[*Org. Biomol. Chem.* **12**, 6590-6597 (2014)] [Lab. of Pharmaceutical & Medicinal Chemistry]

A New Class of High-contrast Fe(II) Selective Fluorescent Probes Based on Spirocyclized Scaffolds for Visualization of Intracellular Labile Iron Delivered by Transferrin.

Masato NIWA, Tasuku HIRAYAMA*, Kensuke OKUDA* and Hideko NAGASAWA*

Iron is an essential metal nutrient that plays physiologically and pathologically important roles in biological systems. Herein, we report a new class of Fe^{2+}-selective fluorescent probes based on the spirocyclization of hydroxymethylrhodamine and hydroxymethylrhodol scaffolds controlled by using our recently established *N*-oxide chemistry as a Fe^{2+}-selective switch of fluorescence response. The spirocyclization strategy improved the turn-on rate dramatically, and reducing the size of the substituents of the *N*-oxide group enhanced the reaction rate against Fe^{2+}. These new probes showed significant enhancements in the fluorescence signal against not only the exogenously loaded Fe^{2+}but also the endogenous Fe^{2+}levels. Furthermore, we succeeded in monitoring the accumulation of labile iron in the lysosome induced by transferrin-mediated endocytosis with a turn-on fluorescence response.

[*Drug Des. Devel. Ther.* **8**, 701-717 (2014)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Optimization of Biguanide Derivatives as Selective Antitumor Agents Blocking Adaptive Stress Responses in the Tumor Microenvironment.

Kosuke NARISE, Kensuke OKUDA*, Yukihiro ENOMOTO, Tasuku HIRAYAMA* and Hideko NAGASAWA*

Adaptive cellular responses resulting from multiple microenvironmental stresses, such as hypoxia and nutrient deprivation, are potential novel drug targets for cancer treatment. We focused on developing anticancer agents targeting the tumor microenvironment (TME). In this study, thirteen new compounds, designed and synthesized on the basis of the arylmethylbiguanide scaffold of phenformin, were used in structure activity relationship studies of inhibition of hypoxia inducible factor (HIF)-1 and unfolded protein response (UPR) activation and of selective cytotoxicity under glucose-deprived stress conditions, using HT29 cells. The guanidine analog had activities comparable with those of phenformin. Our structural development studies provided promising candidates for a novel anticancer agent targeting the TME for selective cancer therapy, to be subjected to further in vivo study.

[*Free Radic. Res.* **48**, 990-995 (2014)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Histological Detection of Catalytic Ferrous Iron with the Selective Turn-on Fluorescent Probe RhoNox-1 in a Fenton Reaction-based Rat Renal Carcinogenesis Model.

Takahiro MUKAIDE, Yuka HATTORI, Nobuaki MISAWA, Satomi FUNAHASHI, Li JIANG, Tasuku HIRAYAMA*, Hideko NAGASAWA* and Shinya TOYOKUNI

Iron overload of a chronic nature has been associated with a wide variety of human diseases, including infection, carcinogenesis, and atherosclerosis. Recently, a highly specific turn-on fluorescent probe (RhoNox-1) specific to labile ferrous iron [Fe(II)], but not to labile ferric iron [Fe(III)], was developed. In this study, we applied this probe to frozen sections of an established Fenton reaction-based rat renal carcinogenesis model with an iron chelate, ferric nitrilotriacetate (Fe-NTA), in which catalytic iron induces the Fenton reaction specifically in the renal proximal tubules, presumably after iron reduction. Notably, this probe reacted with Fe(II) but with neither Fe(II)-NTA, Fe(III) nor Fe(III)-NTA *in vitro*.

[*Chemistry* **20**, 4156-4162 (2014)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Facile One-pot Synthesis of [1, 2, 3]Triazolo[1, 5-*a*]pyridines from 2-acylpyridines by Copper(II)-catalyzed Oxidative N-N Bond Formation.

Tasuku HIRAYAMA*, Satoshi UEDA, Takahiro OKADA, Norihiko TSURUE, Kensuke OKUDA* and Hideko NAGASAWA*

An efficient and simple method for the synthesis of various [1, 2, 3]triazolo[1, 5-*a*]pyridines has been established. The method involves a copper(II)-catalyzed oxidative N-N bond formation that uses atmospheric oxygen as the terminal oxidant following hydrazonation in one pot. The use of ethyl acetate as the solvent dramatically promotes the oxidative N-N bond-formation reaction and enables the application of oxidative cyclization in the efficient one-pot reaction. A mechanism for the reaction was proposed on the basis of the results of a spectroscopic study.

[*J. Heterocycl. Chem.* **51**, 518–522 (2014)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 78: Synthesis of *N*-[2-([1,2,4]Oxadiazol-5-yl)cyclohepten-1-yl]formamide Oximes and their Evaluation as Inhibitors of Platelet Aggregation.

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

N-[2-([1,2,4]oxadiazol-5-yl)cyclohepten-1-yl]formamide oximes were synthesized by fusion of (6,7,8,9-tetrahydro-5*H*-cyclohepta[1,2-*d*]pyrimidin-4-yl)amidines with hydroxylamine hydrochloride through a subsequent rearrangement reaction. Effects of the products as well as the structurally related *N*-[4-([1,2,4]oxadiazol-5-yl)-2,3-dihydro[1] benzoxepin-5-yl]formamide oximes and *N*-[4-([1,2,4]oxadiazol-5-yl)-2,3-dihydro[1]benzothiepin-5-yl]formamide oximes on platelet aggregation were evaluated.

[*J. Heterocycl. Chem.* **51**, 661–668 (2014)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 76: Synthesis and Anti-platelet Evaluation of 2,4-Disubstituted 5,6-Dihydro[1]benzofuro[3',2':2,3]oxepino[4,5-*d*]pyrimidines.

Kensuke OKUDA*, Jun-ichi TAKANO, Takashi HIROTA, Kenji SASAKI, Yuta NISHINA and Hiroyuki ISHIDA

Reaction of several Vilsmeier reagents with 5-amino-2,3-dihydro[1]benzofuro[3,2-*b*]oxepin-4-carbonitrile gave tetracyclic 2-substituted 4-chloro-5,6-dihydro[1]benzofuro[3',2':2,3]oxepino[4,5-*d*]pyrimidines. The structure of one of these, the 4-chloro-2-phenyl derivative, was confirmed by X-ray crystallography. Treatment of the 4-chloro derivatives with simple amines as nucleophile afforded 2-substituted 4-amino derivatives. A pentacyclic compound was also obtained by dehydrative ring closure. These products were evaluated for anti-platelet activity and some showed potency comparable to aspirin.

[*J. Heterocycl. Chem.* **51**, 788–793 (2014)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 83: Synthesis of 2,4-Disubstituted 5,6-Dihydro[1]benzofuro[3',2':2,3]oxepino[4,5-*d*]pyrimidines and 2,4,5-Trisubstituted 5,6-Dihydro[1]benzofuro[2',3':5,6]pyrano[4,3-*d*]pyrimidines from 4-Chloro-5,6-dihydro[1]benzofuro[3',2':2,3]oxepino[4,5-*d*]pyrimidines.

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

Treatment of 2-substituted 4-chloro-5,6-dihydro[1]benzofuro[3',2':2,3]oxepino[4,5-*d*]pyrimidines with simple alcohols and thiols as nucleophile afforded 2-substituted 4-alkoxy (or sulfanyl) derivatives. In the case of alkoxide nucleophiles, rearranged reaction products were also obtained. X-ray crystallography was used to support the structure assignment of the rearranged product.

[*J. Heterocycl. Chem.* **51**, 891–898 (2014)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 80: Synthesis and Evaluation of Effects on *in vitro* Pentosidine Formation of 5,6-Dihydro[1]benzothieno[3',2':2,3]thiepino[4,5-*d*]pyrimidine and Related Compounds.

Kensuke OKUDA*, Yutaka ITSUJI, Takashi HIROTA and Kenji SASAKI

Reaction of 3-(3-cyanopropylthio)[1]benzothiophene-2-carbonitrile with *tert*-BuONa gave 5-amino-1,2-dihydro[1]benzothieno[3,2-*d*]thieno[2,3-*b*]pyridine and 5-amino-2,3-dihydro[1]benzothieno[3,2-*b*]thiepin-4-carbonitrile. The latter compound served as a convenient scaffold for the synthesis of the new heterocycles, [1]benzothieno[3',2':2,3]thiepino[4,5-*d*]pyrimidines. All of our new tetracyclic products were evaluated for *in vitro* inhibitory activity on the formation of pentosidine, which is one of representative advanced glycation end products.

[*J. Heterocycl. Chem.* **51**, 911–920 (2014)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 82: Synthesis and Evaluation of Anti-platelet Aggregation Activity of 2,4-Disubstituted 5,6-Dihydro[1]benzothiepino[5,4-*d*]pyrimidine and Related Compounds.

Kensuke OKUDA*, Takashi HIROTA and Kenji SASAKI

We have synthesized a large number of tricyclic 2-substituted 4-alkylamino-5,6-dihydro[1]benzothiepino[5,4-*d*]pyrimidines as part of our research to develop new effective anti-platelet drugs. A variety of alkyl and aryl groups were used as substituents at the 2-position. Evaluation of the effects of the newly synthesized compounds on collagen-induced platelet aggregation revealed several promising anti-platelet candidates with potencies superior to aspirin.

[*J. Heterocycl. Chem.* **51**, 972–981 (2014)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 75: Synthesis of 2,4-Disubstituted 5,6-Dihydro[1]benzoxepino[5,4-*d*]pyrimidines and 12-Substituted 1,2,4,5-Tetrahydro[1]benzoxepino[4,5-*e*]imidazo[1,2-*c*]pyrimidines as Potential Anti-platelet Aggregators.

Kensuke OKUDA*, Yuko YAMAMOTO, Takashi HIROTA and Kenji SASAKI

Libraries of tricyclic 2-substituted 4-alkylamino-5,6-dihydro[1]benzoxepino[5,4-*d*]pyrimidines and tetracyclic 12-substituted 1,2,4,5-tetrahydro[1]benzoxepino[4,5-*e*]imidazo[1,2-*c*]pyrimidines were synthesized as part of our research to develop new effective anti-platelet drugs. Several alkyl and aryl groups were used as substituents at the 2-position. Evaluation of the effects of the newly synthesized compounds on collagen-induced platelet aggregation revealed several promising anti-platelet candidates with potencies superior to aspirin.

[*J. Heterocycl. Chem.* **51**, 1528–1530 (2014)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 81: Synthesis and Evaluation of Pentacyclic 1,2,4,5-Tetrahydro[1]benzothieno[2',3':6,7]thiepino[4,5-*e*]imidazo[1,2-*c*]pyrimidine and Related Compounds as Potential Anti-platelet Aggregators.

Kensuke OKUDA*, Yutaka ITSUJI and Takashi HIROTA

Dehydrative ring closure reactions were carried out on fused 4-(2-hydroxyethylamino (or 2-hydroxyethyloxy or 2-hydroxyethylthio)pyrimidines to give fused 2,3-dihydroimidazo[1,2-*c*] (or 2,3-dihydrooxazolo[3,2-*c*] or 2,3-dihydrothiazolo[3,2-*c*])pyrimidines. This reaction produced the pentacyclic dehydrated ring-closure derivatives from the 2-hydroxyethylamino-derivative and 2-hydroxyethylthio-derivative, respectively. In contrast, 2-hydroxyethyloxy-derivative gave the rearrangement product. Effects of the synthesized compounds on collagen-induced platelet aggregation were also evaluated.

[*J. Heterocycl. Chem.* **51**, 1607–1614 (2014)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 79: Synthesis of 2,4-Disubstituted 6,7-Dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidines as Potential Anti-platelet Aggregators.

Kensuke OKUDA*, Takashi HIROTA and Kenji SASAKI

Libraries of tricyclic 2-substituted 4-alkylamino-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidines were synthesized as part of our research to develop new effective anti-platelet drugs. Several alkyl and aryl groups were used as substituents at the 2-position. Evaluation of the effects of the newly synthesized compounds on collagen-induced platelet aggregation revealed several promising anti-platelet candidates with potencies superior to aspirin.

[*Chem. Eur. J.* **20**, 510-516 (2014)] [Lab. of Organic Chemistry]

Iron-Catalyzed Friedel-Crafts Benzylation Using Benzyl TMS Ethers at Room Temperature.

Yoshinari SAWAMA,* Yuko SHISHIDO, Takahiro KAWAJIRI, Ryota GOTO, Yasunari MONGUCHI and Hironao SAJIKI*

Friedel–Crafts benzylations between unactivated arenes and benzyl alcohol derivatives are clean and straightforward processes to construct biologically useful di- and triarylmethanes. We have established an efficient iron-catalyzed Friedel–Crafts benzylation method at room temperature that uses benzyl TMS ethers as substrates, which are poorly reactive under common nucleophilic substitution conditions. The reaction seems to progress through iron-catalyzed self-condensation of the benzyl TMS ether to the corresponding dibenzylic ether. The use of excess arene relative to benzyl TMS ether produced mono-benzylated arene (di- and tri-arylmethane products), whereas the use of excess benzyl TMS ether versus arene provided bis-benzylated arene (polyarylated products) in high yields and regioselectivities. In previous methods, the latter double Friedel–Crafts benzylations hardly proceed.

[*Heterocycles* **88**, 233-243 (2014)] [Lab. of Organic Chemistry]

Cu/HP20-Catalyzed Solvent-Free Huisgen Cycloaddition at Ordinary Temperatures.

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

We have developed an environmentally friendly and highly efficient solvent-free Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction using a polymer-supported copper catalyst (Cu/HP20). Substrates poorly soluble in common organic solvents are also applicable to the present cycloaddition reaction without any solvents and provide the corresponding 1,4-triazole in high yields.

[*RSC Adv.* **4**, 8657-8660 (2014)] [Lab. of Organic Chemistry]

Effect of Sodium Acetate in Atom Transfer Radical Addition of Polyhaloalkanes to Olefins.

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

The atom transfer radical addition of polyhaloalkanes, such as bromotrichloromethane and polyfluoroalkyl iodine, to olefins smoothly proceeds in the presence of sodium acetate as an efficient auxiliary agent in dimethoxyethane. The present transition metal- and peroxide-free methodology is applicable to a broad scope of substrates.

[*Adv. Synth. Catal.* **356**, 313-318 (2014)] [Lab. of Organic Chemistry]

Palladium on Carbon-catalyzed Gentle and Quantitative Combustion of Hydrogen at Room Temperatures.

Yasunari MONGUCHI, Takashi IDA, Toshihide MAEJIMA, Takayoshi YANASE, Yoshinari SAWAMA, Yasushi SASAI, Shin-ichi KONDO and Hironao SAJIKI*

A gentle oxidation of hydrogen in the presence of oxygen in various solvents was achieved under Pd/C-catalyzed conditions at ordinary pressures and temperatures. A quantitative generation of water toward the consumed oxygen was observed. The stability of H_2O_2, which would form as an intermediate, was increased in cold CF_3CO_2H even in the presence of Pd/C, and 64% H_2O_2 based on the consumed oxygen was detected. A mechanistic study revealed that the single electron transfer and generation of the hydroxyl radical are involved in the combustion process. The reactive oxygen species generated during the process was effectively utilized for the chemical oxidation of sulfides and phosphines to afford the corresponding sulfoxides and phosphine oxides, respectively.

[*Chem. Eur. J.* **20**, 2631-2636 (2014)] [Lab. of Organic Chemistry]

Chemoselective and Direct Functionalization of Methyl Benzyl Ethers and Unsymmetrical Dibenzyl Ethers Using Iron Trichloride.

Yoshinari SAWAMA,* Ryota GOTO, Saori NAGATA, Yuko SHISHIDO, Yasunari MONGUCHI and Hironao SAJIKI*

Methyl and benzyl ethers are widely utilized as protected alcohols due to their chemical stability, such as the low reactivity of the methoxy and benzyloxy groups as leaving groups under nucleophilic conditions. We have established the direct azidation of chemically stable methyl and benzyl ethers derived from secondary and tertiary benzyl alcohols. The present azidation chemoselectively proceeds at the secondary or tertiary benzylic positions of methyl benzyl ethers or unsymmetrical dibenzyl ethers and is also applicable to direct allylation, alkynylation, and cyanation reactions, as well as the azidation.

[*Tetrahedron* **70**, 4790-4798 (2014)] [Lab. of Organic Chemistry]

Systematic Evaluation of the Palladium-Catalyzed Hydrogenation under Flow Conditions.

Tomohiro HATTORI, Aya TSUBONE, Yoshinari SAWAMA, Yasunari MONGUCHI* and Hironao SAJIKI*

Four types of heterogeneous Pd catalysts (10% Pd/C, 10% Pd/HP20, 0.5% Pd/MS3A, and 0.3% Pd/BN) were applied to the flow hydrogenation to systematically evaluate the appropriate conditions for the reduction of a wide variety of reducible functionalities. The use of 10% Pd/C and 10% Pd/HP20 allowed the hydrogenation of various reducible functionalities by a single-pass of the substrateeMeOH solution through the catalyst cartridge, while 0.5% Pd/MS3A and 0.3% Pd/BN catalyzed a novel chemoselective hydrogenation; only alkene, alkyne, azide, and nitro functionalities could be reduced with other coexisting reducible functionalities intact.

[*Green Chem.* **16**, 3439-3443 (2014)] [Lab. of Organic Chemistry]

Rhodium on Carbon-Catalyzed Hydrogen Scavenger- and Oxidant-free Dehydrogenation of Alcohols in Aqueous Media.

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

The efficient and catalytic dehydrogenation of alcohols is a clean approach for preparing carbonyl compounds accompanied only by the generation of hydrogen gas. We have accomplished the heterogeneous rhodium-on-carbon catalyzed dehydrogenation of secondary, as well as primary, alcohols to the corresponding ketones and carboxylic acids in water under basic conditions.

[*Adv. Synth. Catal.* **356**, 1866-1872 (2014)] [Lab. of Organic Chemistry]

Palladium on Carbon-Catalyzed One-Pot N-Arylindole Synthesis: Intramolecular Aromatic Amination, Aromatization, and Intermolecular Aromatic Amination.

Yasunari MONGUCHI,* Takahisa MARUMOTO, Haruki TAKAMATSU, Yoshinari SAWAMA and Hironao SAJIKI*

Indole and indoline derivatives were selectively and temperature dependently synthesized via the intramolecular cross-coupling reaction between the amino and aromatic bromine functionalities of 2-bromophenetylamine derivatives in the presence of 10% palladium on carbon (Pd/C), 1,1'-bis(diphenylphosphino)ferrocene (DPPF), and NaO*t*-Bu in mesitylene at 140 and 200 °C, respectively. The neutralization using acetic acid after formation of the indoline derivatives effectively promoted their aromatization, and the corresponding indole derivatives were obtained at 140 °C. Furthermore, various aryl groups were also introduced to the N-1 position of the indole, pyrrole, and carbazole by their direct intramolecular arylation with aryl halides and the one-pot protocol for the *N*-arylindole synthesis from 2-bromophenetylamine was developed.

[*Tetrahedron* **70**, 4540-4546 (2014)] [Lab. of Organic Chemistry]

Efficient Partial Hydrogenation of Trichloromethyl to Geminal-Dichloromethyl Groups in Platinum on Carbon-Catalyzed System.

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

While gem-dichloromethyl groups can be directly synthesized by the mono-dechlorination of the corresponding trichloromethyl groups, the suppression control of the over-reduction to form chloromethyl or methyl functionalities is quite difficult.We have established the efficient and widely applicable monodechlorination method of the trichloromethyl groups to form the corresponding *gem*-dichloromethyl groups using platinum on carbon in dimethylacetamide as a specific solvent at 25 °C under a hydrogen atmosphere.

[*Tetrahedron Lett.* **55**, 3160-3162 (2014)] [Lab. of Pharmaceutical Synthetic Chemistry]

2-Chloroanthraquinone-catalyzed aerobic photo-oxidative synthesis of diacylamines from benzylamides.

Izuho ITOH, Yoko MATSUSAKI, Akitoshi FUJIYA, Norihiro TADA, Tsuyoshi MIURA and Akichika ITOH*

In this Letter, the aerobic photo-oxidative green synthesis of diacylamines from benzylamides in the presence of molecular oxygen and catalytic amounts of 2-chloroanthraquinone under visible light irradiation from a fluorescent lamp was reported.

[*Synlett* **25**, 1453-1457 (2014)] [Lab. of Pharmaceutical Synthetic Chemistry]

Aerobic photooxidative carbon-carbon bond formation between tertiary amines and carbon nucleophiles using 2-chloroanthra-9,10-quinone.

Tomoaki YAMAGUCHI, Tomoya NOBUTA, Norihiro TADA, Tsuyoshi MIURA, Tatsushi NAKAYAMA, Bunji UNO and Akichika ITOH*

Carbon-carbon bonds were formed between tertiary amines and carbon nucleophiles such as nitroalkanes, ketones, trimethylsilyl cyanide, or indole under aerobic photooxidative conditions by using 2-chloroanthra-9,10-quinone as an organocatalyst. This reaction uses harmless visible-light irradiation with molecular oxygen as the terminal oxidant.

[*Tetrahedron Lett.* **55**, 6543-6546 (2014)] [Lab. of Pharmaceutical Synthetic Chemistry]

Aerobic photooxidative synthesis of benzimidazoles from aromatic aldehydes and diamines using catalytic amounts of magnesium iodide.

Yoshitomo NAGASAWA, Yoko MATSUSAKI, Toshiyuki HOTTA, Tomoya NOBUTA, Norihiro TADA, Tsuyoshi MIURA and Akichika ITOH*

This Letter proposes a safe, mild, and environmentally benign method for the synthesis of benzimidazoles from aromatic aldehydes and diamines by aerobic photooxidation using irradiation with visible light, a catalytic amounts of magnesium iodide, which serves as both a Lewis acid and an oxidant, and molecular oxygen as the terminal oxidant.

[*Synlett* **25**, 2613-2616 (2014)] [Lab. of Pharmaceutical Synthetic Chemistry]

Aerobic photooxidative synthesis of phenols from arylboronic acids using 2-propanol as solvent.

Keita MATSUI, Takafumi ISHIGAMI, Tomoaki YAMAGUCHI, Eiji YAMAGUCHI, Norihiro TADA, Tsuyoshi MIURA and Akichika ITOH*

A useful method for the synthesis of phenols from arylboronic acids with hydrogen peroxide generated in situ by aerobic photooxidation is reported. This reaction uses visible-light irradiation and easily handled 2-chloroanthraquinone as an organocatalyst under mild conditions, i.e., an air atmosphere and ambient pressure and temperature. Because of this method is metal- and base-free conditions, it represents an environmentally benign approach to the synthesis of phenols from arylboronic acids.

[*Tetrahedron: Asymmetry* **25**, 974-979 (2014)] [Lab. of Pharmaceutical Synthetic Chemistry]

Asymmetric conjugate addition of malonate to α,β-unsaturated ketones in water using a perfluoroalkanesulfonamide organocatalyst.

Yuji KAMITO, Akira MASUDA, Hiroki YUASA, Norihiro TADA, Akichika ITOH*, Kosuke NAKASHIMA, Shin-ichi HIRASHIMA, Yuji KOSEKI and Tsuyoshi MIURA

Perfluoroalkanesulfonamide organocatalyst efficiently promotes asymmetric Michael additions of malonates to enones in cyclohexane or water to produce the corresponding addition products with excellent yields and with up to 99% ee.

[*Tetrahedron Lett.* **55**, 4619-4622 (2014)] [Lab. of Pharmaceutical Synthetic Chemistry]

Cinchona-diaminomethylenemalononitrile organocatalyst for asymmetric conjugate addition of 1,3-diketone to nitroalkene.

Shin-ichi HIRASHIMA, KosukeNAKASHIMA, Yuki FUJINO, Ryoga ARAI, Takaaki SAKURAI, Masahiro KAWADA, Yuji KOSEKI, Miho MURAHASHI, Norihiro TADA, Akichika ITOH* and Tsuyoshi MIURA

A diaminomethylenemalononitrile organocatalyst with a cinchona motif efficiently promotes the enantioselective conjugate addition of acetylacetone to various nitroalkenes to yield the corresponding addition products in high to excellent yields with up to 89 % ee.

[*RSC Adv.* **4**, 13191-13194 (2014)] [Lab. of Pharmaceutical Synthetic Chemistry]

Molecular-iodine-catalyzed aerobic oxidative synthesis of β-hydroxy sulfones from alkenes.

Atsumasa KARIYA, Tomoaki YAMAGUCHI, Tomoya NOBUTA, Norihiro TADA, Tsuyoshi MIURA and Akichika ITOH*

The synthesis of β-hydroxy sulfones from alkenes and sodium benzene sulfinates under aerobic oxidative conditions was achieved in the presence of a catalytic amount of molecular iodine. Molecular oxygen in air served as the terminal oxidant and the catalytic amount of molecular iodine acted as the sulfonyl radical initiator and peroxide reductant.

[*Synlett* **25**, 884-888 (2014)] [Lab. of Pharmaceutical Synthetic Chemistry]

Photooxidative cleavage of aromatic alkenes into aldehydes using catalytic iodine and molecular oxygen under visible light irradiation.

Akitoshi FUJIYA, Atsumasa KARIYA, Tomoya NOBUTA, Norihiro TADA, Tsuyoshi MIURA and Akichika ITOH*

A method for the photooxidative cleavage of stilbenes was reported to give aldehydes using O_2 as the terminal oxidant, visible light, and a catalytic amount of I_2 and trifluoroacetic acid.

[*Tetrahedron Lett.* **55**, 2703-2706 (2014)] [Lab. of Pharmaceutical Synthetic Chemistry]

Pyrrolidine-diaminomethylenemalononitrile organocatalyst for Michael additions of carbonyl compounds to nitro alkenes under solvent-free conditions.

Kosuke NAKASHIMA, Shin-ichi HIRASHIMA, Masahiro KAWADA, Yuji KOSEKI, Norihiro TADA, Akichika ITOH* and Tsuyoshi MIURA

The novel pyrrolidine-diaminomethylenemalononitrile organocatalyst I promotes the asymmetric conjugate addition of a carbonyl compound to a nitro alkene to afford the corresponding adduct in high yield with ≤99% ee under solvent-free conditions.

[*Tetrahedron Lett.* **55**, 4334-4337 (2014)] [Lab. of Pharmaceutical Synthetic Chemistry]

Solvent-free asymmetric conjugate addition of malonates to enones using a diaminomethylenemalononitrile organocatalyst.

Shin-ichi HIRASHIMA, Takaaki SAKAI, Kosuke NAKASHIMA, Nana WATANABE, Yuji KOSEKI, Kanako MUKAI, Yohei KANADA, Norihiro TADA, Akichika ITOH* and Tsuyoshi MIURA

Diaminomethylenemalononitrile organocatalyst 1 efficiently promotes the asymmetric conjugate addition of malonates to α,β-unsaturated ketones to afford the corresponding addition products in high to excellent yields with up to 98% ee.

[*Fitoterapia* **92**, 9-15 (2014)] [Lab. of Pharmacognosy]

Anti-androgenic activity of hydroxyxanthones in prostate cancer LNCaP cells.

Toshinobu SHAKUI, Kazuhiro IGUCHI, Misako BABA, Kazuyuki HIRANO, Tetsuro ITO, Masayoshi OYAMA*, Munekazu IINUMA, Shigeyuki USUI, and Hideki TOSA

Anti-androgens are used to treat prostate cancer. Here, we report that hydroxyxanthones from a plant extract act as anti-androgens in androgen receptor (AR)-positive prostate cancer LNCaP cells. Anti-androgenic activity of the ethanol extract from Garcinia subelliptica was observed in a luciferase assay using LNCaP/MMTV cells with a stably integrated mouse mammary tumor virus (MMTV) promoter. HPLC-based activity profiling followed by a chemical library-based assay strategy enabled the rapid identification of several active principles bearing a xanthone core substituted with hydroxyl and isoprenyl groups. Among the active compounds, 2-(1,1-dimethyl-allyl)-1,4,5,6-tetrahydroxyxanthone (subelliptenone F) was identified as a potent inhibitor of AR transcriptional activity.

[*Tetrahedron Lett.* **55**, 314-318 (2014)] [Lab. of Pharmacognosy]

Dehydroxylation of stilbenoid oligomers: absolute configuration determination via comparison of experimental and theoretical electronic circular dichroic spectra.

Tetsuro ITO* and Tatsuo NEHIRA

Dehydroxylation of naturally occurring oligomeric resveratrol derivatives resulted in the formation of compounds with dramatically reduced principal stable conformers. In addition, dehydroxylation allowed the determination of the absolute configurations of the original resveratrols via comparison of experimental and theoretical electronic circular dichroic spectra. Notably, the absolute configuration of pauciflorol B was identified using this novel procedure, which is the first application of dehydroxylated derivatives for the determination of the absolute configuration of naturally occurring polyphenols.

[*Tetrahedron* **70**, 5640-5649 (2014)] [Lab. of Pharmacognosy]

Structure elucidation of highly condensed stilbenoids: chiroptical properties and absolute configuration.

Tetsuro ITO*, Hiromi ITO, Tatsuo NEHIRA, Ryuichi SAWA, and Munekazu IINUMA

We investigated the potential roles of the skeleton-based comparative study of electronic circular dichroism spectra for an application of absolute configuration determination of oligostilbenoids. This approach was ultimately achieved followed by the isolation and elucidation of relative configuration of upunaphenol Q (new compound) and vateriaphenol A, namely two octamers are dimeric tetramers of resveratrol. The common building blocks provides further insight into how smaller oligostilbenoids are apparently conserved during downstream metabolites, as well as providing additional impetus to resolve absolute configuration of highly condensed stilbenoids. They also underline the importance of studies on determination of absolute configuration of common building blocks in the chemical library and to provide chiroptical properties.

[*J. Ethnopharmacol.* **155**, 731-735 (2014)] [Lab. of Pharmacognosy]

Effects of *Sceletium tortuosum* in rats.

Melissa J. LORIA, Zulfiqar ALI, Naohito ABE*, Ikhlas A. KHAN, and Kenneth J. SUFKA

ETHNOPHARMACOLOGICAL RELEVANCE: Broad historical and current uses in addition to diverse activity on CNS targets may make *Sceletium tortuosum* a useful therapeutic in a variety of clinical settings. This study sought to more broadly characterize activity of *Sceletium tortuosum* and mesembrine in a number of common, rodent-based assays that model nociception, depression, anxiety, ataxia, and abuse liability. MATERIALS AND METHODS: Male Sprague-Dawley were administered Sceletium tortuosum extract products and behavioral responses were evaluated in the conditioned place preference (CPP), hot plate, forced swim, elevated plus, and rotarod tests.

[*J. Appl. Biomed.* **12**, 291-299 (2014)] [Lab. of Pharmacognosy]

Possible hepatocellular toxicity of EGCG under the influence of an inflammagen.

Ibrahim G. SALEH, Zulfiqar ALI, Naohito ABE*, Farid M. HAMADA, Mohamed F. ADB-ELLAH, Larry A. WALKER, Ikhlas A. KHAN, and Mohammad K. ASHFAQ

Epigallocatechin-3-gallate (EGCG) is widely used as a weight-controlling supplement. Concerns about its safety evoked after cases of hepatotoxicity occurred upon its use. The underlying factors that could be involved in EGCG associated hepatotoxicity are not fully studied. In this study, we investigated the possible impact of lipopolysaccharide (LPS), as an inflammagen, on the effect of EGCG on hepatocytes. HepG2 cells were treated with different concentrations of EGCG (100, 200, 500 μM), with and without LPS (10 nM)-presensitization of the cells. Viability of HepG2 cells decreased with the increased concentrations of EGCG; the viability was even lesser in LPS-presensitized cells.

[*J. Ethnopharmacol.* **151**, 361-364 (2014)] [Lab. of Pharmacognosy]

The effect of *Salvia divinorum* and *Mitragyna speciosa* extracts, fraction and major constituents on place aversion and place preference in rats.

Kenneth J. SUFKA, Melissa J. LORIA, Kevin LEWELLYN, Jordan K. ZJAWIONY, Zulfiqar ALI, Naohito ABE*, and Ikhlas A. KHAN

ETHNOPHARMACOLOGICAL RELEVANCE: Consumer use of botanicals has increased despite, in many instances, the paucity of research demonstrating efficacy or identifying liabilities. This research employed the place preference/aversion paradigm to characterize the psychoactive properties of *Salvia divinorum* ext. (10, 30, 100 mg/kg), salvinorin A (0.1, 0.3, 1.0 mg/kg), *Mitragyna speciosa* MeOH ext. (50, 100, 300 mg/kg), *Mitragyna speciosa* alkaloid-enriched fraction (12.5, 25, 75 mg/kg) and mitragynine (5, 10, 30 mg/kg) in rats.

[*Anal. Sci.* **30**, 519-522 (2014)] [Lab. of Pharmaceutical Analytical Chemistry]

Facile and Effective Pretreatment Using Stop and Go Extraction Tips for LC-MS/MS Analysis of Trace Amounts of DNA Adducts.

Hiroya MURAKAMI, Rieko KAWAMURA, Takayoshi SAKAKIBARA, Yukihiro ESAKA, Yasushi ISHIHAMA and Bunji UNO*

The preparation and application of a stop and go extraction tip (StageTip) used for pre-purification of sample solutions for LC–MS/MS analysis of DNA adducts was simplified and improved to increase throughput while maintaining high adduct selectivity. It was demonstrated that the StageTip composed of two sheets of a poly(styrene-divinylbenzene) copolymer disk was easily prepared and useful for selective extraction of trace amounts of DNA adducts from a sample solution containing a great quantity of normal deoxynucleosides.

[*Chem. Pharm. Bull.* **62**, 88-91 (2014)] [Lab. of Pharmaceutical Analytical Chemistry]

Electrochemical Analysis in a Liposome Suspension Using Lapachol as a Hydrophobic Electro Active Species.

Noriko OKUMURA, Shiori WAKAMATSU and Bunji UNO*

This study demonstrated that the electro-chemical analysis of hydrophobic quinones can be performed in liposome suspension systems. We prepared and analyzed liposome suspensions containing lapachol, which is a quinone-based anti-tumor activity compound. In this suspension system, a simple one redox couple of lapachol is observed. These results are quite different from those obtained in organic solvents. In addition, the pH dependence of redox behaviors of lapachol could be observed in multilamellar vesicle (MLV) suspension system. This MLV suspension system method may approximate the electrochemical behavior of hydrophobic compounds in aqueous conditions. A benefit of this liposome suspension system for electrochemical analysis is that it enables to observe water-insoluble compounds without using organic solvents.

[*J.Chromatogr. A* **1358**, 261-268 (2014)] [Lab. of Pharmaceutical Analytical Chemistry]

Stepwise Elusion Method in Micellar Electrokinetic Chromatography via Sequential Use of Lithium Perfluorooctadecyl Sulfonate and Lithium Dodecyl Sulfate.

Yukihiro ESAKA*, Fumiaki RIN, Miki KOBAYASHI, Ryohei OSAKO, Hiroya MURAKAMI and Bunji UNO

The present stepwise elution method of MEKC is performed by replacing the inlet reservoir of a first running solution containing lithium perfluorooctadecyl sulfonate (LPFOS) with that of a second running solution containing lithium dodecyl sulfate (LDS) during a single separation run in the absence of electroosmotic flow under acidic conditions, where LPFOS micelles work as carriers in first and then LDS micelles turn over. Effective separation of 15 nonionic aromatic compounds was controlled well by adjusting the time in the inlet reservoir, which could not be accomplished with systems using only LPFOS or only LDS, with significant changes in the elution order where necessary. Furthermore, separations with the present stepwise method were easily simulated.

[*Chromatography* **35**, 155-162 (2014)] [Lab. of Pharmaceutical Analytical Chemistry]

Simultaneous Analysis of Polymethoxyflavones and Flavanone Glycosides in Citrus Fruits by Micellar Electrokinetic Chromatography Using Sodium Deoxycholate.

Yukihiro ESAKA*, Hiroya MURAKAMI, Bunji UNO, Hiroko MURATA, Munekazu IINUMA and Toshiyuki TANAKA

Simultaneous MEKC separation of polymethoxyflavones (PMFs) and flavanone glycosides (FGs) was achieved by using sodium deoxycholate (SDC) in the presence of DMSO. Fourteen flavonoids consisting of 4 flavanones, including three FGs and 10 flavones, including nine PMFs, were separated nearly completely using 10 mM phosphate buffer (pH 7.2), 75 mM SDC, 20% DMSO, and 10% acetonitrile as a running solution for MEKC. Additionally, we proposed a novel index that is an alternative to migration time and corrects variation of migration times in measurements successfully. Some of the flavonoids in fruits such as flat lemon and ponkan orange were identified with the help of this index.

[*Chem. Pharm. Bull*., **62,** 538-544 (2014)] [Lab. of Pharmaceutical Enginnering]

Characterization of a doxorubicin liposome formulation by a novel in vitro release test methodology using column-switching high-performance liquid chromatography.

Naozumi OHNISHI, Hiromasa TOMIDA, Yousuke ITO, Kohei TAHARA and Hirofumi TAKEUCHI*

A novel *in vitro* release test methodology for a liposome formulation was developed using a column-switching high-performance liquid chromatography (HPLC) system. Doxorubicin (DXR) liposome formulations were used as a model. To evaluate the release profile, this system can be used for determining the released and encapsulated DXR in the liposome formulation separately. Comparison with a conventional *in vitro* release test methodology by dialysis revealed that the methodology developed by column-switching HPLC had no rate-limiting process of membrane permeation of the drug (which is occasionally observed in the dialysis method). The developed method did not require a large amount of sample or a complicated pretreatment. In addition, the developed column-switching HPLC system was applicable for characterization of the encapsulation profile of liposome formulations.

[*Journal of the Society of Powder Technology, Japan* **51**, 16-24 (2014)] [Lab. of Pharmaceutical Enginnering]

Preparation of Co-ground Mixture of Erythritol and Micronized Crospovidone Using a Ball Mill for Orally Disintegrating Tablets.

Eri KATSUNO, Yoshiko TAKEUCHI, Kohei TAHARA and Hirofumi TAKEUCHI*

The purpose of this study was to prepare a co-ground mixture of erythritol and micronized crospovidone (M-CPVP) to prepare the orally disintegrating tablets (ODTs) by directly compressing. The co-ground mixture was prepared by ball milling. Several processing time for ball mill and the different ratio of M-CPVP/erythritol were tested to determine the appropriate agglomerates for designing the ODTs. The ODTs containing co-ground M-CPVP/erythritol mixture showed rapid disintegration (<30s) and adequate hardness of the tablets (tensile strength>1.0MPa). On the other hand, the powder mixture without this co-processing had poor compactibility, and the tableting trouble such as capping was observed. We could also demonstrate that ODTs containing ethenzamide, as an active ingredient, could be prepared using the co-ground M-CPVP/erythritol.

[*Sci. Rep.*, **4**, 1-8 (2014)] [Lab. of Pharmaceutical Enginnering]

Involvement of Autophagy in Antitumor Activity of Folate-appended Methyl-β-cyclodextrin.

Risako ONODERA*, Keiichi MOTOYAMA, Nao TANAKA, Ayumu OHYAMA, Ayaka OKAMATSU, Taishi HIGASHI, Ryusho KARIYA, Seiji OKADA and Hidetoshi ARIMA

Autophagy, the major lysosomal pathway for recycling intracellular components including organelles, is emerging as a key process regulating tumorigenesis and cancer therapy. Most recently, we newly synthesized folate-appended methyl-β-cyclodextrin (FA-M-β-CyD), and demonstrated the potential of FA-M-β-CyD as a new antitumor drug. In this study, we investigated whether anticancer activity of FA-M-β-CyD in folate receptor-α (FR-α)-positive tumor cells is involved in autophagy. FA-M-β-CyD induced the formation of autophagic vacuoles, which were partially colocalized with mitochondria, in KB cells. Taken together, these results suggest that FR-α-expressing cell-selective cytotoxic activity of FA-M-β-CyD could be mediated by the regulation of autophagy, rather than the induction of apoptosis.

[*J. Drug. Target.*, **22**, 211-219 (2014)] [Lab. of Pharmaceutical Enginnering]

Potential Use of Complex of Doxorubicin with Folate-conjyugated Methyl-β-cyclodextrin for Tumor-selective Cancer Chemotherapy

Keiichi MOTOYAMA, Risako ONODERA*, Ayaka OKAMATSU, Taishi HIGASHI, Ryusho KARIYA, Seiji OKADA and Hidetoshi ARIMA

In the present study, to expand the application of folate-conjugated M-β-Cyclodextrin (FA-M-β-CyD) for cancer chemotherapy, we evaluated the potential of FA-M-β-CyD as a tumor-targeting anticancer drug carrier at a low dose. Antitumor activity of DOX was increased by the complexation with FA-M-β-CyD, but not with folate-conjugated β-CyD (FA-β-CyD) or M-β-CyD in KB cells, a folate receptor-α (FR-α)-expressing cell line. The DOX/FA-M-β-CyD complex showed markedly high antitumor activity, compared to DOX alone and DOX/M-β-CyD complex, after an intravenous administration to FR-α-expressing tumor cell-bearing mice. These findings suggest that FA-M-β-CyD could be useful as a tumor-selective carrier for anticancer drugs.

[*J. Control. Release*, **193**, 35-41 (2014)] [Lab. of Pharmaceutical Enginnering]

Potential use of fucose-appended dendrimer/α-cyclodextrin conjugates as NF-κB decoy carriers for the treatment of lipopolysaccharide-induced fulminant hepatitis in mice.

Chiho AKAO, Takahiro TANAKA, Risako ONODERA*, Ayumu OHYAMA, Nana SATO, Keiichi MOTOYAMA, Taishi HIGASHI and Hidetoshi ARIMA

The purpose of the present study is to treat lipopolysaccharide (LPS)-induced fulminant hepatitis by NF-κB decoy complex with fucose-appended dendrimer (G2) conjugate with α-cyclodextrin (Fuc-S-α-CDE (G2)). Fuc-S-α-CDE (G2, average degree of substitution of fucose (DSF2))/NF-κB decoy complex significantly suppressed nitric oxide and TNF-α production from LPS-stimulated NR8383 cells by adequate physicochemical properties and fucose receptor-mediated cellular uptake. Intravenous injection of Fuc-S-α-CDE (G2, DSF2)/NF-κB decoy complex extended the survival of LPS-induced fulminant hepatitis model mice. These results suggest that Fuc-S-α-CDE (G2, DSF2) has the potential for a novel Kupffer cell-selective NF-κB decoy carrier.

[*J. Phar. Nutri. Sci.*, **4**, 37-42 (2014)] [Lab. of Pharmaceutical Physical Chemistry]

Synthesis of Amphiphilic Blockcopolymer Using Mechanically Produced Macromonomers Possessing Anhydrate as a Terminal Group and Its Application to Polymeric Micelles.

Shin-ichi KONDO*, Machi OMOTO, Yuka SAWAMA, Yasushi SASAI, Kenjiro TATEMATSU, Yukinori YAMAUCHI and Masayuki KUZUYA

We have synthesized macromonomers by mechanochemical reaction of poly(benzyl methacrylate) (PBzMA) and maleic anhydride (MA). The amphiphilic blockcopolymer was synthesized with macromonomer of PBzMA and amino-terminated polyethylene glycol. The number average molecular weight of the produced amphiphilic blockcopolymer was 33,000. Polymeric micelles were readily prepared from the present amphiphilic blockcopolymer by a dialysis method. The mean diameter of the micelles measured by dynamic light scattering was about 146 nm. It was shown that the present macromonomer mechanically produced can be used for the synthesis of amphiphilic bockcopolymer to form polymeric micelles.

[*J. Photopolym. Sci. Thechnol.* **27**, 385-388 (2014)] [Lab. of Pharmaceutical Physical Chemistry]

Intermolecular Interaction of Cyclodextrin Derivatives Immobilized onto the Self-Assembled Phospholipid Layer Fabricated by Plasma-Assisted Method.

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

We immobilized cyclodextrin derivatives possessing Cy3 or Cy5 (Per-6-ABCD-Cy3 or Per-6-ABCD-Cy5) on the self-assembled phospholipid layer fabricated by plasma-assisted method (LDPE-StA-PC-SA) and observed the fluorescence intensity ascribed to Cy3 and Cy5. Inclusion complex between Per-6-ABCD-Cy3 and Per-6-ABCD-Cy5 immobilized onto the self-assembled phospholipid layer was formed, so that the fluorescence resonance energy transfer was observed. The inclusion complex was decomposed by the addition of polyethylene glycol (PEG). It was suggested that PEG could be detected with the present film and that the amount of PEG might depend on the decrease of fluorescence intensity.

[*J. Photopolym. Sci. Thechnol.* **27**, 369-372 (2014)] [Lab. of Pharmaceutical Physical Chemistry]

Plasma Irradiation to Poly(acrylic acid) Brushes Fabricated on Polystyrene Substrate and its Characterization.

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

We have previously reported the method for fabrication of poly (acrylic acid) (pAAc) brushes on polystyrene (PS) substrate using surface-initiated atom transfer radical polymerization. The resultant sufaces with high density pAAc brushes exhibited nonfouling properties against protein adsorption. In this study, we examined the effects of argon plasma irradiation to the pAAc-grafted PS substrate on the surface hydrophilicity and the protein adsorption property. The argon plasma irradiation caused the decarboxylation in pAAc brushes so that the protein nonfouling properties were lost even by short plasma irradiation. These results indicated that the the protein adsorption onto pAAc-grafted surfaces could be easily controlled by plasma irradiation and this plasma technique would be applicable for fabricating substrates for protein and cell arrasy system.

[*J. Photopolym. Sci. Thechnol.* **27**, 389-392 (2014)] [Lab. of Pharmaceutical Physical Chemistry]

A New Drug Delivery System Using Plasma-Irradiated Polysaccharide.

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

We attempted to control the drug release from a double-compressed tablet containing theophylline core with outer layer which was consisted of hydroxylpropyl methyl cellulose acetate succinate and *myo*-inositol, or hydroxypropyl methyl cellulose phthalate and lactose. By use of difference in degradation properties of organic compounds, the oxygen plasma-irradiation varied outer layer so as to cause release of drugs at different rates, depending on plasma operational condition. The scanning electron microscope analysis suggested that concurrent occurrence of cross-link reaction and the micropores formation on outer layer converted rapid-release system into sustained-release system.

[*J. Toxicol. Sci*. **39**, 173-177 (2014)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

Gene Expression Differences in the Duodenum of 129/Sv and DBA/2 Mice Compared with that of C57BL/6J Mice.

Shunji IMAI, Maki TOKUMOTO, Yasuyuki FUJIWARA, Akiko HONDA, Hasegawa, Yoshiyuki SEKO, Jin-Yong LEE, Hisamitsu NAGASE* and Masahiko SATOH

We compared the cadmium (Cd) concentration in the liver and kidney of different strains of mice after exposure to 50 ppm Cd for 30 days via drinking water. Cd concentration in the liver and kidney of C57BL/6J mice were higher than those of 129/Sv and DBA/2 mice. Microarray analyses were performed to compare the expression levels of transport-related genes in the duodenum among the three strains of mice. The expression levels of 9 and 11 genes were elevated more than 2.0-fold and 13 and 12 genes were reduced less than 0.5-fold in 129/Sv mice and DBA/2 mice, respectively. Among these low expressed genes, 10 genes were common between the two strains. These results suggest that some of those genes might be involved in Cd absorption and its toxicity.

[*Endocrinology* **155**, 4275-4286 (2014)] [Lab. of Hygienic Chemistry & Molecular Toxicology]

A Mollusk Retinoic Acid Receptor (RAR) Ortholog Sheds Light on the Evolution of Ligand Binding.

Juliana GUTIERREZ-MAZARIEGOS, Eswar Kumar NADENDLA, Daniela LIMA, Keely PIERZCHALSKI, Jace W. JONES, Maureen KANE, Jun-Ichi NISHIKAWA, Youhei HIROMORI, Tsuyoshi NAKANISHI*, Miguel M. SANTOS, L. Filipe C. CASTRO, William BOURGUET, Michael SCHUBERT and Vincent LAUDET

Here we present the identification and characterization of a retinoic acid receptor (RAR) from the mollusk *Nucella lapillus* (NlRAR). NlRAR specifically binds to DNA response elements organized in direct repeats as a heterodimer with retinoid X receptor, but does not bind all-*trans* retinoic acid (atRA) or other retinoids. Furthermore, NlRAR is unable to activate the transcription of reporter genes in response to stimulation by retinoids and to recruit coactivators in the presence of these compounds. Our data suggest that, RAR in mollusks has lost its affinity for atRA, highlighting the evolutionary plasticity of its ligand-binding pocket.

[*J. Neurophysiol. Neurol. Disord.* **2**, 1-8 (2014)] [Lab. of Molecular Biology]

Caffeic Acid Phenethyl Ester Ameliorates Depression- and Anxiety-like Behaviors of Mice Exposed to Chronic Mild Stress.

Atsushi TORATANI, Haruko SOGA, Hidefumi FUKUMITSU, Hitomi SOUMIYA, Yoshiko FURUKAWA, Shoei FURUKAWA.

Depression- and anxiety-like symptoms appeared in mice when they were kept in cages and sequentially subjected to leaning, drenching, and rotation within 1-2 days for 3 weeks (chronic mild stress: CMS). Caffeic acid phenethyl ester (CAPE), a component of propolis, showed a preventive effect against both symptoms when administered during the stress loading, and CAPE also displayed a therapeutic effect against both symptoms when administered after the stress loading. Furthermore, CAPE restored the CMS-induced decrease in the level of the phosphorylated forms of ERK1/2 and CREB in the hippocampus to a normal level. These results suggest that CAPE is a promising tool for therapy of mood disorders through activation of the hippocampal signaling cascade.

[*Cell Mol. Neurobiol.* **34**, 1199-1208 (2014)] [Lab. of Molecular Biology]

Imipramine ameliorates pain-related negative emotion via induction of brain-derived neurotrophic factor.

Seiko YASUDA, MitsuhiroYOSHIDA, Hirotaka YAMAGATA, Yasutake IWANAGA, Hiromi SUENAGA, Kozo ISHIKAWA, Masako NAKANO, Satoshi OKUYAMA, Yoshiko FURUKAWA, Shoei FURUKAWA*, Toshizo ISHIKAWA.

We aimed to characterize the antidepressant effects of imipramine (IMI) without analgesia based on brain-derived neurotrophic factor (BDNF)/TrkB-mediated signaling and gene expression in chronic pain. Present results show that IMI reduces pain-related negative emotion without influencing pain and that this effect is diminished by denervation of 5-hydroxytryptamine (5-HT) neurons and by anti-BDNF treatment. IMI also normalizes derangement of ERK/CREB coupling, which leads to induction of BDNF. This suggests a possible interaction between 5-HT and BDNF.

[*J. Clin. Biochem. Nutr.* **54**, 129-135 (2014)] [Lab. of Clinical Pharmaceutics]

The Involvement of Endoplasmic Reticulum Stress in Bile Acid-induced Hepatocellular Injury.

Tetsuo ADACHI*, Tomoyuki KAMINAGA, Hiroyuki YASUDA, Tetsuro KAMIYA and Hirokazu HARA

Secondary bile acids produced by enteric bacteria accumulate to high levels in the enterohepatic circulation and may contribute to the pathogenesis of hepatocellular injury. Relative hydrophobicity has been suggested to be an important determinant of the biological properties of these compounds, although the mechanism by which bile acids induce pathogenesis is not fully understood. On the other hand, endoplasmic reticulum (ER) stress has been shown to be involved in the induction and development of various pathogenic conditions. In this report, we demonstrated that the intensities of cytotoxicity and ER stress in HepG2 cells triggered by the bile acids tested were largely dependent on their hydrophobicity. In conclusion, our study demonstrated that bile acids induced ER stress, which in turn stimulated apoptosis in HepG2 cells, in a hydrophobicity-dependent manner.

[*Biol. Pharm. Bull.* **37**, 1042-1049 (2014)] [Lab. of Clinical Pharmaceutics]

Newly Synthesized 'Hidabeni' Chalcone Derivatives Potently Suppress LPS-Induced NO Production *via* Inhibition of STAT1, but not NF-κB, JNK, and p38, Pathways in Microglia.

Hirokazu HARA*, Ryoko IKEDA, Masayuki NINOMIYA, Tetsuro KAMIYA, Mamoru KOKETSU and Tetsuo ADACHI

In this study, to explore chalcone derivatives with potent nitric oxide (NO) inhibitory activity, we synthesized ten compounds based on 'hidabeni' chalcone and examined their effects on LPS-triggered inducible NO synthase (iNOS) expression and NO production. Compounds C4 and C10 potently inhibited NO production. C4 and C10 suppressed LPS-induced iNOS expression via the inhibition of STAT1, but not NF-κB, JNK, and p38, pathways. C4 and C10 also suppressed LPS-induced expression of interferon regulatory factor 1 (IRF-1), which is an important transcription factor involved in iNOS expression. Our findings indicate that these chalcone derivatives are candidate compounds for preventing microglia-mediated neuroinflammation.

[*Hypertension* **114**, e03287 (2014)] [Lab. of Clinical Pharmaceutics]

Plasma Levels of Nitric Oxide Metabolites are Markedly Reduced in Normotensive Men with Electrocardiographically Determined Left Ventricular Hypertrophy.

Fumihiko KAMEZAKI, Masato TSUTSUI, Masao TAKAHASHI, Shinjo SONODA, Tatsuhiko KUDO, Yoshihisa FUJINO, Tetsuo ADACHI*, Haruhiko ABE, Masaaki TAKEUCHI, Toshihiko MAYUMI and Yutaka OTSUJI

In this study, we tested our hypothesis that normotensive subjects with ECG-LVH have reduced nitric oxide production. A total of 840 Japanese male workers were enrolled, and 579 eligible subjects were studied. ECG-LVH was assessed according to the Sokolow-Lyon voltage criteria and the Cornell voltage-duration product. The median level of plasma NOx (nitrite plus nitrate), a marker of systemic nitric oxide production, was markedly lower in the normotensive subjects with ECG-LVH (n=73) than in those without (n=506), and the clinical characteristics were significantly different between the 2 groups (each $P<0.05$).

[*Int. J. Anal. Bio- Sci.* **2**, 155-162 (2014)] [Lab. of Clinical Pharmaceutics]

EC-SOD Levels in Pre-dialysis Sera and the Relationship with HOMA-R.

Yojiro MAEHATA, Kaori MAEHATA, Tetsuo ADACHI*, Akira TANAKA, Naoko IKOSHI, Naotaka KURODA, Naoya KISHIKAWA, Teruo SHIBA, Makoto MATSUSHITA, Takaharu YANAGISAWA, Eisuke MAEHATA and Hiroji SHIMOMURA

The study patients were characterized as having diabetic nephropathy, which was renal insufficiency with glomerular deterioration in the majority of cases. Focusing on a combination of extracellular SOD (EC-SOD) and insulin resistance index (HOMAR) as a new marker in dialysis samples (N = 48), we examined its usefulness as an index of disease status. The results yielded a correlation coefficient of $r = 0.740$ ($p < 0.001$) and regression equation $y = 22.3x + 108.1$, which indicated usefulness.

[*Fitoterapia* **92**, 9-15 (2014)] [Lab. of Pharmaceutics]

Anti-androgenic Activity of Hydroxyxanthones in Prostate Cancer LNCaP Cells.

Toshinobu SHAKUI, Kazuhiro IGUCHI, Tetsuro ITO, Masako BABA, Shigeyuki USUI*, Masayoshi OYAMA, Hideki TOSA, Munekazu IINUMA and Kazuyuki HIRANO

We reported hydroxyxanthones from a plant extract act as anti-androgens in androgen receptor (AR)-positive prostate cancer LNCaP cells. Anti-androgenic activity of the ethanol extract from *Garcinia subelliptica* was observed in a luciferase assay using LNCaP/MMTV cells. HPLC-based activity profiling followed by a chemical library-based assay strategy enabled the rapid identification of several active principles bearing a xanthone core substituted with hydroxyl and isoprenyl groups. Among the active compounds, 2-(1,1-dimethyl-allyl)-1,4,5,6-tetrahydroxyxanthone was identified as a potent inhibitor of AR transcriptional activity. A quantitative RT-PCR analysis revealed that treatment with the compound resulted in a significant reduction in AR-induced gene (*KLK3*) expression. Hydroxyxanthone may be a possible candidate for the development of a new anti-androgenic molecule.

[*Yakugaku Zasshi* **134**, 575-580 (2014)] [Lab. of Pharmaceutics]

The Investigation of Understanding and Comfort of Patients Taking Divigel® 1 mg.

Midori SODA*, Kaori OGAWA, Yaeko HARADA, Suzuko KAWAMOTO, Miyoko TANAKA, Mayuko YAMAGUCHI, Chie TAKAHASHI, Asako UENO, Takashi OSADA, Akiko OGURI and Keiko YAMAMURA

Hormone replacement therapy (HRT) can improve the quality of life (QOL) of patients with menopausal symptoms. In this study, we investigated the understanding of medicines and diseases. Responses of 37 patients taking estradiol gel (Divigel®1mg) indicated that 70% of patients failed to use the gel as prescribed, and they had poor knowledge of both the sites where the gel shouldn't be applied and appropriate measures to take if having forgotten to apply the gel (43% and 11% correct understanding, respectively). Accordingly, pharmacists should facilitate proper adherence to HRT to improve and maintain women's QOL in the perimenopausal period, necessitating they actively provide pharmaceutical care such as preparing useful instructions patients can repeatedly use and periodically checking patients' understanding of their HRT medications.

[*Jpn J. Pharm Health Care Sci.* **40**, 85-93 (2014)] [Lab. of Pharmaceutics]

Development and Implementation System of the Initial Dose Setting for Vancomycin in the Night Shift.

Hiroko HAYASHI, Takashi NIWA, Shuri TAKEICHI, Yoshinori IMANISHI, Yuki TONOGAI, Shinji OKAYASU, Kiyoyuki KITAICHI*, Kimio YASUDA, Nobuo MURAKAMI and Yoshinori ITOH

The aim of this study was to fill the evidence-practice gap by using a newly developed procedure manual for planning the vancomycin dosing schedule and verifying the impact of the implementation of the initial dose setting for vancomycin. 25 patients received an individual dose regimen using the present procedure (intervention group) and 23 patients taking the conventional dose by prescriber (non-intervention group) were enrolled. Clinical efficacy after 3 days of treatment was significantly superior, and the duration for 50% reduction of C-reactive protein and the duration for the reduction in body temperature to <37℃ were significantly shorter in the intervention group as compared with those in the non-intervention group. These findings suggest the present implementation system was useful for promotion of initial dose setting for vancomycin.

[*Mol. Brain.* **7**, 31 (2014)] [Lab. of Pharmaceutics]

Comprehensive Behavioral Study of mGluR3 Knockout Mice: Implication in Schizophrenia Related Endophenotypes.

Ryuta FUJIOKA, Takenobu NII, Akiko IWAKI, Atsushi SHIBATA, Isao ITO, Kiyoyuki KITAICHI*, Masatoshi NOMURA, Satoko HATTORI, Keizo TAKAO, Tsuyoshi MIYAKAWA and Yasuyuki FUKUMAKI

We generated metabotropic glutamate receptor 3 (mGluR3) knockout (KO) mice and conducted comprehensive behavioral analyses. We assessed long-term potentiation (LTP) in the CA1 region in the hippocampi of KO and wild-type mice, and observed no differences in the amplitude of LTP between the two genotypes. In addition, we performed *in vivo* microdialysis measurements of extracellular dopamine in the nucleus accumbens, and observed enhancements in the methamphetamine-induced release of dopamine in KO mice. These results demonstrate that a disturbance in the glutamate-dopamine interaction may be involved in the pathophysiology of schizophrenia-like behavior, such as hyperactivity in mGluR3 KO mice.

[*Oncol. Lett.* **7**, 1665-1668 (2014)] [Lab. of Pharmaceutics]

Effects of 14 frequently Used Drugs on Prostate-specific Antigen Expression in Prostate Cancer LNCaP Cells.

Kazuhiro IGUCHI, Maki HASHIMOTO, Masafumi KUBOTA, Shuji YAMASHITA, Mitsuhiro NAKAMURA, Shigeyuki USUI*, Tadashi SUGIYAMA and Kazuyuki HIRANO

The levels of prostate-specific antigen (PSA) are influenced by a number of drugs, such as non-steroidal anti-inflammatory drugs and statins. In the present study, the drugs prescribed to patients on a repeat prescription collected at the pharmacy of the Gifu Pharmaceutical University were examined for their effects on the levels of PSA expression in LNCaP cells. Among the 14 drugs investigated, betamethasone and dexamethasone was found to increase the levels of PSA mRNA expression in the LNCaP cells. This betamethasone-induced expression was mediated, at least in part, through androgen receptor transcriptional activation. Therefore, it would be interesting to examine whether the serum PSA levels in prostate cancer patients are influenced by betamethasone.

[*Anticancer Res.* **34**, 7271-7277 (2014)] [Lab. of Pharmaceutics]

Polaprezinc Prevents Oral Mucositis in Patients Treated with High-dose Chemotherapy Followed by Hematopoietic Stem Cell Transplantation.

Hiroko HAYASHI, Ryo KOBAYASHI, Akio SUZUKI, Masashi ISHIHARA, Nobuhiko NAKAMURA, Junichi KITAGAWA, Nobuhiro KANEMURA, Senji KASAHARA, Kiyoyuki KITAICHI*, Takeshi HARA, Hisashi TSURUMI, Hisataka MORIWAKI and Yoshinori ITOH

We investigated whether polaprezinc in sodium alginate suspension (P-AG) prevents oral mucositis in patients with hematological malignancy receiving high-dose chemotherapy and radiotherapy followed by hematopoietic stem cell transplantation (HSCT). P-AG dramatically reduced the incidence of oral mucositis as compared to the control group treated with azulene gargle and pain associated with oral mucositis. On the other hand, P-AG had no influence on the incidence of other adverse events, tumor remission rate or the survival rate. Therefore, P-AG was found to be highly effective in preventing oral mucositis.

[*Neurology* **82**, 705-712 (2014)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Evaluation of *SLC20A2* Mutations that Cause Idiopathic Basal Ganglia Calcification in Japan.

Megumi YAMADA, Masaki TANAKA, Mari TAKAGI, Seiji KOBAYASHI, Yoshiharu TAGUCHI, Shutaro TAKASHIMA, Kortaro TANAKA, Tetsuo TOUGE, Hiroyuki HATSUTA, Shigeo MURAYAMA, Yuichi HAYASHI, Masayuki KANEKO, Hiroyuki ISHIURA, Jun MITSUI, Naoki ATSUTA, Gen SOBUE, Nobuyuki SHIMOZAWA, Takashi INUZUKA, Shoji TSUJI and Isao HOZUMI*

To investigate the clinical, genetic, and neuroradiologic presentations of idiopathic basal ganglia calcification (IBGC) in a nationwide study in Japan. Six new mutations in SLC20A2 were found in patients with IBGC: 4 missense mutations, 1 nonsense mutation, and 1 frameshift mutation. Four of them were familial cases and 2 were sporadic cases in our survey. The members in the families with the same mutation had similar patterns of calcification in the brain and the affected members showed similar clinical manifestations.

[*Plos One* **9**, e105435 (2014)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Ezrin Mediates Neuritogenesis via Down-regulation of RhoA Activity in Cultured Cortical Neurons.

Yosuke MATSUMOTO, Masatoshi INDEN*, Atsushi TAMURA, Ryou HATANO, Sachiko TSUKITA and Shinji ASANO

Ezrin, a member of Ezrin/Radixin/Moesin (ERM) proteins links between membrane proteins and actin cytoskeleton, and contributes to maintenance of cellular function and morphology. In cultured hippocampal neurons, suppression of both radixin and moesin showed deficits in growth cone morphology and neurite extensions. We demonstrated that the cultured cortical neurons prepared from the Vil2(kd/kd) mice embryo exhibited impairment of neuritogenesis. Moreover, we observed increased RhoA activity and phosphorylation of myosin light chain 2 (MLC2), as a downstream effector of RhoA in the Vil2(kd/kd) neurons. In addition, inhibition of Rho kinase and myosin II rescued the impairment of neuritogenesis in the Vil2(kd/kd) neurons. These data altogether suggest a novel role of ezrin in the neuritogenesis of the cultured cortical neurons through down-regulation of RhoA activity.

[*Sci*. *Rep*. **4**, 7283 (2014)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

The Homeobox Gene DLX4 Promotes Generation of Human Induced Pluripotent Stem Cells.

Naritaka TAMAOKI, Kazutoshi TAKAHASHI, Hitomi AOKI, Kazuki IIDA, Tomoko KAWAGUCHI, Daijirou HATAKEYAMA, Masatoshi INDEN*, Naoyuki CHOSA, Akira ISHISAKI, Takahiro KUNISADA, Toshiyuki SHIBATA, Naoki GOSHIMA, Shinya YAMANAKA and Ken-ichi TEZUKA

The reprogramming of somatic cells into iPSCs by defined transcription factors has been a well-established technique and will provide an invaluable resource for regenerative medicine. However, the low reprogramming efficiency of human iPSC is still a limitation for clinical application. Here we showed that the reprogramming potential of human dental pulp cells (DPCs) obtained from immature teeth is much higher than those of mature teeth DPCs. Furthermore, immature teeth DPCs can be reprogrammed by OCT3/4 and SOX2, conversely these two factors are insufficient to convert mature teeth DPCs to pluripotent states. Our findings indicate that DLX4 can functionally replace c-MYC and supports efficient reprogramming of immature teeth DPCs.

[*Toxicol Sci.* **139**, 121-132 (2014)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

The Ah Receptor Recruits IKK*α* to its Target Binding Motifs to Phosphorylate Serine-10 in Histone H3 Required for Transcriptional Activation.

Hisaka KURITA*, Michael SCHNEKENBURGER, Jerald L. OVESEN, Ying XIA and Alvaro PUGA

Aryl hydrocarbon receptor (AHR) activation by xenobiotic ligands such as TCDD is key to their toxicity. Following activation and nuclear translocation, AHR heterodimerizes with the ARNT and binds to AHR response elements (AhREs) in the promoter of target genes, such as Cyp1a1. Previously, we showed that concomitant with AHR binding, histone H3 in the Cyp1a1 promoter AhRE cluster became phosphorylated in serine-10 (H3S10), suggesting that the ligand-activated AHR recruited kinases to the AhRE to phosphorylate this residue. Our results showed complexes of AHR, ARNT, and IKKα could be coimmunoprecipitated from nuclei of TCDD treated Hepa1c1c7 and IKKα knockdown inhibited H3S10 phosphorylation in the Cyp1a1 enhancer. We conclude that AHR recruits IKKα to the promoter of its target genes and that AHR-mediated H3S10 phosphorylation.

[*Invest Ophthalmol Vis Sci.* **55**, 7652-7661 (2014)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Eyelid Closure in Embryogenesis is Required for Ocular Adnexa Development.

Qinghang MENG, Maureen MONGAN, Vinicius CARREIRA, Hisaka KURITA*,
Chia-yang LIU, Winston KAO and Ying XIA

Mammalian eye development requires temporary fusion of the upper and lower eyelids in embryogenesis. Failure of lid closure in mice leads to an eye open at birth phenotype. Many genetic mutant strains develop this phenotype. Our results showed some eye abnormalities, such as smaller lens in the Map3k1-null mice and Harderian gland hypoplasia in the Dkk2-null mice, whereas other abnormalities were seen in all mutants examined. The common defects included corneal erosion/ulceration, meibomian gland hypoplasia, truncation of the eyelid tarsal muscles, failure of levator palpebrae superioris extension into the upper eyelid and misplacement of the inferior oblique muscle and inferior rectus muscle. In addition to providing a protective barrier for the ocular surface, eyelid closure in embryogenesis is required for the development of ocular adnexa, including eyelid and extraocular muscles.

[*J. Biol. Chem.* **289**, 10045-10056 (2014)] [Lab. of Microbiology & Immunology]

Threonine 680 Phosphorylation of FLJ00018/PLEKHG2, a Rho Family-specific Guanine Nucleotide Exchange Factor, by Epidermal Growth Factor Receptor Signaling Regulates Cell Morphology of Neuro-2a Cells.

Katsuya SATO, Tsuyoshi SUGIYAMA*, Takahiro NAGASE, Yukio KITADE and Hiroshi UEDA

FLJ00018/PLEKHG2 (F018) is a guanine nucleotide exchange factor for the small GTPases Rac and Cdc42 and mediates actin cytoskeleton reorganization. The function of F018 is regulated by the interaction of heterotrimeric G protein Gβγ subunits. In this study we show that F018 is phosphorylated and activated by β1-AR stimulation-induced EGFR transactivation, and also by direct EGFR stimulation. We identified that Thr-680 is phosphorylated by EGFR stimulation thourgh Ras/MAPK pathway and that the mutant showed a limited response of the Neuro-2a cell morphology to EGF stimulation. Our results provide evidence that stimulation of the Ras/MAPK pathway by EGFR results in F018 phosphorylation at Thr-680, which in turn controls changes in cell shape.

[*J. Gen. Virol.* **95**, 1376-1382 (2014)] [Lab. of Microbiology & Immunology]

Guinea Pig Cytomegalovirus GP129/131/133, Homologs of Human Cytomegalovirus UL128/130/131A, are Required for Viral Entry into Monocytes and Macrophages.

Souichi YAMADA, Saki FUKUCHI, Kaede HASHIMOTO, Yoshiko FUKUI, Mihoko TSUDA, Michiyo KATAOKA,
Harutaka KATANO and Naoki INOUE*

The GP129, GP131 and GP133 genes of guinea pig cytomegalovirus (GPCMV) are homologues of human CMV UL128, UL130 and UL131A, respectively, which are essential for infection of endothelial and epithelial cells, and for viral transmission to leukocytes. Previously we demonstrated that a GPCMV strain lacking the 1.6 kb locus that contains these genes had a growth defect in animals. Here, we demonstrated that the WT strain, but not the 1.6 kb-deleted one, formed capsids in macrophages. To understand the mechanism, we prepared GPCMV strains defective in each of these genes, and found that they were all essential for the infection of macrophages but not of fibroblasts, suggesting the macrophage tropism as a determinants for viral dissemination in vivo.

[*Ann. Clin. Transl. Neurol.* **1**, 570-588 (2014)] [Lab. of Microbiology & Immunology]

Aberrant Fetal Macrophage/Microglial Reactions to Cytomegalovirus Infection.

Makiko SAKAO-SUZUKI, Hideya KAWASAKI, Taisuke AKAMATSU, Shiori MEGURO, Hiroaki MIYAJIMA
H, Toshihide IWASHITA, Yoshihiro TSUTSUI, Naoki INOUE* and Isao KOSUGI

To investigate innate immunity to cytomegalovirus (CMV) infection and its effects on cerebral corticogenesis, pregnant mice were intraplacentally infected with murine CMV (MCMV). MCMV antigens were found frequently in perivascular macrophages, and subsequently in neural stem/progenitor cells (NSPCs). Infection increased expression of inducible nitric oxide synthase and proinflammatory cytokines, resulting in infiltration of activated macrophages. In addition to the infected area, the numbers of both meningeal macrophages and parenchymal microglia increased even in the uninfected areas of brain due to recruitment of their precursors from other sites. MCMV infection globally disrupted the self-renewal of NSPCs. Thus, brain macrophages are crucial for innate immunity during infection in the fetal brain, while their aberrant recruitment may adversely impact on the stemness of NSPCs.

[*J. Perinatal. Med.* **42**, 755-759 (2014)] [Lab. of Microbiology & Immunology]

The IgG Avidity Value for the Prediction of Congenital Cytomegalovirus Infection in a Prospective Cohort Study.

Yasuhiko EBINA, Toshio MINEMATSU Ayako SONOYAMA, Ichiro MORIOKA, Naoki INOUE*, Shinya TAIRAKU, Satoshi NAGAMATA, Kenji TANIMURA, Mayumi MORIZANE, Masashi DEGUCHI and Hideto YAMADA

The maternal IgG avidity value for the prediction of congenital cutomegaloviru (CMV) infection was analyzed. Of 759 CMV IgG-positive women, 14 delivered newborns with congenital CMV infection. CMV IgG avidity indices in the congenital infection group (median 35.1%) were significantly lower than those in the non-congenital infection group (70.4%). A cutoff value of <40% IgG avidity index with 96.1% specificity and 64.3% sensitivity for congenital infection was determined. The highest sensitivity (88.9%), 96.2% specificity, 27.6% positive predictive value, 99.8% negative predictive value, and 96.1% accuracy were found when IgGavidity was measured in <28 weeks of gestation.

[*Brain & Dev*. **36**, 10-15 (2014)] [Lab. of Microbiology & Immunology]

Quantitative Evaluation of Ventricular Dilatation using Computed Tomography in Infants with Congenital Cytomegalovirus Infection.

Kiyomi MATSUO, Ichiro MORIOKA, Mai ODA, Yoko KOBAYASHI, Yuji NAKAMACHI, Seiji KAWANO, Miwako NAGASAWKA, Tsubasa KODA, Tomoyuki YOKOTA, Satoru MORIKAWA, Akihiro MIWA, Akio SHIBATA, Toshio MINEMATSU, Naoki INOUE*, Hideto YAMADA and Kazumoto IIJIMA

To determine the risk factor for hearing impairment early in life due to congenital cytomegalovirus (CMV) infection, ventricle sizes of 21 uninfected infants and those of congenitally infected 11 and 8 infants without and with abnormal auditory brainstem response (ABR), respectively, were assessed by calculating Evans' index (EI) and lateral ventricle width/hemispheric width (LVW/HW) ratio on brain computed tomography (CT) images. EI and LVW/HW ratio were significantly higher in the abnormal ABR group than the other two groups. LVW/HW ratio had a more association with abnormal ABR than EI.

[*J. Infec. Dis.* **209**, 1573-1584 (2014)] [Lab. of Microbiology & Immunology]

Idiopathic Intrauterine Growth Restriction Caused by Cytomegalovirus Infection and Associated Placental Pathology.

Lenore PEREIRA, Matthew PETITT, Alex FONG, Mitsuru TSUGE, Takako TABATA, June FANG-HOOVER, Ekaterina MAIJI, Martin ZYDEK, Yan ZHOU, Naoki INOUE*, Sanam LOGAHVI, Samuel PEPKOWITZ, Lawrence M. KAUVAR and Dotun OGUNYEMI

To investigate possible underlying congenital cytomegalovirus (CMV) infection for idiopathic intrauterine growth restriction (IUGR), maternal and cord sera and placentas from 19 pregnancies, including 9 normal, 7 with IUGR, and 3 with preeclampsia, were analyzed. Among 7 IUGR cases, 2 primary and 3 recurrent CMV infections were identified. CMV replicated in glandular epithelium and lymphatic endothelium in the decidua, cytotrophoblasts, and smooth muscle cells in blood vessels of floating villi and the chorion, suggesting impairment of placental development and function.

[*Virus Res.* **179**, 241-246 (2014] [Lab. of Microbiology & Immunology]

The Highly Conserved HCMV *UL136* ORF Encodes Multiple Protein Isoforms Localizing in the Golgi Apparatus.

Huanan LIAO, Jung-Hyun LEE, Rikita KONDO, Marei KATATA, Ken-Ichi IMADOME, Kenji MIYADO, Naoki INOUE*, Shigeyoshi FUJIWARA and Hiroyuki NAKAMURA

The *UL133-UL138* gene locus of the human cytomegalovirus (HCMV) genome is considered to play certain roles in viral replication, dissemination and latency in a host cell type-dependent manner. Here we characterized UL136 products by preparing a polyclonal antibody against *UL136* products (pUL136). The anti-pUL136 antibody specifically recognized at least five protein isoforms of 29–17 kDa both in infected cells and in cells transfected with a construct expressing pUL136. The putative transmembrane domain of pUL136 was required for localization in the Golgi apparatus, and multiple AUG codons in the gene contributed to the generation of isoforms of the protein.

[*Microbiol. Immunol.* **58**, 72-75 (2014)] [Lab. of Microbiology & Immunology]

The Presence of Antibodies against the AD2 Epitope of Cytomegalovirus Glycoprotein B is Associated with Acute Rejection after Renal Transplantation.

Kei ISHIBASHI, Tadahiko TOKUMOTO, Hiroki SHIRAKAWA, Toshiki OGURO, Tomohiko YANAGIDA, Norio TAKAHASHI, Masanori NOMIYA, Nobuhiro HAGA, Ken AIKAWA, Kazunari TANABE, Naoki INOUE*, Yoshiyuki KOJIMA and Tatsuo SUZUTANI

The aim of this study was to evaluate the association between antibodies against cytomegalovirus (CMV) glycoprotein B (gB) and acute rejection after transplantation. Seventy-seven consecutive renal transplant recipients in a D + /R+ setting were studied. Biopsy-proven rejection occurred in 35% of the recipients. Among these recipients, 85% had antibodies against CMV gB. The rate of acute rejection was significantly higher in recipients with antibodies against gB than in those without them. Antibodies against gB can be a useful predictor of acute rejection in renal transplant recipients in a D + /R+ setting.

[*J. Dermatol.* 41, 181-182 (2014)] [Lab. of Microbiology & Immunology]

Unusually extensive disseminated herpes zoster with multiple ulcer formation in a methotrexate-treated rheumatoid arthritis patient.

Risa KAKUTA, Utako OHKATA, Takeru FUNAKOSHI, Yumi FUJIO, Naoki INOUE*, Shinichi TAKAHASHI, Masayuki AMAGAI and Manabu OHYAMA

An 84-year-old Japanese woman with vesicles/blisters and severely painful ulcers, which started to spread from the left leg 1.5 month prior to her visit, was referred to our clinic. Her medical history included rheumatoid arthritis treated with methotrexate 5mg/week, mizoribine 100mg/day and prednizolone 5mg/day for 20years and four episodes of herpes zoster (HZ). The swabs obtained from her vesicles detected varicella zoster virus (VZV). Despite intensive antiviral treatment, the patient developed VZV-viremia and eventually died. Virological investigation denied the possible emergence of antiviral resistant virus. This case emphasizes the necessity of preemptive management of VZV.

[*J. Clin. Virol.* **61**, 448-452 (2014)] [Lab. of Microbiology & Immunology]

Evidence for Human Herpesvirus-6B Infection of Regulatory T-cells in Acute Systemic Lymphadenitis in an Immunocompetent Adult with the Drug Reaction with Eosinophilia and Systemic Symptoms Syndrome: A Case Report.

Sohtaro MINE, Koyu SUZUKI, Yuko SATO, Hitomi FUKUMOTO, Michiyo KATAOKA, Naoki INOUE*, Chiho OHBAYASHI, Hideki HASEGAWA, Tetsutaro SATA T, Masashi FUKUYAMA and Harutaka KATANO

A medication to treat respiratory symptoms caused a fatal case of drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome with human herpesvirus-6B (HHV-6B)-associated lymphadenitis and virus-associated hemophagocytic syndrome. The lymph node structure was disrupted with infiltration of large lymphocytes containing HHV-6B. The infected cells were CD3+CD4+CD25+FoxP3+ T cells, suggesting a significant role of HHV-6 infection of regulatory T-cells in the pathogenesis of DRESS syndrome.

[*Clin. Infec. Dis.* **59**, 545-548 (2014)] [Lab. of Microbiology & Immunology]

Molecular and Virological Evidence of Viral Activation from Chromosomally Integrated HHV-6A in a Patient with X-SCID.

Akifumi ENDO, Ken WATANABE, Tamae OHYE, Kyoko SUZUKI, Tomoyo MATSUBARA, Norio SHIMIZU, Hiroki KURAHASHI, Tetsushi YOSHIKAWA, Harutaka KATANO, Naoki INOUE*, Kohsuke IMAI, Masatoshi TAKAGI, Tomohiro MORIO and Shuki MIZUTANI

It has been unclear whether chromosomally integrated human herpesvirus 6 (ciHHV-6) can be activated with pathogenic effects on the human body. We present molecular and virological evidence of ciHHV-6A activation in a patient with X-linked severe combined immunodeficiency. These findings have significant implications for the management of patients with ciHHV-6.

[*FASEB J.*, **28**, 440-452 (2014)] [Lab. of Biochemistry]

Antiobese function of platelet-activating factor: increased adiposity in platelet-activating factor receptor-deficient mice with age.

Junko SUGATANI, Satoshi SADAMITSU, Masahiko YAMAGUCHI, Yasuhiro YAMAZAKI, Ryoko HIGA, Yoshiki HATTORI, Takahiro UCHIDA, Akira IKARI*, Wataru SUGIYAMA, Tatsuo WATANABE, Satoshi ISHII, Masao MIWA and Takao SHIMIZU

Platelet-activating factor receptor (PAFR)-deficient mice developed a more severe obese state characterized by higher body mass (~25%) and epididymal fat mass (~55%) with age than that of wild-type (WT) littermates. PAFR-deficient mice did not show changes in the expression of critical genes involved in anabolic and catabolic metabolism in adipose, liver, and muscle tissues between 6 and 36 wk. We found that obesity in PAFR-deficient mice resulted from impaired brown adipose tissue (BAT) activity and suggest that the antiobese function of PAF occurs through β3- adrenergic receptor/uncoupling protein 1 expression in BAT.

[*Biol. Open.*, **3**, 12-21 (2014)] [Lab. of Biochemistry]

Functional coupling of chloride-proton exchanger ClC-5 to gastric H^+,K^+-ATPase.

Yuji TAKAHASHI, Takuto FUJII, Kyosuke FUJITA, Takahiro SHIMIZU, Taiga HIGUCHI, Yoshiaki TABUCHI, Hisato SAKAMOTO, Ichiro NAITO, Koji MANABE, Shinichi UCHIDA, Sei SASAKI, Akira IKARI*, Kazuhiro TSUKADA and Hideki SAKAI

It has been reported that chloride-proton exchanger ClC-5 and vacuolar-type H^+-ATPase are essential for endosomal acidification in the renal proximal cells. Here, we found that ClC-5 is expressed in the gastric parietal cells which secrete actively hydrochloric acid at the luminal region of the gland, and that it is partially localized in the intracellular tubulovesicles in which gastric H^+,K^+-ATPase is abundantly expressed. ClC-5 was co-immunoprecipitated with H^+,K^+-ATPase, but not with endogenous Na^+,K^+-ATPase. Our results suggest that ClC-5 and H^+,K^+-ATPase are functionally associated and that they may contribute to gastric acid secretion.

[*J. Biol. Chem.*, **289**, 13112-13123 (2014)] [Lab. of Biochemistry]

Tight junctional localization of claudin-16 is regulated by syntaxin 8 in renal tubular epithelial cells.

Akira IKARI*, Chie TONEGAWA, Ayumi SANADA, Toru KIMURA, Hideki SAKAI, Hisayoshi HAYASHI, Hajime HASEGAWA, Masahiko YAMAGUCHI, Yasuhiro YAMAZAKI, Satoshi ENDO, Toshiyuki MATSUNAGA and Junko SUGATANI.

Claudin-16 (CLDN16) regulates the paracellular reabsorption of Mg^{2+} in the thick ascending limb of Henle's loop. However, the mechanism regulating the tight junctional localization of CLDN16 remains unknown. In yeast two-hybrid systems, CLDN16 bound to syntaxin 8 (STX8), a target soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor. An association between CLDN16 and STX8 was observed in rat renal homogenates and Madin-Darby canine kidney cells. Recycling assays indicated that STX8 siRNA decreased the trafficking of CLDN16 to the plasma membrane without affecting endocytosis. We suggest that STX8 mediates the recycling of CLDN16 and constitutes an important component of the CLDN16 trafficking machinery in the kidney.

[*Biochim. Biophys. Acta.*, **1843**, 2079-2088 (2014)] [Lab. of Biochemistry]

Nuclear distribution of claudin-2 increases cell proliferation in human lung adenocarcinoma cells.

Akira IKARI*, Ryo WATANABE, Tomonari SATO, Saeko TAGA, Shun SHIMOBABA, Masahiko YAMAGUCHI, Yasuhiro YAMAZAKI, Satoshi ENDO, Toshiyuki MATSUNAGA and Junko SUGATANI

Claudin-2 is expressed in human lung adenocarcinoma tissue and cell lines, although it is absent in normal lung tissue. However, the role of claudin-2 in cell proliferation and the regulatory mechanism of intracellular distribution remain undefined. Proliferation of human adenocarcinoma A549 cells was decreased by claudin-2 knockdown together with a decrease in the percentage of S phase cells. This knockdown decreased the expression levels of ZONAB and cell cycle regulators. Nuclear claudin-2 formed a complex with ZO-1, ZONAB, and cyclin D1. We suggest that nuclear distribution of claudin-2 is up-regulated by dephosphorylation and claudin-2 serves to retain ZONAB and cyclin D1 in the nucleus, resulting in the enhancement of cell proliferation in lung adenocarcinoma cells.

[*Drug Metab. Dispos.*, **42**, 1708-1718 (2014)] [Lab. of Biochemistry]

Threonine-290 regulates nuclear translocation of the human pregnane X receptor through its phosphorylation/dephosphorylation by Ca^{2+}/calmodulin-dependent protein kinase II and protein phosphatase 1.

Junko SUGATANI, Yoshiki HATTORI, Yuji NOGUCHI, Masahiko YAMAGUCHI, Yasuhiro YAMAZAKI and Akira IKARI*

The human pregnane X receptor (hPXR) is recognized as a xenobiotic-sensing nuclear receptor that transcriptionally regulates the gene expression of drug-metabolizing enzymes and transporters. Our study elucidates the mechanism by which the localization of hPXR is regulated through threonine-290. A phosphomimetic mutation at threonine-290 retained hPXR in the cytoplasm of HepG2, HuH6, and SW480 cells *in vitro* and the mouse liver *in vivo* even after treatment with rifampicin. We suggest that phosphorylation at threonine-290 by CaMKII may impair the function of hPXR by repressing its translocation to the nucleus.

[*Anticancer Drugs*, **25**, 868-877 (2014)] [Lab. of Biochemistry]

Induction of aldo-keto reductases (AKR1C1 and AKR1C3) abolishes the efficacy of daunorubicin chemotherapy for leukemic U937 cells.

Toshiyuki MATSUNAGA*, Ayano YAMAGUCHI, Yoshifumi MORIKAWA, Chihiro KEZUKA, Hiroaki TAKAZAWA, Satoshi ENDO, Ossama EL-KABBANI, Kazuo TAJIMA, Akira IKARI and Akira HARA

Development of daunorubicin (DNR) resistance upregulated expression of aldo-keto reductase (AKR) 1C1 and AKR1C3 in human leukemic U937 cells, and the contribution of the two AKRs toward the DNR sensitivity was assessed using gene expression and RNA-interference techniques and specific inhibitors. AKR1C1 and AKR1C3 did not interfere with the cell differentiation caused by DNR, whereas their upregulation facilitated reduction of the anticancer drug and a reactive oxygen species (ROS)-derived lipid aldehyde 4-hydroxy-2-nonenal. These results suggest crucial roles of AKR1C1 and AKR1C3 in the acquisition of DNR resistance of leukemic cells by metabolizing both DNR and cytotoxic aldehydes derived from ROS-linked lipid peroxidation.

[*Toxicol. Appl. Pharmacol.*, **278**, 180-189 (2014)] [Lab. of Biochemistry]

Exposure to 9,10-phenanthrenequinone accelerates malignant progression of lung cancer cells through up-regulation of aldo-keto reductase 1B10.

Toshiyuki MATSUNAGA*, Yoshifumi MORIKAWA, Mariko HAGA, Satoshi ENDO, Midori SODA, Keiko YAMAMURA, Ossama EL-KABBANI, Kazuo TAJIMA, Akira IKARI and Akira HARA

Here, we show that treatment of human lung cancer A549 cells with a diesel exhaust quinone 9,10-phenanthrenequinone (9,10-PQ) at its concentrations of 2 and 5 μM elevated the potentials for proliferation, invasion, metastasis and tumorigenesis, all of which were almost completely inhibited by an antioxidant N-acetyl-l-cysteine, inferring a crucial role of ROS in the overgrowth and malignant progression of the cells. The treatment with 9,10-PQ remarkably increased expression of aldo-keto reductase (AKR) member 1B10 and matrix metalloproteinase-2. The metalloproteinase upregulation was almost completely abolished by AKR1B10 inhibitors, inferring that AKR1B10 is a key regulator involved in overgrowth and malignant progression of lung cancer cells.

[*Free Radic. Res.*, **48**, 1371-1385 (2014)] [Lab. of Biochemistry]

Nitric oxide confers cisplatin resistance in human lung cancer cells through upregulation of aldo-keto reductase 1B10 and proteasome.

Toshiyuki MATSUNAGA*, Yukiko YAMAJI, Toshimasa TOMOKUNI, Hiromi MORITA, Yoshifumi MORIKAWA, Ayaka SUZUKI, Ayano YONEZAWA, Satoshi ENDO, Akira IKARI, Kazuhiro IGUCHI, Ossama EL-KABBANI, Kazuo TAJIMA and Akira HARA

We here show that nitric oxide (NO) reduces damage of human lung cancer A549 cells caused by cisplatin (CDDP) through the suppression of apoptotic alterations and elevated proteolytic activity of 26S proteasome, indicating the possibility that NO participates in mechanisms underlying acquisition of the chemoresistance. Treatment with CDDP and NO promoted the induction of aldo-keto reductases (AKR) 1B10 as well as peroxynitrite formation. Collectively, these results suggest the NO functions as a key regulator controlling AKR1B10 expression and 26S proteasome function leading to gain of the CDDP resistance.

[*Drug Metab. Dispos.*, **42**, 803-812 (2014)] [Lab. of Biochemistry]

Cloning and characterization of four rabbit aldo-keto reductases featuring broad substrate specificity for xenobiotic and endogenous carbonyl compounds: relationship with multiple forms of drug ketone reductases.

Satoshi ENDO*, Toshiyuki MATSUNAGA, Yuki ARAI, Akira IKARI, Kazuo TAJIMA, Ossama EL-KABBANI, Sigeru YAMANO, Akira HARA and Yukio KITADE

Multiple forms of reductases for several drug ketones were isolated from rabbit liver, but their interrelationship and physiologic roles remain unknown. We isolated cDNAs for four aldo-keto reductases (AKR1C30, AKR1C31, AKR1C32, and AKR1C33), which share high amino acid sequence identity with the partial sequences of two rabbit naloxone reductases. The four enzymes correspond to the multiple drug ketone reductases, and may function in the metabolisms of steroids, isatin and reactive carbonyl compounds, and bile acid synthesis.

[*Bioorg. Med. Chem.*, **22**, 5220-5233 (2014)] [Lab. of Biochemistry]

Synthesis of non-prenyl analogues of baccharin as selective and potent inhibitors for aldo-keto reductase 1C3.

Satoshi ENDO*, Dawei HU, Toshiyuki MATSUNAGA, Yoko OTSUJI, Ossama EL-KABBANI, Mohmad KANDEEL, Akira IKARI, Akira HARA, Yukio KITADE and Naoki TOYOOKA

Inhibitors of AKR1C3 are regarded as promising therapeutics for the treatment of prostatic and breast cancers. Baccharin was shown to be potent (K_i 56 nM) and highly isoform-selective inhibitor of AKR1C3. In this study, a series of derivatives of baccharin were synthesized, and their inhibitory activities for the enzyme were evaluated. Among them, two benzyl ether derivatives, **6m** and **6n**, showed an equivalent inhibitory potency to baccharin. The molecular docking of **6m** in AKR1C3 has allowed the design and synthesis of **14** with improved potency (K_i 6.4 nM) and selectivity comparable to baccharin. Additionally, **14** significantly decreased the cellular metabolism of androsterone and cytotoxic 4-oxo-2-nonenal by AKR1C3 at much lower concentrations than baccharin.

[*Biol. Pharm. Bull.*, **37**, 1848-1852 (2014)] [Lab. of Biochemistry]

Probing AKR1C30 and AKR1C31 with site-directed mutagenesis: identifying the roles of residues 54 and 56 in the binding of substrates and inhibitors.

Satoshi ENDO*, Yuki ARAI, Toshiyuki MATSUNAGA, Akira IKARI, Ossama EL-KABBANI, Akira HARA and Yukio KITADE

Five rabbit AKRs that participate in the reduction of drug ketones and ketosteroids have recently been cloned and characterized. Among them, AKR1C30 and AKR1C31 show the highest amino acid sequence identity of 91%, but markedly differ in their substrate specificity. AKR1C30 reduces ketotifen and naloxone, whereas AKR1C31 does not reduce the two drugs. Residue A54 is located adjacent to the catalytic residue Y55 of AKR1C30. When we performed the mutagenesis of A54 to the corresponding residue of AKR1C31, the A54L mutation produced an enzyme that had almost the same substrate specificity as AKR1C31. Thus, the difference in the properties between the two enzymes can be attributed to their residue difference at position 54.

[*Cell Rep.* **6**, 366-376 (2014)] [Lab. of Pharmacology]

Critical Role for Mast Cell Stat5 Activity in Skin Inflammation.

Tomoaki ANDO, Wenbin XIAO, Peisong GAO, Siavash NAMIRANIAN, Kenji MATSUMOTO, Yoshiaki TOMIMORI, Hong HONG, Hirotaka YAMASHITA*, Miho KIMURA, Jun-ichi KASHIWAKURA, Tissa R. HATA, Kenji IZUHARA, Michael F. GURISH, Axel ROERS, Nicholas M. RAFAELS, Kathleen C. BARNES, Colin JAMORA, Yuko KAWAKAMI, and Toshiaki KAWAKAM

Phospholipase C-β3 (PLC-β3)-deficient mice spontaneously developed eczematous skin lesions, in which hyperkeratosis and infiltration of inflammatory cells were shown with IgE elevation. The atopic dermatitis (AD)-like skin lesions depended on mast cell because *PLCb*$^{-/-}$; *Kit*$^{Wsh/Wsh}$ mice did not develop the phenomena. PLC-β3 inhibits the proliferation of hematopoietic stem cells and myeloid cells by interacting with signal transducer and activator of transcription 5 (Stat5). Mast cell-specific deletion of *Stat5* gene ameliorated the dermatitis. STAT5 phosphorylation and/or PLC-β3 expression may be clinical target for AD.

[*Biol. Pharm. Bull*. **37**, 1014-1020 (2014)] [Lab. of Pharmacology]

Collagen Gel Contraction Assay Using Human Bronchial Smooth Muscle Cells and Its Application for Evaluation of Inhibitory Effect of Formoterol.

Yusuke SAKOTA, Yuji OZAWA, Hirotaka YAMASHITA, Hiroyuki TANAKA and Naoki INAGAKI*

Normal human bronchial smooth muscle cells were embedded in collagen gel. The gels were contracted by stimulation of acetylcholine and histamine in a concentration-dependent manner. Formoterol, a long-acting β2-adrenoceptor agonist, inhibited histamine-induced collagen gel contraction. Fluticasone is steroidal preparation for inhalation. Although 10^{-10} M formoterol failed to inhibit gel contraction, it apparently inhibited the gel contraction after treatment with fluticasone for 4 and 12 hours. Additionally, 4 hour simultaneous pre-treatment with 10^{-8} M formoterol and fluticasone partially but significantly recovered the inhibitory effect of 10^{-8} M formoterol. The collagen gel contraction assay using human bronchial smooth muscle cells is useful for evaluating the effects of bronchodilating drugs.

[*Free Radic. Biol. Med.* **72**, 124-133 (2014)] [Lab. of Molecular Pharmacology]

Temporal Activation of Nrf2 in the Penumbra and Nrf2 Activator-mediated Neuroprotection in Ischemia-reperfusion Injury.

Toshinori TAKAGI, Akira KITASHOJI, Takao IWAWAKI, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Shinichi YOSHIMURA, Toru IWAMA and Hideaki HARA*

Oxidative stress plays a critical role in mediating tissue injury and neuron death during ischemia-reperfusion injury (IRI). The Keap1-Nrf2 defense pathway serves as a master regulator of endogenous antioxidant defense, and Nrf2 has been attracting attention as a target for the treatment of IRI. In this study, we evaluated Nrf2 expression in IRI using OKD (Keap1-dependent oxidative stress detector) mice and investigated the neuroprotective ability of an Nrf2 activator. Our findings indicate that earlier Nrf2 activation protects neurons, possibly via effects on astrocytes.

[*Cell Death Dis.* **5**, e1332 (2014)] [Lab. of Molecular Pharmacology]

Fluvoxamine Alleviates ER Stress *via* Induction of Sigma-1 Receptor.

Tsubasa OMI, Hitoshi TANIMUKAI, Daisuke KANAYAMA, Yukako SAKAGAMI, Shinji TAGAMI, Masayasu OKOCHI, Takashi MORIHARA, M. SATO, Kanta YANAGIDA, Akira KITASHOJI, Hideaki HARA*, Kazunori IMAIZUMI, Tangui MAURICE, Nathalie CHEVALLIER, Stephan MARCHAL, Masatoshi TAKEDA and Takashi KUDO

Fluvoxamine (Flv) is a selective serotonin reuptake inhibitor with a high affinity for sigma-1 receptor (Sig-1R). In the present study, we show that treatment of neuroblastoma cells with Flv induces Sig-1R expression by increasing ATF4 translation directly, through its own activation, without involvement of the PERK pathway. This suggests that Flv could be a feasible therapy for cerebral diseases caused by endoplasmic reticulum stress.

[*PLoS ONE* **9(8)**, 1315-1328 (2014)] [Lab. of Molecular Pharmacology]

Diabetes Mellitus Aggravates Hemorrhagic Transformation after Ischemic Stroke *via* Mitochondrial Defects Leading to Endothelial Apoptosis.

Keisuke MISHIRO, Takahiko IMAI, Sou SUGITANI, Akira KITASHOJI, Yukiya SUZUKI, Toshinori TAKAGI, Huayue CHEN, Yasunori OUMI, Kazuniro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

Chronic hyperglycemia aggravated hemorrhagic transformation after stroke through mitochondrial dysfunction and morphological alteration, partially via matrix metalloproteinase 9 activation, leading to caspase-dependent apoptosis of endothelial cells of diabetic mice. Mitochondria-targeting therapy may be a clinically innovative therapeutic strategy for diabetic complications in the future.

[*Neuroscience* **277**, 123-131, (2014)] [Lab. of Molecular Pharmacology]

Glycoprotein Nonmetastatic Melanoma Protein B (GPNMB) as a Novel Neuroprotective Factor in Cerebral Ischemia-reperfusion Injury.

Yusuke NAKANO, Yukiya SUZUKI, Toshinori TAKAGI, Akira KITASHOJI, Yoko ONO, Kazuhiro TSURUMA, Shinichi YOSHIMURA, Masamitsu SHIMAZAWA, Toru IWAMA and Hideaki HARA*

Glycoprotein nonmetastatic melanoma protein B (GPNMB) protected neurons against IRI, and phosphor-Akt and phosphor-ERK might be a part of the protective mechanisms, and that the neuroprotective effect of GPNMB was seemingly induced by the extracellular sequence of GPNMB. In conclusion, these findings indicate that GPNMB has neuroprotective effects against IRI, via phosphorylation of ERK1/2 and Akt, suggesting that GPNMB may be a therapeutic target for ischemia-reperfusion injuries.

[*Curr. Neurovasc. Res.* **11**, 302-311 (2014)] [Lab. of Molecular Pharmacology]

The Phosphodiesterase III Inhibitor Cilostazol Ameliorates Ethanol-induced Endothelial Dysfunction.

Toshinori TAKAGI, Keisuke MISHIRO, Masamitsu SHIMAZAWA, Shinichi YOSHIMURA, Toru IWAMA and Hideaki Hara*

Intracranial hemorrhage (ICH) remains a devastating disease, and heavy alcohol consumption is an underlying risk factor. The aim of this study was to study the mechanism of ethanol-induced endothelial cell damage and to evaluate the protective effect of cilostazol against ethanol-induced damage. Our results indicate that cilostazol protected endothelial cells against ethanol-induced endothelial dysfunction by inhibiting reactive oxygen spices-mediated activation of matrix metalloproteinase 9.

[*Pharmacol. Pharm.* **5**, 37-42 (2014)] [Lab. of Molecular Pharmacology]

Crocetin Prevents Amyloid $\beta_{1\text{-}42}$-induced Cell Death in Murine Hippocampal Cells.

Yuta YOSHINO, Mitsue ISHISAKA, Naofumi UMIGAI, Masamitsu SHIMAZAWA, Kazuhiro TSURUMA and Hideaki HARA*

Crocetin is an aglycon of carotenoid extracted by saffron stigmas (*Crocus sativus L.*) and known to have a potent anti-oxidative effect. In this study, we investigated the effect of crocetin on hippocampal HT22 cell death induced by $A\beta_{1\text{-}42}$. Furthermore, to clarify the mechanism underlying the protective effects of crocetin against $A\beta_{1\text{-}42}$- induced cell death, we measured reactive oxygen species (ROS) production by CM-H2DCFDA kit assay. Crocetin at 1 -10 μM protected HT22 cells against $A\beta_{1\text{-}42}$-induced neuronal cell death and decreased ROS production increased by $A\beta_{1\text{-}42}$. These results that crocetin has the potent neuroprotective effect against $A\beta_{1\text{-}42}$-induced cytotoxicity in hippocampal cells by attenuating oxidative stress, suggest that crocetin may provide a useful therapeutic strategy against Aβ-related disorders.

[*Neurosci. Lett.* **559**, 174-178 (2014)] [Lab. of Molecular Pharmacology]

SA4503, a Sigma-1 Receptor Agonist, Suppresses Motor Neuron Damage in *in Vitro* and *in Vivo* Amyotrophic Lateral Sclerosis Models.

Yoko ONO, Hirotaka TANAKA, Masafumi TAKATA, Yuki NAGAHARA, Yasuhiro NODA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Isao HOZUMI and Hideaki HARA*

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease. Recently, it has been reported that a mutation in the sigma-1 receptor causes juvenile ALS. Therefore, the function of the sigma-1 receptor may be important in the pathology of ALS. In the present study, we investigated the effect of SA4503, a sigma-1 receptor agonist, against in in vitro and in vivo ALS models. Our results indicate that SA4503 is effective in suppressing motor neuron degeneration and symptom progression in ALS.

[*Sci. Rep.* **4**:5223 (2014)] [Lab. of Molecular Pharmacology]

Damage of Photoreceptor-derived Cells in Culture Induced by Light Emitting Diode-derived Blue Light.

Yoshiki KUSE, Kenjiro OGAWA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

Our eyes are increasingly exposed to light from the emitting diode (LED) light of video display terminals (VDT) which contain much blue light. The present study aims to clarify the mechanism underlying blue LED light-induced photoreceptor cell damage. The LED light induced cell damage was wavelength-, but not energy-dependent and may cause more severe retinal photoreceptor cell damage than the other LED light.

[*Stem Cells Transl. Med.* **3**:42-53 (2014)] [Lab. of Molecular Pharmacology]

Progranulin, a Major Secreted Protein of Mouse Aadipose-derived Stem Cells, Inhibits Light-induced Retinal Degeneration.

Kazuhiro TSURUMA, Mika YAMAUCHI, Sou SUGITANI, Tomohiro OTSUKA, Yuta OHNO, Yuki NAGAHARA, Yuka IKEGAME, Masamitsu SHIMAZAWA, Shinichi YOSHIMURA, Toru IWAMA and Hideaki HARA*

Adipose tissue stromal vascular fraction contains mesenchymal stem cells, which show protective effects when administered to damaged tissues, mainly through secreted trophic factors. We examined the protective effects of adipose-derived stem cells (ASCs) and ASC-conditioned medium (ASC-CM) against retinal damage and identified the neuroprotective factors in ASC-CM. ASCs and mature adipocytes were isolated from mouse subcutaneous tissue. Our findings suggest that ASC-CM and progranulin have neuroprotective effects in the light-induced retinal-damage model. Progranulin may be a potential target for the treatment of the degenerative diseases of the retina.

[*Invest. Ophthalmol. Vis. Sci.* **55**, 6851-6860 (2014)] [Lab. of Molecular Pharmacology]

Role of Metallothioneins 1 and 2 in Ocular Neovascularization.

Shinsuke NAKAMURA, Masamitsu SHIMAZAWA, Yuki INOUE, Shinsuke TAKATA, Yasushi ITO, Kazuhiro TSURUMA, Tsunehiko IKEDA, Akiko HONDA, Masahiko SATOH and Hideaki HARA*

Purpose:The incidence of blindness is increasing, in part, because of the abnormal ocular neovascularization. Anti-VEGF therapies have yielded impressive results; however, they are not a cure for blindness. Recently, metallothioneins (MTs) 1 and 2 have been implicated in the process of angiogenesis. Therefore, we investigated whether MT 1 and MT 2 were also involved in ocular neovascularization. Methods:The concentrations of MT 1 and MT 2 (hereafter MT-1/2) were observed by ELISA. We examined the role of MT-1/2 in ocular neovascularization by using both an oxygen-induced retinopathy (OIR) model and a laser-induced choroidal neovascularization (CNV) model. These results indicate that MTs 1 and 2 are involved in retinal and choroidal neovascularization, and that MT-1/2 might be a new therapeutic target in diseases in which ocular angiogenesis is implicated.

[*PLoS ONE* **9**, e84387 (2014)] [Lab. of Molecular Pharmacology]

Systemic Simvastatin Rescues Retinal Ganglion Cells from Optic Nerve Injury Possibly through Suppression of Astroglial NF-κB Activation.

Seita MORISHITA, Hidehiro OKU, Taeko HORIE, Masahiro TONARI, Teruyo KIDA, Akiko OKUBO, Tetsuya SUGIYAMA, Shinji TAKAI, Hideaki HARA* and Tsunehiko IKEDA

Neuroinflammation is involved in the death of retinal ganglion cells (RGCs) after optic nerve injury. The purpose of this study was to determine whether systemic simvastatin can suppress neuroinflammation in the optic nerve and rescue RGCs after the optic nerve is crushed. We conclude that systemic simvastatin can reduce the death of RGCs induced by crushing the optic nerve possibly by suppressing astroglial NF-κB activation.

[*Exp. Eye Res.* **129**, 24-30 (2014)] [Lab. of Molecular Pharmacology]

Hydroxyl Radicals Cause Fluctuation in Intracellular Ferrous Ion Levels upon Light Exposure during Photoreceptor Cell Death.

Tomoyo IMAMURA, Tasuku HIRAYAMA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Hideko NAGASAWA, Hideaki HARA*

Iron accumulation is a potential pathogenic event often seen in age-related macular degeneration (AMD) patients. In this study, we focused on the relationship between AMD pathology and concentrations of ferrous ion, which is a highly reactive oxygen generator in biological systems. These results suggest that light exposure decreases ferrous ion levels and enhances iron uptake in photoreceptor cells. Ferrous ion may be involved in light-induced photoreceptor cell death through production of hydroxyl radicals.

[*J. Neurosci. Res.* **92**, 329-337 (2014)] [Lab. of Molecular Pharmacology]

Protective Effects of Placental Growth Factor on Retinal Neuronal Cell Damage.

Yuki INOUE, Masamitsu SHIMAZAWA, Shinsuke NAKAMURA, Tomoyo IMAMURA, Sou SUGITANI, Kazuhiro TSURUMA and Hideaki HARA*

Placental growth factor (PlGF) is a member of the vascular endothelial growth factor family. Although it has been reported that PlGF protects against neuronal damage in the brain, little is known about the effects of PlGF in the retina. Therefore, we investigated the effects of PlGF on retinal neuronal cells. Our data suggest that PlGF may be an important protective factor in the retina.

[*Eur. J. Pharmacol.* **738**, 74-82 (2014)] [Lab. of Molecular Pharmacology]

Tissue Kallikrein (Kallidinogenase) Protects against Retinal Ischemic Damage in Mice.

Tomomi MASUDA, Masamitsu SHIMAZAWA, Fumiya ISHIZUKA, Shinsuke NAKAMURA, Kazuhiro TSURUMA and Hideaki HARA*

Ocular ischemic syndrome is likely stem from retinal ischemia, and which causes visual disorder. The pathological mechanism of ocular ischemic syndrome is still unknown, therefore the optimal treatment for ocular ischemic syndrome remains to be established. Then, this study aimed to evaluate the effects of tissue-derived kallidinogenase in retinal ischemia protection in mice. Our findings suggest that kallidinogenase may prevent ischemia/reperfusion-induced retinal damage, might be through eNOS activation.

[*Biol. Pharm. Bull.* **37**, 424-430 (2014)] [Lab. of Molecular Pharmacology]

Protective Effect of SUN N8075, a Free Radical Scavenger, against Excessive Light-induced Retinal Damage in Mice.

Kazuki OJINO, Masamitsu SHIMAZAWA, Yuta OHNO, Tomohiro OTSUKA, Kazuhiro TSURUMA and Hideaki HARA*

Although dry age-related macular degeneration (AMD) is one of the major causes of blindness, no effective therapies are developed. In this study, we investigated the effects of SUN N8075, a radical scavenger with neuroprotective properties, against light-induced retinal damage used as the model of dry AMD in mice. Our findings suggest that the systemic administration of SUN N8075 has protective effects on excess light-induced photoreceptor degeneration, *via* inhibition of oxidative stress.

[*Chin. J. Pract. Ophthalmol.* **32**, 512-516 (2014)] [Lab. of Molecular Pharmacology]

Effect of Anti-glaucoma Ophthalmic Solutions on Cultured Human Conjunctival Cells.

Wenzhong LIN, Yan LU, Yan XU, Wei HE, Kazuhide KAWASE, Tetsuya YAMAMOTO, Masamitsu SHIMAZAWA and Hideaki HARA*

The aim of this study was to probe the effects of anti-glaucoma ophthalmic solutions containing preservatives on cultured human conjunictival cells. Anti-glaucoma ophthalmic solutions with preservative (benzalkonium chloride or benzododecinium bromide) may have defferent effect on cultured human conjunctival cells, and the ones with low or free preservative may have less effect on cultured human conjunctival cells.

[*J. Pharmacol. Sci.* **123**, 209-218 (2013)] [Lab. of Molecular Pharmacology]

The Protective Effects of a Dietary Carotenoid, Astaxanthin, against Light-induced Retinal Damage.

Tomohiro OTSUKA, Masamitsu SHIMAZAWA, Tomohiro NAKANISHI, Yuta OHNO, Yuki INOUE, Kazuhiro TSURUMA, Takashi ISHIBASHI and Hideaki HARA*

Dietary carotenoids exhibit various biological activities, including antioxidative activity. In particular, astaxanthin, a type of carotenoid, is well known as a powerful antioxidant. We investigated whether astaxanthin would protect against light-induced retinal damage. Our findings suggest that astaxanthin has protective effects against light-induced retinal damage via the mechanism of its antioxidative effect.

[*BMC Complement. Altern. Med.* **14**:120 (2014)] [Lab. of Molecular Pharmacology]

Protective Effects of Bilberry and Lingonberry Extracts against Blue Light-emitting Diode Light-induced Retinal Photoreceptor Cell Damage *in Vitro*.

Kenjirou OGAWA, Yoshiki KUSE, Kazuhiro TSURUMA, Saori KOBAYASHI, Masamitsu SHIMAZAWA and Hideaki HARA*

Blue light is a high-energy or short-wavelength visible light, which induces retinal diseases such as age-related macular degeneration and retinitis pigmentosa. Bilberry and lingonberry contain high amounts of polyphenols and thus confer health benefits. This study aimed to determine the protective effects and mechanism of action of bilberry extract (B-ext) and lingonberry extract (L-ext) and their active components against blue light-emitting diode (LED) light-induced retinal photoreceptor cell damage. Our findings suggest that B-ext and L-ext containing high amounts of polyphenols exert protective effects against blue LED light-induced retinal photoreceptor cell damage mainly through inhibition of ROS production and activation of pro-apoptotic proteins.

[*BMC Nephrol.* **15**:98 (2014)] [Lab. of Molecular Pharmacology]

Effect of Chitosan Chewing Gum on Reducing Serum Phosphorus in Hemodialysis Patients: a Multi-center, Randomized, Double-blind, Placebo-controlled Trial.

Tadao AKIZAWA, Yoshinari TSURUTA, Yoichi OKADA, Yoshihiro MIYAUCHI, Akio SUDA, Hiroshi KASAHARA, Nobuhiro SASAKI, Yoshitaka MAEDA, Takako SUZUKI, Noriaki MATSUI, Jun NIWAYAMA, Toshiaki SUZUKI, Hideaki HARA*, Yasushi ASANO, Sadao KOMEMUSHI and Masafumi FUKAGAWA

HS219 (40 mg chitosan-loaded chewing gum) is designed to bind salivary phosphorus as an add-on to available phosphorus binders. We performed a randomized, placebo-controlled, double-blind study to evaluate the efficacy and safety of HS219 in hemodialysis (HD) patients with hyperphosphatemia as an add-on to phosphorus binders. The chitosan-loaded chewing gum HS219 does not affect serum and salivary phosphorus levels in Japanese HD patients with hyperphosphatemia. Our findings do not support previous findings that 20 mg of chitosan-loaded chewing gum reduces serum and salivary phosphorus levels.

[*Food Funct.* **13**, 2-6 (2014)] [Lab. of Molecular Pharmacology]

The Antimicrobial Effect of the Extract of *Canavalia gladiata* on Periodontal Pathogen.

Tomomi MASUDA, Hideki MIYAKUBO, Munekazu IINUMA and Hideaki HARA*

Canavalia gladiata is using for prevention bad breath, not only food. Recently, Porphyromonas gingivalis starts to garner attention. Porphyromonas gingivalis produced potent protease, called gingipain, and is involved with a large part of the periodontal pathogen. In this study, we examined the antimicrobial effect of extract Canavalia gladiate on periodontal pathogen. The finding indicates that Canavalia gladiate has the antimicrobial effect on periodontal pathogen.

[*J. Com. Pharm. Pharm. Sci.* **6**, 57-61 (2014)] [Lab.of Pharmacy Practice & Social Science]

Factors Influencing Pharmacies' Adoption of a System for Preventing Dispensing Errors.

Saori MATSUNAMI, Syuji YAMASHITA, Masafumi KUBOTA, Shigeharu TANEI, Kazuhiro IGUCHI, Yoshihiro NOGUCHI, Emi GOTO and Tadashi SUGIYAMA*

We mailed 200 community pharmacies a questionnaire about adoption of computerized checking systems for preventing dispensing errors, frequency and contents of dispensing errors and so on. We found that 42.0% and 14.8% of the pharmacies had adopted systems for powder drug dispensing and tablet dispensing, respectively. Powder systems were adopted in pharmacies of various scales. In contrast, tablet systems were adopted in only pharmacies that were part of a chain. In pharmacies where such systems were introduced, serious dispensing error rarely occurred. Some additional functions, such as confirming the patient's medication history, indicating drug information, and time keeping, were introduced into the tablet system. Our findings suggest that the usefulness of a checking system for preventing dispensing errors increases when the system performs additional functions.

[*J. Jpn. Soc. Health care Manag.*,**16**, 90 - 96(2014)] [Lab.of Pharmacy Practice & Social Science]

A survey of journal articles related to computerized support systems for cancer chemotherapy in Journal of Japanese Society of Hospital Pharmacists and Japanese Journal of Pharmaceutical Health Care and Science.

Makoto NAKASHIMA and Tadashi SUGIYAMA*

We surveyed journal articles related to computerized support systems for prescriptions by physicians, prescription checks by pharmacists, and drug mixing in cancer chemotherapy. The articles were divided into 2 groups on the basis of whether the computerized systems were linked with the order entry system. With regard to functions that supported prescriptions by physicians were mostly confirmed in articles reported system linked with the order entry system. A support system for prescriptions by physicians leads to proper use of anticancer agents and decreases the working time spent in prescription checks by pharmacists. Therefore, a computerized system that support prescriptions is useful in safety management and efficiency of clinical practice.

[*Jpn. J. Drug Inform.* **16**, 90-96 (2014)] [Lab.of Pharmacy Practice & Social Science]

Introducing computer systems supporting works related to cancer chemotherapy and evaluating their effect (the 2nd report): Computer systems linked to electronic medical records.

Makoto NAKASHIMA, Takuya GOTO, Yuka Aizawa2, Mie Kominami and Tadashi SUGIYAMA*

We introduced two computer systems that utilized data imputed into electronic medical records. The first system was used to check cancer chemotherapy prescriptions, whereas the second system was a preparation support system that facilitates precise mixing of anticancer drugs. Using the prescription checking system, the time required for checking was reduced significantly compared to without using the system. Using the preparation support system, the preparation time required was prolonged significantly compared to that without using the system. However, questionnaire survey revealed that prolonged time was in the allowable range to ensure safety. In conclusion, it is considered that the prescription checking system introduced efficient checking of prescriptions, and that the preparation support system introduced an improvement in the accuracy of preparation.

[*Jpn. J. Pharm. Health Care Sci.* **40**, 734-741 (2014)] [Lab.of Pharmacy Practice & Social Science]

Prognostic factors in patients with unresectable pancreatic cancer treated with gemcitabine: a retrospective analysis.

Masahiro HATORI, Daiki TSUJI, Keisei TAKU, Takashi DAIMON, Mana KAMEZATO
Midori IKEDA, Ryo MAKUTA, Hideki HAYASHI*, Kazuyuki INOUE and Kunihiko ITOH

We retrospectively analyzed the medical records of 88 patients who received gemcitabine mono-therapy for unresectable pancreatic cancer. Multivariate analysis identified gemcitabine total dose (≤ 9075 mg; hazard ratio HR, 3.10; *P* = 0.001), absence of second-line therapy (HR, 6.30; *P* < 0.001), stage IV-b (HR, 4.97; *P* = 0.005), neutrophil counts (> 3979.5 /μL; HR, 3.43; *P* = 0.003), lymphocyte counts (≤ 1155.5 /μL; HR, 2.94; *P* = 0.010), and carcinoembryonic antigen (CEA) levels (> 5.95 ng/mL; HR, 2.57; P = 0.034) as prognostic factors. These prognostic factor could help to select treatment for patients in clinical practice, and these risk-adapted treatment strategies shoul be further investigated in a prospective study.

[*Chemotherapy*, **59**, 407-413 (2014)] [Lab.of Pharmacy Practice & Social Science]

Retrospective analysis of severe neutropenia in patients receiving concomitant administration of docetaxel and clarithromycin.

Daiki TSUJI, Mana KAMEZATO, Takashi DAIMON, Keisei TAKU, Masahiro HATORI, Midori IKEDA,
Hideki HAYASHI*, Kazuyuki INOUE, Takashi ETO and Kunihiko ITOH

The aim of this study was to evaluate whether the risk of severe neutropenia induced by docetaxel was increased by concomitant administration of clarithromycin. Patients with advanced lung cancer receiving docetaxel were identified from an electronic medical record system and divided into 2 groups: concomitant administration of clarithromycin and no concomitant administration of clarithromycin. Multivariate analysis showed that co-administration of clarithromycin [odds ratio (OR) 4.98; *P* = 0.004], pre-treatment absolute neutrophil count (OR 2.62; *P* = 0.011) and female gender (OR 2.75; *P* = 0.029) resulted in an increase in the incidence of grade 4 neutropenia.

[*Epilepsy Res.*, **108**, 1046-1051 (2014)] [Lab.of Pharmacy Practice & Social Science]

4217C>A polymorphism in carbamoyl-phosphate synthase 1 gene may not associate with hyperammonemia development during valproic acid-based therapy.

Kazuyuki INOUE, Eri SUZUKI, Toshiki TAKAHASHI, Yoshiaki YAMAMOTO, Rei YAZAWA,
Yukitoshi TAKAHASHI, Katsumi IMAI, Kou MIYAKAWA, Yushi INOUE, Daiki TSUJI,
Hideki HAYASHI* and Kunihiko ITOH

Polymorphisms in the genes encoding carbamoyl-phosphate synthase 1 (CPS1) and N-acetylglutamate synthase (NAGS) were recently reported to be risk factors for the development of hyperammonemia during valproic acid-based therapy. This study aimed to examine the influence of patient characteristics, including polymorphisms in *CPS1* 4217C>A and *NAGS* -3064C>A, on the development of hyperammonemia in Japanese pediatric epilepsy patients. *CPS1* 4217C>A polymorphism may not be associated with the development of hyperammonemia in Japanese population.

[*Biol. Pharm. Bull.*, **37**, 461-465 (2014)] [Lab.of Pharmacy Practice & Social Science]

Endothelin-1 receptors in rat tissues: characterization by bosentan, ambrisentan and CI-1020.

Yoshinari YOKOYAMA, Ayaka OSANO, Hideki HAYASHI*, Kunihiko ITOH, Takashi OKURA,
Yoshiharu DEGUCHI, Yoshihiko ITO and Shizuo YAMADA

The present study aimed to characterize comparatively endothelin-1 (ET-1) receptors in rat tissues by radioligand binding assay using [^{125}I]ET-1 and to examine receptor binding after oral administration of bosentan. The nonlinear least squares regression analysis revealed the presence of high- and low-affinity ET-1 receptor sites in these tissues for ambrisentan and CI-1020. Oral administration of bosentan caused a dose-dependent decrease in specific [^{125}I]ET-1 binding in the rat lung, kidney and bladder, suggesting significant binding of the tissue ET-1 receptors in vivo. In conclusion, it has been shown that a significant amount of pharmacologically relevant ET-1 receptors may exist in rat tissues and that ET-1 receptor antagonists such as bosentan at pharmacological doses may exert some pharmacological effects by binding these ET-1 receptors.

[*Cancer Sci.*, **105**, 396-401 (2014)] [Lab.of Pharmacy Practice & Social Science]

Identification of anti-CD98 antibody mimotopes for inducing antibodies with antitumor activity by mimotope immunization.

Misa SAITO, Masahiro KONDO, Motohiro OHSHIMA, Kazuki DEGUCHI, Hideki HAYASHI*, Kazuyuki INOUE, Daiki TSUJI, Takashi MASUKO and Kunihiko ITOH

We isolated linear and constrained mimotopes from HBJ127, a tumor-suppressing anti-CD98 heavy chain mAb, and determined their abilities for induction of antitumor activity equal to that of the parent antibody. We detected elevated levels of antipeptide responses, but failed to detect reactivity against native CD98-expressing HeLa cells in sera of immunized mice. Phage display panning and selection of mimotope-immunized mouse spleen-derived antibody Fab library showed that HeLa cell-reactive Fabs were successfully retrieved from the library. This finding indicates that native antigen-reactive Fab clones represented an undetectable minor population in mimotope-induced antibody repertoire.

[*J. Pharmacol. Sci.*, **124**, 86-91 (2014)] [Lab.of Pharmacy Practice & Social Science]

Bladder Endothelin-1 Receptor Binding of Bosentan and Ambrisentan.

Ayaka OSANO, Yoshinari YOKOYAMA, Hideki HAYASHI*, Kunihiko ITOH, Takashi OKURA, Yoshiharu DEGUCHI, Yoshihiko ITO and Shizuo YAMADA

The present study aimed to characterize bladder endothelin-1 (ET-1) receptor binding of clinically used ET-1 receptor antagonists by using [(^{125})I]ET-1. Bosentan and ambrisentan significantly increased the dissociation constant for bladder [(^{125})I]ET-1 binding without affecting maximal number of binding sites (B_{max}). Thus, bosentan and ambrisentan seem to bind to bladder ET-1 receptor in a competitive and reversible manner. Oral administration of bosentan caused a dose-dependent decrease in B_{max} for bladder [(^{125})I]ET-1 binding, suggesting significant binding of bladder ET-1 receptors in vivo. A significant amount of pharmacologically relevant ET-1 receptors may exist in the bladder. These receptors may be implicated in the pathogenesis of lower urinary tract symptoms and may also be promising targets for the development of therapeutic agents.

[*Ther. Drug Monit.*, **36**, 406-409 (2014)] [Lab.of Pharmacy Practice & Social Science]

Influence of *uridine diphosphate glucuronosyltransferase 2B7* -161C>T polymorphism on the concentration of valproic acid in pediatric epilepsy patients.

Kazuyuki INOUE, Eri SUZUKI, Rei YAZAWA, Yoshiaki YAMAMOTO, Toshiki TAKAHASHI, Yukitoshi TAKAHASHI, Katsumi IMAI, Seiichi KOYAMA, Yushi INOUE, Daiki TSUJI, Hideki HAYASHI* and Kunihiko ITOH

This study aimed to examine the relationships between plasma valproic acid (VPA) concentrations and the -161C>T polymorphism in uridine diphosphate glucuronosyltransferase (UGT) 2B7 genes in pediatric epilepsy patients. Significant differences in adjusted plasma VPA concentrations were observed between carriers of CC, CT, and TT genotypes in the UGT2B7 -161C>T polymorphism. Patients with the CC genotype had lower adjusted plasma VPA concentrations than those with CT or TT genotype. These data suggest that the UGT2B7 -161C>T polymorphism in pediatric epilepsy patients affects VPA concentration.

[*J. Pharm. Biomed. Anal.*, **89**, 227-232 (2014)] [Lab.of Pharmacy Practice & Social Science]

Simultaneous microdetermination of bosentan, ambrisentan, sildenafil, and tadalafil in plasma using liquid chromatography/tandem mass spectrometry for pediatric patients with pulmonary arterial hypertension.

Yoshinari YOKOYAMA, Miho TOMATSURI, Hideki HAYASHI*, Keita HIRAI, Yasuo ONO, Yuto YAMADA, Kenichiro TODOROKI, Toshimasa TOYO'OKA, Hiroshi YAMADA and Kunihiko ITOH

A simultaneous, selective, sensitive, and rapid liquid chromatography/tandem mass spectrometry (LC–MS/MS) method was developed and validated for the quantification of bosentan, ambrisentan, sildenafil, and tadalafil in 50 μL of human blood plasma. This validated method was applied to a clinical pharmacokinetic study in pediatric patients with pulmonary arterial hypertension (PAH) following the oral administration of PAH drugs. These results indicate that this method is suitable for assessing the risk/benefit of combination therapy in the pediatric population and useful for therapeutic drug monitoring for PAH treatment.

[*Gan To Kagaku Ryoho* **41**, 975-979 (2014)] [Lab. of Clinical Pharmacy]

Analysis of Factors Influencing the Occurrence of Infusion Reaction after Initial Treatment with Rituximab.

Masahiro YASUDA, Tomoya TACHI, Michi UMEDA, Kazumasa USUI, Katsuhiro NAGAYA, Tomohiro OSAWA, Atushi ICHIHASHI, Hideko GOTO, Senji KASAHARA, Takeshi TAKAHASHI, Hitomi TERAMACHI* and Chitoshi GOTO

We investigated factors influencing the occurrence of infusion reactions after initial treatment with rituximab for B-cell non-Hodgkin's lymphoma. The "2,000 U/mL or less group"of soluble interleukin-2 receptor levels and the "over 2,000 U/mL group"showed significant different ($p = 0.014$). The "double value or less group"of a standard value (211 IU/L) and "over double value group"showed significantly different lactate dehydrogenase levels ($p = 0.017$). The "lower limit or less group"of the standard value (men: 13 g/dL, women: 12 g/dL) and the "over lower limit group"showed significantly different hemoglobin levels ($p = 0.020$).

[*Gifubyoyaku* .**56**, 4-7 (2014)] [Lab. of Clinical Pharmacy]

Investigation for Factors Affecting Clearance of Teicoplanin.

Satoshi AOYAMA, Hitomi TERAMACHI*, Yuko NAKASIMA, Tomoya TACHI, Tomohiro OSAWA, Masahiro FUKUTA, Takashi MIZUI, Teruo TSUCHIYA and Chitoshi GOTO

Teicoplanin (TEIC) is a renally excreted drug. For clinical treatment with TEIC, factors requiring dose revision are not well defined, apart from renal insufficiency. In this study, TEIC pharmacokinetic parameters in Japanese cancer patients were compared with those in non-cancer patients. The two-compartment Bayesian pharmacokinetic program was used to analyze the parameters in 14 cancer patients (aged 73.4 ± 9.3 years, mean ± SD) and 6 non-cancer patients (aged 75.3 ± 3.9 years) The cancer patients showed 1.3 times higher TEIC clearance (0.0121 ± 0.0043 L/hr/kg) than non-cancer patients (0.0091 ± 0.0017 L/hr/kg) ($p<0.05$) with increasing clearance by Cockroft-Gault (CrCL). Also the direction of malignancy and male gender are a tendency higher than non-malignancy. These findings suggest that the measurement of TEIC serum concentration is required for patients with malignancy.

[*Oncol. Lett.* **8**, 2318-2324 (2014)] [Lab. of Clinical Pharmacy]

Oral Anticancer Agent Medication Adherence by Outpatients.

Michio KIMURA, Eiseki USAMI, Mina IWAI, Toshiya NAKAO, Tomoaki YOSHIMURA, Hiromi MORI, Tadashi SUGIYAMA and Hitomi TERAMACHI*

In the present study, medication adherence and factors affecting adherence were examined in patients taking oral anticancer agents. In June 2013, 172 outpatients who had been prescribed oral anticancer agents by Ogaki Municipal Hospital (Ogaki, Gifu, Japan) completed a questionnaire survey, with answers rated on a five-point Likert scale. The factors that affect medication adherence were evaluated using a customer satisfaction (CS) analysis. In 36.0% (62 out of 172) of the cases, there was insufficient medication adherence; 64.5% of those cases (40 out of 62) showed good medication compliance (4-5 point rating score). The percentage of patients with good medication compliance was 87.2% (150 out of 172). Through the CS analysis, three items, the interest in the drug, the desire to consult about the drug and the condition of the patient, were extracted as items for improvement.

[*J. Pharm. Health Care Sci.* **40**, 632-642 (2014)] [Lab. of Clinical Pharmacy]

Effect of Patient Education on Discharge for Use of Medication Notebook on Purchasing Over-the-counter Drugs and Health Foods –a Randomized Controlled Trial–.

Tomoya TACHI*, Kazuhide TANAKA, Shoko ASANO, Takafumi YOKOI, Kazumasa USUI, Misa KATO, Yoshihiro NOGUCHI, Tomohiro OSAWA, Atsushi ICHIHASHI, Masahiro YASUDA, Takashi MIZUI, Chitoshi GOTO and Hitomi TERAMACHI

We investigated the effect of patient education on discharge for medication-notebook use on purchasing OTC drugs and health foods. Patients aged ≥20 years who were hospitalized at the Ophthalmology Department of Gifu Municipal Hospital within a certain period were allocated randomly to an intervention group with the education and a control group without it, and questionnaire surveys were done before the education and at 2 months after discharge. The rate of patients who got to use the notebook on purchasing OTC drugs or health foods in the intervention group (30.0%) was high compared to that in the control group (0%) ($p = 0.020$).

[*SAGE Open Med.* **2**, 2050312114563318 (2014)] [Lab. of Clinical Pharmacy]

Influence of Angiotensin II Receptor Blocker Combination Tablet Prescription on Drug Number and Cost.

Hitomi TERAMACHI*, Tatsuya TAKAHASHI, Tomoya TACHI, Yoshihiro NOGUCHI, Hiroyuki NAGASAWA, Yoko INO, Takashi MIZUI, Chitoshi GOTO and Teruo TSUCHIYA

We conducted a survey on the use of angiotensin II receptor blocker–containing combination tablets as anti-hypertensive drugs, in particular angiotensin II receptor blocker/diuretic and angiotensin II receptor blocker/calcium channel blocker combinations. We performed a retrospective study of patients who visited the outpatient clinic of Gifu Municipal Hospital and received anti-hypertensive agents between June 2006 and December 2011. No reductions in the number of prescribed drugs or drug cost were seen following a change in prescription to an angiotensin II receptor blocker/diuretic. Patients receiving an angiotensin II receptor blocker/calcium channel blocker had a significant reduction in the number of prescribed drugs and a slight decrease in drug cost.

[*J. Pharm. Commun.* **12**, 5-10 (2014)] [Lab. of Clinical Pharmacy]

Evaluation of Lecture to Students in Clinical Training by Pharmacists using Multivariate Analysis and CS (Customer Satisfaction) Analysis.

Tomoya TACHI, Chitoshi GOTO, Kosuke SAITO, Yuki OHNO, Masahiro YASUDA, Takashi MIZUI, Kenji KOBAYASHI, Makoto SAHASHI, Yoshihiro NOGUCHI and Hitomi TERAMACHI*

The aims of the study are to clarify factors influencing lecture satisfaction of pharmacy students using multivariate analyses and the influencing degree, and to extract improving items of lectures using customer satisfaction (CS) analysis, in lectures to pharmacy students by pharmacists. At the department of pharmacy in Gifu Municipal Hospital, lectures by pharmacists and questionnaire survey were performed for pharmacy students in 2012 and 2013 academic years. From the multivariate analyses, it was clarified that “ease to understand” and “rousing interest” particularly influenced “lecture satisfaction”. From CS analysis, “rousing interest” and “characterization” were particularly extracted as improving items of lectures.

[*J. Threm. Anal. Calorim.* **115**, 2089-2097 (2014)] [Lab. of Clinical Pharmacy]

Molecular Dynamics and Energetic Perceptions of Substrate Recognition by Thymidylate Kinase.

Mahmoud KANDEEL, Yoshihiro NOGUCHI*, Kentaro OH-HASHI, Hye-Sook KIM and Yukio KITADE

Plasmodium deoxyguanylate pathways are an attractive area of investigation for future metabolic and drug discovery studies due to their unusual substrate specificities. We investigated the energetic contribution to thymidylate kinase substrate binding, and the forces underlying ligand recognition. The binding constant varied from 8×10^4 M^{-1} at 290 K to 6×10^4 M^{-1} at 310 K for dGMP, and from 16×10^4 M^{-1} at 290 K to 4×10^4 M^{-1} at 310 K for TMP. ΔC_p was estimated as -1.75 kJ mol^{-1} K^{-1} for TMP and +2 kJ mol^{-1} K^{-1} for dGMP. In comparison with TMP, the binding of dGMP to PfTMK produced less favorable enthalpy change, positive or favorable entropic contribution at lower temperature, positive heat capacity change, negative ΔS^{o}_{HE}, positive ΔS_{other}, higher total solvent-exposed surface area and more or less rigid body binding. These changes indicate unfavorable conditions for proper binding and lower conformational changes, and suboptimal structural reordering during dGMP binding.

[*Clin. Pharmacol. Ther.* **95**, 533-541 (2014)] [Lab. of Drug Informatics]

Significant differences in drug-lag in clinical development among various strategies used for regulatory submissions in Japan.

Takahiro UENO, Yasuko ASAHINA, Ayumi TANAKA, Hiroaki YAMADA, Mitsuhiro NAKAMURA* and Yoshiaki UYAMA

Although the number of global clinical trials (GCTs) conducted in multiple countries including Japan has increased recently, it is not clear how much these GCTs help in reducing the lag in drug development (LDD: difference between the submission dates for new drug applications (NDAs) in the United States and Japan). We examined the effects of various clinical development strategies on LDD because the development period depends on what types of clinical trials were conducted for the Japanese NDA. The inclusion of GCTs in the clinical development strategy is also important; simultaneously, the smaller sample size of the Japanese population should be taken into consideration.

[*Anesth. Analg.* **118**, 125-131 (2014)] [Lab. of Drug Informatics]

Hypergravity exposure for 14 days increases the effects of propofol in rats.

Chihiro IWATA, Chikara ABE, Mitsuhiro NAKAMURA* and Hironobu MORITA

It is thought that the gravitational environment of space exploration alters the effects of anesthetics; however, no evidence has as yet been reported. In the present study, we sought to provide direct evidence showing that hypergravity exposure for 14 days increases anesthetic effects and to examine the possible causes. The effects of propofol were compared between rats raised in 1g and 3g environment by measuring time taken to induce the burst suppression in an electroencephalogram, nadir of arterial blood pressure, and time taken for the appearance of the righting response to noxious electrical stimulations. The time course of plasma propofol concentrations was also examined. The results provide evidence that hypergravity exposure for 14 days increases the effects of propofol. It is suggested that the results were not caused by differences in plasma propofol concentrations but by increased sensitivity, which was mediated via the vestibular system.

[*J. Invest. Dermatol.* **134**, 712-718 (2014)] [Lab. of Drug Informatics]

Interferon-gamma decreases ceramides with long-chain fatty acids: possible involvement in atopic dermatitis and psoriasis.

Chisato TAWADA, Hiroyuki KANOH, Mitsuhiro NAKAMURA*, Yoko MIZUTANI, Tomomi FUJISAWA, Yoshiko BANNO and Mariko SEISHIMA

We aimed to identify the mechanism underlying the alteration of fatty acids (FAs) chain length of ceramides (CERs) in these diseases. Mass spectrometry analysis of CERs in the human stratum corneum showed that the proportion of CERs with long-chain FAs was significantly lower in atopic dermatitis (AD) and psoriasis patients than in healthy controls, and this reduction was more pronounced in psoriasis than in AD. Using cultured human keratinocytes and epidermal sheets, our results suggest that IFN-γ decreases CERs with long-chain FAs through the downregulation of elongase of long-chain fatty acids (ELOVL) and ceramide synthase and that this mechanism may be involved in the CER profile alteration observed in psoriasis and AD.

[*JJOMT.* **62**, 322-327 (2014)] [Lab. of Drug Informatics]

Study on the work-related stress among hospital pharmacist.

Ryoichi INABA, Atsushi HIOKI, Yoshihiro KONDO, Hiroki NAKAMURA and Mitsuhiro NAKAMURA*

This study was designed to evaluate the work-related stress among hospital pharmacists. A self-administered questionnaire survey on the related determinants was performed among 314 hospital pharmacists. There were no significant defferences between males (83.3%) and females (85.4%) in the proportion of pharmacists who feel stressful at work. The total health risks read from the figure for judgements of the work-related stress in males and females were 102.3 and 97.4 for 100 of the standard group, respectively. After adjusted for age and carrier of drug compounding, total scores of stress items concerning ‘basic duty’ and ‘employment and future’ were significantly higher in male pharmacists than in female pahmacists ($p<0.05$). These results suggest that there were significant differences in the work-related stress felt by pharmacisits between males and females.

[*YAKUGAKU ZASSHI* **134**, 299-304 (2014)] [Lab. of Drug Informatics]

Evaluation of the association between the use of oral anti-hyperglycemic agents and hypoglycemia in Japan by data mining of the Japanese Adverse Drug Event Report (JADER) database.

Ryogo UMETSU, Yuri NISHIBATA, Junko ABE, Yukiya SUZUKI, Hideaki HARA, Hideko NAGASAWA, Yasutomi KINOSADA and Mitsuhiro NAKAMURA*

The aim of the present study was to evaluate the risk of hypoglycemia due to oral anti-hyperglycemic agents (OHAs) use by using the Japanese Adverse Drug Event Report (JADER) database. To this end, reports of hypoglycemia events included in the JADER database between 2004 and 2012 were analyzed by calculating the reporting odds ratio (OR). Among OHAs, sulfonylureas showed the highest adjusted OR (adjusted OR, 10.13; 95% confidence interval, 9.08-11.26). The adjusted ORs for meglitinides, biguanide, thiazolidinedione, alpha-glucosidase inhibitors, and dipeptidyl peptidase-4 inhibitors were significantly lower than that of sulfonylureas. Data mining of the JADER database was useful for analyzing OHA-associated hypoglycemia events.

[*J. Gifu Byoyaku.* **55**, 10-14 (2014)] [Lab. of Drug Informatics]

A survey of the attiude of pharmacist and pharmaceutical company on the new program of registered drug sellers.

Mitsuhiro NAKAMURA*, Yoshihiro KONDO, Honami SUZUKI, Yuri NISHIBATA, Hiroaki URANISHI, Rie NISHIWAKI, Hitomi TERAMACHI, Tadashi HORIUCHI and Teruo TSUCHIYA

The new over-the-counter (OTC) drug sales program were introduced in accordance with the revised pharmaceutical affairs law from 2009.This law has permitted registered sellers to sell the OTC drugs of category2, and 3. We conducted a questionnaire survey against 413 pharmacists and 50 pharmaceutical companies to evaluate the role of registered seller in community phramacies in the case of recommendation of OTC drugs for self-medicaiton by patients. Many pharmacists and the compacies concerned about the knowledge of medicine and disease that registered drug sellers actually have. Therefore, it would be necessary to develop an educational system for registered drug sellers.

[*J. Gifu Byoyaku.* **55**, 15-17 (2014)] [Lab. of Drug Informatics]

Survey of consumer views on the revision of over-the-counter drug sales system.

Mitsuhiro NAKAMURA*, Yoshihiro KONDO, Rie NISHIWAKI, Honami SUZUKI, Yuri NISHIBATA, Hitomi TERAMACHI, Tadashi HORIUCHI and Teruo TSUCHIYA

Over-the-counter (OTC) drugs were classified into category 1, 2, and 3 by the revised pharmaceutical law introduces in 2009, and new qualifications for the registration of drug sellers was established. The aim of this study was to investgate the views of consumers concerning the revision of the OTC drug sales system. We conducted a questionnaire survey against 312 consumers (male, 184; female, 28). The questionnaire found that 56% of the consumers did not know the term of “registered drug seller.” They realized that pharmacists and/or registered drug seller acquired a special knowledge of OTC drugs and provide appropriate information on safe use of the OTC drugs. These results suggested that appropriate pharmaceutical education programs, which were designed for the achievement of a comprehensive knowledge of self-medication, might be needed for consumers.

[*J. Gifu Byoyaku.* **56**, 8-14 (2014)] [Lab. of Drug Informatics]

Optimization of sample preparation procedure and analytical column selection in the measurement of anti-apileptic drugs in human plasma with high performance liquid chromatography.

Tomofumi OHMORI, Mami KAWAI, Mitsuhiro NAKAMURA*, Kazumi TANIGUCHI, Kazuhiro IGUCHI, Yoshinori ITOH, Shigeyuki USUI and Kazuyuki HIRANO

Several methods for the assay of anti-epileptic drugs, using high performance liquid chromatography (HPLC) with UV detection have been reported to detect therapeutic concentrations in plasma. However, these methods have limited throughput and restrict sensitivity. We evaluated the possibility of using a high throughput high-resolution ODS column compatible with aqueous compounds. Furthermore, solid-phase extraction (SPE) and liquid-liquid extraction was evaluated in sample preparation procedure. SPE followed by HPLC-UV, using a short high resolution octadecyl silica column compatible with aqueous compounds, was useful in the development of a robust and specific analysis of anti-epileptic drugs in human plasma.

[*J. Community Pharm. Pharm. Sci.* **6**, 150-156 (2014)] [Lab. of Community Pharmacy]

Dispensing Errors Made by Pharmacy Students during Pharmacy-based Practical Training at Gifu Pharmaceutical University Pharmacy.

Kazuhiro IGUCHI*, Shuji YAMASHITA, Emi ITO, Yoshihiro NOGUCHI, Masafumi KUBOTA, Mitsuhiro NAKAMURA, Hitomi TERAMACHI, and Tadashi SUGIYAMA

Prescription dispensing is one of the core practice items for pharmacy students during pharmacy-based practical training. Here, we analyzed the dispensing errors reported by pharmacy students and pharmacists in Gifu Pharmaceutical University Pharmacy. There were 2,697 dispensing errors made by 103 pharmacy students. The most common type of dispensing errors was dispensing the wrong quantity. In particular, the wrong amount of single-dose powder packets, (e.g., magnesium oxide and pantethine) was more frequently found to be dispensed by students than by pharmacists. These results will help when proposing preventative actions for reducing the dispensing errors made by students during pharmacy-based practical training.

[*Jpn. J. School Health* **56**, 11-20 (2014)] [Lab. of Community Pharmacy]

Factors Associated with Medicine Use Behavior among Junior and Senior High School Students.

Chihiro SAKAI*, Tetsuro KAWABATA, Kazuya HISHIDA, Meijin LI and Yukiko IMADE

The main purpose of this study was to clarify the factors associated with medicine use behavior among junior and senior high school students. Valid respondents were 326 students in the 9th grade of five public junior high schools and 1,369 students in the 10th grade of seven public high schools in Hyogo prefecture. The data were collected between September and October in 2011, using an anonymous self-administered questionnaire. The results of this study suggest that an attitude is one of the most important factors associated with medicine use behavior in junior and senior high school students. Further the results suggest that it is important to enhance students' family-related self-esteem and problem-focused coping skills to promote an appropriate medicine use behavior.

[生薬学雑誌 **68**, 43-52 (2014)] [Lab. of Herbal Garden]

Studies on Gentianae Radix, Gentianae scabra Radix and Gentianae macrophyllae Radix

Ryohei NOMURA, Keiko ARIMOTO, Rie ISHIHARA, Michiho ITO, Mamoru OKASAKA, Tomonari KANAYA, Yoichi KAWANISHI, Akiko KAWABATA, Eiji SAKAI, Yasuo SHIMADA, Yoshitaka TAKAI, Takamori TAGAMI, Kayoko TOKURA, Yoichi HISATA, Masataka MORIYASU, Yutaka YAMOMOTO and Tsuguo YOKOKURA

According to the Japanese Pharmacopoeia Sixteenth Edition (JP16), the origin of Gentianae Radix is the root and rhizome of *Gentiana lutea* (Gentianaceae), and that of Gentianae sucabra Radix is the root and rhizomae of *Gentiana scabra*, *G. manshurica* and *G. triflora* (*Gentianaceae*). These gentian herbs are used as ingredients of kanpo formuli and bitter stomachics.
However the quantitative test for these gentian herbs is not desigenated in JP16 nor non-JPS 2012. In this study, an analytical method using HPLC for Gentianae Radix, Gentianae scabra Radix and Gentianae mocrophyllae Radix was elaborated, and was applied to market samples.

[*JPS Conf. Proc.***1**,019008-1-019008-4 (2014)] [Lab. of Mathematics]

Dynamics of a Skew Tent Map in the Nonlinear Frobenius-Perron Equation.

Daisuke KATSURAGI

Return maps of the mean field in globally coupled map Lattices (GCML) with a large system size were compared with those at the limit in a large system size. We adopted a nonlinear Frobenius-Perron equation (NFPE) for the limit in the large system size, and used a skew tent map as a chaotic map to simplify calculations in the NFPE. The return maps of the mean field for direct numerical calculations in the GCML usually fluctuate from those for numerical calculations in the NFPE. However, at some coupling strengths, there are totally different return maps between the GCML and the NFPE. We show that this strongly depends on the initial conditions at some coupling strengths.

[*NSSU Journal of Sport Sciences.* **3**, 21-35 (2014)] [Lab. of Health & physical Education]

A study of the transition process of the system for Kendo techniques—focus on the shinai—.

Taichi SAKAMOTO*,Yusuke YANO and Makoto YAGISAWA

The transition process of the system for Kendo techniques were investigated. As the resules, *Shinai* used before Tominaga Kengo's "*Mottomo Jissaitekina Gakusei Kendo no Sui*" (1926) which completed the target-based technique system in Kendo, were similar to those used in the period when Chiba Shusaku's *Kenjyutsu 68 tte* was explicitly stated.The type of *shinai* adopted in Tominaga Kengo's "*Mottomo Jissaitekina Gakusei Kendo no Sui"* (1926) was also similar to those used in the past. In regards to the sword's size; however, a *shinai* with a longer length of 3-shaku 9-sun (approx. 120.0 centimeters) comes to be acceptable in addition to those of the conventional length of 3-shaku 8-sun (approx. 117.3 centimeters).

紀 要 論 文

(1) 信田智哉, 多田教浩, 三浦剛, 伊藤彰近：光と酸素或いは過酸化水素を用いる酸化反応の開発に関する研究.
[*岐阜薬科大学紀要* **63**, 33-42 (2014)]

(2) 杉原光, 竹内洋文：プランルカスト水和物の経口持続性製剤化を目指した胃内滞留性製剤の設計に関する研究
[*岐阜薬科大学紀要* **63**, 1-10 (2014)]

(3) 田中彦孝, 鶴間一寛, 嶋澤雅光, 原英彰：膜貫通糖タンパク質 GPNMB の ALS 病態に対する神経保護因子としての可能性.
[*岐阜薬科大学紀要* **63**, 11-21 (2014)]

(4) 中村信介, 鶴間一寛, 嶋澤雅光, 原英彰：カリジノゲナーゼの血管新生抑制作用.
[*岐阜薬科大学紀要* **63**, 22-32 (2014)]

総　　説

(1) 奥田健介, 平山祐, 永澤秀子：低酸素・低栄養環境関連分子を標的とする創薬研究の現状.
[*放射線生物研究* **49**, 341-357 (2014)]

(2) 佐治木弘尚：特定官能基を標的とした接触還元法の開発.
[*有機合成化学協会誌*, **72**, 39-50 (2014)]

(3) Yuki YABE, Yoshinari SAWAMA, Yasunari MONGUCHI and Hironao SAJIKI: New Aspect of Chemoselective Hydrogenation Utilizing Heterogeneous Palladium Catalysts Supported by Nitrogen- and Oxygen-Containing Macromolecule.
[*Catal. Sci. Technol.* **4**, 260-271 (2014)]

(4) 多田教浩, 伊藤彰近：光と分子状酸素を利用する酸化反応.
[*ファルマシア* **50**, 517-521 (2014)]

(5) 竹内洋文：粒子設計を基盤とする新しい医薬品製剤に関する研究
[粉砕, No.57, 27-35 (2014)]

(6) Masatoshi INDEN：The Causative Gene of Parkinsonism and its Medical Treatment Strategy.
[*Yakugaku Zasshi* **134**, 1253-1258 (2014)]

(7) 稲垣直樹：アトピー性皮膚炎とかゆみ.
[*薬学図書館* **59**, 84-90 (2014)]

(8) 山下弘高, 稲垣直樹：アトピー性皮膚炎の基礎データ.
[*漢方と最新治療* **23**, 327-332 (2014)]

(9) 山下弘高, 田中宏幸, 稲垣直樹：食物アレルギーと食品添加物.
[*臨床免疫・アレルギー科* **62**, 405-410 (2014)]

(10) Mitsue ISHISAKA and Hideaki HARA：The roles of diacylglycerol kinases in the central nervous system: review of genetic studies in mice.

[*J. Pharmacol. Sci.* **124**, 336-343 (2014)]

(11) Yuka IKEGAME, Kentaro YAMASHITA, Shigeru NAKASHIMA, Yuichi NOMURA, Shingo YONEZAWA, Yoshitaka ASANO, Jun SHINODA, Hideaki HARA and Toru IWAMA : Fate of graft cells: what should be clarified for development of mesenchymal stem cell therapy for ischemic stroke?
[*Front. Cell. Neurosci.* **8**, 322 (2014)]

(12) 嶋澤雅光, 原英彰：視覚を用いた脳科学研究, 小胞体(ER)ストレスと緑内障.
[*脳 21* **17**, 66-72 (2014)]

(13) 嶋澤雅光：緑内障の病態と薬物治療および今後の展望.
[*ファルマシア*, **50**, 207-211 (2014)]

(14) 小川健二郎, 原英彰：網膜障害に対する食品色素成分の有効性.
[*FFI ジャーナル* **219**, 114-125 (2014)]

(15) 田中彦孝, 原英彰：ALS の克服を目指して―ALS のバイオマーカー―
[*日薬理誌* **143**, 313 (2014)]

(16) Yuri NISHIO and Masako TSUZUKI: Phonological Features of Japanese EFL Speakers from the Perspective of Intelligibility
[*JACET Journal*, **58**. 57-78 (2014)]

(17) Adam SERAG: The Impact of Using L1 in EFL Classroom on Foreign Language Acquisition in Japan
[*The 2013 JALT National Conference Proceedings, JALT*, 211-221 (2014)]

(18) Yuri NISHIO and Masako TSUZUKI: E-learning to Improve English Phonological Features Affecting the Accuracy and Intelligibility of Japanese English Learners
[*AILA World Congress 2014 Abstract Book*, 273 (2014)]

(19) Yuri NISHIO: Relations Between Realization of English Intonation by the Japanese Future English Teachers and Intelligibility
[*The JACET 53rd (2014) International Convention Book*, 67 (2014)]

著　　書

(1) 永澤秀子(分担執筆)：28. 酸化ストレス適応応答を標的としたがん治療創薬
[*酸化ストレスの医学*（診断と治療社）pp.228-246 (2014)]

(2) Akichika ITOH, Tsuyoshi MIURA, Norihiro TADA (分担執筆)：Oxidation of Carbon-Halogen Bonds.
[*Comprehensive Organic Synthesis, 2nd ed.* pp.744-769 (2014)]

(3) 田中稔幸，酒井英二，大山雅義，阿部尚仁 (分担執筆)：
[*薬草ガイドブック野外編* (日本植物園協会) (2014)]

(4) 田中稔幸，大山雅義，阿部尚仁 (分担執筆)：
[*小石川植物園後援会ニュースレター* 第 48 号 (小石川植物園後援会) (2014)]

(5) 竹内洋文：粒子径測定、比表面積測定
[*薬剤学実験法必携マニュアル* (南江堂) pp.77-86 (2014)]

(6) 笹井泰志 (分担執筆) : 第 2 章第 3 節[12] 高分子表面のプラズマ処理による細胞培養基板の機能化設計と開発.
[*動物細胞培養の手法と細胞死・増殖不良・細胞変異を防止する技術* (技術情報協会) pp. 151-155 (2014)]

(7) Hirokazu HARA and Elias AIZENMAN (分担執筆)：Chapter 4. Oxidative stress and neuronal zinc signaling.
[*Zinc Signals in Cellular Functions and Disorder* (ed. by Fukada and Kambe, Springer Japan) pp. 55-87 (2014)]

(8) 山田晴生, 足立哲夫, 山田裕一, 今井裕一(分担執筆) : 腎性貧血に ESA 製剤の切り替え投与に伴う炎症指標の推移.
[*腎とフリーラジカル　第12 集* (東京医学社) pp. 58-61 (2014)]

(9) 山田晴生, 足立哲夫, 山田裕一, 今井裕一(分担執筆) : Teriparatide 投与による腎での extracellular superoxide dismutase (EC-SOD)産生誘導.
[*腎とフリーラジカル　第12 集* (東京医学社) pp. 86-89 (2014)]

(10) 神谷哲朗, 服部脩平, 原　宏和, 山田晴生, 足立哲夫 (分担執筆) : 低酸素状態下の COS7 細胞における EC-SOD 発現調節機構としてのエピジェネティクス.
[*腎とフリーラジカル　第12 集* (東京医学社) pp. 100-103 (2014)]

(11) 臼井茂之 (分担執筆) :
[*2014 年版 実習に行く前の 覚える医薬品集－服薬指導に役立つ－* (廣川書店) (2014)]

(12) 北市清幸 (分担執筆) : アレルギーに使用される健康食品素材・成分と薬剤の関連について
[*日本食品安全協会会報* **9(1)**, 18-19 (2014)]

(13) 北市清幸 (分担執筆), 中野一子 (分担執筆) : 糖尿病に関係する健康食品と薬の相互作用について
[*日本食品安全協会会報* **9(4)**, 11-13 (2014)]

(14) 保住　功 (分担執筆) :「神経症候群 V」(*第2 版*) Fahr 病
[*神経症候群 V* (*第 2 版*) **30**, 750-755 (2014)]

(15) 杉山剛志 (分担執筆) : 第 2 章 4. 微細藻類由来多糖による自然免疫活性化作用.
[*エンドトキシン・自然免疫研究17 －エンドトキシン・自然免疫の展開 : 新しい機序、診断、応用－* (医学図書出版) pp.57-60 (2014)]

(16) 松永俊之（分担執筆）:
[*薬学生のための病態検査学　第2 版* (南江堂) (2014)]

(17) 稲垣直樹 (分担執筆) : 総論 3-2 漢方薬の効かせ方―現代薬理からの解析.
[*小児漢方治療の手引き* (日本小児医事出版社) pp. 20-22, (2014)]

(18) Masamitsu SHIMAZAWA and Hideaki HARA (分担執筆)：ER Stress.
[*Neuroprotection and Neuroregeneration for Retinal Diseases* (Springer Japan) pp.67-83(2014)]

(19) 原英彰(分担執筆) : 第 4 章 目が劇的によくなる「食品」　ブルーベリー　－抗酸化作用で緑内障、糖尿病網膜症に効果が期待－.
[*目が劇的によくなる最強辞典　－専門家が教える 26 の視力回復・健康法－*(マキノ出版) pp. 152-156,(2014)]

(20) 原英彰, 柿野衛(分担執筆) : 第 3 章 便秘解消　2 沈香（ジンコウ）葉エキスの便秘改善作用
[*女性の疾患と美容のための機能性素材の開発*、(シーエムシー出版) pp. 61-69 (2014)]

(21) 杉山正 (分担執筆) :
[*2014 年版 実習に行く前の 覚える医薬品集－服薬指導に役立つ－* (廣川書店) (2014)]

(22) 杉山正 (分担執筆) :
[*射剤・経口製剤に代わる新しい薬剤投与デバイスの開発* (技術情報協会) (2014)]

(23) 寺町ひとみ (分担執筆) :
[*モデル・コアカリキュラムに沿った わかりやすい薬局実務実習テキスト第4 版* (じほう) (2014)]

(24) 舘知也 (分担執筆) :

[*モデル・コアカリキュラムに沿った わかりやすい薬局実務実習テキスト第4 版* (じほう) (2014)]

(25) 酒井英二 (分担執筆) :
[*薬草ガイドブック野外編* (日本植物園協会) (2014)]

(26) 酒井英二 (分担執筆) : 特集　薬草の宝庫　伊吹山をたずねて
[*大塚薬報*（大塚ホールディングス) No.696, pp. 4-15 (2014)]

(27) 酒井英二 (分担執筆) : 特集　知の宝庫”薬用植物園”を活用しよう
[*aromatopia*（フレグランスジャーナル) No.126, pp. 2-6 (2014)]

そ　の　他

(1) 竹内 洋文 :（巻頭言）薬学教育の進展と製剤機械技術学会,
[*製剤機械技術*,　23，2-3　(2014)]

(2) 竹内洋文 : 製剤と粒子設計の進展
[*粉体技術* 6，1-6 (2014)]

(3) 田原耕平、竹内洋文 : 平成 24 年度「日本薬局方の試験法等に関する研究」研究報告 高分子医薬における吸入製剤の *in vitro* 吸入特性評価
[*医薬品医療機器レギュラトリーサイエンス* 45(6), 529-533, (2014)

(4) 足立哲夫, 原　宏和, 神谷哲朗, 仲原理紗, 安田浩之 : コーヒー含有ポリフェノールのエピジェネティクス制御能.
[*全日本コーヒー協会平成 25 年度研究助成成果報告書* (2014)]

(5) 保住　功 :「達人に訊け！岐阜薬科大学のあなたの健康に役立つ話」健康長寿は運動から
[*中日新聞プラス* (2014/6/10)]

(6) 保住　功 :「達人に訊け！岐阜薬科大学のあなたの健康に役立つ話」パーキンソン病の予防と治療は“ラジオ体操”から
[*中日新聞プラス* (2014/6/22)]

(7) 保住　功 :「達人に訊け！岐阜薬科大学のあなたの健康に役立つ話」筋萎縮性側索硬化症 (ALS)に運動、その思いそして創薬研究へ
[*中日新聞プラス* (2014/7/4)]

(8) 保住　功 :「達人に訊け！岐阜薬科大学のあなたの健康に役立つ話」解き明かされていく生命現象　その 1 : ヒト遺伝子の解明～次世代シーケンサーの到来～
[*中日新聞プラス* (2014/10/16)]

(9) 保住　功 :「達人に訊け！岐阜薬科大学のあなたの健康に役立つ話」解き明かされていく生命現象　その 2 : iPS 細胞の作製～再生医療の未来～
[*中日新聞プラス* (2014/10/21)]

(10) 保住　功 :「達人に訊け！岐阜薬科大学のあなたの健康に役立つ話」解き明かされていく生命現象　その 3 : 集団から個の重視へ　～エビデンスとナラディブ～
[*中日新聞プラス* (2014/10/30)]

(11) 稲垣直樹 (研究分担) , 田中宏幸(研究協力), 山下弘高(研究協力) : マウスモデルを用いた掻痒発現機序の解析と治療戦略の構築.
[*厚生労働科学研究費補助金 (免疫アレルギー疾患予防・治療研究事業)平成25 年度・研究報告書*　434-436

(2014)]

(12) 舘知也：チーム医療における薬剤師の新たな取り組み：血圧テレモニタリング
[*ファルマシア* ***4*** *号*, 50 (2014)]

(13) 寺町ひとみ：6 年制の卒業生を送って－岐阜薬科大学における 6 年制教育の構築－
[*薬剤学* **74**, 301-304 (2014)]

(14) 酒井英二 (分担研究)：内部形態写真及び植物体栽培情報に関する研究.
[*厚生労働省科学研究費補助金 (創薬基盤推進研究事業　薬用植物栽培並びに関連産業振興を指向した総合情報データベースの拡充と情報整備に関する研究)平成 25 年度 総括・分担研究報告書* (2014)]

(15) 酒井英二，田中俊弘，後藤稔治，川村智子：
[*乗鞍スカイライン沿線植物群落－モニタリング調査報告書（平成 25 年度）(2014)*]

(16) 葛城大介：「統計」入試問題研究 WG 報告 - 英国 S1 レベルからの出題，を受けての大学定期試験への導入.
[*2013 年度数学教育学会春季年会発表論文集*, 249-251 (2014)]

(17) 葛城大介：数学における入学前教育の検証.
[*2014 年度数学教育学会秋季例会発表論文集*, 86-88 (2014)]

(18) 杉浦春雄，酒井信江，杉浦浩子：大学生の対人ストレスコーピングと友人関係の築き方が友人関係満足感に与える影響.
[*健康レクリエーション研究* **10**, 5-12 (2014)]

(19) 杉浦春雄，杉浦浩子：自尊感情と対人ストレスコーピング，対人恐怖心性と自己愛傾向の関連性.
[*健康レクリエーション研究* **10**, 13-19 (2014)]

(20) 杉浦浩子，小森美咲，杉浦春雄：大学生における楽観性とストレス認知との関連.
[*健康レクリエーション研究* **10**, 33-41 (2014)]

(21) 杉浦浩子，曽我祐子，杉浦春雄：看護学生における楽観性・非観性傾向の違いが認知的評価に与える影響.
[*健康レクリエーション研究* **10**, 43-56 (2014)]

(22) 八木沢誠，新里知佳野，坂本太一，古澤伸晃，向本敬洋，楠本恭久：剣道における審判員の注視点について.
[*日本体育大学紀要* **43**, 27-35 (2014)]