[*Org. Biomol. Chem.* **11**, 3030-3037 (2013)] [Lab. of Pharmaceutical & Medicinal Chemistry]

FRET-based Imaging of Transbilayer Movement of Pepducin in Living Cells by Novel Intracellular Bioreductively Activatable Fluorescent Probes.

Mieko Tsuji,Satoshi Ueda,Tasuku Hirayama,Kensuke Okuda,Yoshiaki Sakaguchi,Aoi Isono and Hideko Nagasawa*

To elucidate the mechanisms of direct transmembrane penetration of pepducins, which are artificial lipopeptide G protein-coupled receptor (GPCR) modulators, we developed two types of FRET-based probes, Pep13-FL-SS-Dab (**13**) targeting the inner leaflet of the lipid bilayer and Pep13-Dab-SS-FL (**14**) targeting the cytosol, respectively. When they are internalized into the cytosol, intracellular glutathione can cleave the disulfide bond to release the quencher, which results in a turn-on fluorescence signal. Using these probes, we performed live cell imaging of transbilayer movements of pepducins on MCF-7 cells for the first time. The results suggested that the lipid moiety of the probes facilitated pepducin flipping across and tethering to the membrane. The present study raises the possibility of applying the probe architecture for direct intracellular drug delivery.

[*Chem. Sci.* **4**, 1250-1256 (2013)] [Lab. of Pharmaceutical & Medicinal Chemistry]

A Highly Selective Turn-on Fluorescent Probe for Iron(II) to Visualize Labile Iron in Living Cells.

Tasuku Hirayama, Kensuke Okuda and Hideko Nagasawa*

The physiological and pathophysiological functions of iron have not been sufficiently explored, partially due to a lack of methods for visualizing intracellular labile iron. In this edge article, we present a novel turn-on fluorescent probe (RhoNox-1) for the selective detection of Fe^{2+} based on *N*-oxide chemistry. Spectroscopic studies combined with DFT calculations and electrochemical studies revealed that fluorescence quenching of RhoNox-1 occurred in physiological conditions, which was attributed to non-radiative deactivation of the excited state of tertiary amine *N*-oxide substituted xanthene involving a twisted internal charge transfer (TICT) process and partially due to photo-induced electron transfer (PET) from the *N*-oxide group. RhoNox-1 showed significant enhancement of the fluorescence signal in Fe^{2+}-loaded cells *via*selective Fe^{2+}-mediated deoxygenation of the *N*-oxide group and also successfully detected basal and endogenous labile Fe^{2+} in living cells.

[*J. Heterocycl. Chem.* **50**, E9–E11 (2013)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic *N*-Heterocyclic Compounds. Part 73: Synthesis and Evaluation of 5-Substituted 1,2-dihydrofuro[2,3-*c*]isoquinolines as Inducers of Lipoprotein Lipase mRNA Expression.

Kensuke Okuda*, Masahiko Yoshida, Takashi Hirota, and Kenji Sasaki

Several 5-substituted 1,2-dihydro[2,3-*c*]isoquinoline derivatives were synthesized as part of our research to develop new diabetes drugs. Amines and sulfanyls were used as substituents at the 5-position. Evaluation of the effects of the newly synthesized compounds on lipoprotein lipase mRNA expression in 3T3-L1 preadipocytes revealed one promising candidate with potency comparable to that of troglitazone.

[*Synth. Commun.* **43**, 1619–1625 (2013)] [Lab. of Pharmaceutical & Medicinal Chemistry]

Polycyclic N-Heterocyclic Compounds. Part 77: Synthesis of [1]Benzothieno[3',2':2,3]oxepino[4,5-d]pyrimidines and Their Evaluations as Anti-Platelet Aggregation.

Kensuke Okuda*, Takashi Nikaido, Takashi Hirota, and Kenji Sasaki

Reaction of 3-(3-cyanopropoxy)[1]benzothiophene-2-carbonitrile with sodium hydride gave 5-amino-1,2-dihydro[1]benzothieno[3,2-*d*]furo[2,3-*b*]pyridine and 5-amino-2,3-dihydro[1]benzothieno[3,2-*b*]oxepin-4-carbonitrile. The latter compound served as a convenient scaffold for the synthesis of the new heterocycles [1]benzothieno[3',2':2,3]oxepino[4,5-*d*]pyrimidines and the parent 1,2,4,5-tetrahydro[1]benzothieno[2',3':6,7]oxepino[4,5-*e*]imidazo[1,2-*c*]pyrimidine heterocyclic system. The new compounds described in this report were evaluated as inhibitors of platelet aggregation *in vitro*.

[*ChemCatChem* **13**, 3629-2635 (2013)] [Lab. of Organic Chemistry]

Chemoselective Hydrogenation Catalyzed by Pd on Spherical Carbon.

Hiroyoshi ESAKI, Tomohiro HATTORI, Aya TSUBONE, Satoko MIBAYASHI, Takao SAKATA, Yoshinari SAWAMA, Yasunari MONGUCHI, Hidehiro YASUDA, Kazuto NOSAKA, and Hironao SAJIKI*

We have developed a highly chemoselective hydrogenation method using a novel palladium catalyst supported on spherical carbon (0.5% Pd/SC). The 0.5% Pd/SC exhibited a novel catalytic activity and could achieve the chemoselective hydrogenation of alkynes, alkenes, azides, nitro groups, and aliph. *O*-*tert*-butyldimethylsilyl (TBS) ethers without hydrogenolysis of benzyl esters, benzyl ethers, nitriles, arom. ketones, N-carbobenzyloxy (*N*-Cbz) protective groups, and arom. *O*-TBS ethers.

[*Org. Lett.* **15**, 5282-5285 (2013)] [Lab. of Organic Chemistry]

Iron-Catalyzed Ring-Opening Azidation and Allylation of *O*-Heterocycles.

Yoshinari SAWAMA*, Kyoshiro SHIBATA, Yuka SAWAMA, Masato TAKUBO, Yasunari MONGUCHI, Norbert KRAUSE and Hironao SAJIKI*

We have established the first catalytic C-C and C-N bond formation reactions of *O*-heterocycles (e.g., THF, phthalan, and lactone derivs.) using iron trichloride as a catalyst in the presence of $TMSN_3$ or allylsilanes accompanied by the ring opening of *O*-heterocycles. The reactions smoothly proceeded at room temperature to give the corresponding primary saturated alcoholos from the 2-substituted tetrahydrofurans, *ortho*-substituted benzyl alcohols from phthalanes, and saturated carboxylic acids from lactones in high yields.

[*J. Org. Chem.* **78**, 8980-8985 (2013)] [Lab. of Organic Chemistry]

Mechanism Study of Copper-mediated One-pot Reductive Amination of Aryl Halides Using Trimethylsilyl Azide.

Toshihide MAEJIMA, Moriatsu UEDA, Jun NAKANO, Yoshinari SAWAMA, Yasunari MONGUCHI* and Hironao SAJIKI*

Reaction mechanisms of the copper-mediated amination of aryl halides with trimethylsilyl azide ($TMSN_3$) were analyzed on the basis of the time-course study using reaction monitoring FT-IR, trapping an intermediary aryl azide by the Huisgen reaction, and the anal. of the generated N_2 gas during the reaction. This amination would proceed through multiple pathways via aryl radicals and copper(I) azide.

[*Adv. Synth. Catal.* **355**, 1529-1534 (2013)] [Lab. of Organic Chemistry]

Platinum on Carbon-Catalyzed H-D Exchange Reaction of Aromatic Nuclei Due to Isopropyl Alcohol-Mediated Self-Activation of Platinum Metal in deuterium oxide.

Yoshinari SAWAMA, Tsuyoshi YAMADA, Yuki YABE, Kosuke MORITA, Kyoshiro SHIBATA, Masahiro SHIGETSURA, Yasunari MONGUCHI and Hironao SAJIKI*

An efficient and simple deuteration method of arenes using the platinum on carbon-isopropanol-cyclohexane-deuterium oxide combination under hydrogen gas-free conditions was accomplished. Since the hydrogen-deuterium exchange reaction cannot be promoted without isopropanol, zerovalent platinum metal (on carbon) is self-activated by the in situ-generated very low amountof hydrogen or hydrogen-deuterium gas derived from isopropanol or isopropanol-d_1. The present hydrogen gas-free method is safe from the viewpoint of process chemistry and various arenes possessing a variety of reducible functionalities within the molecules could be effectively and directly deuterium-labeled without undesired reduction.

[*ChemCatChem* **5**, 2360-2366 (2013)] [Lab. of Organic Chemistry]

Easily-Controlled Chemoselective Hydrogenation Using Palladium on Boron Nitride.

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

The hydrogenation catalyzed heterogeneously by palladium on boron nitride (Pd/BN) in methanol realized the chemoselective hydrogenation of only azides, alkenes, and alkynes in the presence of other reducible functionalities such as benzyl ethers, aryl halides, aryl ketones, and nitro groups. Furthermore, the totally chemoselective semihydrogenation of alkynes could also be achieved without the reduction of other coexisting reducible functionalities, which include azides and alkenes, by using Pd/BN in pyridine as a solvent.

[*Org. Lett.* **15**(6), 1306-1309 (2013)] [Lab. of Organic Chemistry]

Chemoselective Hydrogenation Reaction of Unsaturated Bonds in the Presence of an *o*-Nitrobenzenesulfonyl Group.

Akinori KAWANISHI, Chiyako MIYAMOTO, Yuki YABE, Makoto INAI, Tomohiro ASAKAWA, Yoshitaka HAMASHIMA, Hironao SAJIKI* and Toshiyuki KAN

Chemoselective hydrogenation of unsaturated compounds bearing an *o*-nitrobenzenesulfonyl (Ns)-amide moiety, affording the corresponding saturated compounds, was accomplished efficiently without loss of the nitro group by using the Pd/MS3A catalyst and a H_2 balloon. Partial hydrogenation of alkynes bearing an Ns group to corresponding *cis* alkenes was achieved with the combination of the Pd/BN catalyst and an additive (diethylenetriamine or acetic acid).

[*Adv. Synth. Catal.* **355**, 517-528 (2013)] [Lab. of Organic Chemistry]

Lewis Acid-Catalyzed Ring-Opening Functionalizations of 1,4-Epoxy-1,4-dihydronaphthalenes.

Yoshinari SAWAMA*, Yuta OGATA, Koichi KAWAMOTO, Hiroyuki SATAKE, Kyoshiro SHIBATA, Yasunari MONGUCHI, Hironao SAJIKI and Yasuyuki KITA

We have accomplished the Lewis acid-catalyzed carbon-carbon and carbon-nitrogen bond formations assocompaneid with the ring opening of 1,4-epoxy-1,4-dihydronaphthalenes using nucleophiles such as allyltrimethylsilanes, trimethylsilyl cyanide andtrimethylsilyl azide, by using the stabilization effect of the cation intermediate based on the introduction of appropriatesubstituents into the bridgehead positions of 1,4-epoxy-1,4-dihydronaphthalenes to give the corresponding unique and multi-functionalized naphthalene derivatives. The present reactions could provide excellent regioselective functionalization methods using unsymmetrical substrates, which are quite difficult to achieve using transition metal-induced procedures.

[*Green Chem.* **15**, 490-495 (2013)] [Lab. of Organic Chemistry]

Solvent-free Huisgen Cyclization Using Heterogeneous Copper Catalysts Supported on Chelate Resin.

Yasunari MONGUCHI, Kei NOZAKI, Toshihide MAEJIMA, Yutaka SHIMODA, Yoshinari SAWAMA, Yoshiaki KITAMURA, Yukio KITADE and Hironao SAJIKI*

Copper catalysts supported on chelate resins bearing iminodiacetate moieties (DIAION CR11) or polyamine moieties (DIAION CR20) as chelating functional groups (12% Cu/CR11 and 7% Cu/CR20, respectively) were developed. 12% Cu/CR11 effectively catalyzed the Huisgen cycloaddition of mono-substituted alkynes to azides in the presence of triethylamine under totally solvent-free conditions to afford the corresponding 1,4-disubstituted 1,2,3-triazoles in excellent yields and in a completely regioselective manner. Furthermore, the Huisgen cycloaddition was found to effectively proceed without addition of triethylamine by the use of 7% Pd/CR20 as a catalyst.

[*Angew. Chem. Int. Ed*. **52**, 1515-1519 (2013)] [Lab. of Organic Chemistry]

Efficient Generation of *ortho*-Naphthoquinone Methides from 1,4- Epoxy-1,4-dihydronaphthalenes and Their Annulation with Allyl Silanes.

Yoshinari SAWAMA*, Yuko SHISHIDO, Takayoshi YANASE, Koichi KAWAMOTO, Ryota GOTO, Yasunari MONGUCHI, Yasuyuki KITA and Hironao SAJIKI*

We have established a $FeCl_3$-catalyzed method for the synthesis of 1-naphthoquinone-2-methides from 1-siloxymethyl-1,4-epoxy-1,4-dihydronaphthalenes and the further transformation of the products in an annulation reaction with various allyl silanes to afford biologival useful dihydronaphthopyran derivsatives. Various products were directly and effectively obtained via the continuous sequence of reactions, including an exceptional hetero-Diels-Alder reaction of α,β-unsaturated carbonyl compounds and allyl silanes. This methodology can be expected to contribute to the synthesis of natural products and novel bioactive agents.

[*Chem. Eur. J.* **19**, 484-488 (2013)] [Lab. of Organic Chemistry]

Site-Selective Deuterated-Alkene Synthesis Using Palladium on Boron Nitride.

Yuki YABE, Yoshinari SAWAMA, Yasunari MONGUCHI and Hironao SAJIKI*

We have developed a new Et_3N-mediated H-D exchange reaction of alkynes to prepare alkynes-d_1 in a mixture of D_2O and THF at room temperature and the Pd/BN-catalyzed (BN = boron nitride) regioselective systematic reduction and reductive deuteration gave various deuterated terminal alkenes from unlabeled alkynes or deuterated alkyne derivatives in excellent yields and with high D contents and regioselectivities. A variety of reducible functionalities, such as nitro groups, benzyl ethers, TBS ethers, and silanes, are well tolerated under the reaction conditions and the wide variety of deuterated products obtainable by this method are expected to be useful building blocks for new deuterated materials such as deuterium-labeled drugs, deuterated polymers, and tracers.

[*SYNTHESIS (PSP)* **45**, 40-44 (2013)] [Lab. of Organic Chemistry]

A Practical Protocol for the Hiyama Cross-Coupling Reaction Catalyzed by Palladium on Carbon.

Yasunari MONGUCHI, Takayoshi YANASE, Shigeki MORI and Hironao SAJIKI*

A method for the palladium on carbon (Pd/C) catalyzed cross-coupling reaction between aryl halides and trialkoxy(aryl)silanes in the presence of a small amount. of water is established using tris(4-fluorophenyl)phosphine as the ligand. A range of biaryl compounds is prepared using this protocol.

[*Tetrahedron Lett.* **54**, 6218-6221 (2013)] [Lab. of Pharmaceutical Synthetic Chemistry]

Aerobic Photooxidative Cleavage of 1,3-Diketones to Carboxylic Acids Using 2-Chloroanthraquinone.

Yuma TACHIKAWA, Lei CUI, Yoko MATSUSAKI, Norihiro TADA, Tsuyoshi MIURA and Akichika ITOH*

We developed direct aerobic photooxidation of 1,3-diketones to corresponding carboxylic acids in the presence of a catalytic amount of 2-chloroanthraquinone under visible light irradiation from fluorescent lamps. When benzoylacetones were used as substrates, the corresponding carboxylic acids were obtained in good-to-high yields, regardless of the presence of an electron-donating or electron-withdrawing group on the benzene ring. Furthermore, 2-naphthoic acid and 3-thiophenecarboxylic acid were obtained in good yields. Dibenzoylmethane was also oxidized to benzoic acid in good yield. Furthermore, 1,3-oxoester was converted to benzoic acid in moderate yield. In addition, 1,3-cyclohexanedione and 2-hydroxyacetophenone were oxidized to corresponding carboxylic acids albeit in low yields, respectively. Under these conditions, acetophenone functioned as a poor substrate.

[*Photochem. Photobiol. Sci.* **12**, 417-420 (2013)] [Lab. of Pharmaceutical Synthetic Chemistry]

Aerobic Photooxidative Cleavage of Epoxides to Carboxylic Acids Using Magnesium Bromide.

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

We developed an aerobic photooxidative cleavage of epoxides to carboxylic acids using a catalytic quantity of magnesium bromide and molecular oxygen as the terminal oxidant, under photoirradiation with a high-pressure mercury lamp. Reactions of styrene oxides bearing substituents at the para-position proceeded smoothly to afford the corresponding carboxylic acids, respectively in good to high yields. In addition, cis-stilbene oxide, 1-acetyl-2-phenyloxirane, and chalcone epoxide were converted to corresponding carboxylic acids in good yields. Furthermore, β-methylstyrene epoxide and 2-vinylnaphthalene epoxide were converted to benzoic acid and 2-naphthoic acid, respectively, in moderate yields. In contrast, trans-stilbene oxide was a poor substrate. Aliphatic epoxides were converted to the corresponding carboxylic acids, albeit in low yields. It is noteworthy that gram scale reaction can be proceeded in 48% yield under non-optimized condition.

[*Tetrahedron Lett.* **54**, 4896-4899 (2013)] [Lab. of Pharmaceutical Synthetic Chemistry]

Asymmetric Conjugate Addition of Aldehydes to Vinyl Sulfone Using a Diaminomethylenemalononitrile Organocatalyst.

Yohei KANADA, Hiroki YUASA, Kosuke NAKASHIMA, Miho MURAHASHI, Norihiro TADA, Akichika ITOH, Yuji KOSEKI and Tsuyoshi MIURA*

Diaminomethylenemalononitrile organocatalyst promotes the asymmetric conjugate addition of branched aldehydes to vinyl sulfone to afford the corresponding adducts with all-carbon quaternary stereocenters in excellent yields with up to 91% ee. We selected methyl and methoxy substituents as the representative electron-donating group and halogen substituents as the electron-withdrawing groups on the benzene ring. The reactions of branched aldehydes with 1,1-bis(phenylsulfonyl)ethene proceeded smoothly and resulted in the corresponding adducts in excellent yields with 82–91% ee. The conjugate addition of *N*-Boc α-aminophenylacetaldehyde gave the corresponding adduct in excellent yield with low enantioselectivity.

[*Chem. Lett.* **42**, 1151-1153 (2013)] [Lab. of Pharmaceutical Synthetic Chemistry]

Asymmetric Conjugate Addition of Malonates to Enones Using Pperfluorobutanesulfonamide Organocatalyst.

Yuji KAMITO, Akira MASUDA, Hiroki YUASA, Norihiro TADA, Akichika ITOH, Yuji KOSEKI and Tsuyoshi MIURA*

Perfluorobutanesulfonamide organocatalyst efficiently promotes asymmetric conjugate additions of malonates to α,β-unsaturated ketones to afford the corresponding adducts in excellent yields with up to 99% ee. We selected bromo and nitro substituents as representative electron-withdrawing groups on the benzene ring and methyl and methoxy substituents as the electron-donating group. The reactions of enones with substituents smoothly proceeded to give the corresponding adducts in high yields with excellent enantioselectivitie. Moreover, we examined the reactions with enone possessing a naphthalene skeleton to afford the corresponding adduct with 94% ee.

[*Tetrahedron Lett.* **54**, 256-258 (2013)] [Lab. of Pharmaceutical Synthetic Chemistry]

Calcium Iodide Catalyzed Photooxidative Oxylactonization of Oxocarboxylic Acids Using Molecular Oxygen as Terminal Oxidant.

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

We developed aerobic photooxidative oxylactonization of oxocarboxylic acids catalyzed by calcium iodide using molecular oxygen as the terminal oxidant under photo irradiation. Electron-deficient and electron-rich substrates gave the corresponding oxolactones in good yields. Sterically hindered substrates possessing two methyl groups on the aromatic ring gave the corresponding oxolactones in modest yields. 4-(2-Naphthoyl)butyric acid gave the corresponding oxolactone in good yield. Unfortunately, 4-acetylbutyric acid, 3-benzoylpropionic acid, and 5-benzoylpentanoic acid were poor substrates.

[*Synlett* **24**, 607-610 (2013)] [Lab. of Pharmaceutical Synthetic Chemistry]

Catalytic Aerobic Photooxidative Cleavage of Carbon-carbon Triple Bonds Using Carbon Tetrabromide.

Tomoaki YAMAGUCHI, Tomoya NOBUTA, Yasuhisa KUDO, Shin-ichi HIRASHIMA, Norihiro TADA, Tsuyoshi MIURA and Akichika ITOH*

We developed the aerobic photooxidative cleavage of carbon–carbon triple bonds to carboxylic acids in the presence of catalytic amounts of carbon tetrabromide under photoirradiation with a high-pressure mercury lamp. Generally, the corresponding carboxylic acids are obtained in good to high yields regardless of the electron-donating or withdrawing group on the benzene ring. In addition, the ethynyl group is more easily oxidized to carboxylic acid than the methyl group, and 4-methylbenzoic acid was obtained in 52% yield. Furthermore, internal alkynes were also oxidized in moderate to good yields. Unfortunately, no 2-picolinic acid was obtained. On the other hand, 3-ethynylthiphene was converted to 3-thiophenecarboxylic acid albeit in low yield.

[*Tetrahedron Lett.* **54**, 162-165 (2013)] [Lab. of Pharmaceutical Synthetic Chemistry]

Efficient Generation of Hydrogen Peroxide by Aerobic Photooxidation of 2-Propanol Using Anthraquinone-2-carboxylic Acid and One-pot Epoxidation of α,β-Unsaturated Ketones.

Lei CUI, Sohei FURUHASHI, Yuma TACHIKAWA, Norihiro TADA, Tsuyoshi MIURA and Akichika ITOH*

We developed an efficient method for the generation of hydrogen peroxide by aerobic photooxidation of 2-propanol using anthraquinone-2-carboxylic acid and molecular oxygen in air and visible light from fluorescent lamps. One-pot epoxidation of α,β-unsaturated ketones using the generated hydrogen peroxide is also reported. In these reactions, hydrogen peroxide was generated for 10 h, and subsequently, the α,β-unsaturated ketone was epoxidized in the presence of 1.0 M aqueous KOH. trans-Chalcone and derivatives with substituents such as electron withdrawing and donating groups are good substrates for this reaction, which afforded corresponding epoxides in high yields. The epoxide of trans-4-phenyl-3-buten-2-one, which has one aliphatic substituent, was also obtained in 71% yield. Furthermore, aliphatic substrates participate effectively in this reaction.

[*Synthesis* **45**, 2684-2688 (2013)] [Lab. of Pharmaceutical Synthetic Chemistry]

Facile Aerobic Photooxidation of Alcohols Using 2-Chloroanthraquinone under Visible Light Irradiation.

Yoshiko SHIMADA, Kasumi HATTORI, Norihiro TADA, Tsuyoshi MIURA and Akichika ITOH*

We report a facile photooxidation of alcohols to obtain carboxylic acids and ketones using easily handled 2-chloroanthraquinone as an organocatalyst under visible light irradiation in an air atmosphere. The reaction conditions are mild, such as an air atmosphere and ambient pressure and temperature. Both substrates with an electron-withdrawing and electron-donating group in aromatic nucleus gave the corresponding carboxylic acids in good yields in an air atmosphere and using visible light irradiation from fluorescent lamps in the presence of K_2CO_3 (Method A) or TFA/H_2O (Method B); however, the substrate with a strong electron-withdrawing group gave few carboxylic acids. We also examined an aliphatic alcohol, and obtained the corresponding carboxylic acid in low yields.

[*Adv. Synth. Catal.* **355**, 2203-2207 (2013)] [Lab. of Pharmaceutical Synthetic Chemistry]

Metal-free Direct C-H Perfluoroalkylation of Arenes and Heteroarenes Using a Photoredox Organocatalyst.

Lei CUI, Yoko MATSUSAKI, Norihiro TADA, Tsuyoshi MIURA, Bunji UNO and Akichika ITOH*

We report visible-light-induced trifluoromethylation of arenes and heteroarenes using sodium trifluoromethanesulfinate catalyzed by anthraquinone-2-carboxylic acid. This reaction is the metal-free trifluoromethylation of arenes and heteroarenes catalyzed by a photoredox organocatalyst. Perfluoroalkylated arenes were also produced using sodium perfluoroalkylsulfinate. Electron-rich arenes gave the corresponding products in good yields. In addition, some substituted heteroarenes were also obtained in good yields. In contrast, we got trace amounts of product when benzene was used as substrate, and nitrobenzene didn't react at all. For further studies, we used various sodium perfluoroalkylsulfinates and found that pentafluoroethyl (C_2F_5) and heptafluoropropyl (C_3F_7) groups could be substituted on 1,3,5-trimethoxybenzene in good yields.

[*Org. Lett.* **15**, 574-577 (2013)] [Lab. of Pharmaceutical Synthetic Chemistry]

Molecular Iodine Catalyzed Cross-dehydrogenative Coupling Reaction between Two sp3 C-H Bonds Using Hydrogen Peroxide.

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

A useful method for molecular iodine catalyzed oxidative C-C bond formation between tertiary amines and a carbon nucleophile using hydrogen peroxide as the terminal oxidant is reported. This is the first report of a molecular iodine catalyzed cross-dehydrogenative coupling (CDC) reaction between two sp^3 C-H bonds. In general, the corresponding aza-Henry products were obtained in good yields with nitromethane as the coupling partner, regardless of whether there was an electron-donating or electron-withdrawing group on the *N*-aryl group aromatic ring. Using nitroethane, C-C bond formations proceeded smoothly to afford the desired products in moderate to good yields. Unfortunately, *N*,*N*-dimethyl-*p*-toluidine was poor substrete.

[*RSC Adv.* **3**, 10189-10192 (2013)] [Lab. of Pharmaceutical Synthetic Chemistry]

Molecular-iodine-catalyzed Aerobic Photooxidative C-C Bond Formation Between Tertiary Amines and Carbon Nucleophiles.

Tomoya NOBUTA, Akitoshi FUJIYA, Tomoaki YAMAGUCHI, Norihiro TADA, Tsuyoshi MIURA and Akichika ITOH*

This paper reports a useful method for molecular-iodine-catalyzed aerobic photooxidative C–C bond formation between tertiary amines and carbon nucleophiles. This reaction provides a practical method for C–C bond formation through the use of molecular iodine, harmless visible light irradiation, and molecular oxygen as the terminal oxidant. In general, the corresponding aza-Henry products were obtained in good to high yields using nitromethane and nitroethane regardless of whether an electron-donating or electron-withdrawing group was present on the aromatic ring of the *N*-aryl moiety. Unfortunately other tertiary amines, such as *N*,*N*-dimethyl-*p*-toluidine and *N*-benzyl-*N*-methylaniline, were poor substratas.

[*Molecules* **18**, 14529-14542 (2013)] [Lab. of Pharmaceutical Synthetic Chemistry]

Perfluoroalkanesulfonamide Organocatalysts for Asymmetric Conjugate Additions of Branched Aldehydes to Vinyl Sulfones.

Kosuke NAKASHIMA, Miho MURAHASHI, Hiroki YUASA, Mariko INA, Norihiro TADA, Akichika ITOH, Shin-ichi HIRASHIMA, Yuji KOSEKI and Tsuyoshi MIURA*

Asymmetric conjugate additions of branched aldehydes to vinyl sulfones promoted by sulfonamide organocatalyst 6 or 7 have been developed, allowing facile synthesis of the corresponding adducts with all-carbon quaternary stereocenters in excellent yields with up to 95% ee. A range of electron-withdrawing substituents such as bromo and fluoro moieties, and electron-donating substituents such as methyl and methoxy groups on the aromatic ring of branched aldehydes provided the corresponding adducts in excellent yields with good enantioselectivities (83%–92% ee). The additions of branched aldehydes possessing a naphthalene motif, to vinyl sulfone proceeded smoothly to afford the corresponding adducts in excellent yields with 92% ee, respectively

[*Phytochem. Lett.* **6**, 215-218 (2013)] [Lab. of Pharmacognosy]

Flavonoids isolated from the leaves of *Melicope triphylla* and their extracellular-superoxide dismutase-inducing activity.

Masayoshi OYAMA*, Ken-ichi NAKASHIMA, Tetsuro KAMIYA, Manami HABA, Tetsuro ITO, Hiroko MURATA, Toshiyuki TANAKA, Tetsuo ADACHI, Munekazu IINUMA and Takeshi KINOSHITA

Two novel flavonoids, named meliflavones A (**1**) and B (**2**), were isolated from the leaves of *Melicope triphylla* (Lam.) Merr., along with thirteen known compds. (**3**–**15**). Four of the polymethoxyflavonoids bearing a prenyloxy (3-methylbut-2-enyloxy) function (**1**, **3**–**5**) induced the expression of extracellular-superoxide dismutase (EC-SOD) in a human leukemic U937 cell-based assay.

[*Phytochem. Lett.* **6**, 193-197 (2013)] [Lab. of Pharmacognosy]

Absolute Configuration and Conformational Analysis of *C*-glucoside of a Resveratrol Trimer: Structure of Hopeaside E from *Hopea utilis*.

Tetsuro ITO, Ryosuke HOSHINO, Yasumasa HARA, Masayoshi OYAMA* and Munekazu IINUMA

A new glucoside of the resveratrol trimer (hopeaside E) was isolated from the stem wood of *Hopea utilis*. The glucoside structure is partially composed of balanocarpol (resveratrol dimer) after oxidative condensation of the (*E*)-resveratrol-10-*C*-β-glucopyranoside. The structure elucidation was achieved by spectroscopic analysis including NMR experiments, and the absolute configuration was determined on the basis of the comparative configurational analysis with the β-$_D$-glucopyranosyl group. Conformational analysis was also performed by considering deshielding effects due to aromatic rings using computational methods of molecular modeling. The aglycone has six asymmetric carbons with two aliphatic hydroxyl groups attached to them that has not been reported in any other resveratrol derivative studies.

[*Chem. Pharm. Bull*., **61**, 551-558(2013)] [Lab. of Pharmacognosy]

Isolation of Six Isoprenylated Biflavonoids from the Leaves of *Garcinia subelliptica*.

Tetsuro ITO, Renpei YOKOTA, Tatsuya WATARAI, Koki MORI, Masayoshi OYAMA*, Hideko NAGASAWA, Hideaki MATSUDA and Munekazu IINUMA

Six new biflavonoids were isolated from the leaves of *Garcinia subelliptica*. The new biflavonoids are rare mono-isoprenylated derivatives that have a flavone-(3′–8″)-flavone core (amentoflavone type) and a flavanone-(3–8″)-flavone core (morelloflavone type). The absolute configurations of the morelloflavone-type biflavonoids were confirmed by circular dichroism. The biflavonoids with an isoprenyloxy group and a 2-hydroxy-3-methyl-3-butenyl group, and the morelloflavone-type biflavonoids with a C_5 unit are the first examples in nature. We found that amentoflavone, one of the major biflavonoids, strongly inhibited hypoxia-inducible factor-1 in human embryonic kidney 293 cells under hypoxic conditions.

[*Phytochem. Lett.* **6**, 667-670 (2013)] [Lab. of Pharmacognosy]

Novel Isolation of Resveratrol Dimer *O*-glucosides with Enantiomeric Aglycones from the Leaves of *Shorea cordifolia*.

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

Two *O*-glucosides of resveratrol dimers, ampelopsin F-11b-*O*-β-glucopyranosides with enantiomeric aglycones (cordifolosides A and B) and an enantiomer of the aglycone [(−)-ampelopsin F] were isolated from the leaves of *Shorea cordifolia* (Dipterocarpaceae). These structures were identified on the basis of spectroscopic evidence and their absolute configurations were elucidated using circular dichroism data. This is the first report on oligostilbenoids that demonstrates the co-occurrence of diastereomeric *O*-glucosides with enantiomeric aglycones in this family.

[*Bunseki Kagaku* **62**, 167-171 (2013)] [Lab. of Pharmaceutical Analytical Chemistry]

High-performance Liquid Chromatographic Estimation of the π-π Charge-transfer Interaction Ability of Electron Acceptors Using Phenyl-modified Silica-gel Column.

Bunji UNO,* Satoshi MAEKAWA, Tatsushi NAKAYAMA1, Hiroya MURAKAMI1, and Yukihiro ESAKA

The intermolecular π-π charge-transfer (CT) complex formation ability of electron acceptors such as *p*-chloranil, TCNE, TCNQ, DDQ, and TCNB has been evaluated as retention times of HPLC using a phenyl-modified silica-gel column as a stationary phase with mobile phase consisting of heptane and benzene. It is found that there is a good linear correlation between the retention times of the acceptors and the formation constants (K_{CT}) for the CT complexes with pyrene. On the other hand, half-wave reduction potentials ($E_{1/2}$) as experimental LUMO energies of acceptors are well correlated with the intermolecular CT band energies based on Mulliken' CT theory, but unfavorably explain the K_{CT} values. Therefore, the retention times obtained by the HPLC system are considered as a direct indicator value of the intermolecular π-π type CT ability of electron acceptors.

[*J. Phys. Chem. B* **117**, 10834-10845 (2013)] [Lab. of Pharmaceutical Analytical Chemistry]

Formal Redox Potentials of Organic Molecules in Ionic Liquids on the Basis of Quaternary Nitrogen Cations as Adiabatic Electron Affinities.

Kunimasa SETO, Tatsushi NAKAYAMA, and Bunji UNO*

Formal redox potentials $E^{\circ\prime}$ involving neutral species and radical anions in ionic liquids (ILs) are discussed from the point of view of the adiabatic electron affinity as a molecular property. It is found that the $E^{\circ\prime}$ values of the 1,4-benzoquinone (BQ)/$BQ^{\bullet-}$ redox couple in the ammonium and pyridinium ILs are not influenced by the measurement conditions, and that they remain considerably dependent on the nature and concentration of the electrolyte when measured using the traditional method involving molecular solvents combined with a supporting electrolyte (0.1–0.5 M). Notably, the $E^{\circ\prime}$ values obtained in the ammonium IL correlate well with the calculated standard redox potentials and are linearly fitted with high correlation over all classes of compounds using a single regression equation based on Koopmans' theorem.

[*Int J Pharm* **453**, 329-335 (2013)] [Lab. of Pharmaceutical Enginnering]

Drug delivery to the ocular posterior segment using lipid emulsion via eye drop administration: Effect of emulsion formulations and surface modification.

Lin YING, Kohei TAHARA and Hirofumi TAKEUCHI*

This work explored submicron-sized lipid emulsion as potential carriers for intraocular drug delivery to the posterior segment via eye drops. The effects of physicochemical properties of lipid emulsion on drug delivery were evaluated in vivo using mice. Different formulations of submicron-sized lipid emulsions were prepared using a high pressure homogenization system. Using coumairn-6 as a model drug and fluorescent marker, fluorescence could be observed in the retina after administration of the lipid emulsion. The fluorescence intensity observed after administration of medium chain triglycerides containing the same amount of coumarin-6 was much lower than that observed after administration of lipid emulsions. The inner oil property and phospholipid emulsifier did not affect the drug delivery efficiency to the retina.

[*Asian Journal of Pharmaceutical Sciences* **8**, 104-109 (2013)] [Lab. of Pharmaceutical Enginnering]

Preparation of bromfenac-loaded liposomes modified with chitosan for ophthalmic drug delivery and evaluation of physicochemical properties and drug release profile.

Toshimasa TSUKAMOTO, Kohei HIRONAKA, Takuya FUJISAWA, Daiki YAMAGUCHI, Kohei TAHARA, Yuichi TOZUKA and Hirofumi TAKEUCHI*

The purpose of this study was to design a submicron-sized liposomal non-steroidal anti-inflammatory drug (NSAID) preparation that targets the retina via topical instillation of eye drops. Bromfenac (BRF)-loaded liposomes were prepared using the calcium acetate gradient method. Liposome sizes and encapsulation efficiencies were optimized by screening several liposome formulations of lipid, drug concentration, and buffer solution. BRF entrapment efficiency was greater than 90% using this method, and was low using conventional hydration methods. High initial BRF loading using the pH gradient method caused aggregation of liposomes.

[*Powder Technology* **240**, 2-6 (2013)] [Lab. of Pharmaceutical Enginnering]

Dry powder formulation with α-glycosyltransferase-treated stevia for the effective absorption of hydrophobic bioactive compounds in crude drugs.

Yuichi TOZUKA, Masaaki IMONO, Hiromasa UCHIYAMA, Kohei TAHARA, Shigemi TAZAWA, Yoko ARAKI and Hirofumi TAKEUCHI*

The purpose of this study was to prepare a functional dry powder capable of effectively improving the bioavailability of hydrophobic bioactive ingredients in crude drugs. A dry powder formulation from an ethanol extract of Brazilian green propolis was achieved in the presence of α-glycosyltransferase-treated stevia (Stevia-G). The resulting powder dispersed easily into an aqueous medium, and the average particle size of the suspension was about 350 nm. Propolis is known as a mixture of more than 200 ingredients; therefore, the suspension contains particles of hydrophobic compounds as well as dissolved molecules of several other compounds.

[*Journal of Drug Delivery Science and Technology* **23**, 471-475 (2013)] [Lab. of Pharmaceutical Enginnering]

Design of rapidly disintegrating drug delivery films for oral doses with hydoxypropyl methylcellulose.

Hirofumi. TAKEUCHI*, Rie YAMAKAWA, Tomoka NISHIMATSU, Yoshiko TAKEUCHI, Kazuhisa HAYAKAWA and Naoaki MARUYAMA

The purpose of this study was to develop pharmaceutical thin films that disintegrate rapidly in the oral cavity. Films containing acetaminophen as a model drug were prepared by the solution/solvent casting method. HPMC as a polymeric film former, MCC and pregelatinized starch as disintegrants, and micronized L-HPC as both a film former and a disintegrant were examined. The disintegration time and the tensile strength were measured. The surface morphologies of the films were compared using SEM. The addition of disintegrants or especially the optimal content of L-HPC into the films shortened the disintegration time and affected the tensile strength.

[*Journal of Pharmaceutics* **2013**, 6 (2013)] [Lab. of Pharmaceutical Enginnering]

Quantum Dot-Loaded Liposomes to Evaluate the Behavior of Drug Carriers after Oral Administration.

Kohei TAHARA, Shiho FUJIMOTO, Fumihiko FUJII, Yuichi TOZUKA, Takashi JIN and Hirofumi TAKEUCHI*

We have developed submicron-sized liposomes modified with a mucoadhesive polymer to enhance peptide drug absorption after oral administration. Liposomal behavior in the gastrointestinal tract is a critical factor for effective peptide drug delivery. The purpose of this study was to prepare quantum dot- (QD-) loaded submicron-sized liposomes and examine liposomal behavior in the body after oral administration using in vivo fluorescence imaging. Two types of CdSe/CdZnS QDs with different surface properties were used: hydrophobic (unmodified) QDs and hydrophilic QDs with glutathione (GSH) surface modifications. QD- and GSH-QD-loaded liposomes were prepared by a thin film hydration method. Transmission electron microscopy revealed that QDs were embedded in the liposomal lipid bilayer.

[*Eur J Pharm Biopharm* **83**, 364-369 (2013)] [Lab. of Pharmaceutical Enginnering]

Retinal drug delivery using eyedrop preparations of poly-l-lysine-modified liposomes.

Hitoshi SASAKI, Keiichi KARASAWA, Kohei HIRONAKA, Kohei TAHARA, Yuichi TOZUKA and Hirofumi TAKEUCHI

The purpose of this study was to develop surface-modified liposomes that enhance the efficiency of eye drop drug delivery to the retina. Various molecular weights and concentrations of the water-soluble cationic polymer poly-l-lysine (PLL) were used to modify the surface of submicronized (100nm) liposomes. Physicochemical properties of surface-modified liposomes were determined in vitro, and the efficiency of drug delivery to the retina was investigated in vivo. Using coumarin-6 as a model drug and fluorescent marker, we show that liposome surface modification by PLL dramatically increased delivery to mouse retina segments after eye drop administration. However, when PLL of high molecular weight (>30,000) was used at higher concentrations (>0.05%), aggregation of surface-modified liposomes increased particle size and hampered distribution to inner ocular tissues. As a result, the efficiency of drug delivery of these aggregated surface-modified liposomes was the same as unmodified liposomes.

[*Int J Pharm* **458**, 9-14 (2013)] [Lab. of Pharmaceutical Enginnering]

A novel approach to monitor coating amount by short-wavelength near-infrared spectroscopy using a tracer with a long-chain hydrocarbyl group.

Takahiro OZAWA, Makoto YOKOYAMA, Tetsuya HOSONO, Takuya NAGATO, Kohei TAHARA and Hirofumi TAKEUCHI*

Investigation into the use of near-infrared (NIR) as a Process Analytical Technology has been conducted for in-process monitoring of coating amounts for oral pharmaceutical products. However, the low specificity of NIR spectra has made it time consuming and costly to establish quantitative calibration models for commercial production. Here we revealed that long-chain hydrocarbyl group compounds containing saturated hydrocarbon chains, such as cetyl and stearyl, exhibit specific and strong absorption in the short wavelength (SW)-NIR region (800-1100nm) with limited interference from peaks corresponding to other components. To simplify the quantitative model, we used cetanol as a model tracer of coating amount to enhance detection sensitivity and analytical precision.

[*Int J Pharm* **441**, 67-74 (2013)] [Lab. of Pharmaceutical Enginnering]

Rapid determination of the encapsulation efficiency of a liposome formulation using column-switching HPLC.

Naozumi OHNISHI, Eiichi YAMAMOTO, Hiromasa TOMIDA, Kenji HYODO, Hiroshi ISHIHARA, Hiroshi KIKUCHI, Kohei TAHARA and Hirofumi TAKEUCHI*

The feasibility of a rapid automated method for determination of the encapsulation efficiency (EE) of a liposome formulation using a column-switching HPLC system was confirmed by employing several types of liposome formulations containing doxorubicin (DXR). A suspension of DXR liposome was injected directly into an online solid-phase extraction (SPE) system comprising a Diol SPE column and an ODS SPE column connected in series. Free (not encapsulated) DXR was trapped on the Diol SPE column, whereas encapsulated DXR was eluted without interaction. The eluted encapsulated DXR was trapped on the ODS SPE column after being extracted from the inner phase of the liposome by mixing with an organic solvent.

[*J Pharm Sci* **102**, 1281-1289 (2013)] [Lab. of Pharmaceutical Enginnering]

Surface modification of liposomes using polymer-wheat germ agglutinin conjugates to improve the absorption of peptide drugs by pulmonary administration.

Mitsutaka MURATA, Takashi. YONAMINE, Shota TANAKA, Kohei TAHARA, Yuichi TOZUKA and Hirofumi TAKEUCHI*

In this study, we investigated the feasibility of a system based on liposomal surface modification with a novel mucoadhesive polymer-lectin conjugate for the pulmonary delivery of therapeutic peptides and proteins. We covalently attached wheat germ agglutinin (WGA), a ligand that specifically interacts with alveolar epithelial cells, to carbopol (CP), a mucoadhesive polymer, using the carbodiimide method and then evaluated the efficacy and potential toxicity of CP-WGA surface-modified liposomes in vivo and in vitro. In association studies, CP-WGA modification enhanced the interaction with A549 lung epithelial cells compared with unmodified or CP-modified liposomes. This increased association was dependent on temperature and the surface concentration of free WGA.

[*Powder Technology* **241**, 60-66 (2013)] [Lab. of Pharmaceutical Enginnering]

Orally disintegrating tablets prepared by a co-processed mixture of micronized crospovidone and mannitol using a ball mill to improve compactibility and tablet stability.

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

The purpose of this study was to prepare orally disintegrating tablets (ODTs) by directly compressing a mixture of sugar alcohol (mannitol) and micronized crospovidone (M-CPVP). When the mixture of mannitol and M-CPVP was co-processed by ball milling, the physicochemical properties of the resultant tablets were considerably improved, particularly their stability during storage. Several types of coground mixtures using a different ratio of M-CPVP/mannitol and processing time were tested to determine the appropriate aggregates for designing the ODTs. Without this co-processing, the powder mixture had poor compactibility, and the stability of the tablet was inferior, probably due to the high hygroscopicity of M-CPVP. The ODTs containing coground M-CPVP/mannitol showed good stability for six months under humid conditions.

[*Chem Pharm Bull (Tokyo)* **61**, 962-966 (2013)] [Lab. of Pharmaceutical Enginnering]

Development of a novel and simple method to evaluate disintegration of rapidly disintegrating tablets.

Yohei HOASHI, Yuichi TOZUKA and Hirofumi TAKEUCHI*

The purpose of this study was to develop and test a novel and simple method for evaluating the disintegration time of rapidly disintegrating tablets (RDTs) in vitro, since the conventional disintegration test described in the pharmacopoeia produces poor results due to the difference of its environmental conditions from those of an actual oral cavity. Six RDTs prepared in our laboratory and 5 types of commercial RDTs were used as model formulations. Using our original apparatus, a good correlation was observed between in vivo and in vitro disintegration times by adjusting the height from which the solution was dropped to 8 cm and the weight of the load to 10 or 20 g. Properties of RDTs, such as the pattern of their disintegrating process, can be assessed by verifying the load. These findings confirmed that our proposed method for an in vitro disintegration test apparatus is an excellent one for estimating disintegration time and the disintegration profile of RDTs.

[*Drug Dev Ind Pharm* **39**, 259-265 (2013)] [Lab. of Pharmaceutical Enginnering]

Solventless dry powder coating for sustained drug release using mechanochemical treatment based on the tri-component system of acetaminophen, carnauba wax and glidant.

Yohei HOASHI, Yuichi TOZUKA and Hirofumi TAKEUCHI*

Solventless dry powder coating methods have many advantages compared to solvent-based methods: they are more economical, simpler, safer, more environmentally friendly and easier to scale up. The purpose of this study was to investigate a highly effective dry powder coating method using the mechanofusion system, a mechanochemical treatment equipped with high compressive and shearing force. Acetaminophen (AAP) and carnauba wax (CW) were selected as core particles of the model drug and coating material, respectively. Sustained AAP release was observed by selecting appropriate processing conditions for the rotation speed and the slit size. The dissolution rate of AAP processed with CW substantially decreased with an increase in talc content up to 40% of the amount of CW loaded.

[*Int J Pharm* **455**, 132-137 (2013)] [Lab. of Pharmaceutical Enginnering]

A completely solvent-free process for the improvement of erythritol compactibility.

Yohei HOASHI, Yuichi TOZUKA and Hirofumi TAKEUCHI*

Objective: We obtained improvement of erythritol compactibility by formulating composite particles composed of erythritol and porous silica using a twin-screw kneader. Methods: Erythritol-based tablets formulated with composite particles were directly compacted, and we estimated their hardness and the friability. The compression properties of the erythritol powder bed including composite particles were estimated using a Heckel analysis and force-displacement profiles, and we investigated the physical states of the composite particles by powder X-ray diffractometry, a thermal analysis and a nitrogen gas adsorption study. Results: A direct-compacted erythritol tablet formulated with composite particles, prepared at the melting temperature of erythritol (120°C), exhibited high hardness and low friability. A pressure transmission study revealed the higher plasticity and lower elasticity of an erythritol powder bed formulated with composite particles prepared at 120°C.

[*Biomacromolecules.* **14**, 4420-4428 (2013)] [Lab. of Pharmaceutical Enginnering]

Design and Evaluation of Folate-appended α-, β-, and γ-cyclodextrins Having a Caproic Acid as a Tumor Selective Antitumor Drug Carrier *in Vitro* and *in Vivo*.

Ayaka OKAMATSU, Keiichi MOTOYAMA, Risako ONODERA*, Taishi HIGASHI, Takahiro KOSHIGOE, Yasutaka SHIMADA, Kenjiro HATTORI, Tomoko TAKEUCHI and Hidetoshi ARIMA

We designed and evaluated the FA-appended three kinds of CyDs possessing a caproic acid as a spacer between FA and a CyD molecule (Fol-c1-CyDs) as a tumor targeting carrier for antitumor drugs. Antitumor activity of doxorubicin (DOX) was increased by the complexation with Fol-c1-β-CyD, but not with Fol-c1-α-CyD or Fol-c1-γ-CyD in KB cells. Also, Fol-c1-β-CyD increased antitumor activities of paclitaxel and vinblastine, but not 5-fluorouracil. The Fol-c1-β-CyD/DOX complex showed much higher antitumor activity than DOX alone after intravenous administrations to tumor-bearing mice with a negligible change of the blood chemistry values. These findings suggest that Fol-c1-β-CyD could be useful as a tumor-selective carrier for antitumor drugs.

[*Int. J. Pharm.* **452**, 116-123 (2013)] [Lab. of Pharmaceutical Enginnering]

Involvement of Cholesterol Depletion from Lipid Rafts in Apoptosis Induced by Methyl-β-cyclodextrin.

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

Methyl-β-cyclodextrin (M-β-CyD), which is widely used as a lipid rafts disrupting agent, is known to induce cytotoxicity at high concentration. In the present study, we investigated the potential of M-β-CyD as an antitumor drug. M-β-CyD markedly caused apoptotic cell-death in KB cells, Ihara cells and M213 cells, through cholesterol depletion in cell membranes. The DNA content and mitochondrial transmembrane potential in KB cells were significantly decreased after treatment with M-β-CyD. M-β-CyD drastically inhibited the tumor growth after intratumoral injection to Colon-26 cells-bearing mice. These results strongly suggest that M-β-CyD induced apoptosis in tumor cells and had the potential a novel antitumor agent and/or its lead compound.

[*Bioconjug. Chem.* **24**, 724-733 (2013)] [Lab. of Pharmaceutical Enginnering]

Folate-appended β-cyclodextrin as a Promising Tumor Targeting Carrier for Antitumor Drugs *in Vitro* and *in Vivo*.

Ayaka OKAMATSU, Keiichi MOTOYAMA, Risako ONODERA*, Taishi HIGASHI, Takahiro KOSHIGOE, Yasutaka SHIMADA, Kenjiro HATTORI, Tomoko TAKEUCHI and Hidetoshi ARIMA

We first prepared heptakis-6-folic acid (FA)-appended β-cyclodextrin (β-CyD) possessing two caproic acids between FA and a β-CyD molecule as a spacer (Fol-c2-β-CyD) and evaluated the potential as a novel tumor targeting carrier for antitumor drugs through a complexation. Fol-c2-β-CyD increased *in vitro* antitumor activities of antitumor drugs such as doxorubicin (DOX), vinblastine, and paclitaxel in KB cells, but not in A549 cells, a FR-α-negative cell line. The complex of DOX with Fol-c2-β-CyD markedly increased antitumor activity of DOX after intravenous administration to mice subcutaneously inoculated Colon-26 cells, a FR-α-positive cell line. These findings suggest that Fol-c2-β-CyD could be useful as a promising antitumor drug carrier.

[*Sci. Rep.* **3**, 1104, 1-9 (2013)] [Lab. of Pharmaceutical Enginnering]

Potential Use of Folate-appended Methyl-β-cyclodextrin as an Anticancer Agent.

Risako ONODERA*, Keiichi MOTOYAMA, Ayaka OKAMATSU, Taishi HIGASHI and Hidetoshi ARIMA

To obtain a tumor cell-selectivity of methyl-β-cyclodextrin (M-β-CyD), we newly synthesized folate-appended M-β-CyD (FA-M-β-CyD), and evaluated the potential of FA-M-β-CyD as a novel anticancer agentin vitroandin vivo. Potent antitumor activity and cellular association of FA-M-β-CyD were higher than those of M-β-CyD in KB cells, folate receptor (FR)-positive cells. FA-M-β-CyD drastically inhibited the tumor growth after intratumoral or intravenous injection to FR-positive Colon-26 cells-bearing mice. The antitumor activity of FA-M-β-CyD was comparable and superior to that of doxorubicin after both intratumoral and intravenous administrations, respectively, at the same dose, in the tumor-bearing mice. Importantly, an intravenous administration of FA-M-β-CyD to tumor-bearing mice did not show any significant change in blood chemistry values. These results strongly suggest that FA-M-β-CyD has the potential as a novel anticancer agent.

[*J. Photopolym. Sci. Thechnol.* **26**, 545-548 (2013)] [Lab. of Pharmaceutical Physical Chemistry]

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

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

In this paper, we immobilized the cyclodextrin derivatives onto the self-assembled phospholipid layer. We immobilized per-6-amino-β-cyclodextrin as a model compound on LDPE-StA-PC-SA, and labeled the immobilized per-6-amino-β-cyclodextrin with fluorescein-4-isothiocyanate. In this experimental condition, self-assembled phospholipid layer with 3 or 5 layer was obtained. It was also shown that the triple PC layer was stable up to 60 ºC. We could successfully immobilize per-6-amino-β-cyclo-dextrin onto LDPE-StA-PC-SA film. Per-6-amino-β-cyclodextrin was easily labeled with fluorescein-4-isothiocyanate. We are now actively elaborating the application of biosensor using LDPE-StA-PC-SA film immobilizing cyclodextrin derivatives.

[*J. Photopolym. Sci. Thechnol.* **26**, 559-562 (2013)] [Lab. of Pharmaceutical Physical Chemistry]

Preparation of Enzyme-immobilized Filter Paper Using Plasma Surface Treatment.

Yasushi SASAI*, Daishi MISHIMA, Tomomi RIKIHISA, Shin-ichi KONDO, Yukinori YAMAUCHI and Masayuki KUZUYA

In this study, the trypsin-immobilized filter paper with protease activity was designed. The surface of cellulose fiber in filter paper was modified with vinylmethylether-maleic acid copolymer (VEMAC) by plasma-based method to immobilize trypsin with high activity retention. The trypsin was covalently immobilized to carboxyl group of VEMAC on cellulose fiber through 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride/N-hydroxysuccinimide (EDC/NHS) coupling reaction. The enzyme activity of the immobilized trypsin and its thermal stability were considerably improved, indicating that VEMAC worked as an effective interface between trypsin and cellulose fiber.

[*J. Photopolym. Sci. Thechnol.* **26**, 529-532 (2013)] [Lab. of Pharmaceutical Physical Chemistry]

Construction of Matrix-type Drug Delivery System Using Solid Phase Polymerization Initiated by Plasma-induced Radicals.

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

In this study, we aimed to construct the high functional surface of low-substituted hydroxypropyl cellulose (L-HPC) powder by vibratory miximng of reactive surface radicals formed with plasma treatment and solid-state yinyl monomer, N-vinylacetamide (NVA). The block copolymer, L-HPC-*block*-poly-NVA, was successfully synthesized on the L-HPC surface by radical polymerization of NVA, which was initiated by the plasma-irradiated radicals located on the surface. Drug release properties from tablets prepared with or without NVA grafted L-HPC had been studied and compared for the different contents of NVA. The drug was more slowly released from the tablets with the increase of the content of NVA.

[*Chemosphere*, **90**, 57-64 (2013)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Practical Method for PCB Degradation Using Pd/C–H2–Mg System.

Akiko IDO, Shinji ISHIHARA, Akira KUME, Tsuyoshi NAKANISHI, Yasunari MONGUCHI, Hironao SAJIKI and Hisamitsu NAGASE*

We have reported a catalytic degradation method of polychlorinated biphenyls (PCBs) based on a palladium on carbon (Pd/C)-catalyzed dechlorination in the presence of Et3N under ambient hydrogen pressure and temperature. In this study, we demonstrate a more practical system using magnesium metal instead of Et3N for the dechlorination of a variety of aromatic chlorides. The method was applicable for the complete degradation of a variety of PCB mixtures, such as Aroclor 1242, 1248, 1254 and PCBs removed from a capacitor toproduce only biphenyl and magnesium chloride as the maritime component, both of which are less toxic and easily separable. Moreover, the Pd/C could be recovered and reused at least five times without any loss of catalytic activity. The present Pd/C–Mg–H2 system is a simple, safe, inexpensive, and environmentally-benign degradation method of PCBs.

[*J. Toxicol. Sci.*, **38**, 151-153(2013)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

Microarray analysis of neonatal brain exposed to cadmium during gestation and lactation.

Akiko HONDA, Chiho WATANABE, Minoru YOSHIDA, Hisamitsu NAGASE* and Masahiko SATOH

DNA microarray containing 30,000 genes was used to monitor the transcriptional response of the neonatal brain after cadmium (Cd) exposure. C57BL/6J pregnant mice were exposed to Cd (10 ppm) during gestation and lactation via drinking water. In a comparison between the Cd-exposed neonatal brain and control, three genes including transferrin receptor (Tfrc) were up-regulated and one gene was down-regulated.

[*J. Toxicol. Sci.*, **38**, 155-157 (2013)] [Lab. of Hygienic Chemistry and Molecular Toxicology]

DNA microarray analysis of hepatic gene expression in mice exposed to cadmium for 30 days.

Maki TOKUMOTO, Tomoaki OHTSU, Shunji IMAI, Akiko HONDA, Hisamitsu NAGASE*and Masahiko SATOH

Although cadmium causes hepatotoxicity, its molecular mechanism is unclear. In the present study, transcriptional responses in the liver of C57BL/6J mice given 50 ppm cadmium as a drinking water for 30 days were evaluated with DNA microarray. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were not elevated following the administration of cadmium. Cadmium increased the expressions of 2 genes and reduced those of 15 genes in the liver of mice before the leading to hepatotoxicity.

[*J.Funct.Foods.* **5**, 601-606 (2013)] [Lab. of Molecular Biology]

Medium-chain Fatty Acid-containing Dietary Oil Alleviates the Depression-like Behaviour in Mice Exposed to Stress due to Chronic Forced Swimming

Hisami SHINOHARA, Hidefumi FUKUMITSU, Akira SETO and Shoei FURUKAWA*

Antidepressant-like effects of medium-chain fatty acid-containing dietary oil were examined by using mice forced to swim. This stress induced depressive symptoms and decreased the ratio of phosphorylated (p) extracellular signal-regulated kinases (ERK)1/2 to ERK1/2 in the hippocampus, demonstrating that our model prepared in mice was comparable to the models using rats. Consequently, the intake of medium- and long-chain triacylglycerols (MLCTs) resulted in a reduction in the immobility time in the forced swim test. Moreover, the ratio of pERK 1/2 to ERK1/2 was higher in mice fed the MLCT diet than in those fed the long-chain triacylglycerols. These results are the first evidence showing that MLCTs have a preventive effect against forced swimming-induced depression-like symptoms.

[*Evid Based complement Alternat Med.2013 403503* (2013)] [Lab. of Molecular Biology]

Neurite Outgrowth in PC12 Cells Stimulated by Components from Dendranthema × Grandiflorum cv. "Mottenohoka" Is Enhanced by Suppressing Phosphorylation of p38MAPK.

Atsuyoshi NISHINA, Hirokazu KIMURA, Hiroyuki TSUKAGOSHI, Kunihisa KOZAWA, Mamoru KOKETSU, Masayuki NINOMIYA and Shoei FURUKAWA*

Components from Dendranthema × grandiflorum cv. "Mottenohoka" that promote neurite outgrowth of PC12 cells were identified as acacetin and luteolin. The effects on PC12 cells were evaluated by immunoblot and immunostaining. Slight neurite outgrowth in PC12 cells was observed within 2 days of culture by luteolin or acacetin. However, NGF-stimulation induced remarkable neurite outgrowth in comparison. Neurite outgrowth by luteolin or acacetin was significantly enhanced by pretreatment with SB203580 (a p38MAPK inhibitor). The results of this study into the phosphorylation of ERK 1/2 and p38MAPK by flavonoids suggest that the inhibition of p38MAPK phosphorylation may effectively enhance neurite outgrowth.

[*Biochem.Biophys.Res.Commuun.* **432**, 456-459 (2013)] [Lab. of Molecular Biology]

Intracellular Interaction of Newly Synthesized Nerve Growth Factor and its Receptors.

Hiroshi NOMOTO, Hisako NOTSU, Yukio KATO, Keigo EKINAGA and Shoei FURUKAWA*

In autocrine cells, both a ligand and its receptors are synthesized in the same cell, but their intracellular interaction is not well known. We examined it using PC84 cells, a mutant PC12 cell line expressing (NGF. We have already reported that the intracellularprecursor of TrkA was phosphorylated and that MAP kinase was phosphorylated in PC84 cells. In this paper we found that the NGF receptors, TrkA and p75NTR, existed mainly as precursors, and most p75NTR localized inside PC84 cells. The phosphorylation of MAP kinase was also observed even when PC84 cells were incubated with anti-NGF antibody to block the extracellular interaction. These results suggest the possibility that newly synthesized NGF could interact intracellularly with the receptors in PC84 cells.

[*Biomed. Res.* **34**, 259-267 (2013)] [Lab. of Molecular Biology]

Anxiolytic-like Effect of 2-Decenoic Acid Ethyl Ester in Stress-Induced Anxiety-like Model Mice.

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

We developed trans-2-decenoic acid ethyl ester (DAEE) as a stable and small molecule with BDNF-like activities, and tested its activities on a stress-induced anxiety-like mouse model. Mice were subjected to 3 sets of sequential leaning, drenching, and rotation as chronic mild stresses applied for 1-2 days over a 3-week period; and the anxiety-like symptom was evaluated by use of the elevated plus-maze test. A daily administration of DAEE competed against the anxiety-like symptom when administered during the stress-loading, and became therapeutic when administered after the stress-loading. This activity was accompanied by amelioration of the stress-induced reduction of BDNF and neurotrophin-3 mRNAs and phosphorylated ERK1/2 in the hippocampus. These results demonstrated that DAEE behaved like an anxiolytic and ameliorated anxiety-like symptom, suggesting that DAEE may be a promising candidate for a novel anxiolytic with a new mechanism of action.

[*Biomed. Res.* **34**, 231-239 (2013)] [Lab. of Molecular Biology]

Neurotrophin-3 Influences the Number and the Laminar Fate of Cortical Progenitors in the Developing Cerebral Cortex of Mice through the MEK/ERK1/2 Signaling Pathway.

Masanari OHTSUKA, Hitomi SOUMIYA, Masami HANAI, Shoei FURUKAWA and Hidefumi FUKUMITSU*

The laminar formation in the developing cerebral cortex requires precisely regulated generation of phenotype-specific neurons. We investigated whether neurotrophin-3 (NT3) is involved in this formation by using intrautero injection of NT3 to lateral ventrilcule of E13.5 mouse embryos. NT3 increased the number of newly generated neurons and altered the neuronal phenotypes in the position and the transcription factors-expression profiles; the neuronal phenotypes originally committed for layer IV neurons were altered toward for layers II/III neurons. The former effects were observed when the parent progenitor cells were exposed to NT3 in the G1- to S-phase, whereas the latter effects were observed with exposure in the G1-phase. Taken together with aggitional observations suggested that NT3 is involved in the cortical laminar formation through the intercellular MEK/ERK pathway.

[*Neurochem. Res.* **38**, 2397-2407 (2013)] [Lab. of Molecular Biology]

Neurite Outgrowth of PC12 Cells by 4'-O-β-D-Glucopyranosyl-3', 4-Dimethoxychalcone from Brassica Rapa L. 'Hidabeni' was Enhanced by Pretreatment with p38MAPK Inhibitor.

Atsuyoshi NISHINA, Hirokazu KIMURA, Hiroyuki TSUKAGOSHI, Kunihisa KOZAWA, Mamoru KOKETSU, Masayuki NINOMIYA, Daisuke SATO, Yuki OBARA and Shoei FURUKAWA*

The cellular effects of eleven compounds including chalcone glycosides isolated from Brassica rapa L. 'hidabeni' and their synthetic derivatives were studied in rat pheochromocytoma PC12 cells. 4'-O-β-D-Glucopyranosyl-3', 4-dimethoxychalcone (A2) increased the levels of the phosphorylated ERK 1/2, p38 MAPK, and JNK/SAPK, but did not affect Akt. NGF increased the levels of phosphorylated ERK1/2, JNK/SAPK, and Akt but not p38MAPK. Signals evoked by A2 shared characteristics with those induced by NGF. Although the neuritogenic activity of A2 was weak, this effect was enhanced by pre-treatment with a p38MAPK inhibitor, suggesting that the phosphorylation of p38MAPK down-regulated neurite outgrowth.

[*FEBS J.* **280**, 3313-3327 (2013)] [Lab. of Clinical Pharmaceutics]

Zinc-induced Modulation of SRSF6 Activity Alters Bim Splicing to Promote Generation of The Most Potent Apoptotic Isoform BimS.

Hirokazu HARA*, Tatsuya TAKEDA, Nozomi YAMAMOTO, Keisuke FURUYA, Kazuya HIROSE, Tetsuro KAMIYA and Tetsuo ADACHI

Bim, a pro-apoptotic BH3-only Bcl-2 family proteins, undergoes alternative splicing to produce three dominant splicing variants (BimEL, BimL, and BimS). Zn^{2+} triggered alterations in Bim splicing and induced preferential generation of BimS, but not BimEL and BimL. Analysis using *Bim* mini-gene revealed that predicted binding sites of the SR-protein SRSF6 are located in the intronic region adjacent to exon 4. The mutations in the binding sites abolished generation of BimS mRNA derived from the mutated *Bim* mini-gene. In addition, SRSF6 directly bound to the binding site and Zn^{2+} suppressed the binding. Our findings suggest that Zn^{2+} inhibits the activity of SRSF6 and promotes elimination of exon 4, leading to preferential generation of BimS.

[*Biol. Pharm. Bull.* **36**, 585-591 (2013)] [Lab. of Clinical Pharmaceutics]

Protective Effects of Apomorphine against Zinc-induced Neurotoxicity in Cultured Cortical Neurons.

Hirokazu HARA*, Asuka MAEDA, Tetsuro KAMIYA and Tetsuo ADACHI

There is evidence that excessive Zn^{2+} release from presynaptic terminals following brain injuries such as ischemia and severe epileptic seizures induces neuronal cell death. Pretreatment with apomorphine (Apo) dose- and time-dependently ameliorated Zn^{2+} neurotoxicity. In addition, pretreatment with Apo prevented intracellular NAD^+ and ATP depletion caused by Zn^{2+} exposure. Dopamine receptor antagonists did not influence Apo protection against Zn^{2+} neurotoxicity. N-acetylcysteine, a thiol compound, partially reduced Apo protection. Entry of Zn^{2+} into neurons is thought to a critical step of Zn^{2+} neurotoxicity. Interestingly, we found that pretreatment with Apo decreased elevation of intracellular Zn^{2+} levels after Zn^{2+} exposure. Taken together, these results suggest that the protective effects of Apo are regulated, at least in part, by its oxidized products, and preventing intracellular accumulation of Zn^{2+} contributes to Apo protection against Zn^{2+} neurotoxicity.

[*J. Clin. Biochem. Nutr.* **52**, 101-105 (2013)] [Lab. of Clinical Pharmaceutics]

Effect of Endoplasmic Reticulum (ER) Stress Inducer Thapsigargin on the Expression of Extracelular-Superoxide Dismutase in Mouse 3T3-L1 Adipocytes.

Tetsuro KAMIYA*, Hirokazu HARA and Tetsuo ADACHI

Endoplasmic reticulum (ER) stress is related to metabolic disorders. It is known that inflammatory adipocytokines and oxidative stress are increased, while anti-inflammatory adipocytokines such as adiponectin are decreased in adipocytes during above conditions. Extracellular-superoxide dismutase (EC-SOD) is an anti-inflammatory enzyme that protects cells from oxidative stress. It is speculated that the regulation of EC-SOD might lead to the suppression of metabolic disorders. We observed the reduction of EC-SOD and adiponectin in 3T3-L1 adipocytes treated with thapsigargin, an ER stress inducer. Moreover, eukaryotic translation initiation factor 2α signaling cascade plays a pivotal role in the reduction of EC-SOD in 3T3-L1 adipocytes during ER stress conditions.

[*Free Radic. Biol. Med.* **61C**, 197-205 (2013)] [Lab. of Clinical Pharmaceutics]

Epigenetic Regulation of Extracellular-Superoxide Dismutase in Human Monocytes.

Tetsuro KAMIYA*, Masatomo MACHIURA, Junya MAKINO, Hirokazu HARA, Isao HOZUMI and Tetsuo ADACHI

Extracellular-superoxide dismutase (EC-SOD) plays an important role in normal redox homeostasis. It has been reported that epigenetic factors, such as DNA methylation and histone modification, are involved in several kinds of gene regulation. We investigated the involvement of epigenetic factors in EC-SOD expression and determined high levels of DNA methylation within promoter and coding regions of EC-SOD in THP-1 cells compared to those in U937 cells. Treatment with a DNA methyltransferase inhibitor significantly induced the expression of EC-SOD in THP-1 cells. Moreover, we detected histone acetylation during differentiation. Further, pretreatment with histone acetyltransferase inhibitors significantly suppressed the TPA-inducible EC-SOD expression. We also determined the epigenetic suppression of EC-SOD in peripheral blood mononuclear cells. These findings provide novel evidence that cell-specific and TPA-inducible EC-SOD expression are regulated by epigenetics in human monocytes.

[*J. Nat. Prod.* **76**, 1285-1290 (2013)] [Lab. of Clinical Pharmaceutics]

Luteolin Suppresses the Differentiation of THP-1 Cells through the Inhibition of NOX2 mRNA Expression and the Membrane Translocation of $p47^{phox}$.

Junya MAKINO, Ryohei NAKANISHI, Tetsuro KAMIYA*, Hirokazu HARA, Masayuki NINOMIYA, Mamoru KOKETSU and Tetsuo ADACHI

Luteolin is known to have several biological activities. Cluster for differentiation (CD) families are expressed during pathological processes of atherosclerosis. We investigated whether luteolin and three other flavonoids, chrysin, apigenin, and tricetin, blocked TPA-triggered induction of CD families, which were induced through the activation of PKC, MEK/ERK, and NOX-derived ROS. Luteolin blocked TPA-triggered induction of CD families in THP-1 cells. Luteolin completely blocked ROS generation, whereas it did not inhibit MEK/ERK phosphorylation. Moreover, pretreatment with luteolin suppressed TPA-triggered activation of NOX2. It is revealed that luteolin suppresses TPA-triggered induction of CD families by the prevention of NOX2 activation.

[*Int. J. Food Sci. Nutr.* **64**, 407-414 (2013)] [Lab. of Clinical Pharmaceutics]

Consumption of Polyphenol-rich Juar Tea Increases Endothelium-bound Extracellular Superoxide Dismutase Levels in Men with Metabolic Syndrome: Link with LDL Oxidizability.

Harumi UTO-KONDO, Makoto AYAORI, Yoshimi KISHIMOTO, Tetsuo ADACHI*, Shunichi TAKIGUCHI, Emi YAKUSHIJI, Makoto SASAKI, Tomohiro KOMATSU, Kazuo KONDO and Katsunori IKEWAKI

Endothelium-bound extracellular superoxide dismutase (eEC-SOD), a major antioxidative enzyme in the vasculature, is involved in anti-atherogenesis by inhibiting low-density lipoprotein (LDL) oxidation. The objective was to investigate whether the polyphenol-rich juar tea had beneficial effects on LDL oxidation and eEC-SOD levels in patients with metabolic syndrome (MetS). Although there was no change in LDL oxidizability after consumption of either tea, juar tea significantly increased eEC-SOD levels by 16%, whereas barley tea significantly decreased levels by 15%. It is noteworthy that the changes in eEC-SOD were positively associated with those in LDL oxidizability after tea consumption.

[*Med. Biol.* **157,** 134-141 (2013)] [Lab. of Clinical Pharmaceutics]

The Nutritive Power and Antioxidant Potential of Brown Rice Power with Five Grain Ingredients: The Expected Benefit of the High ORAC Value.

Kazuo AKUTSU, Hiroshi MORI, Takaharu YANAGISAWA, Hiroshi KAYAHARA, Yuki UEKI, Naoyuki HIRAKATA, Takahiro IMAZATO, Tetsuo ADACHI*, Hiroji SHIMOMURA and Eisuke MAEHATA

We investigated the mitigation of oxidative stresses using a brown rice formula containing five grain ingredients (brown rice, black soy beans, small adzuli beans, edible adlay and sesame). Tests in U.S. verified that the oxygen radical absorbance capacity (ORAC) of this formula was as high as 21 μmol TE (Trolox equivalent)/g, and it was found to contain abundant water-soluble vitamins and minerals. These characteristics are conductive to higher antioxidant potential. We evaluated the use of this product in rheumatoid arthritis patients in as attempt to bolster the body's resilience to oxidative stresses.

[*Biomed. Rep*. **1**, 614-618 (2013)] [Lab. of Pharmacokinetics]

Suppression of Metallothionein 3 Gene Expression by Androgen in LNCaP Prostate Cancer Cells.

Takashi OTSUKA, Aki HAMADA, Kazuhiro IGUCHI, Shigeyuki USUI, and Kazuyuki HIRANO*

Prostate tissue has a high zinc concentration, which may correlate with prostate cancer progression. We investigated the effect of dihydrotestosterone (DHT) on the gene expression of metallothioneins (MTs) and zinc transporters in prostate cancer. The MT3 gene expression in LNCaP cells was suppressed by DHT in a dose−dependent manner. However, it increased in a culture medium containing androgen−deficient FBS. Bicalutamide increased the gene expression of MT3 and partially reversed the suppression of MT3 gene expression induced by DHT. In PC‐ 3 cells lacking androgen receptors, DHT and bicalutamide exerted no effect on MT3 gene expression. These results suggest that MT3 gene expression is downregulated by androgen.

[*Jpn.J.Pharm.Health Care Sci.* **39**, 156-165 (2013)] [Lab. of Pharmacokinetics]

A Novel Comparative Evaluation for the Quality of Oral Generic Drugs.

Masato TERASHITA , Kazuhiro IGUCHI, Shigeyuki USUI and Kazuyuki HIRANO*

We propose a statistical and objective method to evaluate the quality of generic drugs using the coefficient of variances (CVs) calculated from their pharmacokinetic parameters. CVs were estimated from AUC, Cmax, Tmax, t1/2, and MRT which were provided as publicly-available drug information. The generic drug assessment tool (G-DAT), an original equation derived from the ratio of CVs of corresponding generic to original drugs, and the quality of generic drugs was statistically evaluated. The proposed comparative evaluation showing equivalent pharmacokinetics of generic to original drugs can be used as one solution to reduce anxieties and increase the reliability of generic drugs.

[*Gan To Kagaku Ryoho.* **40**, 349-354 (2013)] [Lab. of Pharmacokinetics]

Evaluation of the efforts of pharmaceutical care services before medical examination at an outpatient cancer chemotherapy clinic.

Chiaki YOSHIMI, Maya YAMADA, Hironori FJII, Minako NISHIGAKI, Hirotoshi IIHARA, Kiyoyuki KITAICHI*, Mayu TANAKA, Sayoko KURAHASHI, Takao TAKAHASHI, Kazuhiro YOSHIDA, Yoshinori ITOH

In the outpatient cancer chemotherapy clinic of Gifu University Hospital, we evaluated the efforts of the pharmaceutical care services (PCS) to all patients. As a consequence, the time spent for patient education and monitoring and the number of proposals on prescriptions significantly increased. After PCS, the percentage of the acceptance of proposals was reached to 94%. PCS also improved the control of chemotherapy-induced nausea and vomiting, peripheral neuropathy and skin rash. These results suggest that PCS by pharmacists would be beneficial to progress the quality of outpatient cancer chemotherapy.

[*Mol. Clin. Oncol.* **1**, 41-46 (2013)] [Lab. of Pharmacokinetics]

Difference in the emetic control among highly emetogenic chemotherapy regimens: Implementation for appropriate use of aprepitant.

Shinya AOKI*, Hirotoshi IIHARA, Minako NISHIGAKI, Yoshinori IMANISHI, Keita YAMAUCHI, Masashi ISHIHARA, Kiyoyuki KITAICHI, Yoshinori ITOH

Although antiemetic medication based on the emetogenicity of the cancer chemotherapy regimen is recommended, emetic control varies even among highly emetogenic chemotherapy (HEC). In the present study, we retrospectively investigated the rates of emetic control by a combination of aprepitant (APR), granisetron, 5-HT3 antagonist, and dexamethasone in various HEC regimens. The analized data suggest that the rate of emetic control was varied between HEC regimens as expected. APR would be benefical to improve emetic control in HEC regimens if it used appropriately. These results suggest that further intervention of emetic control in HEC regimens would be needed.

[*Biol. Trace. Elem. Res.* **151**, 9-13 (2013)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

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

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

The remarkable calcification of the basal ganglia and cerebellum has been traditionally called Fahr's disease, but this nomenclature is criticized for including heterogeneous diseases. To determine the pattern of some biological metals in the hair of patients with Fahr's disease, we investigated the levels of 24 bioelements in the hair of 28 patients (17 males and 11 females) with Fahr's disease and compared them with those of three age-, sex-, and living region-matched controls (84 controls in total). Although Fahr's disease has been considered to be a heterogenous entity, the significant tendencies of several bioelements in the hair of patients in this study suggest metabolic disorders of bioelements, especially biometals, on the background.

[*Geriatr. Gerontol. Int.* **13**, 706-710 (2013)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

High Frequency of Calcification in Basal Ganglia on Brain Computed Tomography Images in Japanese Older Adults.

Megumi YAMADA, Takahiko ASANO, Kouichirou OKAMOTO, Yuichi HAYASHI, Masayuki KANEMATSU, Hiroaki HOSHI, Yasuhisa AKAIWA, Takayoshi SHIMOHATA, Masatoyo NISHIZAWA, Takashi INUZUKA and Isao HOZUMI*

To investigate the frequency of calcification in the basal ganglia and the dentate nuclei in the cerebellum, and compare the difference in age and area, we examined the brain computed tomography (CT) images of all patients in two representative university hospitals in Japan. Compared with previous reports, the frequency of calcification of the basal ganglia in this study markedly increased. This might be because of the increased number of older adults and the increased sensitivity of CT.

[*Brain Res.* **1531**, 75-83 (2013)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Localization of Type-III Sodium-dependent Phosphate Transporter 2 in the Mouse Brain.

Masatoshi INDEN, Masaki IRIYAMA, Mari TAKAGI, Masayuki KANEKO and Isao HOZUMI*

Type-III sodium-dependent phosphate transporters 1 and 2 (PiT-1 and PiT-2, respectively) are proteins encoded by *SLC20A1* and *SLC20A2*, respectively. The aim of this study is to clarify the distribution of PiT-2 expression in the mouse brain. Our biochemical and immunohistochemical analyses using a polyclonal antibody (Ab) and a monoclonal Ab showed that PiT-2 was ubiquitously expressed throughout the brain. PiT-2 was expressed in neurons, astrocytes and vascular endothelial cells. Our results indicate that PiT-2 plays an important role in the maintenance of cellular P_i homeostasis in neurons, astrocytes, and endothelial cells. This finding is a milestone in the study of the function of PiT-2 in the brains of patients with Fahr's disease.

[*J. Neurol.* **260**, 1611-1616 (2013)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Is There a Difference in Gastric Emptying between Myotonic Dystrophy Type 1 Patients with and without Gastrointestinal Symptoms?

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

Gastrointestinal symptoms are frequent complaints in patients with myotonic dystrophy type 1 (MyD1) and may be associated with reduced gastrointestinal motility caused by smooth muscle dysfunction. Although previous studies have found delayed gastric emptying (GE) in MyD1 patients, the relationship between GE and symptoms has been unclear. These findings suggest that impairment of GE evolves over time and that the progression of delayed GE and skeletal muscle impairment are independent. Smooth muscle impairment may be affected at an earlier stage in MyD1.

[*J. Neurol.* **260**, 2380-2386 (2013)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Cardiac Sympathetic Function in the Patients with Amyotrophic Lateral Sclerosis: Analysis Using Cardiac [^{123}I] MIBG Scintigraphy.

Yuji TANAKA, Megumi YAMADA, Akihiro KOUMURA, Takeo SAKURAI, Yuichi HAYASHI, Akio KIMURA, Isao HOZUMI and Takashi INUZUKA

Amyotrophic lateral sclerosis (ALS), which is the most serious form of degenerative motor neuron disease in adults, is characterized by upper and lower motor neuron degeneration, skeletal muscle atrophy, paralysis, and death. Some patients with respiratory-dependent ALS die of sudden cardiac arrest or anoxic encephalopathy following circulatory collapse, which may be associated with sympathetic hyperactivity. These results suggested that some patients with ALS have sympathetic hyperactivity at the time of diagnosis. ALS patients may suffer from chronic cardiac sympathetic hyperactivity, which is associated with sudden cardiac death and stress induced cardiomyopathy. Increased WR in cardiac [(^{123})I] MIBG scintigraphy may be a predictive factor in ALS.

[*Chemistry Letters* **42**, 1051-1052 (2013)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Discovery of Synthetic Methoxy-substituted 4-Phenylbutyric Acid Derivatives as Chemical Chaperones.

Seisuke MIMORI, Yasunobu OKUMA, Masayuki KANEKO*, Koichi KAWADA, Yasuyuki NOMURA, Yasuoki MURAKAMI and Hiroshi HAMANA

In this study, we evaluated the chemical chaperone activity of synthetic 4-phenylbutyric acid (4-PBA) derivatives. These derivatives have a methoxy group at the benzene ring and/or longer or shorter fatty acid portions. Several 4-PBA derivatives demonstrated higher antiaggregation activity than 4-PBA. Moreover, 4-(4-methoxyphenyl)butanoic acid (7b) showed protective effects against endoplasmic reticulum stress-induced neuronal cell death.

[*Bioorg. Med. Chem. Lett.* **23**, 6015-6018 (2013)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

4-Phenylbutyric Acid Protects against Neuronal Cell Death by Primarily Acting as a Chemical Chaperone rather than Histone Deacetylase Inhibitor.

Seisuke MIMORI, Hiroyasu OHTAKA, Yukari KOSHIKAWA, Koichi KAWADA, Masayuki KANEKO*, Yasunobu OKUMA, Yasuyuki NOMURA, Yasuoki MURAKAMI, Hiroshi HAMANA

This letter describes the mechanism behind the protective effect of 4-phenylbutyric acid (4-PBA) against endoplasmic reticulum (ER) stress-induced neuronal cell death using three simple 4-(p-substituted phenyl) butyric acids (4-PBA derivatives). Their relative human histone deacetylase (HDAC) inhibitory activities were consistent with a structural model of their binding to HDAC7, and their ability to suppress neuronal cell death and activity of chemical chaperone in vitro. These data suggest that 4-PBA protects against neuronal cell death mediated by the chemical chaperone activity rather than by inhibition of histone deacetylase.

[*J. Neurosci. Res.* **91**, 62-72 (2013)] [Lab. of Medical Therapeutics & Molecular Therapeutics]

Therapeutic Effects of Human Mesenchymal and Hematopoietic Stem Cells on Rotenone-treated Parkinsonian Mice.

Masatoshi INDEN*, Kazuyuki TAKATA, Kaneyasu NISHIMURA, Yoshihisa KITAMURA, Eishi ASHIHARA, Kanji YOSHIMOTO, Hiroyoshi ARIGA, Osamu HONMOU and Sun SHIMOHAMA

To appreciate the potential applications of stem cell technology in neurodegenerative diseases, including Parkinson's disease (PD), it is important to understand the characteristics of the various types of stem cells. In this study, we designed a set of experiments to compare the ability of three types of human stem cells—mesenchymal stem cells (MSCs), bone marrow $CD34^+$ cells (BM), and cord blood $CD34^+$ cells (CB)—using rotenone-treated NOD/SCID mice. The beneficial effects of intravenous delivery of stem cells into rotenone mice may result not only from a neurotrophic effect but also from endogenous brain repair mechanisms and the potential of intravenous delivery of stem cells derived from an autologous source for clinical applications in PD.

[*Immunopharmacol Immunotoxicol.* **35**, 1-7 (2013)] [Lab. of Microbiology & Immunology]

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

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

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

[*Cell Signal.* **25**, 41-49 (2013)] [Lab. of Microbiology & Immunology]

Identification of a Rho Family Specific Guanine Nucleotide Exchange Factor, FLJ00018, as a Novel Actin-Binding Protein.

Katsuya SATO, Hiroaki HANDA, Masashi KIMURA, Yukio OKANO, Hitoshi NAGAOKA, Takahiro NAGASE, Tsuyoshi SUGIYAMA*, Yukio KITADE and Hiroshi UEDA

FLJ00018/PLEKHG2 is a guanine nucleotide exchange factor for the Rho family small GTPases. FLJ00018 is directly activated by heterotrimeric G protein Gβγ subunits. Using two-hybrid screening, we have identified non-muscle cytosolic actin as a binding partner of FLJ00018. We found that there were two actin-binding regions in FLJ00018 at the N-terminal region (150-283 amino acids) and at the C-terminal region (465-1386 amino acids). The overexpression of non-muscle cytosolic actin attenuated the FLJ00018-induced serum response element-dependent gene transcription. These results suggest that non-muscle cytosolic actin may be a negative regulator of FLJ00018 through its interaction with the Dbl homology domain.

[*Inflammation.* **36**, 372-378 (2013)] [Lab. of Microbiology & Immunology]

Inhibitory Mechanism of 10-Hydroxy-*trans*-2-decenoic Acid (Royal Jelly Acid) against Lipopolysaccharide- and Interferon-β-induced Nitric Oxide Production.

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

Royal jelly acid, 10-hydroxy-*trans*-2-decenoic acid (10H2DA), is a major lipid component of royal jelly. In this study, the ability of 10H2DA to inhibit LPS-induced nitric oxide (NO) production was investigated. LPS-induced NO production and inducible NO synthase (iNOS) gene transcription were inhibited by 10H2DA. LPS-stimulated interferon (IFN)-β production, IFN regulatory factor-1 induction and IFN-stimulated response element activation were unaffected by 10H2DA. IFN-β-induced NO production, however, was significantly inhibited by 10H2DA. These results and our previous study suggest that 10H2DA inhibits LPS- and IFN-β-induced NO production via inhibition of NF-κB activation induced by LPS or IFN-β.

[*Biomed Res.* **34**, 205-214 (2013)] [Lab. of Microbiology & Immunology]

Inhibitory Effect of 10-Hydroxydecanoic Acid on Lipopolysaccharide-induced Nitric Oxide Production via Translational Downregulation of Interferon Regulatory Factor-1 in RAW264 Murine Macrophages.

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

In this study, we investigated the effect of 10-hydroxydecanoic acid (10HDA), a saturated fatty acid of 10H2DA, on LPS-induced cytokines/chemokines and NO production. 10HDA inhibited LPS-induced NO production. LPS-induced activation of interferon (IFN)-stimulated response element was inhibited by 10HDA. We found that IRF-1 mRNA level in the polysomal fraction was significantly decreased by 10HDA. Further, LPS-induced phosphorylation of Akt and 4E-BP1, which control mRNA translation, was markedly decreased. These results suggest that 10HDA inhibited LPS-induced NO production through inhibiting IRF-1 translation. These findings elucidate a novel mechanism for anti-inflammatory activity of medium-chain fatty acid 10HDA.

[*Vaccine* **31**, 3199-3205 (2013)] [Lab. of Microbiology & Immunology]

Effects of Immunization of Pregnant Guinea Pigs with Guinea Pig Cytomegalovirus Glycoprotein B on Viral Spread in the Placenta.

Kaede HASHIMOTO, Souichi YAMADA, Harutaka KATANO, Saki FUKUCHI S, Yuko SATO, Minami KATO, Tokyofumi YAMAGUCHI, Kohji MORIISHI and Naoki INOUE*

Cytomegalovirus (CMV) is the most common cause of congenital virus infection. Although glycoprotein B (gB) vaccines have been reported to reduce the incidence and mortality of congenital infection, the mechanisms of protection remain unclear. To understand the gB vaccine protection mechanisms, we analyzed the spread of challenged viruses in the placentas and fetuses of guinea pig dams immunized with recombinant adenoviruses expressing guinea pig CMV gB and β-galactosidase. We found that antibodies against gB protected against infection mainly at the interface of the placenta rather than from the placenta to the fetus. The development of strategies to block cell-to-cell viral spread in the placenta is required for effective congenital CMV vaccines.

[*Int. J. Infec. Dis*. 17, e1092-1097 (2013)] [Lab. of Microbiology & Immunology]

Polymorphisms in Toll-like Receptor 2 are Associated with Congenital Cytomegalovirus Infection.

Rumi TANIGUCHI, Shin KOYANO, Tatsuo SUZUTANI, Keiji GOISHI,Yushi ITO, Ichiro MORIOKA, Akira OKA, Hiroyuki NAKAMURA, Hideto YAMADA, Takashi IGARASHI and Naoki INOUE*

The risk factors for cytomegalovirus (CMV) infection in utero and for progression to a severe clinical outcome remain uncertain. In this study, associations between the single nucleotide polymorphisms (SNPs) of Toll-like receptor (TLR)-2, -4, and -9 genes and congenital CMV infection or disease were evaluated. The CC genotype at SNP rs3804100 in the TLR-2 gene was significantly associated with congenital CMV infection but not with congenital CMV disease. Furthermore, the AG genotype at SNP rs1898830 in the TLR-2 gene tended to be identified less frequently in children with congenital CMV infection. Thus, TLR-2 polymorphisms may have some association with congenital CMV infection, although the mechanism underlying this effect remains to be clarified.

[*Arch. Dis. Child Fetal Neonatal Ed* **9**8, F182 (2013)] [Lab. of Microbiology & Immunology]

Newborn Screening of Congenital Cytomegalovirus Infection Using Saliva can be Influenced by Breast Feeding.

Shin KOYANO, Naoki INOUE*, Tsunehisa NAGAMORI, Hiroyuki MORIUCHI and Hiroshi AZUMA

As the early identification of congenitally cytomegalovirus infected newborns may allow early intervention and antiviral treatment options, it is important to establish newborn CMV screening programs. Since newborn screening assays using dried blood spots were shown to have a limitation in their sensitivity, an assay using saliva specimen were reported. To validate the assay, we examined the presence of CMV DNA in breast milk and saliva specimens collected within 6 days after parturition). Some saliva specimens collected within 30 min after breastfeeding were CMV positive due to the viral secretion to breast milk, suggesting that the timing of specimen collection is critical to ensure proper implementation of saliva-based screening programs. Alternatively, CMV screening can be done by collecting urine onto filter paper placed in diaper, as we reported recently.

[*Herpesviridae* **4**, 2 (2013)] [Lab. of Microbiology & Immunology]

Human Cytomegalovirus Induces Apoptosis in Neural Stem/Progenitor Cells Derived from Induced Pluripotent Stem Cells by Generating Mitochondrial Dysfunction and Endoplasmic Reticulum Stress.

Hiroyuki NAKAMURA, Huanan Liao, Kaori MINAMI, Masashi TOYODA, Hidenori AKUTSU, Yoshitaka MIYAGAWA, Hajime OKITA, Nobutaka KIYOKAWA, Akihiro UMEZAWA, Ken-ichi IMADOME, Naoki INOUE* and Shigeyoshi FUJIWARA

An induced pluripotent stem cell (iPSC) line was established from the human fibroblast line by introducing the Yamanaka's four factors and then induced to differentiate into neural stem/progenitor cells (NSPCs). iPSC-derived NSPCs were susceptible to HCMV infection, allowed the expression of both early and late viral gene products, and underwent apoptosis. Several lines of evidence indicated the involvement of mitochondrial dysfunction and endoplasmic reticulum (ER) stress in the HCMV-induced apoptosis. Thus, iPSC-derived NSPCs are thought to be a useful model to study HCMV neuropathogenesis.

[*J. Clin. Virol.* **58**, 474-478 (2013)] [Lab. of Microbiology & Immunology]

Cytomegalovirus (CMV) Glycoprotein H-based Serological Analysis in Japanese Healthy Pregnant Women, and in Neonates with Congenital CMV Infection and their Mothers.

Kazufumi IKUTA, Toshio MINEMATSU, Naoki INOUE*, Takahiko KUBO, Kimisato ASANO, Kei ISHIBASHI, Takashi IMAMURA, Hidetaka NAKAI, Tetsushi YOSHIKAWA, Hiroyuki MORIUCHI, Sigeyoshi FUJIWARA, Shin KOYANO and Tatsuo SUZUTANI

Seroprevalence against CMV and IgG subclasses were determined in 344 serum samples from healthy pregnant women in Japan. Thirty-two percent of them were seronegative, while 66% of seropositive women had IgG3 antibodies against one epitope on glycoprotein H (gH). Only a single genotype determined by CMV gH neutralizing epitope was found in the urine from the 18 congenital CMV cases. Two (11%) of the cases had infection via maternal CMV reinfection, indicating that maternal humoral immunity did not prevent infection with another gH subtype strain.

[*BMC Neurol.* **13,** 200 (2013)] [Lab. of Microbiology & Immunology]

Detection of Human Herpesviruses in the Cerebrospinal Fluid from Patients Diagnosed with or Suspected of Having Progressive Multifocal Leukoencephalopathy.

Kazuo NAKAMICHI, Naoki INOUE*, Toshio SHIMOKAWA, Ichiro KURANE, Chang-Kweng LIM and Masayuki SAIJO

Progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease caused by JC virus (JCV), occurs mainly in immunocompromised patients. While JCV DNA is detected in the cerebrospinal fluid (CSF) from a certain proportion of patients suspected of having PML, JCV-negative patients may also develop brain lesions. This study assessed the prevalence of six herpesviruses in the CSF from patients diagnosed with or suspected of PML. Herpesvirus DNAs were detected in the CSF specimens from 29 of 255 patients. HSV-1 and CMV were detected in JCV-negative patients, whereas VZV and EBV were detected in both CSF JCV-positive and -negative individuals.

[*J. Clin. Microbiol.* 51, 356-359 (2013)] [Lab. of Microbiology & Immunology]

Neonatal Herpes Encephalitis Caused by a Virologically Confirmed Acyclovir-Resistant HSV-1.

Satsuki KAKIUCHI, Shigeaki NONOYAMA, Hajime WAKAMATSU, Kazuhiro KOGAWA, Lixin WANG, Hitomi KINOSHITA-YAMAGUCHI, Mutsuyo TAKAYAMA-ITO, Chang-Kweng LIM, Naoki INOUE*, Masashi MIZUGUCHI, Takashi IGARASHI and Masayuki SAIJO

A neonate with herpes simplex virus 1 encephalitis was treated with intravenous acyclovir. During the course of therapy, the infection became intractable to the treatment and a mutation in the viral thymidine kinase gene (nucleotide G375T, amino acid Q125H) developed. This mutation was demonstrated *in vitro* to confer acyclovir resistance.

[*Biochim. Biophys. Acta*. **1833**, 2617-2627 (2013)] [Lab. of Biochemistry]

Hyperosmolarity-induced up-regulation of claudin-4 mediated by NADPH oxidase-dependent H_2O_2 production and Sp1/c-Jun cooperation.

Akira IKARI*, Kosuke ATOMI, Yasuhiro YAMAZAKI, Hideki SAKAI, Hisayoshi HAYASHI, Masahiko YAMAGUCHI and Junko SUGATANI

Claudin-4 is exclusively localized in the tight collecting ducts in the renal tubule. We examined what molecular mechanism is involved in the regulation of claudin-4 expression. In rat kidney slices, Madin-Darby canine kidney cells, and mouse inner medullary collecting duct cells, hyperosmolarity increased the expression level of claudin-4 and the production of reactive oxygen species, which were inhibited by an NADPH oxidase inhibitor and a scavenger of H_2O_2. Western blotting, immunofluorescence, and chromatin immunoprecipitation analysis showed that hyperosmolarity increases nuclear Sp1/c-Jun complex and the association of the complex with the Sp1 binding site, resulting in the segment-specific expression of claudin-4 in the kidney.

[*Biochem. Pharmacol.* **86**, 632-644 (2013)] [Lab. of Biochemistry]

A Penicillium sp. F33 metabolite and its synthetic derivatives inhibit acetyl-CoA:1-O-alkyl-sn-glycero-3-phosphocholine acetyltransferase (a key enzyme in platelet-activating factor biosynthesis) and carrageenan-induced paw edema in mice.

Yasuhiro YAMAZAKI, Kengo YASUDA, Tensei MATSUYAMA, Takuya ISHIHARA, Ryoko HIGA, Taira SAWAIRI, Masahiko YAMAGUCHI, Masahiro EGI, Shuji AKAI, Toshio MIYASE, Akira IKARI*, Masao MIWA and Junko SUGATANI

Acetyl-CoA:1-O-alkyl-sn-glycero-3-phosphocholine (lyso-PAF) acetyltransferase is a key enzyme in the biosynthesis of PAF in inflammatory cells. Substances which inhibit this enzyme are of therapeutic interest. We screened for new inhibitors of lyso-PAF acetyltransferase with anti-inflammatory effects and isolated novel acetyltransferase inhibitor identified as dihydrofumigatin (2-methoxy-1,3,4-trihydroxy-5-methylbenzene) from high resolution mass spectrometer and NMR data.

[*Chem. Biol. Interact.*, **202**, 234-242 (2013)] [Lab. of Biochemistry]

Pathophysiological Roles of Aldo-Keto Reductases (AKR1C1 and AKR1C3) in Development of Cisplatin Resistance in Human Colon Cancers.

Toshiyuki MATSUNAGA*, Aki HOJO, Yumi YAMANE, Satoshi ENDO, Ossama EL-KABBANI and Akira HARA

We established the cisplatin (CDDP)-resistant phenotypes of human colon HCT15 cells and monitored expressions of aldo-keto reductases (AKRs) 1A1, 1B1, 1B10, 1C1, 1C2 and 1C3. Among the six AKRs, AKR1C1 and AKR1C3 are highly induced with the CDDP resistance and their overexpression in the parental HCT15 cells mitigated the cytotoxicity of the drug. The resistant cells also showed an enhancement in proteolytic activity of proteasome accompanied by overexpression of its catalytic subunits (PSMβ9 and PSMβ10) and pretreatment of the resistant cells with a potent proteasome inhibitor Z-Leu-Leu-Leu-al augmented the CDDP sensitization elicited by the AKR inhibitors. Collectively, these results suggest the involvement of up-regulated AKR1C1, AKR1C3 and proteasome in CDDP resistance of colon cancers and support a chemotherapeutic role for their inhibitors.

[*Anticancer Drugs.* **24**, 473-483 (2013)] [Lab. of Biochemistry]

Sphingosine Kinase 1 Plays a Role in the Upregulation of CD44 Expression Through Extracellular Signal-Regulated Kinase Signaling in Human Colon Cancer Cells.

Satomi KAWAHARA, Yoko OTSUJI, Mitsuhiro NAKAMURA, Masashi MURAKAMI, Takashi MURATE, Toshiyuki MATSUNAGA*, Hiroyuki KANOH, Mariko SEISHIMA, Yoshiko BANNO and Akira HARA

We used two colon cancer cell lines (oxaliplatin-resistant RKO and -sensitive HCT116) to examine the relationship between sphingosine kinase (SPHK) 1 activity and CD44 expression. The levels of SPHK1, CD44 and phosphorylated-extracellular signal-regulated kinase (ERK) were much higher in the RKO cells than in the HCT116 cells. The SPHK1 inhibition suppressed CD44 expression in RKO cells, and overexpression of SPHK1 enhanced the expression of both CD44 and phosphorylated-ERK in HCT116 cells. Exogenous sphingosine-1-phosphate (S1P) increased ERK phosphorylation and CD44 expression in HCT116 cells, indicating that SPHK1 acts as a regulator of CD44 expression through the ERK signaling pathway in human colon cancer cells.

[*Arch. Biochem. Biophys.* **529**, 131-139 (2013)] [Lab. of Biochemistry]

Characterization of a Rabbit Morphine 6-Dehydrogenase and Its Two Isoforms Which Function as NAD^+-Dependent 3α(17β)-Hydroxysteroid dehydrogenases.

Satoshi ENDO*, Toshiyuki MATSUNAGA, Airi FUJIMOTO, Sho KUMADA, Yuki ARAI, Yoko MIURA, Hiroshige MIKAMO, Ossama EL-KABBANI, Shigeru YAMANO, Munekazu IINUMA and Akira HARA*

Mammalian morphine 6-dehydrogenase (M6DH) converts morphine into a reactive electrophile, morphinone. We isolated cDNAs for the three rabbit AKRs (AKR1C26, 1C27 and 1C28). Among them, only AKR1C26 oxidized morphine. The three AKRs showed NAD^+-dependent dehydrogenase activity towards other non-steroidal alicyclic alcohols and 3α/17β-hydroxysteroids. The kinetic constants for the substrates suggest that at least AKR1C26 and AKR1C28 act as NAD^+-dependent 3α/17β-hydroxysteroid dehydrogenases. AKR1C27 differed from AKR1C28 in its low affinity for the substrates and low inhibitor sensitivity, despite their 95% sequence identity. We also showed that Tyr118 and Phe310 in AKR1C27 have played key roles in ligand binding.

[*Chem. Biol. Interact.*, **202**, 146-152 (2013)] [Lab. of Biochemistry]

Modulation of Activity and Inhibitor Sensitivity of Rabbit Aldose Reductase-Like Protein by Oxidized Glutathione and SH-Reagents.

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

In this study, we found that the reductase activity of AKR1B19 was activated to about 5-fold immediately after the addition of 10 μM SH-reagents such as *p*-chloromercuriphenylsulfonic acid. The activity was also activated by incubation with glutathione disulfide (GSSG) for 1h. The activated enzyme was converted into the native enzyme by further incubation with dithiothreitol and glutathione. The activation was abolished by the C299S mutation of AKR1B19. In the reduction of 4-oxo-2-nonenal, the catalytic efficiency of cys299-modified enzyme was increased. Thus, AKR1B10 may be modulated by cellular ratio of GSSG/glutathione and more efficiently act as a detoxifying enzyme for the cytotoxic aldehyde under oxidatively stressed conditions.

[*Biol. Pharm. Bull.*, **36**, 1514-1518 (2013)] [Lab. of Biochemistry]

Substrate Specificity and Inhibitor Sensitivity of Rabbit 20α-Hydroxysteroid Dehydrogenase.

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

In this study, we examined the substrate specificity of rabbit 20α-hydroxysteroid dehydrogenase (AKR1C5), which plays a role in the termination of pregnancy by progesterone inactivation. AKR1C5 moderately reduced the 3-keto group of only 5α-dihydrosteroids with 17β- or 20α/β-hydroxy group. In contrast, the enzyme reversibly and efficiently catalyzed the reduction of various 17- and 20-ketosteroids, including estrogen precursors and tocolytic 5β-pregnane-3,20-dione. In addition to the progesterone inactivation, the formation of estrogens and metabolism of the tocolytic steroid by AKR1C5 may be related to its role in rabbit parturition. AKR1C5 also reduced various carbonyl compounds, including isatin, an antagonist of the C-type natriuretic peptide receptor, and 4-oxo-2-nonenal, suggesting its roles in controlling the bioactive isatin and detoxification of cytotoxic aldehydes.

[*Biochem. Pharmacol.* **86**, 1366-1375 (2013)] [Lab. of Biochemistry]

Rabbit 3-Hydroxyhexobarbital Dehydrogenase Is a NADPH-Preferring Reductase with Broad Substrate Specificity for Ketosteroids, Prostaglandin D2, and Other Endogenous and Xenobiotic Carbonyl Compounds.

Satoshi ENDO*, Toshiyuki MATSUNAGA, Atsuko MATSUMOTO, Yuki ARAI, Satoshi OHNO, Ossama EL-KABBANI, Kazuo TAJIMA, Yukio KITADE, Yasuo BUNAI, Shigeru YAMANO and Akira HARA

We have examined the specificity for coenzymes and substrates and tissue distribution of rabbit 3-hydroxyhexobarbital dehydrogenase (3HBD). 3HBD reduced broad substrate specificity for various carbonyl compounds, including steroids and prostaglandin D_2. The overexpression of the enzyme in the cells decreased the cytotoxicity of 4-oxo-2-nonenal. The results suggest that 3HBD is an NADPH-preferring reductase, and plays roles in the metabolisms of steroids, prostaglandin D_2 and xenobiotics, as well as a defense system, protecting against reactive carbonyl compounds.

[*Bioorg. Med. Chem.*, **21**, 6378-6384 (2013)] [Lab. of Biochemistry]

Synthesis and Structure-Activity Relationship of 2-Phenyliminochromene Derivatives as Inhibitors for Aldo-Keto Reductase (AKR) 1B10.

Satoshi ENDO*, Dawei HU, Miho SUYAMA, Toshiyuki MATSUNAGA, Kenji SUGIMOTO, Yuji MATSUYA, Ossama EL-KABBANI, Kazuo KUWATA, Akira HARA, Yukio KITADE and Naoki TOYOOKA

AKR1B10 Inhibitors are regarded as promising therapeutics for the treatment of cancer. Recently, we have discovered a chromene-3-carboxamide derivative (1) as the potent competitive inhibitor. In this study, 18 derivatives of 1 were synthesized and their inhibitory potency against AKR1B10 evaluated. Among them, 7-hydroxy-2-(4-methoxyphenylimino)-2*H*-chromene-3-carboxylic acid benzylamide (5n) was the most potent inhibitor (K_i = 1.3 nM). The molecular docking and site-directed mutagenesis suggest that the hydrogen-bond interactions between the 7-hydroxyl group of 5n and the catalytic residues of the enzyme, together with a π-stacking interaction of the benzylamide moiety of 5n with Trp220, are important for the potent inhibition.

[*J. Trad. Med.* **30**, 114-123 (2013)] [Lab. of Pharmacology]

Assessment of Relief from Pruritus Due to Kampo Medicines by Using Murine Models of Atopic Dermatitis.

Hirotaka YAMASHITA*, Toshiaki MAKINO, Naoki INAGAKI, Mitsuhiko NOSE and Hajime MIZUKAMI

Kampo medicines are used as an alternative medical option for the treatment of chronic allergic diseases such as atopic dermatitis. Since kampo medicines should be used according to the particular physical and mental conditions, the use of distinctive animal models is necessary for the experimental assessment of the efficacy of kampo medicines. In this study, we assessed 7 kampo medicines that have been used for atopic dermatitis patients in 2 types of atopic dermatitis-like murine pruritus models. One kampo medicine, shofusan, suppressed the persisting scratching behavior induced by repeated hapten treatment. The results indicate that certain kampo medicines improve chronic itching sensation to the same extent as or better than antiallergic medicines.

[*J.Invest. Dermatol.* **133**, 2695-2705 (2013)] [Lab. of Pharmacology]

Mast Cells are Required for Full Expression of Allergen/SEB-induced Skin Inflammation.

Tomoaki ANDO, Kenji MATSUMOTO, Siavash NAMIRANIAN, Hirotaka YAMASHITA*, Haley GLATTHORN, Miho KIMURA, Brandon R DOLAN, James J LEE, Stephen J GALLI, Yuko KAWAKAMI, Colin JAMORA and Toshiaki KAWAKAMI

Repeated epicutaneous applications of a house dust mite extract and Staphylococcal enterotoxin B to murine skin induced eczematous lesions, and global gene expression patterns in the skin were very similar to human atopic dermatitis (AD) skin. In this model, mast cells and T cells, but not eosinophils, were required for the dermatitis. The clinical severity of dermatitis correlated with the numbers of mast cells. Consistent with the idea that Th2 cells played a predominant role in allergic diseases, the receptor for the Th2-promoting cytokine thymic stromal lymphopoietin and the high-affinity IgE receptor were required to attain maximal clinical scores. Therefore, this clinically relevant model provides mechanistic insights into the pathogenic mechanism of human AD.

[*Stroke* **44**, 2862-2868 (2013)] [Lab. of Molecular Pharmacology]

Cilostazol Ameliorates Warfarin-induced Hemorrhagic Transformation after Cerebral Ischemia in Mice.

Akira KITASHOJI, Yusuke EGASHIRA, Keisuke MISHIRO, Yukiya SUZUKI, Hideki ITO, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

Although long-term treatment with the oral anticoagulant warfarin is widely used to prevent cardioembolic ischemic stroke, it has been reported that warfarin can exacerbate hemorrhagic transformation (HT) after cerebral ischemia. We investigated whether cilostazol, a phosphodiesterase-III inhibitor, suppressed the warfarin-induced HT after cerebral ischemia in mice. HT volume was exacerbated by warfarin treatment, and cilostazol (3 mg/kg, IP) suppressed this exacerbation. Furthermore, cilostazol improved survival rate and upregulated the expression of tight junction proteins and vascular endothelial cadherin. This result suggested that cilostazol administration in patients with acute ischemic stroke might reduce HT.

[*J. Neurol.* **260**, 1782-1797 (2013)] [Lab. of Molecular Pharmacology]

ITIH4 and Gpx3 Are Potential Biomarkers for Amyotrophic Lateral Sclerosis.

Hirotaka TANAKA, Masamitsu SHIMAZAWA, Masafumi TAKATA, Hideo KANEKO, Kazuhiro TSURUMA, Tsunehiko IKEDA, Hitoshi WARITA, Masashi AOKI, Mitsunori YAMADA, Hitoshi TAKAHASHI, Isao HOZUMI, Hiroshi MINATSU, Takashi INUZUKA and Hideaki HARA*

The diagnosis of amyotrophic lateral sclerosis (ALS) is difficult due to lack of definitive biomarkers. We identified two candidate proteins—inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4) and glutathione peroxidase 3 (Gpx3). The 120kDa ITIH4 increased at the onset of the disease and the 85kDa ITIH4, a cleaved form, at the end-stage in the sera of the mutant superoxide dismutase-1 $(SOD1)^{H46R}$ rats. Expression of the 85kDa ITIH4 was substantial in ALS patient. The Gpx3 protein levels in the sera of $SOD1^{H46R}$ rats were upregulated pre-symptom and gradually decreased as the disease progressed. The Gpx3 protein levels were lower in the sera of the patients. These results indicate that ITIH4 and Gpx3 are potential biomarkers for ALS.

[*Br. J. Pharmacol.* **170**, 341-351 (2013)] [Lab. of Molecular Pharmacology]

Fasudil, a Rho Kinase Inhibitor, Limits Motor Neuron Loss in Experimental Models of Amyotrophic Lateral sclerosis.

Masafimi TAKATA, Hirotaka TANAKA, Masataka KIMURA, Yuki NAGAHARA, Kousuke TANAKA, Koh KAWASAKI, Minoru SETO, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder with no effective treatment. In this study, we investigated the effect of fasudil on experimental models of ALS. Hydroxyfasudil prevented motor neuron cell death in *in vitro* and fasudil slowed disease progression, increased survival time and reduced motor neuron loss, in mutant superoxide dismutase 1 $(SOD1^{G93A})$ mice. As the mechaniasm, fasdil suppressed both the increase in ROCK activity and phosphorylated phosphatase and tensin homologue deleted on chromosome 10 (PTEN). These findings indicate that fasudil may be effective at suppressing motor neuron degeneration and symptom progression in ALS. Hence, fasudil may have potential as a therapeutic agent for ALS treatment.

[*J.Neuroinflammation* **10**: 105 (2013)] [Lab. of Molecular Pharmacology]

The Growth Factor Progranulin Attenuates Neuronal Injury Induced by Cerebral Ischemia-Reperfusion Through the Suppression of Neutrophil Recruitment.

Yusuke EGASHIRA, Yukiya SUZUKI, Yukio AZUMA, Toshinori TAKAGI, Keisuke MISHIRO, Sou SUGITANI, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Shinichi YOSHIMURA, Masanori KASHIMATA, Toru IWAMA and Hideaki HARA*

The aim of this study was to investigate the possible ameliorative effects of progranulin (PGRN) against ischemia-reperfusion (I/R) injury in mice. We found that recombinant-PGRN (r-PGRN) treatment at 2 h after MCAO resulted in a reduction in the infarct volume and decreased brain swelling. Furthermore, r-PGRN treatment suppressed neutrophil recruitment into the I/R brain, and this led to a reduction of nuclear factor-κB and matrix metalloproteinase-9 activation. From these results, PGRN exerted ameliorative effects against I/R-induced inflammation, and these effects may be due to the inhibition of neutrophil recruitment into the I/R brain.

[*Sci. Rep.* **3**: 3177 (2013)] [Lab. of Molecular Pharmacology]

Involvements of Mincle and Syk on the Changes to Innate Immune after Ischemic Stroke.

Yukiya SUZUKI, Yusuke NAKANO, Keisuke MISHIRO, Toshinori TAKAGI, Kazuhiro TSURUMA, Mitsuhiro NAKAMURA, Shinichi YOSHIMURA, Masamitsu SHIMAZAWA and Hideaki HARA*

Macrophage-inducible C-type lectin, Mincle, is one of the innate immune receptor C-type lectin-like receptor (CLR) to response against dying cells. In the present study, we showed that Mincle, its ligand SAP130, and its downstream phospho-Syk/Syk were upregulated after ischemia, and that Mincle is expressed in immune and non-immune cells in the ischemic brains of mice and human. We treated mice with piceatannol, a Syk inhibitor, and consequently the infarct volume and swelling were suppressed by piceatannol. The levels of phospho-Syk, MMP9 and ICAM-1 were downregulated, and the level of Claudin5 was uplegurated in piceatannol-treated groups. These data indicate that innate immune system, such as Mincle and Syk plays a pivotal role in the pathogenesis after the ischemia and reperfusion.

[*Curr. Neurovasc. Res.* **10**, 39-48 (2013)] [Lab. of Molecular Pharmacology]

Tissue Plasminogen Activator Prevents Restoration of Tight Junction Proteins Through Upregulation of Angiopoietin-2.

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

We examined the temporal profiles of changes in the expressions of tight junction proteins (TJPs) after focal cerebral ischemia/reperfusion in mice. We also examined the effects of delayed treatment with tissue plasminogen activator (tPA) on the expressions of TJPs and angiopoietin (Ang) -1/2/Tie2. The expressions of TJPs were significantly decreased in the early phase of ischemia/reperfusion, and then gradually recovered. A delayed treatment with tPA decreased the expressions of TJPs and increased Ang-2 expression. These findings suggest that delayed tPA treatment prevents recovery of TJPs following focal cerebral ischemia/reperfusion, partially via upregulation of Ang-2.

[*Biochem. Biophys. Res. Commun.* **434**, 904-909 (2013)] [Lab. of Molecular Pharmacology]

A Sigma-1 Receptor Antagonist (NE-100) Prevents Tunicamycin-Induced Cell Death via GRP78 Induction in Hippocampal Cells.

Yoko ONO, Hirotaka TANAKA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA and Hideaki HARA*

In the present study, we investigated whether *N*, *N*-dipropyl-2-[4-methoxy-3-(2-phenylethoxy)-phenyl]-ethylamine monohydrochloride (NE-100), sigma-1 receptor antagonist, might suppress neuronal cell death that is induced by endoplasmic reticulum (ER) stress. NE-100 protected the ER stress-induced cell death of murine hippocampal HT22 cells. In addition, NE-100 attenuated the upregulation of C/EBP homologous protein (CHOP) induced by ER stress and upregulated the expression of both the 50-kDa activating transcription factor 6 (p50ATF6) and the 78-kDa glucose-regulated protein (GRP78). These findings suggest that NE-100 suppresses ER stress-induced cell death *via* CHOP expression by the upregulation of GRP78 through ATF6 pathway, independent sigma-1 receptor antagonist effect.

[*Neurosci. Med.* **4**, 117-122 (2013)] [Lab. of Molecular Pharmacology]

Increased Seizure Susceptibility in a Mouse with Diacylglycerol Kinase β Deficiency.

Mitsue ISHISAKA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Yasuhito SHIRAI, Naoaki SAITO and Hideaki HARA*

Diacylglycerol kinase (DGK) is an enzyme that converts diacylglycerol to phosphatidic acid. In the present study, we investigated the involvement of DGK*β* in seizure using DGK*β* knockout (KO) mice. Seizures were more severe in DGK*β* KO mice than in wild-type (WT) mice after pentylenetetrazol (PTZ) treatment and after kainic acid treatment, but there were no differences in latency to seizure. There were fewer parvalbu-min-positive interneurons in the hippocampal CA3 area in DGK*β* KO mice than in control WT mice, which might partly account for the increased seizure susceptibility displayed by DGK*β* KO mice. These results suggest that DGK*β* may play a pivotal role in the development of the relevant interneurons, and that on inherent deficiency of DGK*β* in-creases the animal's sensitivity to seizure-inducing stimuli.

[*J. Neurochem.* **125**, 111-124 (2013)] [Lab. of Molecular Pharmacology]

Role of Endoplasmic Reticulum Stress in Light-Induced Photoreceptor Degeneration in Mice.

Tomohiro NAKANISHI, Masamitsu SHIMAZAWA, Sou SUGITANI, Takashi KUDO, Shunsuke IMAI, Yuta INOKUCHI, Kazuhiro TSURUMA and Hideaki HARA*

Exposure to excessive levels of light induces photoreceptor apoptosis and can be a causative factor in age-related macular degeneration (AMD). We investigated the roles of endoplasmic reticulum (ER) stress in light-induced cell death in the murine retina and murine photoreceptor cells (661W). Excessive light exposure induced retinal dysfunction, photoreceptor degeneration, and apoptosis. Furthermore, the accumulation of polyubiquitinated proteins and the transcriptional expression of ER stress-related factors were increased in light-exposed retinas. Treatment with BiP inducer X (BIX), an ER stress inhibitor, reduced light-induced photoreceptor cell death. These data indicate that the ER stress may play a pivotal role in light exposure-induced retinal damage.

[*Mol. Neurodegeneration* **8**:4(2013)] [Lab. of Molecular Pharmacology]

Kinetics of Neurodegeneration Based on a Risk-related Biomarker in Animal Model of Glaucoma.

Takuya HAYASHI, Masamitsu SHIMAZAWA Hiroshi WATABE, Takayuki OSE, Yuta INOKUCHI, Yasushi ITO, Hajime YAMANAKA, Shin-ichi URAYAMA, Yasuyoshi WATANABE, Hidehara HARA* and Hirotaka ONOE

Neurodegenerative diseases progress slowly and steadily over years or decades. However, there is no established “biomarker model” by which one can quantitatively predict the progress of neurodegeneration. We show predictability of a model with risk-based kinetics of neurodegeneration, whereby neurodegeneration proceeds as probabilistic events depending on the risk. We used several experimental glaucomatous animals and repeatedly measured IOP as well as white matter integrity by diffusion tensor imaging (DTI) as a biomarker of axonal degeneration. Our findings indicate that the variable-risk model, using a risk-related biomarker, could predict the spatiotemporal progression of neurodegeneration. This model, virtually equivalent to survival analysis, may allow us to estimate possible effect of neuroprotection in delaying progress of neurodegeneration.

[*Invest. Ophthalmol. Vis. Sci.* **54**, 5807-5816 (2013)] [Lab. of Molecular Pharmacology]

Toll-like Receptor 4 Mediates Retinal Ischemia/Reperfusion Injury Through Nuclear Factor-κB and Spleen Tyrosine Kinase Activation.

Fumiya ISHIZUKA#, Masamitsu SHIMAZAWA#, Yuki INOUE, Yusuke NAKANO, Hiromi OGISHIMA, Shinsuke NAKAMURA, Kazuhiro TSURUMA, Hiroyuki TANAKA, Naoki INAGAKI and Hideaki HARA*
# : Contributed equally

This study aimed to determine whether toll-like receptor (TLR) 4 is involved with injury in an ocular ischemic syndrome mice model and to clarify the downstream pathway of TLR4. TLR4 knock-out (KO) mice significantly inhibited the histologic damage induced by ischemia/reperfusion (I/R). A spleen tyrosine kinase inhibitor, Piceatannol, inhibited the histologic and functional retinal damage, and reduced the phosphorylation level of nuclear factor-κB induced by I/R. These data indicate that TLR4 has a pivotal role in the pathogenesis of ocular ischemic syndrome, and Syk and NF-κB are key molecules in TLR4 signaling in retinal ischemia.

[*Invest. Ophthalmol. Vis. Sci.* **54**, 3815-3829 (2013)] [Lab. of Molecular Pharmacology]

Role of Heparin-binding Epidermal Growth Factor-like Growth Factor in Light-Induced Photoreceptor Degeneration in Mouse Retina.

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

We investigated the role of heparin-binding epidermal growth factor-like growth factor (HB-EGF) in light-induced photoreceptor degeneration using forebrain-specific *Hb-egf* knockout (KO) mice. *Hb-egf* and pro-HB-EGF levels were increased after light exposure. Exposure to light reduced the a- and b-wave amplitudes of the dark-adapted electroretinogram, and also outer nuclear layer (ONL) thickness, in *Hb-egf* KO mice. Treatment with HB-EGF improved both the a- and b-wave amplitudes and the thickness of the ONL. HB-EGF also protected against light-induced cell death and reduced reactive oxygen species (ROS) production in 661W cells. These data suggest that HB-EGF plays a pivotal role in light-induced photoreceptor degeneration.

[*PloS ONE* **8(3)**, e60517 (2013)] [Lab. of Molecular Pharmacology]

Mild Endoplasmic Reticulum Stress Promotes Retinal Neovascularization via Induction of BiP/GRP78.

Shinsuke NAKAMURA#, Haruka TAKIZAWA#, Masamitsu SHIMAZAWA#, Yuhei HASHIMOTO, Sou SUGITANI, Kazuhiro TSURUMA and Hideaki HARA* #: Contributed equally

The goal of the present study was to clarify the relationship between endoplasmic reticulum (ER) stress and pathological neovascularization in the retina. Exposure to tunicamycin and thapsigargin increased the proliferation and migration of human retinal microvascular endothelial cells (HRMEC). Tunicamycin enhanced the protein level on the cell surface, and increased the formation of a BiP/T-cadherin immunocomplex. In oxygen-induced retinopathy model mice, retinal neovascularization was accelerated by treatments with ER stress inducers. In conclusion, ER stress may contribute to the formation of abnormal vasculature in the retina via BiP complexation with T-cadherin, which then promotes endothelial cell proliferation and migration.

[*Exp. Eye Res.* **116**, 254-264 (2013)] [Lab. of Molecular Pharmacology]

The Potential Neuroprotective Effect of Human Adipose Stem Cells Conditioned Medium against Light-induced Retinal Damage.

Sou SUGITANI, Kazuhiro TSURUMA, Yuta OHNO, Yoshiki KUSE, Mika YAMAUCHI, Yusuke EGASHIRA, Shinichi YOSHIMURA, Masamitsu SHIMAZAWA, Toru IWAMA and Hideaki HARA*

Human adipose-derived stem cells (hASCs) are reported to secrete various factors that have neuroprotective effects. In the present study, we examined whether hASC-conditioned medium (hASC-CM) was effective against experimental degenerative retinal disease. hASC-CM inhibited photoreceptor degeneration and retinal dysfunction after exposure to light. Several proteins secreted by hASCs, such as the tissue inhibitor of metalloproteinase-1 and the secreted protein acidic and rich in cysteine, protected against light-induced damage *in vitro* and *in vivo*. These showed that hASC-CM has neuroprotective effects against light-induced retinal damage and hASCs have a therapeutic potential in retinal degenerative diseases *via* their secreted proteins.

[*Exp. Eye Res.* **113**, 19-25 (2013)] [Lab. of Molecular Pharmacology]

Thickness Mapping of the Inner Retina by Spectral-Domain Optical Coherence Tomography in an *N*-methyl-D-aspartate-Induced Retinal Damage Model.

Yuta OHNO, Shuichi MAKITA, Masamitsu SHIMAZAWA, Kazuhiro TSURUMA, Yoshiaki YASUNO and Hideaki HARA*

Spectral-domain optical coherence tomography (SD-OCT) is an interferometric optical tomography technique and provides high resolution and noninvasive visualization of retinal morphology. The purpose of this study was to assess the utility of thickness maps and quantitative thickness measurements of the ganglion cell complex (GCC) obtained by SD-OCT of a mouse model of *N*-methyl-D-aspartate (NMDA)-induced retinal damage. The GCC thickness measured using OCT sectional images correlated with the thickness measured using histological images in NMDA-treated retina. In conclusion, GCC thickness mapping is a useful method for evaluating NMDA-induced retinal degeneration in mice.

[*Exp. Eye Res.* **111**, 1-8 (2013)] [Lab. of Molecular Pharmacology]

Establishment of the Ocular Hypertension Model Using the Common Marmoset.

Masamitsu SHIMAZAWA, Shinsuke NAKAMURA, Miki MIWA, Kazuhiro TSURUMA, Makoto AIHARA, Katsuki NAKAMURA and Hideaki HARA*

The purpose of this study was to establish an experimental glaucoma model in the common marmoset (*Callithrix jacchus*). Chronic intraocular pressure (IOP) elevation was induced by laser trabeculoplasty. Mean IOP values were over 40 mmHg in laser-treated eyes. In ophthalmoscopy, deepened and enlarged optic disc cupping, depending on the extent of IOP elevation and duration, were observed in laser-treated eyes. Histological examination showed marked atrophy with deepened and enlarged cupping of optic disc, thinning of retinal nerve fiber layer and retinal ganglion loss in the retina, and axonal atrophy and loss in the optic nerve, depending on the extent of IOP elevation and duration. In conclusion, we succeeded in producing an experimental glaucoma model in the common marmoset, and this model may be useful in elucidating the pathophysiological mechanism for glaucoma.

[*Eur. J. Pharmacol.* **702**, 56-61 (2013)] [Lab. of Molecular Pharmacology]

Bimatoprost Protects Retinal Neuronal Damage via Akt Pathway.

Norihiro TAKANO, Kazuhiro TSURUMA, Yuta OHNO, Masamitsu SHIMAZAWA and Hideaki HARA*

Worldwide, prostaglandin analogs, such as bimatoprost, have become the major therapeutic class for medical treatment of glaucoma, however, the detailed mechanism of the direct action of bimatoprost on retinal ganglion cells (RGC) has rarely been understood. In this study, we elucidated the mechanism of the protective effects of bimatoprost on RGC against oxidative stress. Bimatoprost significantly reduced l-buthionin-(S,R)-sulfoximine (BSO) plus glutamate- and serum deprivation-induced death in concentration-dependent manners in *in vitro*. In an *in vivo* study, bimatoprost reduced *N*-methyl-D-aspartate -induced RGC death. Bimatoprost induced activation of Akt and ERK. These findings indicate that bimatoprost has protective effects on *in vitro* and *in vivo* retinal damage, suggesting that the mechanism underlying may be *via* the Akt pathway, which may modulate the ERK pathway.

[*Eur. J. Pharmacol.* **703**, 1-10 (2013)] [Lab. of Molecular Pharmacology]

Crocetin, a Carotenoid Derivative, Inhibits Retinal Ischemic Damage in Mice.

Fumiya ISHIZUKA, Masamitsu SHIMAZAWA, Naofumi UMIGAI, Hiromi OGISHIMA, Shinsuke NAKAMURA, Kazuhiro TSURUMA and Hidehara HARA*

We evaluated the protective effects of crocetin against the retinal ischemia induced by ligation of both the pterygopalatine artery and the external carotid artery in anesthetized mice. The histological analysis revealed that ischemia/reperfusion (I/R) decreased the cell number in the ganglion cell layer (GCL) and the thickness of inner nuclear layer (INL), and that crocetin inhibited GCL and INL. Electroretinogram measurements revealed that crocetin prevented the I/R-induced reductions in a- and b-wave amplitudes. In addition, crocetin decreased the numbers of apoptotic cells and 8-hydroxy-2-deoxyguanosine-positive cells, and phosphorylations of mitogen-activated protein kinases, nuclear factor-kappa B, and c-Jun present in the retina after I/R. These findings indicate that crocetin prevented ischemia-induced retinal damage through its inhibition of oxidative stress.

[*Pharmacol. Res. Perspectives* **1**, e00006 (2013)] [Lab. of Molecular Pharmacology]

Cilostazol Prevents Retinal Ischemic Damage Partly *via* Inhibition of Tumor Necrosis Factor-α-induced Nuclear Factor-kappa B/Activator Protein-1 Signaling Pathway.

Fumiya IHIZUKA#, Masamitsu SHIMAZAWA#, Yusuke EGASHIRA, Hiromi OGISHIMA, Shinsuke NAKAMURA, Kazuhiro TSURUMA and Hideaki HARA* #: Contributed equally

Cilostazol is widely used to treat ischemic symptoms of peripheral vascular disease. We evaluated the protective effects of cilostazol in a murine model of ocular ischemic syndrome. The histological analysis revealed that ischemia/reperfusion (I/R) decreased the cell number in the ganglion cell layer and the thicknesses of the inner plexiform layer and inner nuclear layer, and cilostazol attenuated these decreases. Additionally, cilostazol prevented the hyperpermeability of blood vessels and the increased expression of tumor necrosis factors-α (TNF-α) and the phosphorylation levels of nuclear factor-kappa B (NF-κB). These findings indicate that cilostazol may prevent I/R-induced retinal damage partly through inhibition of TNF-α-induced NF-κB activation.

[*Neurosci. Lett.* **535**, 95-99 (2013)] [Lab. of Molecular Pharmacology]

Effects of Roscovitine, a Cell Cycling-Dependent Kinase Inhibitor, on Intraocular Pressure of Rabbit and Retinal Ganglion Cell Damage.

Hiroyoshi KASAI, Tomoyo IMAMURA, Kazuhiro TSURUMA, Yuji TAKAHASHI, Takashi KURASAWA, Haruhisa HIRATA, Masamitsu SHIMAZAWA and Hideaki HARA*

Glaucoma is characterized by increased intraocular pressure (IOP) and the death of retinal ganglion cells. Roscovitine, a cell cyclin-dependent kinase (CDK) inhibitor, has racemic isomers. In this study, we investigated the effects of both the R-isomer and the S-isomer on the IOP of rabbits and on the death of cultured retinal ganglion cells. In the *in vivo* rabbit experiment, instillation of both isomers significantly lowered the IOP. In the *in vitro* cell experiment, the only R-isomer amplified the effects of tunicamycin, an endoplasmic reticulum stress inducer, and increased oxygen‐glucose deprivation-induced cell death. These results suggest that the S-isomer of roscovitine may be useful as an agent for lowering IOP and its neuroprotective effects.

[*Pharmacol. Pharm.* **4**, 377-384 (2013)] [Lab. of Molecular Pharmacology]

Comparison of Efficacy and Safety Evaluation of Latanoprost Formulations with and without Benzalkonium Chloride.

Hiroyoshi KASAI, Yumiko AOYAMA, Takashi KURASAWA, Tomoyo IMAMURA, Kazuhiro TSURUMA, Hideaki HARA*, Haruhisa HIRATA and Tetsuya YAMAMOTO

This study investigated the safety and pharmacological effects of latanoprost formulations with benzalkonium chloride (latanoprost with BAK) and without BAK (NP). Cytotoxicity tests *in vitro* revealed that NP was less toxic than latanoprost with BAK and significantly inhibited H_2O_2-induced cell damage while latanoprost with BAK did not. NP was safer than latanoprost with BAK with respect the results obtained in the in vitro cytotoxicity test. There was no difference observed between latanoprost with BAK and NP in the IOP lowering effect in monkeys and healthy volunteers. Taken together, these results indicate that NP is as effective as latanoprost with BAK, and is more likely to maintain ocular surface health than latanoprost with BAK.

[*Food Chem.* **139**, 129-137 (2013)] [Lab. of Molecular Pharmacology]

Maqui Berry (*Aristotelia chilensis*) and the Constituent Delphinidin Glycoside Inhibit Photoreceptor Cell Death Induced by Visible Light.

Junji TANAKA, Takashi KADEKARU, Kenjirou OGAWA, Shoketsu HITOE, Hiroshi SHIMODA and Hideaki HARA*

The protective effects of maqui berry (*Aristotelia chilensis*) extract (MBE) and its major anthocyanins [delphinidin 3,5-*O*-diglucoside (D3G5G) and delphinidin 3-*O*-sambubioside-5-*O*-glucoside (D3S5G)] against light-induced murine photoreceptor cells (661W) death were evaluated. MBE, D3G5G, and D3S5G significantly inhibited the cell death and the production of reactive oxygen species (ROS). Furthermore, MBE significantly suppressed the light-induced phosphorylation of p38. These findings indicate that MBE and its anthocyanidins suppress the light-induced photoreceptor cell death by inhibiting ROS production, suggesting that the inhibition of phosphorylated-p38 may be involved in the underlying mechanism.

[*J. Agric. Food Chem.* **61**, 10345-10353 (2013)] [Lab. of Molecular Pharmacology]

The Protective Effects of Bilberry and Lingonberry Extracts against UV Light-induced Retinal Photoreceptor Cell Damage in Vitro.

Kenjirou OGAWA, Kazuhiro TSURUMA, Junji TANAKA, Mamoru KAKINO, Saori KOBAYASHI, Masamitsu SHIMAZAWA and Hideaki HARA*

We investigated the protective mechanisms of the bilberry extract (B-ext) and lingonberry extract (L-ext) against ultraviolet A (UVA)-induced retinal photoreceptor cell damage. B-ext, L-ext, and constituents improved cell viability and suppressed reactive oxygen species (ROS) generation. B-ext and cyanidin inhibited phosphorylation of p38 mitogen-activated protein kinase, and B-ext also inhibited phosphorylation of c-Jun N-terminal kinase by UVA. L-ext, trans-resveratrol, and procyanidin alleviated the reduction of phosphorylated Akt levels by UVA. Finally, a cotreatment with B-ext and L-ext showed an additive effect on cell viability. Our findings suggest that both B-ext and L-ext endow protective effects against UVA-induced retinal damage.

[*Life Sci.* **92**, 17-25 (2013)] [Lab. of Molecular Pharmacology]

Purple Rice Extract and Its Constituents Suppress Endoplasmic Reticulum Stress-induced Retinal Damage in Vitro and in Vivo.

Junji TANAKA, Tomohiro NAKANISHI, Hiroshi SHIMODA, Seikou NAKAMURA, Kazuhiro TSURUMA, Masamitsu SHIMAZAWA, Hisashi MATSUDA, Masayuki YOSHIKAWA and Hideaki HARA*

The protective effects of maqui berry (*Aristotelia chilensis*) extract (MBE) and its major anthocyanins [delphinidin 3,5-*O*-diglucoside (D3G5G) and delphinidin 3-*O*-sambubioside-5-*O*-glucoside (D3S5G)] against light-induced murine photoreceptor cells (661W) death were evaluated. Viability of 661W after light treatment was improved by addition of MBE, D3G5G, and D3S5G. Intracellular radical activation was reduced by MBE and its anthocyanins. MBE significantly suppressed the light-induced phosphorylation of p38. These findings indicate that MBE and its anthocyanidins suppress the light-induced photoreceptor cell death by inhibiting ROS production, suggesting that the inhibition of phosphorylated-p38 may be involved in the underlying mechanism.

[*Jpn. J. Pharm. Health Care Sci.* **39**, 104-109 (2013)] [Lab.of Pharmacy Practice and Social Science]

Retrospective Survey of the Efficacy of Erlotinib for Non-small Cell Lung Cancer Patients Previously Treated with Gefitinib.

Makoto NAKASHIMA, Ryoko OHNISHI, Mie NOMURA, Takuya GOTO, Rie MORI, Takahiro KUMAGAI, Nobuyuki MISHIMA, Tatsuo KATO and Tadashi SUGIYAMA*

We surveyed the efficacy of erlotinib for patients who had been previously treated with gefitinib. The subjects comprised non-small cell lung cancer patients being treated with erlotinib, who had previously been treated with gefitinib. A total of 11 patients were included. The minimum value of time to treatment failure (TTF) was 12 days and average TTF was more than 136 days. Three patients who discontinued gefitinib treatment because of hepatic disorder did not develop severe hepatic disorder with erlotinib treatment. The minimum value of TTF for these 3 patients was 182 days and the average TTF was more than 250 days. In conclusion, erlotinib treatment is one option for patients previously treated with gefitinib.

[*Jpn. J. Pharm. Health Care Sci.* **39**, 286-293 (2013)] [Lab.of Pharmacy Practice and Social Science]

A retrospective survey of implementation status of non-daily administration of gefitinib to control associated adverse reactions.

Makoto NAKASHIMA, Mie NOMURA, Takuya GOTO, Rie MORI, Yuka AIZAWA, Takahiro KUMAGAI, Nobuyuki MISHIMA, Tatsuo KATO and Tadashi SUGIYAMA*

We surveyed the implementation status of non-daily administration of gefitinib to control associated adverse reactions. The most common reasons for complementation with non-daily administration were hepatic disorder, anorexia, and dermatitis. The levels of aspartate aminotransferase and alanine aminotransferase were significantly lower during non-daily administration than during daily administration of gefitinib. Control of adverse reactions was essential for the continuation of gefitinib treatment over a long period of time. Therefore, non-daily administration of gefitinib could be considered as a useful treatment option for the control of adverse reactions induced by gefitinib.

[*Jpn. J. Pharm. Health Care Sci.* **39**, 294-303 (2013)] [Lab.of Pharmacy Practice and Social Science]

Survey of the efficacy and cost of administering aprepitant to non-small cell lung cancer patients treated with combination therapy of carboplatin and paclitaxel every 3 weeks.

Makoto NAKASHIMA, Takuya GOTO, Mie NOMURA, Rie MORI, Yuka AIZAWA, Takahiro KUMAGAI, Tatsuo KATO and Tadashi SUGIYAMA*

We evaluated the adequacy of aprepitant (APR), an antiemetic drug, to 3w-CP therapy in patients treated with 3w-CP therapy. With regard to efficacy, nausea that had developed in the delayed phase and anorexia that had developed in the delayed and post-delayed phases were significantly suppressed by the coadministration of APR. With regard to safety, the incidence of hot flashes, hiccups and hyperglycemia were increased significantly by the coadministration of APR. These results suggest that the use of APR during 3w-CP therapy would be beneficial to prevent the impairment of quality of life as well as the losing motivation for continuing chemotherapy by inhibiting the incidence of nausea, vomiting, and anorexia especially in the delayed and the post-delayed phases.

[*Jpn. J. Pharm. Health Care Sci.* **39**, 552-557 (2013)] [Lab.of Pharmacy Practice and Social Science]

A case of a patient with non-small cell lung cancer in whom periodic testing for D-dimer led to early detection of pulmonary embolus caused by bevacizumab and early initiation of anticoagulation therapy.

Makoto NAKASHIMA, Ryoko OHNISHI, Tatsuo KATO, Nobuyuki MISHIMA, Morihito TERASHI and Tadashi SUGIYAMA*

We report a case of patient with non-small cell lung cancer in whom periodic testing for D-dimer led to early detection of pulmonary embolus caused by bevacizumab. We noted markedly elevated levels of D-dimer by testing for D-dimer once a month, and thus, we could initiate anticoagulation therapy before the patient suffer from symptom of pulmonary embolus. As a result, the thrombus could be dissolved rapidly. Therefore, we suggest that testing for D-dimer before initiating chemotherapy and periodic testing could facilitate the early detection of pulmonary embolus and thus early initiation of anticoagulation therapy.

[*Jpn. J. Pharm. Health Care Sci.* **39**, 294-303 (2013)] [Lab.of Pharmacy Practice and Social Science]

A comprehensive dispensing support system for community pharmacists.

Saori MATSUNAMI, Syuji YAMASHITA, Masafumi KUBOTA, Shigeharu TANEI, Kazuhiro IGUCHI, Yoshihiro NOGUCHI, Naoki MIZUNO, Kiyoshi SAKURAI and Tadashi SUGIYAMA*

We developed a dispensing support system for community pharmacists that uses an iPhone in conjunction with a prescription database. This system integrates three functions: verification of drug tablet dispensing, recording photographs of dispensed medicines, and displaying patients' medication records. We compared the rate of incidents between system-operated and non-system-operated dispensing; the error rates were 1.12% and 2.00%, respectively, and this difference was significant. System-operated dispensing completely prevented medication incident related to drug names. The average waiting time for patients were 606 seconds and 612 seconds, respectively, and this difference was not significant. Therefore, this dispensing support system for community pharmacists appears to be particularly useful for preventing dispensing errors.

[*Jpn. J. Pharm. Health Care Sci.* **39**, 52-60 (2013)] [Lab. of Clinical Pharmacy]

Factors Analysis in Communication Skills by Fifth-year Pharmacy Students after Long-term Practical Training.

Hitomi TERAMACHI*, Tomoya TACHI and Teruo TSUCHIYA

In the present study, an anonymous questionnaire and the TePSS-31 scale for measuring pharmacists' communication skills were conducted on 76 fifth-year students in the 2011 academic year after completing either term I, II or III of practical training at hospitals and pharmacies. Most students reported high levels of overall satisfaction with both hospital- and pharmacy-based practical training and also gave high evaluations regarding improvements in their medical communication skills, observing how pharmacist instructors dealt with patients, and direct patient interaction, demonstrating that effective training was achieved. The high internal consistency of the TePSS-31 was reconfirmed, and factor analysis of the 31 scale items identified the following four skill subscales: "information collection and acceptance", "encouraging others", "dealing with patients positively", and "expressive behavior".

[*Jpn. J. Ther. Drug Monit.* **30**, 1-5 (2013)] [Lab. of Clinical Pharmacy]

Investigation for Factors Affecting Clearance of Vancomycin.

Satoshi AOYAMA, Hitomi TERAMACHI*, Takeyuki MIYAZATO, Tsuyoshi SUGIYAMA, Hiroshi MORI, Teruo TSUCHIYA and Chitoshi GOTO

It has been shown that Japanese cancer patients treated with Vancomycin (VCM) for treatment of methicillin-resistant Staphylococcus aureus infection frequently have lower serum VCM concentration than expected, despite having normal renal function. Therefore, in this study, VCM pharmacokinetic parameters in Japanese cancer patients were compared with those in non-cancer patients for patients with normal renal function and those with impaired renal function. The two-compartment Bayesian pharmacokinetic program was used to analyze the parameters in patients. The cancer patients with normal renal function showed 1.5 times higher VCM clearance (0.097 ± 0.032 L/hr/kg) than non-cancer patients with normal renal function (0.064 ± 0.016 L/hr/kg) ($p<0.05$). These findings suggest that the measurement of VCM serum concentration is required for patients with malignancy.

[*Pharmazie* **68**, 217-220 (2013)] [Lab. of Clinical Pharmacy]

Risk Factors Contributing to Urinary Protein Expression Resulting from Bevacizumab Combination Chemotherapy.

Hitomi TERAMACHI*, Hitomi SHIGA, Natsuki KOMADA, Kento TAMURA, Masahiro YASUDA, Michi UMEDA, Tomoya TACHI, Chitoshi GOTO and Teruo TSUCHIYA

In this study, the risk factors for urinary protein expression resulting from bevacizumab combination chemotherapy were investigated. The subjects were patients aged ≥ 20 years who had received bevacizumab combination chemotherapy at Gifu Municipal Hospital between February 2010 and February 2011, and 24 and 10 were assigned to the urinary protein non-expression and expression group, respectively. The results of multivariate logistic regression analysis revealed a significant difference in systolic blood pressure (≥ 130 mmHg) between the two groups (OR: 14.499, 95%CI: 1.326-158.577). This finding shows that systolic blood pressure (≥ 130 mmHg) is a risk factor for urinary protein expression resulting from bevacizumab combination chemotherapy.

[*Pharmazie* **68**, 706-710 (2013)] [Lab. of Clinical Pharmacy]

Risk Factors for *Clostridium Difficile*-associated Diarrhea and the Effectiveness of Prophylactic Probiotic Therapy.

Takashi MIZUI, Hitomi TERAMACHI*, Tomoya TACHI, Kento TAMURA, Hitomi SHIGA, Natsuki KOMADA, Michi UMEDA, Akihide KODA, Satoshi AOYAMA, Chitoshi GOTO and Teruo TSUCHIYA

This study was conducted to identify the risk factors contributing to *C. difficile*-associated diarrhea and to evaluate the clinical benefit of probiotics in its prevention. The study included 2716 patients at least 20 years old who received an injected antibiotic at any time between February 2010 and February 2011; 2687 and 29 patients were assigned to the non-*C. difficile*-associated and the *C. difficile*-associated diarrhea group, respectively. Multivariate logistic regression analysis revealed that antibiotic therapy for ≥ 8 days, intravenous hyperalimentation, proton pump inhibitor use and H2 blocker use were risk factors for *C. difficile*-associated diarrhea. Prophylactic probiotic therapy was not shown to suppress the occurrence of *C. difficile*-associated diarrhea.

[*Pharmazie* **68**, 909-915 (2013)] [Lab. of Clinical Pharmacy]

Pharmacoeconomic Analysis of DPP-4 Inhibitors.

Hitomi TERAMACHI*, Hiroki OHTA, Tomoya TACHI, Manabu TOYOSHIMA, Takashi MIZUI, Chitoshi GOTO and Teruo TSUCHIYA

This study was a comparative survey of the usage, treatment effectiveness, and cost of Dipeptidyl peptidase-4 (DPP-4) inhibitors. The subjects were patients prescribed DPP-4 inhibitors (sitagliptin, vildagliptin, and alogliptin) at Gifu Municipal Hospital between February 2010 and August 2011, and HbA1c and concomitant antidiabetic agents were surveyed for 12 weeks after the start of DPP-4 inhibitors. A cost-effectiveness analysis showed that the cost required for a 0.1% decrease in HbA1c for 12 weeks was the lowest with vildagliptin (2,478 yen; decrease in HbA1c: 0.75% +/- 0.85%). In a cost analysis with a virtual cohort of 1000 patients, the number of patients who achieved the treatment target (HbA1c 6.5%) was estimated with respect to a virtual cohort created based on the HbA1c level (7.59 +/- 1.13%) at baseline of 307 patients. The results suggest that vildagliptin provides a superior cost-benefit.

[*Pharmazie* **68**, 977-982 (2013)] [Lab. of Clinical Pharmacy]

Impact of Levofloxacin Dosage Adjustments by Dispensing Pharmacists on Adverse Reactions and Costs in the Treatment of Elderly Patients.

Tomoya TACHI*, Hitomi TERAMACHI, Shoko ASANO, Kazuhide TANAKA, Masahiro FUKUTA, Tomohiro OSAWA, Satoshi AOYAMA, Masahiro YASUDA, Takashi MIZUI, Chitoshi GOTO and Teruo TSUCHIYA

Upon receiving a prescription of levofloxacin for patients aged ≥ 75 years, pharmacists evaluate the patients' kidney function and adjusted the appropriate dosage at the time of dispensation. The comparative study was performed at Gifu Municipal Hospital in Japan from March to August 2011, and included an intervention (142 patients) and a control group (98 patients). In the intervention and control groups, levofloxacin-induced adverse reactions developed in 6 of 142 (4.2%) and 13 of 98 (13.3%) patients, respectively ($p < 0.05$). The intergroup difference in the total cost per patient was ¥465.6. Dose adjustment of levofloxacin at the time of dispensation by the pharmacist for patients aged ≥ 75 years resulted in a decrease in the incidence of adverse reactions and cost.

[*J. Pharm. Pract.* **26**, 409-414 (2013)] [Lab. of Clinical Pharmacy]

Pharmaceutical Care for Patients Undergoing S-1 Plus Cisplatin Therapy for Unresectable Recurrent Gastric Cancer.

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

We examined the adverse gastrointestinal events associated with tegafur/gimeracil/oteracil potassium (S-1) plus cisplatin therapy for unresectable recurrent gastric cancer and risk factors for discontinuing therapy due to adverse events. A total of 65 subjects who had received S-1 plus cisplatin therapy for gastric cancer at Ogaki Municipal Hospital were examined. We found that the risk factors for discontinuation of the therapy due to adverse events were serum albumin (Alb) level less than 3.5 g/dL, creatinine clearance (CrCl) rate less than 78 mL/min, and performance status more than 1. Moreover, grade 3 or 4 nonhematological toxicities (including malaise and anorexia) were significantly higher in subjects with Alb less than 3.5 g/dL and CrCl less than 78 mL/min.

[*J. Com. Pharm. Pharm. Sci.* **5**, 124-131 (2013)] [Lab. of Clinical Pharmacy]

Study on the Safety and Continuity of Multiple Tyrosine Kinase Inhibitors in Chemotherapy for Advanced/metastatic Renal Cell Carcinoma.

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

We retrospectively studied the safety and continuity of sunitinib and sorafenib therapy for advanced/metastatic renal cell carcinoma (mRCC), for pharmaceutical care practices. Suspention or dose reduction of sunitinib was because of thrombocytopenia in 33.3% and hand-foot syndrome (HFS), and the relative dose intensity (RDI) was 60.7%. Suspention or dose reduction of sorafenib was because of HFS in 46.6%, and the RDI was 87.5%. It may be difficult for Japanese to sustain treatment of sunitinib therapy at standard dose. Therefore, altering the dose and administration method for individual patients at the outset of treatment appears to be advisable.

[*Yakugaku Zasshi* **133**, 1223-1233 (2013)] [Lab. of Clinical Pharmacy]

Economic Evaluation of Adjustments of Levofloxacin Dosage by Dispensing Pharmacists for Patients with Renal Dysfunction.

Tomoya TACHI*, Misa KATO, Tomohiro OSAWA, Akihide KODA, Masahiro FUKUTA, Kazuhide TANAKA, Satoshi AOYAMA, Masahiro YASUDA, Takashi MIZUI, Chitoshi GOTO and Hitomi TERAMACHI

In our study, we used questionnaires that were administered to pharmacists and doctors at the hospital to investigate their respective working times and the cost of the program, in order to comprehensively analyze the clinical resource costs of the hospital and evaluate the economic burden of the program for levofloxacin. In addition, we studied the pharmacists' and doctors' attitudes toward the program and the circumstances of prescriptions for patients with renal dysfunction. For cost estimation, we used data from this study as well as those of our previous study that suggested that the levofloxacin program was economically beneficial. Furthermore, their attitudes toward the program and circumstances of prescriptions for patients with renal dysfunction were clarified.

[*J. Jpn. Soc. Hosp. Pharm.* **49**, 653-658 (2013)] [Lab. of Clinical Pharmacy]

Evaluation of the Role of Full-time Clinical Pharmacists in the Hematology Ward.

Masahiro YASUDA, Michi UMEDA, Tomoya TACHI*, Kenji KOBAYASHI, Hitomi TERAMACHI, Teruo TSUCHIYA and Chitoshi GOTO

We evaluated the role of full-time clinical pharmacists in the hematology ward. We classified the tasks of pharmacists into 13 items and analyzed scores of "contribution to clinical staffs" and "contribution to patients" about the tasks from April to August 2012. "Confirmation of patients' background and drugs, and planning and suggestion of prescription based on the evaluation" was the most (18.9%) in the tasks of pharmacists. The roles of full-time clinical pharmacists were important and they resulted in direct participatin to a medical team.

[*Parasitol. Int.* **62**, 368-371 (2013)] [Lab. of Clinical Pharmacy]

Synthesis of carbocyclic pyrimidine nucleosides and their inhibitory activities against *Plasmodium falciparum* thymidylate kinase.

Yoshihiro NOGUCHI*, Yuri YASUDA, Makoto TASHIRO, Tadashi KATAOKA, Yoshiaki KITAMURA, Mahmoud KANDEEL and Yukio KITADE

Plasmodium falciparum thymidylate kinase (*Pf* TMK) is a promising antimalarial target due to its unique substrate specificity. We have designed and synthesized enantioselective 2′, 3′-dideoxycarbocyclic pyrimidine nucleosides based on our previous results and screened them for inhibitory activity against *Pf* TMK. The most potent inhibitor showed Ki^{TMP} and Ki^{dGMP} values of 14 and 20 μM, respectively. The fluorinated dideoxy derivative exhibited lower Ki^{TMP} and higher Ki^{dGMP} compared with that of the parent compound (Ki^{TMP}, Ki^{dGMP} equals 20 and 7 μM, respectively). The modification of carbocyclic pyrimidine nucleosides is a promising strategy for developing powerful *Pf* TMK inhibitors.

[*Biochim. Biophys. Acta.* **1833**, 1006-1016 (2013)] [Lab. of Drug Informatics]

Sphingosine kinase 1 expression is downregulated during differentiation of Friend cells due to decreased c-MYB.

Naoki MIZUTANI, Misa KOBAYASHI, Sayaka SOBUE, Masatochi ICHIHARA, Hiromi ITO, Koji TANAKA, Soichiro IWAKI, Satoshi FIJII, Yuri ITO, Keiko TAMIYA-KOIZUMI, Akira TAKAGI, Tetsuhito KOJIMA, Tomoki NAOE, Motoshi SUZUKI, Mitsuhiro NAKAMURA*, Yoshiko BANNO, Yoshinori NOZAWA and Takashi MURATE.

Mouse Friend cells showed higher SPHK1 but not SPHK2 expression compared with other mouse cell lines. A Sphk1 promoter analysis demonstrated the region between -53bp and the first exon as the minimal promoter. Further promoter truncation revealed the importance of a MYB-binding site. Effects of pertussis toxin, a G-protein-coupled receptor inhibitor, and S1P receptor antagonist on Friend cell growth and differentiation were negligible, suggesting the importance of the intracellular SPHK1/S1P signaling in Friend cells.

[J Nat Med **67**, 276-280 (2013)] [Lab. of Herbal Garden]

Identification of *Anogeissu latifolia* Wallich and analysis of refined gum ghatti .

Eiji SAKAI, Tsuyoshi KATAYAMA*, Takeshi OGASAWARA and Mizuo MIZUNO

Gum ghatti was originally used as an alternative to gum arabic having similar properties to those found in gum arabic. Currently, gum ghatti has been used as a food additive due to its excellent emulsification properties. In this study, we obtained gum ghatti nodules and branches with flowers and leaves as botanical specimens which were collected from the same harvesting area. Component analysis of the refined gum ghatti samples revealed that they contained arabinose, galactose, mannose, xylose, rhamnose, and glucuronic acid as constituent sugars, protein, moisture and tannin. As a result of the investigation of botanical specimens, they were identified as *Anogeissus latifolia* Wallich.

[*Mutagenesis.* **28**, 161-168 (2013)] [Lab. of Radiochemsitry]

The Enhancing effect of ethanol on the mutagenic activation of *N*-nitrosomethylbenzylamine by cytochrome P450 2A in the rat oesophagus.

Kenjiro TATEMATSU*, Akihiro KOIDE, Keiichiro MORIMURA, Shoji FUKUSHIMA and Yukio MORI

To elucidate the mechanism underlying the role of ethanol in the enhancement of *N*-nitrosomethylbenzylamine (NMBA)-induced oesophageal carcinogenesis, we evaluated the hepatic and extrahepatic levels of the cytochrome P450 (CYP) and mutagenic activation of environmental carcinogens in F344 rats treated with 10% ethanol in the drinking water or 50% ethanol intragastricly. The results of these experiments showed that the enhancing effect of ethanol on NMBA-induced oesophageal carcinogenesis could be attributed to an increase in the metabolic activation of NMBA by oesophageal CYP2A during the initiation phase, and that this occurred independently of CYP2E1.

紀 要 論 文

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