of ALS (<65 years; early onset) and 22 patients with late-onset ALS (≥65 years; late onset). Our finding suggested that patients with late-onset ALS showed more rapid disease progression than those with early-onset ALS using ΔFS.

[*Biol. Pharm. Bull.* **35**, 84-90 (2012)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Protective Effects of 4-PBA (Phenylbutyrate) Derivatives on the Neuronal Cell Death and Endoplasmic Reticulum Stress.

Seisuke MIMORI, Yasunobu OKUMA, Masayuki KANEKO*, Koichi KAWADA, Toru HOSOI, Koichiro OZAWA, Yasuyuki NOMURA and Hiroshi HAMANA

Sodium 4-phenylbutyrate (4-PBA) possesses *in vitro* chemical chaperone activity and reduces the accumulation of Parkin-associated endothelin receptor-like receptor (Pael-R). In this study, in order to investigate the structure–activity relationships of 4-PBA analogs, we examined the effect of terminal aromatic substituted fatty acids, which act as chemical chaperones, on protein aggregation and ER stress-induced cell death. The results suggest that terminal aromatic substituted fatty acids are potential candidates for the treatment of neurodegenerative diseases.

[*Biol. Pharm. Bull.* **35**, 269-272 (2012)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Possible Involvement of Ubiquitin Ligase HRD1 Insolubilization in Amyloid β Generation.

Masayuki KANEKO*, Ryo SAITO, Yasunobu OKUMA and Yasuyuki NOMURA

Endoplasmic reticulum (ER)-associated degradation (ERAD) selectively retro-transports and degrades unfolded proteins accumulated in the ER. We have demonstrated that the ubiquitin ligase HRD1 involved in ERAD was significantly decreased in the cerebral cortex of Alzheimer's disease patients. Furthermore, the HRD1 level was negatively correlated with amyloid β (Aβ) production levels. Here we found that the HRD1 protein level decrease was due to its insolubilization. Moreover, these protein levels extracted from detergent insoluble fraction were positively correlated with those of SEL1L and Aβs (Aβ40 and Aβ42). Thus, the insolubilization-induced decrease in the HRD1 and SEL1L levels might involve in Aβ generation.

[*Mol. Cell* **47**, 99-110 (2012)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

STT3B-Dependent Posttranslational N-Glycosylation as a Surveillance System for Secretory Protein.

Takashi SATO, Yasuhiro SAKO, Misato SHO, Mamiko MOMOHARA, Mary Ann SUICO, Tsuyoshi SHUTO, Hideki NISHITOH, Tsukasa OKIYONEDA, Koichi KOKAME, Masayuki KANEKO*, Manabu TAURA, Masanori MIYATA, Keisuke CHOSA, Tomoaki KOGA, Saori MORINO-KOGA, Ikuo WADA and Hirofumi KAI

Various signatures of client proteins, including exposure of hydrophobic patches or unpaired sulfhydryls, are coordinately utilized to reduce nonnative proteins in the endoplasmic reticulum (ER). We report here the cryptic N-glycosylation site as a recognition signal for unding of a natively nonglycosylated protein, transthyretin (TTR). Folding and ER-associated degradation (ERAD) perturbation analyses revealed that prolonged TTR unfolding induces externalization of cryptic N-glycosylation site and triggers STT3B-dependent posttranslational N-glycosylation. We postulate that STT3B-dependent posttranslational N-glycosylation is part of a triage-salvage system recognizing cryptic N-glycosylation sites of secretory proteins to preserve protein homeostasis.

[*J. Toxicol. Sci.* **37**, 1049-1057 (2012)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Effects of Hsp90 Inhibitors, Geldanamycin and its Analog, on Ceramide Metabolism and Cytotoxicity in PC12 Cells.

Kaori TOYOMURA, Takeshi SAITO, Syunsuke EMORI, Ikiru MATSUMOTO, Erina KATO, Masayuki KANEKO*, Yasunobu OKUMA, Hiroyuki NAKAMURA and Toshihiko MURAYAMA

The inhibitors of heat shock protein-90 (Hsp90), geldanamycin (GA) and 17-(allylamino)-17-desmethoxygeldanamycin, show various cellular effects including destabilization of Hsp90 clients and expression of other chaperones, *etc*. and modulate cytotoxicity depending on cell types and stimuli. In this study, we investigated the effects of Hsp90 inhibitors on survival of PC12 cells with and without cytotoxic stimuli including orthovanadate, Na_3VO_4. Our results suggest the possible involvement of ceramide metabolism, not AA release, in GA-induced cytotoxicity in PC12 cells.

[*Cell Death Differ.* **19**, 1939-1949 (2012)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Activation of OASIS Family, ER Stress Transducers, is Dependent on its Stabilization.

Shinichi KONDO, Shin-ichiro HINO, Atsushi SAITO, Soshi KANEMOTO, Noritaka KAWASAKI, Rie ASADA, Soutarou IZUMI, Masayuki KANEKO*, Yasuyuki NOMURA, Fumihiko URANO and Kazunori IMAIZUMI

BBF2 human homolog on chromosome 7 (BBF2H7) and old astrocyte specifically induced substance (OASIS), endoplasmic reticulum (ER)-resident transmembrane proteins, have recently been identified as novel ER stress transducers that have roles in chondrogenesis and osteogenesis, respectively. Here, we showed that BBF2H7 and OASIS are notably unstable proteins that are easily degraded via the ubiquitin-proteasome pathway under normal conditions. HRD1, an ER-resident E3 ubiquitin ligase, ubiquitinated BBF2H7 and OASIS under normal conditions, whereas ER stress conditions dissociated the interaction between HRD1 and BBF2H7 or OASIS. These findings suggest that ER stress stabilizes OASIS family members and this is a novel molecular mechanism for the activation of ER stress transducers.

[*Biol. Pharm. Bull.* **35**, 1603-1606 (2012)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Detoxification of 6-Hydroxydopamine-induced Dopaminergic Neurodegeneration by 5,5-Dimethyl-1-pyrroline N-Oxide, a Radical Trapper, in Hemiparkinsonian Rats.

Masatoshi INDEN*, Yoshihisa KITAMURA, Kazuyuki TAKATA, Hiroyuki YASUI, Kanji YOSHIMOTO and Eishi ASHIHARA

5,5-Dimethyl-1-pyrroline N-oxide (DMPO) has been used most frequently as a spin trap agent. Co-microinjection of 4 nmol DMPO, but not 0.4 nmol, significantly prevented 6-hydroxydopamine (6-OHDA)-induced behavioral impairments and dopaminergic neurodegeneration. In ESR analysis, DMPO directly trapped hydroxyl radical (·OH) generated from 6-OHDA and Fe^{2+} in a concentration-dependent manner. These results suggest that DMPO attenuates 6-OHDA-induced dopaminergic neurodegeneration in a rat model of PD via scavenging ·OH, and is a useful tool for biological research of oxidative stresses.

[*J. Pharmacol. Sci.* **119**, 10-19 (2012)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Effect of Selective Serotonin Reuptake Inhibitors via 5-HT_{1A} Receptors on L-DOPA-induced Rotational Behavior in a Hemiparkinsonian Rat Model.

Masatoshi INDEN*, Mari ABE, Hideyuki MINAMINO, Kazuyuki TAKATA, Kanji YOSHIMOTO, Ikuo TOOYAMA and Yoshihisa KITAMURA

Intranigral injection of 6-hydroxydopamine (6-OHDA) in rats caused a significant loss of tyrosine hydroxylase immunoreactivity. However, tryptophan hydroxylase immunoreactivity was unaffected by 6-OHDA. Pre-treatment with fluoxetine significantly suppressed L-Dihydroxyphenylalanine (L-DOPA)-induced rotational behavior. Additionally, fluoxetine suppressed L-DOPA-induced ERK1/2 and histone H3 phosphorylation. These effects of fluoxetine were abolished by pre-treatment with WAY 100135, a 5-HT_{1A} antagonist. These results suggest that fluoxetine may influence motor function in Parkinson's disease via pharmacological modification of interactions between serotonergic and dopaminergic neuronal networks.

[*Innate Immun.* **18**, 429-437 (2012)] [Lab. of Microbiology]

Inhibitory Effect of 10-Hydroxy-*trans*-2-decenoic Acid on LPS-induced IL-6 Production via Reducing IκB-ζ Expression.

Tsuyoshi SUGIYAMA*, Keita TAKAHASHI, Shunji TOKORO, Takaki GOTOU, Paola NERI and Hiroshi MORI

The effect of 10-hydroxy-trans-2-decenoic acid (10H2DA) was investigated on LPS-induced cytokine production in murine macrophage cell line. 10H2DA inhibited LPS-induced IL-6 production dose-dependently, but did not inhibit TNF-α production. 10H2DA inhibited LPS-induced NF-κB activation in a dose-dependent fashion. In addition, 10H2DA inhibited NF-κB activation induced by over-expression of either MyD88 or Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF). We found that lipocalin-2 and granulocyte colony-stimulating factor (G-CSF), which is dependent on IκB-ζ, was also inhibited by 10H2DA. These results suggest that 10H2DA is one of the components of royal jelly to show anti-inflammatory effects and could be a therapeutic drug candidate for inflammatory and autoimmune diseases associated with IκB-ζ and IL-6 production.

[*Cell Immunol.* **273**, 73-78 (2012)] [Lab. of Microbiology]

Inhibition of Interferon-γ-induced Nitric Oxide Production by 10-Hydroxy-*trans*-2-decenoic Acid through Inhibition of Interferon Regulatory Factor-8 Induction.

Keita TAKAHASHI, Tsuyoshi SUGIYAMA*, Shunji TOKORO, Paola NERI and Hiroshi MORI

10-Hydroxy-*trans*-2-decenoic acid (10H2DA) is a major lipid component of royal jelly. In this study, we examined the effect of 10H2DA on interferon (IFN)-γ-induced nitric oxide (NO) production. 10H2DA inhibited IFN-γ-induced NO production and activation of the inducible NO synthase promoter. IFN-γ-induced tumor necrosis factor (TNF)-α production and nuclear factor (NF)-κB activation were inhibited by 10H2DA. IFN-γ-mediated induction of interferon regulatory factor (IRF)-8 was also inhibited by 10H2DA. IFN-γ-induced TNF-α production followed by activation of NF-κB is known to be essential for NO production. Together, 10H2DA inhibited IFN-γ-induced NO production by inhibiting IRF-8 induction and TNF-α production. 10H2DA might modulate IFN-γ-mediated cellular responses by inhibiting the induction of IRF-8 and IRF-8-dependent

[*Biol. Pharm. Bull.* **35**, 917-923 (2012)] [Lab. of Microbiology]

Recombinant Shiga Toxin B Subunit Can Induce Neutralizing Immunoglobulin Y Antibody.

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

Previously, we have shown that chickens immunized with Shiga toxin (Stx) produce Stx-neutralizing egg yolk immunoglobulin Y (IgY) antibody. In the present study, chickens were immunized with recombinant Stx-1 B subunit (rStx-1B) and recombinant Stx-2 B subunit (rStx-2B). Induced anti-rStx-1B and anti-rStx-2B IgY neutralized the toxicity of Stx-1 and Stx-2 against HeLa 229 cells. The neutralizing activity of anti-rStx-1B IgY on Stx-1 was almost 10 times stronger than that of anti-Stx-1 IgY, and that of anti-rStx-2B IgY was 2.6 times stronger than that of anti-Stx-2 IgY. Anti-rStx-1B and anti-rStx-2B IgY reacted with multimeric and monomeric forms of the B subunits in contrast to anti-Stx-1 and anti-Stx-2 IgY that reacted with only the multimeric form. These results indicated that recombinant B subunits were promising antigens for induction of neutralizing antibodies in chickens.

[*Mol. Immunol.* **52**, 299-304 (2012)] [Lab. of Microbiology]

Mechanism of Inhibition of Lipopolysaccharide-induced Interferon-β Production by 2-Aminopurine.

Tsuyoshi SUGIYAMA*, Takaki GOTOU, Kazuya MORIYAMA, Nodoka KAJIURA, Takuya HASEGAWA, Junko TOMIDA, Keita TAKAHASHI, Takayuki KOMATSU, Hiroshi UEDA, Katsuya SATO, Shunji TOKORO, Paola NERI and Hiroshi MORI

We found that 2-AP inhibited IFN-β promoter activation, which was induced by the overexpression of Toll/interleukin-1 receptor domain-containing adaptor inducing IFN-β (TRIF) and the overexpression of TRIF-dependent signaling molecules, including the constitutively active mutant of interferon regulatory factor (IRF)-3. While 2-AP did not affect LPS-induced phosphorylation of IRF-3, nuclear translocation of IRF-3 was inhibited. Moreover, LPS-induced phosphorylation of Akt, another key molecule involved in IRF-3 activation, was inhibited by 2-AP. These results suggest that 2-AP could be a novel lead compound for TLR signaling inhibition specific for TRIF-dependent nuclear translocation of IRF-3.

[*Biochem Biophys Res Commun.* 417, 1127-1132 (2012)] **[Lab. of Microbiology]**

PMA Induces GCMa Phosphorylation and Alters its Stability via the PKC- and ERK-dependent Pathway.

Yuko YASUI, Kazuyo YAMADA, Satoru TAKAHASHI, Mayumi SUGIURA-OGASAWARA, Katsuya SATO, Daisuke MIYAZAWA, Tsuyoshi SUGIYAMA*, Yukio KITADE and Hiroshi UEDA.

The glial cells missing a (GCMa) transcription factor plays a pivotal role in the placental development. We investigated whether GCMa is regulated by PKC-dependent pathway. PMA caused a transient decrease in the endogenous GCMa protein level in the human choriocarcinoma JEG-3 cells that was accompanied by an increase in GCMa phosphorylation. The phosphorylation and degradation of GCMa by PMA treatment was effectively reduced by pretreatment with PKC inhibitors and a MEK inhibitor. Further, we identified the serine residues 328, 378 and 383 to be the phosphorylation sites on GCMa. Our data demonstrate for the first time the GCMa phosphorylation by PKC- and MEK/ERK-dependent mechanism, which is involved in its degradation process.

[*Immunopharmacol Immunotoxicol.* 35, 1-7 (2012)] **[Lab. of Microbiology]**

Antiviral Activity of Acidic Polysaccharides from Coccomyxa Gloeobotrydiformi, a Green Alga, against an in vitro Human Influenza A Virus Infection.

Takayuki KOMATSU, Nobuo KIDO, Tsuyoshi SUGIYAMA* and Takashi YOKOCHI.

The acidic polysaccharide fraction from a green alga Coccomyxa gloeobotrydiformi (CmAPS) was isolated and the antiviral action on an in vitro infection of influenza A virus was examined. The 50% inhibitory concentration of CmAPS on the infection of human influenza A virus strains ranged from 26 to 70 μg/mL and the antiviral activity of CmAPS against influenza A/USSR90/77 (H1N1) was the strongest. The antiviral activity of CmAPS required its presence in the inoculation of virus onto MDCK cells. Pretreatment and post-treatment with CmAPS was ineffective for the antiviral activity. CmAPS inhibited influenza A virus-induced erythrocyte hemagglutination and hemolysis. Taken together, CmAPS was suggested to exhibit the anti-influenza virus activity through preventing the interaction of virus and host cells. The detailed antiviral activity of CmAPS is discussed.

[*Bioorg. Med. Chem.* **20**, 356-367 (2012)] [Lab. of Biochemistry]

Design, Synthesis, and Biological Evaluation of Novel (1-Thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic Acids as Selective Inhibitors for AKR1B1.

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New substituted (1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acids were designed as the inhibitor of AKR1B1 based upon the structure of rhetsinine, a minor alkaloidal component of Evodia rutaecarpa, and twenty derivatives were synthesized and evaluated. In the view of activity and selectivity, the most potent compound was (2-benzyl-6-carboxy-1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid (7t), which showed strong inhibitory effect (IC_{50} = 0.17 μM) and very high selectivity for AKR1B1 against AKR1A1 (311:1) and AKR1B10 (253:1) compared with epalrestat.

[*Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **68**, 400-403 (2012)] [Lab. of Biochemistry]

Structure of the His269Arg Mutant of the Rat Aldose Reductase-like Protein AKR1B14 Complexed with NADPH: Correlation between Structure and Kinetic Constants for the Binding of Coenzyme.

Krithika SUNDARAM, Satoshi ENDO, Toshiyuki MATSUNAGA, Nobutada TANAKA, Akira HARA* and Ossama EL-KABBANI

AKR1B14 plays roles in the detoxification of reactive aldehydes and synthesis of prostaglandin $F_{2\alpha}$. Here, the crystal structure of AKR1B14 H269R mutant complexed with NADPH is described and shows that the negatively charged 2'-phosphate group of the coenzyme forms an ionic interaction with the positively charged guanidinium group of Arg269. We previously reported the mutants of His269 to Arg, Phe and Met showed the fourfold, sevenfold and 127-fold, respectively, of K_m for NADPH, which are in agreement with the present molecular-modelling and X-ray crystallographic studies. This is the first tertiary structure of a mutant of AKR1B14 to be reported in order to investigate the structure-function relationship of the nonconserved His269 and its role in coenzyme binding.

[*Biol. Pharm. Bull.* **35**, 2075-2080 (2012)] [Lab. of Biochemistry]

Inhibition of Human Aldose Reductase-like Protein (AKR1B10) by α- and γ-Mangostins, Major Components of Pericarps of Mangosteen.

Midori SODA, Satoshi ENDO, Toshiyuki MATSUNAGA, Hai-Tao ZHAO, Ossama EL-KABBANI, Munekazu IINUMA, Keiko YAMAMURA and Akira HARA*

AKR1B10 was recently identified as both diagnostic marker and therapeutic target in the treatment of several types of cancer. In this study, we have examined AKR1B10 inhibition by five xanthone derivatives, components of pericarps of mangosteen. γ-Mangostin was found to be the most potent competitive inhibitor (K_i = 5.6 nM), but its 7-methoxy derivative, α-mangostin, was the second potent inhibitor (K_i = 80 nM). Molecular docking and site-directed mutagenesis studies revealed that Phe123, Trp220, Val301 and Gln303 are important for the tight binding of γ-mangostin, and suggested that the 7-methoxy group of α-mangostin impairs the inhibitory potency by altering the orientation of the inhibitor molecule in the substrate-binding site of the enzyme.

[*Eur. J. Med. Chem.* **48**, 321-329 (2012)] [Lab. of Biochemistry]

Design, Synthesis and Evaluation of Caffeic Acid Phenethyl Ester-based Inhibitors Targeting a Selectivity Pocket in the Active Site of Human Aldo-keto Reductase 1B10.

Midori SODA, Dawei HU, Satoshi ENDO, Mayuko TAKEMURA, Jie LI, Ryogo WADA, Syohei IFUKU, Hai-Tao ZHAO, Ossama EL-KABBANI, Shozo OHTA, Keiko YAMAMURA, Naoki TOYOOKA, Akira HARA and Toshiyuki MATSUNAGA*

Inhibitors of AKR1B10 are regarded as promising therapeutics for the treatment of cancer. In this study, we have found that, among honeybee propolis products, caffeic acid phenethyl ester (CAPE) inhibited AKR1B10. Based on a model of docked CAPE in AKR1B10, we found 3-(4-hydroxy-2-methoxyphenyl)acrylic acid 3-(3-hydroxyphenyl)propyl ester (10c) was the most potent competitive inhibitor (K_i = 2.6 nM) with 790-fold selectivity for AKR1B10 over AKR1B1. Additionally, the sub-μM concentration of 10c significantly suppressed the farnesal metabolism and cellular proliferation in AKR1B10-overexpressing cells.

[*Cell Tissue Res.* **347**, 407-417 (2012)] [Lab. of Biochemistry]

9,10-Phenanthrenequinone Promotes Secretion of Pulmonary Aldo-keto Reductases with Surfactant..

Toshiyuki MATSUNAGA*, Mariko HAGA, Gou WATANABE, Yuhki SHINODA, Satoshi ENDO, Yu KAJIWARA, Hiroyuki TANAKA, Naoki INAGAKI, Ossama EL-KABBANI and Akira HARA

In this study, we found that intratracheal infusion of 9,10-phenanthrenequinone (9,10-PQ), a major quinone in diesel exhaust particles, facilitates the secretion of surfactant into rat alveolus. In the cultured rat lung, the 9,10-PQ treatment increased a lower-density surfactant, which contained aldo-keto reductase (AKR) 1C15, suggesting an involvement of AKR1C15 in the quinone-elicited lung damage. In human, treatment of lung type II epithelial A549 cells with 9,10-PQ promoted secretion of AKR1C3 with the surfactant, and the culture medium efficiently reduced 9,10-PQ and produced reactive oxygen species. Exposure of A549 cells to 9,10-PQ also provoked viability loss, which was protected by adding the AKR1C3 inhibitor, suggesting that AKR1C3 secreted in pulmonary surfactants participates in the toxic mechanism triggered by 9,10-PQ.

[*Drug Metab. Pharmacokinet.* **27**, 553-558 (2012)] [Lab. of Biochemistry]

Reduction of Cytotoxic *p*-Quinone Metabolites of *tert*-Butylhydroquinone by Human Aldo-keto Reductase (AKR) 1B10.

Toshiyuki MATSUNAGA*, Satoshi ENDO, Mayuko TAKEMURA, Midori SODA, Keiko YAMAMURA, Kazuo TAJIMA, Takeshi MIURA, Tomoyuki TERADA, Ossama EL-KABBANI and Akira HARA

2-*tert*-Butylhydroquinone (BHQ), an antioxidant used as a food additive, exhibits an anticancer effect, whereas it is carcinogenic in rodents at high doses. BHQ is metabolized into cytotoxic *tert*-butylquinone (BQ), which is further converted to 6-*tert*-butyl-2,3-epoxy-4-hydroxy-5-cyclohexen-1-one (TBEH) through 6-*tert*-butyl-2,3-epoxy-4-benzoquinone (TBE). We found that AKR1B10 was the most efficient catalyst of the stoichiometric reduction of BQ and TBE into less cytotoxic BHQ and TBEH, respectively. Additionally, gene expression of AKR1B10 in HCT116 cells was up-regulated by treatment with BHQ, BQ and TBE. These results suggest a role for the enzyme in protection at least against the toxicity of the two *p*-quinone metabolites of BHQ.

[*Biol. Pharm. Bull.* **35**, 1598-1602 (2012)] [Lab. of Biochemistry]

9,10-Phenanthrenequinone Induces Monocytic Differentiation of U937 Cells through Regulating Expression of Aldo-keto Reductase 1C3.

Toshiyuki MATSUNAGA*, Mika HOSOGAI, Mariko ARAKAKI, Satoshi ENDO, Ossama EL-KABBANI and Akira HARA

A 24-h incubation of human promyelomonocytic U937 cells with 9,10-phenanthrenequinone (9,10-PQ), a major quinone in diesel exhaust particles, provoked apoptotic cell death. Flow cytometric analyses of U937 cells after exposure to 9,10-PQ revealed induction of differentiation that was evidenced by increasing expression of CD11b/CD18, a monocytic differentiation marker. The 9,10-PQ-induced differentiation was significantly sensitized by treating with tolfenamic acid, a selective inhibitor of aldo-keto reductase (AKR) 1C3, suggest that 9,10-PQ treatment acutely leads to apoptosis of U937 cells through promotion of the monocytic differentiation, which is thought to be in part regulated by AKR1C3.

[*J. Nat. Prod.* **75**, 716-21 (2012)] [Lab. of Biochemistry]

Selective Inhibition of Human Type-5 17β-Hydroxysteroid Dehydrogenase (AKR1C3) by Baccharin, a Component of Brazilian Propolis.

Satoshi ENDO*, Toshiyuki MATSUNAGA, Ayano KANAMORI, Youko OTSUJI, Hiroko NAGAI, Krithika SUNDARAM, Ossama EL-KABBANI, Naoki TOYOOKA, Shozo OHTA and Akira HARA

AKR1C3 has been suggested as a therapeutic target in the treatment of prostate and breast cancers. In this study, we found that baccharin (1) is a potent inhibitor with high selectivity against other AKR1C isoforms. Molecular docking and site-directed mutagenesis studies suggested that the nonconserved residues Ser118, Met120, and Phe311 in AKR1C3 are important for determining the inhibitory potency and selectivity of 1. The AKR1C3-mediated metabolism of 17-ketosteroid and farnesal in cancer cells was inhibited by 1. Additionally, 1 suppressed the proliferation of PC3 prostatic cancer cells stimulated by AKR1C3 overexpression. This study is the first demonstration that 1 is a highly selective inhibitor of AKR1C3.

[*Biol. Pharm. Bull.* **35**, 1191-1196 (2012)] [Lab. of Biochemistry]

Molecular Characterization and Mutational Analysis of Recombinant Diadenosine 5',5"-P^1,P^4-Tetraphosphate Hydrolase from *Plasmodium Falciparum*.

Waleed OSMAN, Satoshi ENDO*, Kentaro OH-HASHI, Yoshiaki KITAMURA and Yukio KITADE

Asymmetrical diadenosine 5',5"-P^1,P^4-tetraphosphate (Ap_4A) hydrolase from human malaria parasite *Plasmodium falciparum* was characterized for the first time as a biological target for chemotherapeutic agents against malaria. *Plasmodium falciparum* Ap_4A ($PfAp_4A$) hydrolase catalyzes not only Ap_4A to ATP and AMP, but also diadenosine 5',5″-P^1,P^5-pentaphosphate (Ap_5A) to ATP and ADP. The enzyme showed maximal activity in the presence of 5 mM Mg^{2+} ions. Comparative protein modeling indicated an additional space in the substrate binding site of the parasitic enzyme compared with that of humans. Mutagenic analysis of Pro133 to a smaller residue (Ala) revealed a 5-fold increase in the wild-type K_m value. Furthermore, catalytic activity was markedly affected by introducing a larger residue (Phe), thus creating the potential to develop a specific inhibitor of $PfAp_4A$ hydrolase.

[*Arch. Biochem. Biophys.* **527**, 23-30 (2012)] [Lab. of Biochemistry]

Characterization of Rabbit Aldose Reductase-like Protein with 3β-Hydroxysteroid Dehydrogenase Activity.

Satoshi ENDO*, Toshiyuki MATSUNAGA, Sho KUMADA, Airi FUJIMOTO, Satoshi OHNO, Ossama EL-KABBANI, Dawei HU, Naoki TOYOOKA, Jun'ichi MANO, Kazuo TAJIMA and Akira HARA

We isolated the cDNA for rabbit AKR1B19 that shared an 86% sequence identity to human AKR1B10. AKR1B19 was similar to AKR1B10 in the substrate specificity for various aldehydes and α-dicarbonyl compounds. Meanwhile, AKR1B19 efficiently reduced 3-keto-5α/β-dihydro-C_{19}/C_{21}/C_{24}-steroids into the corresponding 3β-hydroxysteroids. The stereospecific reduction was also observed in the metabolism of 5α/5β-dihydrotestosterones in AKR1B19-expressing cells. Thus, AKR1B19 may function as a 3-ketoreductase in rabbit tissues. Mutations of Phe303 and Met304 in the corresponding residues in AKR1B10 significantly impaired 3-ketoreductase activity, suggesting the two residues play critical roles in recognition of the steroidal substrate.

[*Allergy* **67**, 201-209 (2012)] [Lab. of Pharmacology]

Overcoming Food Allergy through Acquired Tolerance Conferred by Transfer of Tregs in a Murine Model.

Hirotaka YAMASHITA*, Keita TAKAHASHI, Hiroyuki TANAKA, Hiroichi NAGAI and Naoki INAGAKI

Because food allergies result from an absence of inherent mucosal tolerance to food proteins, an understanding of the mechanisms of oral tolerance could aid in the development of novel therapies for food allergies. In this study, we analyzed oral tolerance in food allergy to use two types of murine food allergy model. One was the model for tolerance by pretreatment orally with food antigen of ovalbumin (OVA). The other was the model by adoptive transfer of immune cells from OVA orally treated mice. Anaphylactic responses for oral administration of OVA were ameliorated in both models. The production of OVA-specific immunoglobulins was suppressed completely in the first model, but not in the second model. The tolerance in second model was depended on Tregs. Because we think second type tolerance is closer to clinical situation, we need to analyze detail mechanism.

[*Biol. Pharm. Bull.* **35**, 612-614 (2012)] [Lab. of Pharmacology]

Lactobacillus Acidophilus Strain L-92 Induces $CD4^+CD25^+Foxp3^+$ Regulatory T Cells and Suppresses Allergic Contact Dermatitis.

Mohammad Monir SHAH, Masanao SAIO, Hirotaka YAMASHITA, Hiroyuki TANAKA, Tsuyoshi TAKAMI, Takayuki EZAKI and Naoki INAGAKI*

Lactobacilli are often examined in the management of allergic diseases. In the previous study, we reported *Lactobacillus acidophilus* strain L-92 could suppress 2,4-dinitrofluorobenzene (DNFB) and mite antigen-induced atopic dermatitis-like skin lesions in mice. In this study, BALB/c mice were supplemented daily with L-92 by gavage for 5 weeks in DNFB-induced allergic contact dermatitis model. The percentage of $CD4^+CD25^+Foxp3^+$ T cell (Treg) in spleen and cervical lymph nodes were increased in comparison with control mice. Also, interleukin-10, transforming growth factor-β, and Foxp3 mRNA expressions in mouse ear skin were higher than control mice. These results suggest that L-92 might moderate allergic dermatitis by upregulation of Tregs.

[*J. Pharmacol. Sci.* **118**, 266-274 (2012)] [Lab. of Pharmacology]

Transient Receptor Potential Vanilloid 1 –a Polymodal Nociceptive Receptor– Plays a Crucial Role in Formaldehyde-induced Skin Inflammation in Mice.

Haruki USUDA, Takumi ENDO, Ayumi SHIMOUCHI, Asaka SAITO, Makoto TOMINAGA, Hirotaka YAMASHITA, Hiroichi NAGAI, Naoki INAGAKI and Hiroyuki TANAKA*

Formaldehyde (FA) is irritating to the skin, and causes sick building syndrome (SBS). Previously, we demonstrated that repeated painting with FA on mouse ears caused marked ear swelling and increased mRNA expression of transient receptor potential vanilloid 1 (TRPV1) in the ear. In this study, we exposed TRPV1 knockout mice to FA for 5 weeks to investigate the role of TRPV1. As results, ear swelling and increased expression of neurotrophins mRNA were attenuated in the knockout mice. Painting with a threshold dose of capsaicin to wild-type mice caused marked ear swelling after painting with FA. These data indicated that inflamed tissues after FA application are hypersensitive to various irritations, and TRPV1 may play a pivotal role in SBS.

[*Int. Arch. Allergy Immunol.* **157**, 194-201 (2012)] [Lab. of Pharmacology]

Induction of Thymic Stromal Lymphopoietin Production by Xylene and Exacerbation of Picryl Chloride-induced Allergic Inflammation in Mice.

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

Exposure to chemical compounds in the environment is an aggravating factor for allergic inflammation. In this study, we examined whether organic solvents induce the production of TSLP. Painting with xylene and toluene to earlobe induced the expression of TSLP in the tissue. The level of TSLP in W/W^v mice was as same as in wild-type mice, but diminished in TNF-α or IL-4 receptor knockout mice. Repeated painting of xylene induced an increase in expression of OX40 ligand, and xylene promoted the picryl chloride-induced thickening, both of which were inhibited in TSLP receptor knockout mice. Xylene might trigger the activation of dendritic cells via the production of TSLP from epithelial cells, resulting in an exacerbation of allergic inflammation.

[*Int. Arch. Allergy Immunol.* **158**, 359-368 (2012)] [Lab. of Pharmacology]

The Potential Role of Prostaglandin D_2 in Nasal Congestion Observed in a Guinea Pig Model of Allergic Rhinitis.

Go TAKAHASHI, Hiroyuki TANAKA*, Naoko HIGUCHI, Minoru IKEDA, Naoki INAGAKI and Michitaka SHICHIJO

Allergic rhinitis is the most common allergic disease, with nasal congestion and sneezing. The role of PGD_2 which is the most effective mediator in producing nasal congestion was evaluated on the nasal airflow in guinea pigs rhinitis model using a noninvasive approach. PGD_2 induced an increase in intranasal pressure, and sinusoidal microvessel dilatation appeared around the septum in the nasal mucosa. Relaxation of the nasal mucosa following stimulation of the prostanoid DP-1 receptor was associated with cAMP levels in the tissue, demonstrating that the mechanism of PGD_2-induced nasal congestion is different from other chemical mediators. Consequently, antagonists for the DP-1 receptor would be an alternative approach for the relief of nasal congestion.

[*Allergol. Int.* **61**, 563-572 (2012)] [Lab. of Pharmacology]

Periostin Contributes to the Pathogenesis of Atopic Dermatitis by Inducing TSLP Production from Keratinocytes.

Hiroshi SHIRAISHI, Miho MASUOKA, Shoichiro OHTA, Shoichi SUZUKI, Kazuhiko ARIMA, Kazuto TANIGUCHI, Shigehisa AOKI, Shuji TODA, Tomohiro YOSHIMOTO, Naoki INAGAKI*, Simon J CONWAY, Yutaka NARISAWA and Kenji IZUHARA

Periostin is an extracellular matrix protein induced by Th2 cytokines. To clarify the role of periostin in the pathogenesis of atopic dermatitis (AD), ears of Rag-2-/-γ(c)-/-, and wild-type mice were sensitized repeatedly by painting with house dust mite (HDM). The sensitization induced accumulation of periostin in dermis in wild-type mice but not in Rag-2-/-γ(c)-/- mice. To examine the role of periostin, keratinocytes were culture with wild-type or periostin deficient fibroblasts. Periostin promoted proliferation of keratinocytes and induced TSLP. Periostin might exacerbate the pathogenesis of AD through TSLP production from keratinocytes.

[*J.Clin. Invest.* **122**, 2590-2600 (2012)] [Lab. of Pharmacology]

Periostin Promotes Chronic Allergic Inflammation in Response to Th2 Cytokines.

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Kazuhiko ARIMA, Shigehisa AOKI, Shuji TODA, Naoki INAGAKI*, Yuichi KURIHARA,
Sayaka HAYASHIDA, Satoshi TAKEUCHI, Kenta KOIKE, Junya ONO, Hirokazu NOSHIRO,
Masutaka FURUE, Simon J CONWAY, Yutaka NARISAWA and Kenji IZUHARA

Periostin of matricellular protein interacts with several cell surface integrin molecules. Periostin was produced by Th2 cytokines-stimulated fibroblasts, and interacted with αv integrin on keratinocytes. TSLP was produced by periostin through the NF-κB pathway in keratinocytes. Accordingly, inhibition of periostin or αv integrin prevented the development or progression of allergen-induced skin inflammation. Periostin sets up a vicious circle that links Th2-type immune responses to keratinocyte activation and plays a critical role in the amplification of allergic skin inflammation.

[*Int. Immunopharmacol.* **14**, 224-231 (2012)] [Lab. of Pharmacology]

Oral *Nigella Sativa* Oil Ameliorates Ovalbumin-induced Bronchial Asthma in Mice.

Mohamed Fathy BALAHA, Hiroyuki TANAKA*, Hirotaka YAMASHITA,
Mohamed Nabih Abdel RAHMAN and Naoki INAGAKI

Nigella sativa oil (NSO) is used in folk medicine as a therapy for many diseases including bronchial asthma. We investigated the possible modulating effects of NSO on murine asthma model in which BALB/c mice were sensitized with ovalbumin (OVA) intraperitoneally and exposed as aerosol. Oral treatment with NSO showed significant decrease in airway hyperresponsiveness, and the number of macrophages and eosinophils. We demonstrated NSO inhibited Th2 cytokine production and recovered Th1 cytokine production in BALF. Serum levels of IgE and IgG1 were ameliorated, and IgG2a level was increased, indicating restoration of local Th1/Th2 balance. NSO abrogated the histopathological changes of the lungs, as the images were nearly normal. These results suggest that the treatment with oral NSO could be a promising treatment for bronchial asthma.

[*Sci. Rep.* **2**, 573 (2012)] [Lab. of Molecular Pharmacology]

The Potential of GPNMB as Novel Neuroprotective Factor in Amyotrophic Lateral Sclerosis.

Hirotaka TANAKA, Masamitsu SHIMAZAWA, Masataka KIMURA, Masafumi TAKATA, Kazuhiro TSURUMA,
Mitsunori YAMADA, Hitoshi TAKAHASHI, Isao HOZUMI, Jun-ichi NIWA, Yohei IGUCHI, Takeshi NIKAWA,
Gen SOBUE, Takashi INUZUKA and Hideaki HARA*

Glycoprotein nonmetastatic melanoma protein B (GPNMB) was identified as an amyotrophic lateral sclerosis (ALS)-related factor using DNA microarray analysis with mutant superoxide dismutase ($SOD1^{G93A}$) mice. GPNMB was greatly induced in the spinal cords of ALS patients and a mouse model as the disease progressed. In an NSC34 cell line, glycosylation of GPNMB was inhibited by interaction with $SOD1^{G93A}$, increasing motor neuron vulnerability. Furthermore, GPNMB expression was substantial in the sera of sporadic ALS patients than that of other diseased patients. This study suggests that GPNMB can be a target for therapeutic intervention for suppressing motor neuron degeneration in ALS.

[*Sci. Rep.* **2**, 896 (2012)] [Lab. of Molecular Pharmacology]

Pharmacological Inhibition of TLR4-NOX4 Signal Protects Against Neuronal Death in Transient Focal Ischemia.

Yukiya SUZUKI, Kozo HATTORI, Junya HAMANAKA, Tetsuji MURASE, Yusuke EGASHIRA, Keisuke MISHIRO,
Mitsunori ISHIGURO, Kazuhiro TSURUMA, Yoshinobu HIROSE, Hiroyuki TANAKA, Shinichi YOSHIMURA,
Masamitsu SHIMAZAWA, Naoki INAGAKI, Hideko NAGASAWA, Toru IWAMA and Hideaki HARA*

In the present study, on mice, intracerebroventricular injection of resatorvid (TLR4 signal inhibitor; 0.01 μg) significantly reduced infarct volume and improved neurological score after middle cerebral artery occlusion and reperfusion. Genetic and pharmacological inhibitions of TLR4 each reduced NOX4 expression, leading to suppression of oxidative/nitrative stress and of neuronal apoptosis. These data suggest that resatorvid has potential as a therapeutic agent for stroke since it inhibits TLR4-NOX4 signaling which may be the predominant causal pathway.

[*PLoS ONE* **7(5)**, e37058 (2012)] [Lab. of Molecular Pharmacology]

Diacylglycerol Kinase β Knockout Mice Exhibit Attention-deficit Behavior and an Abnormal Response on Methylphenidate-induced Hyperactivity.

Mitsue ISHISAKA, Kenichi KAKEFUDA, Atsushi OYAGI, Yoko ONO, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Kiyoyuki KITAICHI and Hideaki HARA*

We investigated the function of diacylglycerol kinase β (DGKβ) in the central nervous system, especially in the pathophysiology of attention deficit hyperactivity disorder (ADHD), using DGKβ KO mice. DGKβ KO mice showed attention-deficit behavior in the object-based attention test and it was ameliorated by methylphenidate (MPH, 30 mg/kg, i.p.). In the open field test, DGKβ KO mice displayed a decreased response to the locomotor stimulating effects of MPH, but showed a similar response to an *N*-methyl-d-aspartate (NMDA) receptor antagonist, MK-801 (0.3 mg/kg, i.p.), when compared to WT mice. These findings suggest that DGKβ KO mice showed attention-deficit and hyperactive phenotype, similar to ADHD.

[*PLoS ONE* **7(1)**, e30526 (2012)] [Lab. of Molecular Pharmacology]

An Alteration in the Lateral Geniculate Nucleus of Experimental Glaucoma Monkeys: *In vivo* Positron Emission Tomography Imaging of Glial Activation.

Masamitsu SHIMAZAWA, Yasushi ITO, Yuta INOKUCHI, Hajime YAMANAKA, Tomohiro NAKANISHI, Takuya HAYASHI, Bin JI, Makoto HIGUCHI, Tetsuya SUHARA, Kazuyuki IMAMURA, Makoto ARAIE, Yasuyoshi WATANABE, Hirotaka ONOE and Hideaki HARA*

We examined lateral geniculate nucleus (LGN) degeneration as an indicator for possible diagnosis of glaucoma in experimental glaucoma monkeys using positron emission tomography (PET). Glial activation occured in the LGN at a mild glaucoma stage, and that the LGN degeneration could be detected by a PET imaging with [^{11}C]PK11195 during the moderate experimental glaucoma stage after unilateral ocular hypertension. Therefore, activated glial markers such as peripheral-type benzodiazepine receptor (PBR) in the LGN may be useful in noninvasive molecular imaging for diagnosis of glaucoma.

[*Neuroscience* **205**, 39-48 (2012)] [Lab. of Molecular Pharmacology]

A Broad-spectrum Matrix Metalloproteinase Inhibitor Prevents Hemorrhagic Complications Induced by Tissue Plasminogen Activator in Mice.

Keisuke MISHIRO, Mitsunori ISHIGURO, Yukiya SUZUKI, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

In the present study, we focused on tight junction proteins (TJPs), occludin, zona occludens (ZO)-1, and claudin-5, which are important structural components of the blood-brain barrier, and investigated whether inhibition of matrix metalloproteinases (MMPs) provides a protective effect against hemorrhagic complications induced by tissue plasminogen activator (tPA). GM6001 a broad-spectrum MMP inhibitor significantly reduced tPA-elevated brain hemoglobin, MMP-9, and inhibited the degradation of occludin and zona occludens-1 induced by tPA, but not claudin-5. This suggests that GM6001 may be a useful candidate for combination therapy against the hemorrhagic complications induced by tPA.

[*Neuroscience* **220**, 302-312 (2012)] [Lab. of Molecular Pharmacology]

A Rho Kinase (ROCK) Inhibitor, Fasudil, Prevents Mmatrix Metalloproteinase-9-related Hemorrhagic Transformation in Mice Treated with Tissue Prasminogen Activator.

Mitsunori ISHIGURO, Koh Kawasaki, Yukiya SUZUKI, Fumiya ISHIZUKA, Keisuke MISHIRO, Yusuke EGASHIRA, Ichiro Ikegaki, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Shinichi YOSHIMURA, Toru IWAMA and Hideaki HARA*

We investigated whether fasudil, a Rho kinase (ROCK) inhibitor, would prevent tPA-associated hemorrhagic transformation and extend the reperfusion window in an experimental stroke model in mice. Combination therapy with tissue plasminogen activator (tPA) plus fasudil prevented the development of hemorrhagic transformation. These changes significantly reduced mortality and increased locomotor activity at 7 days after the reperfusion. These findings indicate that fasudil prevents the hemorrhagic transformation induced by focal cerebral ischemia in mice treated with tPA.

[*Brain Res.* **1461**, 87-95 (2012)] [Lab. of Molecular Pharmacology]

The Conditioned Medium of Murine and Human Adipose-derived Stem Cells Exerts Neuroprotective Effects Against Experimental Stroke Model.

Yusuke EGASHIRA, Sou SUGITANI, Yukiya SUZUKI., Keisuke MISHIRO, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Shinichi YOSHIMURA, Toru IWAMA and Hideaki HARA*

This study investigated the possible ameliorative effects of adipose-derived stem cells-conditioned medium (ASC-CM) on experimental ischemic stroke. Intracerebroventricular (i.c.v.) administration of 30- and 100-fold concentrated murine ASC-CM 1 h prior to middle cerebral artery occlusion (MCAO) resulted in a dose-dependent reduction in the infarct volume and the brain swelling. Neuroprotective effects of murine ASC-CM were also confirmed in an *in vitro* model. Similar reduction in the MCAO-induced infarction volume was seen following i.c.v. administration of 100-fold concentrated human ASC-CM or murine ASC-CM. These findings suggest the feasibility of ASC-CM administration as a therapy for acute stage stroke.

[*Eur. J. Pharmacol.* **696**, 83-88 (2012)] [Lab. of Molecular Pharmacology]

Imipramine Protects Mouse Hippocampus Against Tunicamycin-induced Cell Death.

Yoko ONO, Masamitsu SHIMAZAWA, Mitsue ISHISAKA, Atsushi OYAGI, Kazuhiro TSURUMA and Hideaki HARA*

Recently, some reports have suggested that the sigma-1 receptor may play a role in endoplasmic reticulum (ER) stress, and many antidepressants have a high affinity for the sigma-1 receptor. In mouse cultured hippocampal HT22 cells, imipramine, a widely used antidepressant, inhibited cell death induced by tunicamycin, an ER stress inducer. Additionally, NE-100, a selective sigma-1 receptor antagonist, abolished the protective effect of imipramine. Furthermore, in anesthetized mice intracerebroventricular administration of tunicamycin decreased the number of neuronal cells in the hippocampus, and 7 days' imipramine treatment significantly suppressed these reductions. These findings suggest that imipramine protects against ER stress-induced hippocampal neuronal cell death both *in vitro* and *in vivo*. Such protection may be partly due to the sigma-1 receptor.

[*Biochem. Biophys. Res. Commun.* **429**, 186-190 (2012)] [Lab. of Molecular Pharmacology]

Preferential Involvement of Na^+/Ca^{2+} Exchanger Type-1 in the Brain Damage Caused by Transient Focal Cerebral Ischemia in Mice.

Nobutaka MORIMOTO, Satomi KITA, Masamitsu SHIMAZAWA, Hiroko NAMIMATSU, Kazuhiro TSURUMA, Kazuhide HAYAKAWA, Kenichi MISHIMA, Nobuaki EGASHIRA, Takuya IYODA, Ichiro HORIE, Yusuke GOTOH, Katsunori IWASAKI, Michihiro FUJIWARA, Toshio MATSUDA, Akemichi BABA, Issei KOMURO, Kyoji HORIE, Junji TAKEDA, Takahiro IWAMOTO and Hideaki HARA*

Within the brain, three Na^+/Ca^{2+} exchanger (NCX) isoforms (NCX1, NCX2, and NCX3) are widely distributed. We investigated the role of each NCX isoform in ischemic brain damage using NCX isoform-mutant mice ($NCX1^{+/-}$, $NCX2^{+/-}$, and $NCX3^{+/-}$), and demonstrated that the NCX1 isoform may act preferentially to exacerbate the cerebral damage caused by ischemic insult in mice, and that NCX1-selective inhibitors warrant investigation as a potential therapeutic agents for stroke.

[*PLoS ONE* **7(2)**, e32167 (2012)] [Lab. of Molecular Pharmacology]

Morphological and Functional Changes in the Retina after Chronic Oxygen-induced Retinopathy.

Shinsuke NAKAMURA, Shunsuke IMAI, Hiromi OGISHIMA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

The mouse model of oxygen-induced retinopathy (OIR) has been widely used for studies of retinopathy of prematurity (ROP). However, little is known about changes in the chronic phase after ROP. Therefore, in this study, we examined morphological and functional changes in the retina using a chronic OIR model. The chronic OIR model revealed the following: (1) a decrease in oscillatory potential amplitudes, (2) morphological abnormalities in the retinal cells and blood vessels, and (3) an increase in retinal vascular permeability *via* the impairment of the tight junction proteins. These findings suggest that the experimental animal model used in this study is suitable for elucidating the pathogenesis of ROP and may lead to the development of potential therapeutic agents for ROP treatment.

[*Invest. Ophthalmol. Vis. Sci.* **53**, 6729-6737 (2012)] [Lab. of Molecular Pharmacology]

SEMA4A Mutations Lead to Susceptibility to Light Irradiation, Oxidative Stress, and ER Stress in Retinal Pigment Epithelial Cells.

Kazuhiro TSURUMA, Yuhei NISHIMURA, Seiya KISHI, Masamitsu SHIMAZAWA, Toshio TANAKA and Hideaki HARA*

We investigated whether mutant SEMA4A causes retinal dysfunction. Mutant SEMA4A (D345H or F350C) was localized in the ER, whereas wild type (WT) SEMA4A was observed in cell membranes. The expression of 78 kDa glucose-regulated protein (GRP78), a marker of endoplasmic reticulum (ER) stress, was increased by mutant SEMA4A following light irradiation, and phagocytosis was suppressed in mutant SEMA4A-transfected cells. Mutant SEMA4A induced susceptibility to ER stress and oxidative stress. Our results suggest that mutations in SEMA4A may cause susceptibility to light exposure, oxidative stress, and ER stress.

[*Invest. Ophthalmol. Vis. Sci.* **53**, 7896-7903 (2012)] [Lab. of Molecular Pharmacology]

Metallothionein-III Deficiency Exacerbates Light-induced Retinal Degeneration.

Kazuhiro TSURUMA, Hiroki SHIMAZAKI, Yuta OHNO, Yuki INOUE, Akiko HONDA, Shunsuke IMAI, Jinyong LEE, Masamitsu SHIMAZAWA, Masahiko SATOH and Hideaki HARA*

Metallothioneins (MTs) are a family of cysteine-rich proteins, and various physiologic functions have been reported, including protection against metal toxicity and antioxidative potency. We investigated the functional role of MT-III in light-induced retinal damage. The mRNAs of the MTs were increased significantly in murine retina after light exposure. The outer nuclear layer in the MT-III-deficient mice was remarkably thinner compared to light-exposed wild-type (WT) mice, and a- and b-wave amplitudes were decreased; the damage induced in MT-I/-II-deficient mice was comparable to that observed in WT mice. MT-III knockdown by siRNA in 661W exacerbated the cell damage and increased the production of reactive oxygen species in response to light exposure. These findings suggested that MT-III can help protect against light-induced retinal damage compared to MT-I/II.

[*J. Neurosci. Res.* **90**, 1960-1969 (2012).] [Lab. of Molecular Pharmacology]

Involvement of Endoplasmic Reticulum Stress in Optic Nerve Degeneration following *N*-Methyl-D-aspartate-induced Retinal Damage in Mice.

Masamitsu SHIMAZAWA, Akinori MIWA, Yasushi ITO, Kazuhiro TSURUMA, Makoto AIHARA and Hideaki HARA*

We evaluated time-dependent optic nerve degeneration and the role of endoplasmic reticulum (ER) stress in this process following retinal ganglion cell death in mice. Neurofilament heavy (NFH)- and phosphorylated NFH (pNFH)-positive axons were time-dependently decreased in optic nerves after *N*-methyl-D-aspartate (NMDA) injection. Expressions of glucose-regulated protein 78 (*Grp78*)/BiP, *Grp94*, *Calreticulin*, C/EBP homologous protein (*Chop*) in the optic nerve at 14 days after NMDA injection. These findings suggest that the axonal degeneration is dramatic until 7 days after NMDA injection and ER stress may play a pivotal role in the optic nerve degeneration after NMDA-induced retinal damage

[*Mol. Vision* **18**, 2647-2657 (2012)] [Lab. of Molecular Pharmacology]

Induction of Amyloid-$\beta_{1\text{-}42}$ in the Retina and Optic Nerve Head of Chronic Ocular Hypertensive Monkeys.

Yasushi ITO, Masamitsu SHIMAZAWA, Kazuhiro TSURUMA, Chihiro MAYAMA, Kiyoshi ISHII, Hirotaka ONOE, Makoto AIHARA,Makoto ARAIE and Hideaki HARA*

The purpose of this study was to investigate the expression and localization of $A\beta_{1\text{–}42}$ in the retina and the optic nerve head (ONH) of monkeys with experimental glaucoma. $A\beta_{1\text{–}42}$ was upregulated in the nerve fiber layer (NFL) and the ganglion cell layer (GCL) of the retina and the ONH at 11 to 24 weeks after the laser photocoagulation treatment,. The localizations of $A\beta_{1\text{–}42}$ were merged in glial fibrillary acidic protein-positive astroglial cells but not phosphorylated neurofilament heavy- or nonphosphorylated neurofilament heavy-positive axons in the retina and the ONH. These findings indicate that the upregulation of $A\beta_{1\text{–}42}$ after an intraocular pressure elevation could apply to monkeys since the structure of the ONH is more similar to humans than that of rodents.

[*Eur. J. Pharmacol.* **685**, 8-14 (2012)] [Lab. of Molecular Pharmacology]

Candesartan, an Angiotensin II Type 1 Receptor Antagonist, Inhibits Pathological Retinal Neovascularization by Downregulating VEGF Receptor-2 Expression.

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

We examined the efficacy and the mechanism of candesartan, an angiotensin II type 1 (AT_1) receptor antagonist, in suppressing pathological retinal neovascularization. In the oxygen-induced retinopathy (OIR) model, candesartan suppressed the pathological neovascularization in a dose-dependent manner, but did not prevent the physiological angiogenesis. Candesartan reduced the upregulation of vascular endothelial growth factor (VEGF) receptor-2 in the retina, but had no effects in the other angiogenesis-related genes, such as hypoxia-inducible factor (HIF-1α), VEGF-A, and VEGF receptor-1 in the OIR model. These findings indicate that candesartan inhibited the retinal pathological neovascularization, at least in part, by suppressing the expression of VEGF receptor-2, independent of VEGF signaling cascade.

[*J. Pharmacol. Sci.* **118**, 351-362 (2012)] [Lab. of Molecular Pharmacology]

Role of Oxidative Stress in Retinal Photoreceptor Cell Death in *N*-Methyl-*N*-nitrosourea-treated Mice.

Kazuhiro TSURUMA, Mika YAMAUCHI, Yuta INOKUCHI, Sou SUGITANI, Masamitsu SHIMAZAWA and Hideaki HARA*

This study aimed to investigate whether oxidative stress contributes to retinal cell death in a mouse model of photoreceptor degeneration induced by *N*-methyl-*N*-nitrosourea (MNU). In *in vitro* study, MNU induced oxidative radical generation in 661W, photoreceptor–derived cells, and primary retinal cells, but not in RGC-5 cells, a mouse ganglion cell line. Edaravone, a free radical scavenger, at 1 μM reduced MNU-induced radical production in 661W and primary retinal cells. In *in vivo* study, MNU caused photoreceptor cell loss at 7 days after administration, and edaravone inhibited ONL thinning and reduced terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL)-positive cells and the oxidative stress markers. These findings indicate that MNU leads to selective photoreceptor degradation *via* oxidative stress *in vitro* and *in vivo*.

[*Pharmaceutica Analytica Acta* **3**, 154 (2012)] [Lab. of Molecular Pharmacology]

A Three-dimensional Collagen Gel Contraction Monitoring System that Uses a Porcine Trabecular Meshwork for Screening of Anti-intraocular Pressure Agents.

Hiroyoshi KASAI, Mitsue ISHISAKA, Eiichi SHIRASAWA and Hideaki HARA*

In this study, we investigated the effects of various agents on the contractility of cultured Porcine Trabecular Meshwork (pTM) cells using a three-dimensional (3-D) collagen gel assay, in order to develop a screening method for identifying novel anti-intracellular pressure (IOP) agents. Various kinase inhibitors, especially inhibitors of cell cyclin-dependent kinase (rescovitine), rho and Ca^{2+}-dependent protein kinase (Y-27632), tyrosine kinase (tyrphostin AG879), phosphatidylinositol 3-kinase (bisindolylmaleimide I, BIM I), and Ca^{2+}/calmodulin kinase (chelerythrine), strongly inhibited collagen gel contraction. These findings indicate that this *in vitro* 3-D collagen gel contraction monitoring system could be used as a rapid and sensitive screening method for identifying novel agents that induce pTM cell relaxation.

[*Braz. J. Med. Biol. Res.* **45**, 212-215 (2012)] [Lab. of Molecular Pharmacology]

Retinal Protective Effects of Topically Administered Agmatine on Ischemic Ocular Injury Caused by Transient Occlusion of the Ophthalmic Artery.

Samin HONG, Hideaki HARA*, Masamitsu SHIMAZAWA, Kana HYAKKOKU, Chan Yun KIM and Gong Je SEONG

Agmatine, an endogenous polyamine and putative neuromodulator, is known to have neuroprotective effects on various neurons in the central nervous system. We determined whether or not topically administered agmatine could reduce ischemic retinal injury. Transient ocular ischemia induced apoptosis of retinal cells in the entire retinal layer, and topically administered agmatine can significantly reduce this ischemic retinal injury. The proportion of apoptotic cells was definitely decreased. Overall, we determined that topical agmatine application effectively decreases retinal damage in an *in vivo* ocular ischemic injury model. This implies that agmatine is a good candidate as a direct neuroprotective agent for eyes with ocular ischemic diseases.

[*Mol. Nutr. Food Res.* **56**, 713-724 (2012)] [Lab. of Molecular Pharmacology]

Annatto Prevents Retinal Degeneration Induced by Endoplasmic Reticulum Stress *in vitro* and *in vivo*.

Kazuhiro TSURUMA, Hiroki SHIMAZAKI, Ken-ichi NAKASHIMA, Mika YAMAUCHI, Sou SUGITANI, Masamitsu SHIMAZAWA, Munekazu IINUMA and Hideaki HARA*

Annatto (Bixa orellana) seeds have been used as a colorant in butter and in a variety of other foods. In this study, we investigated the amelioration of retinal damage by an acetone extract of annatto (A-ext.), bixin (a main component of annatto), and four bixin derivatives (Bx-1, Bx-2, Bx-3, and Bx-4) that we have synthesized. A-ext., bixin, and Bx-1 treatment inhibited both tunicamycin- and H_2O_2-induced RGC-5 cell (a mouse ganglion cell line) death. Bixin derivatives also inhibited tunicamycin-induced cell death. A-ext., bixin, and Bx-1 significantly inhibited the tunicamycin-induced loss of cells from the ganglion cell layer, and these materials also suppressed the tunicamycin-induced thinning of outer nuclear layer in mice. A-ext., its main component bixin, and bixin derivatives may therefore be useful for preventive and therapeutic treatment of retinal-related diseases.

[*Curr. Neurovasc. Res.* **9**, 102-109 (2012)] [Lab. of Molecular Pharmacology]

Crocetin, a Carotenoid Derivative, Inhibits VEGF-induced Angiogenesis via Suppression of p38 Phosphorylation.

Naofumi UMIGAI, Junji TANAKA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

We evaluated the protective effects of crocetin against angiogenesis induced by vascular endothelial growth factor (VEGF). Crocetin, the aglycone of crocin carotenoids, is found in saffron crocus (Crocus sativus L.) and gardenia fruit (Gardenia jasminoides Ellis). Crocetin significantly suppressed VEGF-induced tube formation by human umbilical vein endothelial cells and migration of human retinal microvascular endothelial cells. It also significantly inhibited phosphorylation of p38 and protected VE-cadherin expression. These findings indicate that crocetin suppresses the VEGF-induced angiogenesis by inhibiting migration and that the inhibition of phosphorylated-p38 and protection of VE-cadherin expression may be involved in its underlying mechanism of action.

[*Phytother. Res.* **26**, 1126-1132 (2012)] [Lab. of Molecular Pharmacology]

Protective Effects of Astaxanthin from *Paracoccus carotinifaciens* on Murine Gastric Ulcer Models.

Kenta MURATA, Atsushi OYAGI, Dai TAKAHIRA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Takashi ISHIBASHI and Hideaki HARA*

The purpose of this study was to investigate the effect of astaxanthin extracted from Paracoccus carotinifaciens on gastric mucosal damage in murine gastric ulcer models. The free radical scavenging activities of astaxanthin were also measured by electron spin resonance (ESR) measurements. Astaxanthin significantly decreased the extent of HCl/ethanol- and acidified aspirin-induced gastric ulcers. Astaxanthin also decreased the level of thiobarbituric acid reactive substance. The ESR measurement showed that astaxanthin had radical scavenging activities against the 1,1-diphenyl-2-picrylhydrazyl radical and the superoxide anion radical. These results suggest that astaxanthin has antioxidant properties and exerts a protective effect against ulcer formation in murine models.

[*Phytother. Res.* **26**, 214-222 (2012)] [Lab. of Molecular Pharmacology]

Purple Rice (*Oryza sativa* L.) Extract and its Constituents Inhibit VEGF-induced Angiogenesis.

Junji TANAKA, Shinsuke NAKAMURA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Hiroshi SHIMODA and Hideaki HARA*

The study evaluated the protective effects of purple rice (Oryza sativa L.) bran extract (PRE) and its constituents, cyanidin and peonidin, against angiogenesis induced by vascular endothelial growth factor (VEGF). The PRE significantly suppressed VEGF-induced tube formation, proliferation and migration in human umbilical vein endothelial cells and human retinal microvascular endothelial cells as well as phosphorylation of phosphorylation of extracellular signal-regulated kinase (p-ERK) and p38. Cyanidin and peonidin also suppressed the proliferation and migration induced by VEGF. These findings indicate that PRE and anthocyanidins suppress VEGF-induced angiogenesis by inhibiting proliferation and migration and suggest that the inhibition of p-ERK and -p38 may be involved in the underlying mechanism.

[*Eur. J. Pharmacol.* **690**, 84-89 (2012)] [Lab. of Molecular Pharmacology]

Oral Administration of Crocetin Prevents Inner Retinal Damage Induced by *N*-Methyl-D-aspartate in Mice.

Yuta OHNO, Tomohiro NAKANISHI, Naofumi UMIGAI, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

Crocetin, an aglycone of crocin, is found in stigmas of the saffron crocus (*Crocus starus* L.) and has been used in traditional medicine. We investigated the effects of oral administration of crocetin on damage induced by *N*-methyl-d-aspartate (NMDA) in the murine retina. NMDA injection decreased the cell number in the ganglion cell layer and crocetin at a dose of 100 mg/kg inhibited this reduction. Crocetin inhibited the increase in number of apoptotic cells and the reduction in the b-wave amplitude of electroretinogram. NMDA injection also induced cleavage of α-spectrin, but crocetin did not affect this process. These findings indicate that oral administration of crocetin prevented NMDA-induced retinal damage *via* inhibition of the caspase pathway.

[*BMC Complement. Altern. Med.* **12**, 192 (2012)] [Lab. of Molecular Pharmacology]

Laxative Effects and Mechanism of Action of Brazilian Green Propolis.

Mamoru KAKINO, Hiroshi IZUTA, Kazuhiro TSURUMA, Yoko ARAKI, Masamitsu SHIMAZAWA, Kenji ICHIHARA and Hideaki HARA*

We investigated the effect and the mechanism of action of extracts of Brazilian green propolis. ,Treatment with a water extract of propolis (WEP), but not with an ethanol extract of propolis (EEP), significantly increased the weight of stools. WEP treatment significantly restored stool frequency and stool weight in clonidine (an α-2 adrenergic receptor agonist)-induced constipation model mice, but not in loperamide (a μ opioid receptor agonist)-induced constipation model mice. This increase was inhibited by an acetylcholine receptor antagonist (atropine), but not by a 5-HT receptor antagonist (GR113808). These findings indicate that WEP has laxative effects both in normal mice and in clonidine-induced constipation model mice. The laxative effects of WEP might be mediated by increased contractional tension of the ileum exerted at least in part *via* activation of an acetylcholine receptor.

[*Pharm. Anal. Acta* **3,** 152 (2012)] [Lab. of Molecular Pharmacology]

Agarwood (*Aquilaria crassna*) Extracts Decrease High-protein High-fat Diet-induced Intestinal Putrefaction Toxins in Mice.

Mamoru KAKINO, Tsuyoshi SUGIYAMA, Hitomi KUNIEDA, Shigemi TAZAWA, Hiroe MARUYAMA, Kazuhiro TSURUMA, Yoko ARAKI, Masamitsu SHIMAZAWA, Kenji ICHIHARA, Hiroshi MORI and Hideaki HARA*

In the present study, we investigated the effects of agarwood (*Aquilaria crassna*) on intestinal toxins, such as indole derivatives and ammonium to investigate the enteral environment. Feeding with Quick Fat (high-protein high-fat diet) increased fecal-containing toxins and delayed carmine egestion in mice. Administrations of water and ethanol of agarwoods decreased fecal-containing toxins and accelerated carmine egestion, and the decrement of fecal-containing toxins was abolished in response to interruption of the administration.

[*J. Community Pharm. Pharm. Sci.* **4**, 23-31 (2012)] [Lab.of Pharmacy Practice and Social Science]

A Questionnaire Survey on Generic Drugs that are Easy to Consume.

Satika MAETA, Syuzi YAMASHITA, Masafumi KUBOTA, Eiji TAKASHIMA, Sayaka HIGASHI, Emi GOTO, Yuko YOSHIMURA and Tadashi SUGIYAMA*

We conducted a questionnaire survey involving 100 patients on the impressions of generic drugs and medical formulations that are easy to consume. Most patients who had consumed generic drugs answered that the quality, effects, and side effects of generic drugs are not different from those of branded drugs. In addition, 77% of the patients answered that the medical formulation that is easiest to consume is a tablet, and over 50% of them responded that tablets of a diameter of 7 mm are easiest to consume. Further, the patients prefer that the information that appears on the package film, namely, the name and quantity of the drug be indicated on all tablets, so that drugs are clearly distinguishable. These findings suggest that to promote the use of generic drugs, pharmacists should inform patients about the availability of some generic drugs that are easy to consume and distinguish.

[*Jpn. J. Pharm. Health Care Sci.* **38**, 273-281 (2012)] [Lab.of Pharmacy Practice and Social Science]

Outcome Measurement of the Review System for Proper Use of Antimicrobial Injections in All Inpatients Established by the Infection Control Team.

Takashi NIWA, Yasutaka SHINODA, Akio SUZUKI, Tomofumi OHMORI, Hirotoshi OHTA, Ayumi FUKAO, Mitsuru YASUDA, Kiyoyuki KITAICHI, Katsuhiko MATSUURA, Tadashi SUGIYAMA*, Nobuo MURAKAMI and Yoshinori ITOH

Since August 2009, Gifu university hospital has established a review system for checking prescriptions in all patients receiving antimicrobial injections according to the intervention and feedback of antimicrobial stewardship (AMS) guideline. The antimicrobial use density, duration of administration, length of hospital stay, and antimicrobial resistance in a year were compared before and after starting the intervention into AMS. The extensive intervention into AMS is effective in reducing the frequency of inappropriate use of antimicrobials, suppressing the occurrence of antimicrobial resistance, and saving medical expenses.

[*J. Jpn. Soc. Hosp. Pharm.* **48**, 869-871 (2012)] [Lab.of Pharmacy Practice and Social Science]

A Retrospective Survey of the Usage Status of Levofloxacin for Tuberculosis Treatment.

Makoto NAKASHIMA, Kimiyasu SANO, Ryoko OHNISHI, Mizuho KOBAYASHI, Toshitaka SUZUKI, Shigeo TASUDA, Takuya GOTO, Nobuyuki MISHIMA, Tatsuo KATO and Tadashi SUGIYAMA*

Levofloxacin (LVFX) is not approved for tuberculosis treatment, but LVFX is one of therapeutic options in circumstances wherein standard therapy cannot be administered. From July 1, 2009 to June 30, 2011, 257 patients underwent medical therapy for tuberculosis, and 43 (16.7%) patients were administrated LVFX. The reason for LVFX administration included alternate treatment because adverse reactions were developed by other antituberculosis drugs in 39 cases, resistance to first-line drug in 6 cases, and other reasons in 3 cases. The success rate of antituberculosis therapy involving LVFX was 83.7%. LVFX was the most frequently administered second-line drug. Through a questionnaire, we asked doctors for the factors involved in choosing a second-line drug. The results indicated that doctors chose the drug based on its efficacy and potential side effects.

[*Jpn. J. Pharm. Health Care Sci.* **38**, 506-512 (2012)] [Lab.of Pharmacy Practice and Social Science]

A Retrospective Survey on Temporal Changes in Pemetrexed-Induced Hematotoxicity in Patients with Non-Small Cell Lung Cancer and Creating an Educational Leaflet on Hematotoxicity.

Makoto NAKASHIMA, Hiromitsu KATO, Takuya GOTO, Mie NOMURA, Yukiko SHIBATA, Takahiro KUMAGAI, Nobuyuki MISHIMA, Tatsuo KATO and Tadashi SUGIYAMA*

We surveyed temporal changes in pemetrexed-induced hematotoxicity in patients with non-small cell lung cancer in order to determine the appropriate time for measuring blood cell counts during administration of pemetrexed monotherapy to outpatients. Next, we investigated the average temporal changes of white blood cells, thrombocytes, hemoglobin after administration of pemetrexed in aforementioned patients, and we plotted graphs of the each average temporal change. Then we created a leaflet using these graphs, and after pharmacists explained to outpatients administered pemetrexed about hematotoxicity using the leaflet. As a result, it was thought that a leaflet was useful for comprehensibility of the need for blood tests and so on.

[*Int. J. Clin. Pract.* **66**, 999-1008 (2012)] [Lab.of Pharmacy Practice and Social Science]

Outcome Measurement of Extensive Implementation of Antimicrobial Stewardship in Patients Receiving Intravenous Antibiotics in a Japanese University Hospital.

Takashi NIWA, Yasutaka SHINODA, Akio SUZUKI, Tomofumi OHMORI, Mituru YASUDA, Hirotoshi OHTA, Ayumi FUKAO, Kiyoyuki KITAICHI, Katsuhiko MATSUURA, Tadashi SUGIYAMA*, Nobuo MURAKAMI and Yoshinori ITOH

The infection control team was involved in the review of individual use of antibiotics in all inpatients (6,348 and 6,507 patients/year during the first and second annual interventions, respectively) receiving intravenous antibiotics, according to the published guidelines, consultation with physicians before prescription of antimicrobial agents. Extensive implementation of antimicrobial stewardship led to a decrease in the inappropriate use of antibiotics, saving in medical expenses, reduction in the development of antimicrobial resistance, and shortening of hospital stay.

[*Jpn. J. Lung Cancer* **52**, 1007-1016 (2012)] [Lab.of Pharmacy Practice and Social Science]

Efficacy and Tolerability of the Bevacizumab/Carboplatin/Paclitaxel Combination Therapy as First-line or Non-first-line Therapy for Non-small-cell Lung Cancer.

Makoto NAKASHIMA, Ryoko OHNISHI, Mizuho KOBAYASHI, Toshitaka SUZUKI, Shigeo YASUDA, Kimiyasu SANO, Takuya GOTO, Nobuyuki MISHIMA, Tatsuo KATO and Tadashi SUGIYAMA*

Bevacizumab/carboplatin/paclitaxel (BEV-CP) combination therapy extends the progression-free survival (PFS) of patients with non-small-cell lung cancer who have never received chemotherapy. However, the efficacy and tolerability of BEV-CP therapy in patients with histories of chemotherapy have not been investigated. In the present study, the efficacy and tolerability of BEV-CP therapy in the first-line therapy (FLT) and non-FLT groups were analyzed retrospectively. The response rate, the disease control rate, the median PFS time, the discontinuation rate between the FLT and non-FLT groups were not statistically significant. Therefore, the efficacy and tolerability of BEV-CP therapy as more than second-line therapy and as FLT were comparable.

[*Anticancer Res.* **32**, 823-829 (2012)] [Lab. of Clinical Pharmacy]

Enhanced Renal Clearance of Vancomycin in Rats with Carcinogen-induced Osteosarcoma.

Izumi SHIMADA, Chieko IWATA, Shino TAGA, Hitomi TERAMACHI*, Masaaki NOMURA, Ken-ichi MIYAMOTO, Hiroyuki TSUCIYA, Takashi WADA, Kazuko KIMURA and Ryo MATSUSHITA

The CLtot and renal clearance (CLr) of vancomycin in the tumor-bearing rats were increased compared to the ones of the control rats without tumor. However, there was no difference in the glomerular filtration rate. The plasma concentrations of interleukin (IL)-1□ and IL-6, were elevated in the tumor-bearing rats. When renal proximal tubular epithelial cells (RPTEC) were exposed to IL-1□, IL-6, and tumor necrosis factor (TNF)-α simultaneously, the excretory ratio increased significantly. These findings suggest that tubular excretion or re-absorption by cytokines might be associated with changes in the vancomycin CLtot enhancement in the tumor-bearing rats.

[*Jpn. J.Pharm.Health Care Sci*. **38**, 282-287 (2012)] [Lab. of Clinical Pharmacy]

Steroid Withdrawal Syndrome after Normal Dose Chemotherapy in Non-Hodgkin's Lymphoma -A Study of the Frequency of Symptoms and the Efficacy of Steroid Tapering.

Eiseki USAMI, Michio KIMURA, Tomoaki YOSHIMURA, Tadashi YASUDA, Hitomi TERAMACHI*, Tadashi SUGIYAMA and Teruo TSUCHIYA

The aim of this retrospective study was to evaluate the prevalence of Steroid Withdrawal Syndrome (SWS) and to assess the relevance of steroid tapering in 116 patients newly diagnosed with non-Hodgkin's lymphoma (NHL). The most significant symptoms of SWS documented were fatigue in 70 patients (60.3%) and anorexia in 32 (27.6%). Four elderly patients (3.4%) suffered with serious SWS symptoms of grade 3. Of the 70 patients suffering with SWS, 22 went on to receive tapered steroid therapy during the subsequent chemotherapy treatment. The average steroid tapering period was 4.4 days and 86.4% patients reported a significant improvement of their SWS symptoms. No serious adverse events were noted.

[*Jpn. J.Pharm.Health Care Sci*. **38**, 392-400 (2012)] [Lab. of Clinical Pharmacy]

Development and Evaluation of Advanced Problem-based Learning Classes as Part of an Integrated Curriculum for Sixth-year University Student.

Hitomi TERAMACHI*, Tomoya TACHI, Tadashi HORIUCHI, Isao HOZUMI, Tadashi SUGIYAMA and Teruo TSUCHIYA

We developed an integrated curriculum involving focused study on four subjects in one month and conducted comprehensive study of advanced problem-based learning (PBL) using case studies. Most students evaluated their understanding of the seven target learning. With regard to PBL classes overall, the one-month focused curriculum, study motivation enhancement, and PBL class utility were also highly evaluated. Covariance structure analysis of these three factors and 'utility of PBL classes' clarified that 'knowledge' and 'communication' were linked to 'issue identification and intervention' and that the PBL classes were beneficial for learning.

[*Jpn. J.Pharm.Health Care Sci*. **38**, 401-408 (2012)] [Lab. of Clinical Pharmacy]

Investigation of Serum Creatinine Rise in Patients after Cisplatin Therapy-influence of Hydration and Patient Characteristics-.

Katsumi TANIZAWA, Makiko TAKAYA, Yuu MIURA, Keiko TAGUCHI, Hideharu ENDO, Hitomi TERAMACHI and Teruo TSUCHIYA*

We intended for the treatment of 266 cycles of 111 patients who had received chemotherapy including more than 60mg/m2 cisplatin in the past. We investigated the infusion solution used for hydration, various patient criteria factors and serum creatinine changes before and after cisplatin dosage. As a result, significant difference was not recognized in age, sex, presence or absence diuretic dosage, quantity of water load in hydration. We infer that these are risk factors for renal function disorder with the cisplatin treatment. Providing Na in excess of 385mEq in hydration seems to be an effective precaution of the renal function disorder.

[*Jpn. J.Pharm. Health Care Sci*. **38**, 513-521 (2012)] [Lab. of Clinical Pharmacy]

Analysis of Relationship between Environmental Improvements of Pharmacy and Human Errors in Preventive Measures for Dispensing Mistakes.

Tomoya TACHI*, Hitomi TERAMACHI, Kento TAMURA, Natsuki KOMADA, Hitomi SHIGA, Keiji IMAI and Teruo TSUCHIYA

We conducted a questionnaire survey for pharmacists working in community pharmacies in order to clarify the relationship between measures for dispensing mistakes by environmental improvements of pharmacies and measures for human errors. The results revealed that the highest risk factor of dispensing mistakes on human factors is psychological and mental situation, which is 69% of the whole causes on human factors. And 7 factors were extracted from factor analysis of 24 items relating to measures for dispensing mistakes by environmental improvements of pharmacies. Covariance structure analysis clarified the relationship between the 7 factors relating to environmental improvements of pharmacies and 3 factors relating to measures for human errors.

[*Jpn. J.Pharm. Health Care Sci*. **38**, 522-533 (2012)] [Lab. of Clinical Pharmacy]

Survey of the Patients' Consciousness Affecting Medication Adherence.

Kennosuke TSUBOI, Hitomi TERAMACHI*, Yumi KUZUYA, Takashi MIZUI, Chitoshi GOTO and Teruo TSUCHIYA

The status of patient medication-taking behavior and factors affecting adherence were investigated through a survey involving 226 patients. The survey items included patient characteristics, medication status, and factors affecting medication adherence. Overall, 73% of patients took medicines as directed. Evaluation by medication adherence status revealed that patients with poor adherence most frequently forgot to take their medicines after lunch and between meals. Then, using Customer Satisfaction analysis and excluding factors related to personality, 4 factors for improvement were selected from the remaining 16 factors. These findings suggest that patient medication adherence increases when the factors of "regularity of life rhythm", "regularity of meals", "trust in pharmacists", and "use of medicine information leaflets" are improved.

[*Jpn. J.Pharm. Health Care Sci*. **38**, 767-779 (2012)] [Lab. of Clinical Pharmacy]

Knowledge and Awareness on Correct Use of Medicine among Elementary, Junior High- and High School Students, and Implementation Status of Education of Medicine at Schools.

Hitomi TERAMACHI*, Hiroki OHTA, Yumi KOHDA, Hideaki KITO, Natsuki KOMADA, Hitomi SHIGA, Kento TAMURA, Tomoya TACHI, Teruo TSUCHIYA and Shingo KATSUNO

We conducted a mail survey with an aim of clarifying the students' current state of knowledge and awareness of the correct use of medicine, and the status of the implementation of medical education at school. This questionnaire survey targeted elementary, junior high- and high school students and teachers of the schools in Japan. We received valid responses from 5,612 students and 146 teachers. Based on the results of this survey, we clarified the involvement with students and medicine, lack of knowledge about medicine, teachers' consciousness and current state of medical education. We hope that these results will contribute to medical education.

[*J. Pharm. Commun.* **10**, 24-35 (2012)] [Lab. of Clinical Pharmacy]

Attitude Survey among Patients and Pharmacists for the Importance and the Utilization of a Medicine Notebook.

Hitomi SHIGA, Hitomi TERAMACHI*, Hitoshi SUZUKI, Natsuki KOMADA, Kento TAMURA, Tomoya TACHI and Teruo TSUCHIYA

Six hundred pharmacists of pharmacies in Gifu Prefecture and 105 patients with possession of a medication notebook in insurance pharmacy responded to a survey. The questionnaire consisted of 22 question items about the degree of importance and utilization of a medication notebook, three comprehensive evaluations of the medication notebook. In addition, we investigated patient's awareness of mobile phone with function of medication notebook. We suggest that some items should be explained to patients mainly. Differences in the recognition toward medication notebook were found between pharmacists and patients, and we need to explain the value of the medication notebook to patients, based on the differences.

[*J. Community Pharm. Pharm. Sci.* **4**, 10-14 (2012)] [Lab. of Clinical Pharmacy]

A Case of Chronic Myeloid Leukemia with Interstitial Pneumonia Associated with Imatinib.

Tomohiro OHSAWA, Masahiro YASUDA, Kennosuke TSUBOI, Yumi KUZUYA, Makoto SAHASHI, Katsutoshi GOTO, Hitomi TERAMACHI*, Teruo TSUCHIYA, Takeshi TAKAHASHI and Kazufumi YONEDA

We experienced a patient who is chronic myeloid leukemia of 67 years old man that developed interstitial pneumonia during the imatinib which is tyrosine kinase inhibitor had been administrated. We were able to detect interstitial pneumonia appropriately. And the steroid treatment and the cancellation of imatinib were able to prevent prolongation and aggravation of interstitial pneumonia. When we administer the imatinib, we need to keep the onset of interstitial pneumonia in our mind. We infer that it is necessary to monitor early case of interstitial pneumonia such as an ascent of CRP, fever and the dry cough, and to measure KL-6 properly. From the course of this case, it was confirmed that we can safely administrate the nilotinib which is another tyrosine kinase inhibitor during steroid treatment in case of the patient onset interstitial pneumonia by the imatinib.

[*J.Community Pharm. Pharm. Sci.* **4**, 62-72 (2012)] [Lab. of Community Pharmaceutics]

Surveillance Study by Analysis of Prescription State and Questionnaire on Awareness of Physicians and Patients about Adverse Reaction and Drug Interaction of Kampo Medicine.

Yoshihiro KONDOH, Rie MATSUSHIMA-NISHIWAKI, Yuji SHIKAMA, Rieko KAWAMURA, Naoko SHIROGUCHI, Tadashi HORIUCHI*, Hitomi TERAMACHI, Teruo TSUCHIYA

We conducted the analyses of 880 prescriptions containing Kampo medicines. There were 30% multiple prescriptions of several Kampo products, the maximum of 6 concomitant preparations, observed. Concomitant Western medicines, few kinds of loop or thiazide diuretics were observed. The results of questionnaire surveillance for 100 patients receiving ethical Kampo products suggested their insufficient and misleading awareness about adverse reactions or interactions by Kampo medicines. This surveillance study suggested that the inappropriate awareness of physicians prescribing or patients receiving ethical Kampo products could lead the probability of severe adverse reactions induced by co-administration medications of Kampo or Western medicines.

[*J. Biochem.* **151**, 599-610 (2012)] [Lab. of Drug Informatics]

Transcriptional Regulation of Neutral Sphingomyelinase 2 in All-trans Retinoic Acid-treated Human Breast Cancer Cell Line, MCF-7.

Hiromi ITO, Koji TANAKA, Kazumi HAGIWARA, Misa KOBAYASHI, Asuka HOSHIKAWA, Naoki MIZUTANI, Akira TAKAGI, Tetsuhiro KOJIMA, Sayaka SOBUE, Masatoshi ICHIHARA, Motoshi SUZUKI, Keiko TAMIYA-KOIZUMI, Mitsuhiro NAKAMURA*, Yishiko BANNO, Yoshinori NOZAWA and Takashi MURATE

Effects of all-trans retinoic acid (ATRA) on sphingomyelinase expression were examined using MCF-7 (ATRA-sensitive) and MDA-MB-231 (ATRA-resistant) breast cancer cells. Chromatin immunoprecipitation (ChIP) assay showed Sp1, RARα and RXRα complex formation in MCF-7 cells regardless of ATRA treatment and ATRA-induced acetylated histone H3 of the 5'-promoter. NSMase2 mRNA expression enhanced by ATRA was due to increased transcription via phosphorylated Sp1 caused by PKCδ activation, followed by chromatin remodelling with histone H3 acetylation.

[*J. Biochem.* **151**, 611-620 (2012)] [Lab. of Drug Informatics]

Role of Down-regulated Neutral Ceramidase during All-trans Retinoic Acid Induced Neuronal Differentiation in SH-SY5Y Neuroblastoma Cells.

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Neutral ceramidase (NCDase) is a critical enzyme for controlling the turnover of ceramide, an important bioactive lipid. We observed that all-trans retinoic acid (ATRA)-induced cellular ceramide accumulation, cell-growth arrest and differentiation accompanied with down-regulation of NCDase in SH-SY5Y cells, without a decrease in sphingosine or sphingosine 1-phosphate. Down-regulation of NCDase through ATRA-induced GATA-2 decrease plays an important role in induction of ceramide accumulation and neuronal differentiation in SH-SY5Y cells.

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Survey on Burnout and Life-style among Medical and Pharmacy Students.

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This study was designed to evaluate the prevalence of burnout and the active condition of life-style among medical and pharmacy students. A self-administered questionnaire survey on the mentioned determinants was performed among 62 medical students and 131 pharmacy students of second year. Prevalence of burnout group among the male medical students was significantly lower than that among the male pharmacy students. On the other hand, there were no significant differences in the prevalence of burnout between the female medical students and pharmacy students. Among the male medical students, the values of age and study time in a day were significantly higher, and the values of sleeping time was significantly lower, compared with the male pharmacy students. These results suggest that there are some differences in the prevalence of burnout and the life-style between medical students and pharmacy students.

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