[*Cancer. Res.*, **66(5)**, 2725-2731 (2006)] [Lab. of Pharm. Chemistry]

HIF-1-dependent Repression of E-cadherin in VHL-null Renal Cell Carcinoma Mediated by TCF3, ZFHX1A, and ZFHX1B.

Balaji KRISHNAMACHARY, David ZAGZAG, Hideko NAGASAWA*, Karin RAINEY, Hiroaki OKUYAMA, Jin H. BAEK, and Gregg L. SEMENZA

A critical event in the pathogenesis of invasive and metastatic cancer is E-cadherin loss of function. Renal clear cell carcinoma (RCC) is characterized by loss of function of the von Hippel-Lindau tumor suppressor (VHL). Loss of E-cadherin expression and decreased cell-cell adhesion in VHL-null RCC4 cells were corrected by enforced expression of VHL, a dominant-negative HIF-1alpha mutant, or a short hairpin RNA directed against HIF-1alpha. In human RCC biopsies, expression of E-cadherin and HIF-1alpha was mutually exclusive. The expression of mRNAs encoding TCF3, ZFHX1A, and ZFHX1B, which repress E-cadherin gene transcription, was increased in VHL-null RCC4 cells in a HIF-1-dependent manner. Thus, HIF-1 contributes to the epithelial-mesenchymal transition in VHL-null RCC by indirect repression of E-cadherin.

[*Radiat Med*, **24(2)**, 98-107 (2006)] [Lab. of Pharm. Chemistry]

Evaluation of Hypoxia-Specific Cytotoxic Bioreductive Agent-Sodium Borocaptate-^{10}B Conjugates, as ^{10}B-Carriers in Boron Neutron Capture Therapy.

Shin-ichiro MASUNAGA, Hideko NAGASAWA*, Keiko GOTOH, Yoshinori SAKURAI, Yoshihiro UTO, Hitoshi HORI, Kenji NAGATA, Minoru SUZUKI, Akira MARUHASHI, Yuko KINASHI and Koji ONO

PURPOSE: To evaluate the usefulness of 5 new ^{10}B-compounds (TX-2091, TX-2095, TX-2097, TX-2100, and TX-2110) as ^{10}B-carriers in boron neutron capture therpy (BNCT). RESULTS: TX-2100 had a significantly stronger radio-sensitizing effect with reactor thermal neutron beams than BSH on both total and Q cells in solid tumors. TX-2100 clearly exhibited a radio-sensitizing effect with gamma-rays not only on total cells but also on Q and hypoxic tumor cells, which was not achieved by BSH. CONCLUSION: A ^{10}B-carrier that acts as a hypoxic cytotoxin on tumor cells as well as having the potential to keep ^{10}B in tumors and sensitize tumor cells more markedly than conventional ^{10}B-carriers, such as TX-2100, is a promising candidate for use in BNCT.

[*Int. J. Hyperthermia.*, **22**, 287-299 (2006)] [Lab. of Pharm. Chemistry]

The Usefulness of Mild Temperature Hyperthermia Combined with a Newly Developed Hypoxia-Oriented ^{10}B Conjugate Compound, TX-2100, for Boron Neutron Capture Therapy.

Shin-ichiro MASUNAGA, Hideko NAGASAWA*, Yoshinori SAKURAI, Yoshihiro UTO, Hitoshi HORI, Kenji NAGATA, Minoru SUZUKI, Akira MARUHASHI, Yuko KINASHI and Koji ONO

RESULTS: ^{10}B biodistribution analyses in tumours, brain, skin, muscles, blood and liver indicated that the administration of TX-2100 plus MTH is most favourable for concentrating a sufficient amount of ^{10}B in tumours and maintaining a high enough ^{10}B concentration during irradiation. In addition, MTH had a stronger sensitizing effect when combined with TX-2100 than with the concurrent administration of its components TX-402 and BSH on both the total and Q cell populations in solid tumours. CONCLUSION: MTH was very effective in combination with the newly-developed TX-2100. The sensitizing effect in combination with MTH should be examined when new ^{10}B-carriers are designed.

[*Br. J. Radiol.*, **79**, 991-998 (2006)] [Lab. of Pharm. Chemistry]

The Usefulness of a Continuous Administration of Tirapazamine Combined with Reduced Dose-Rate Irradiation Using {Gamma}-Rays or Reactor Thermal Neutrons.

Shin-ichiro MASUNAGA, Yoshinori SAKURAI, Kenji NAGATA, Minoru SUZUKI, Akira MARUHASHI, Yuko KINASHI, Hideko NAGASAWA*, Yoshihiro Uto, Hitoshi Hori and Koji ONO

We clarified the usefulness of the continuous administration of tirapazamine (TPZ) in combination with reduced dose-rate irradiation (RDRI) using gamma-rays or reactor thermal neutrons. The sensitivity of both total and Q cells, especially of Q cells, was significantly reduced with RDRI compared with CDRI. Combination of TPZ increased the sensitivity of both populations, with a slightly more remarkable increase in Q cells. Furthermore, the continuous administration of TPZ raised the sensitivity of both total and Q cell populations, especially the former, more markedly than the single administration, whether combined with CDRI or RDRI using gamma-rays or thermal neutrons.

[*Anticancer. Res.*, **26**, 1261-1270 (2006)] [Lab. of Pharm. Chemistry]

Evaluation of Hypoxic Cell Radio-sensitizers in Terms of Radio-sensitizing and Repair-inhibiting Potential Dependency on p53 Status of Tumor Cells and the Effects on Intratumor Quiescent Cells.

Shin-ichiro MASUNAGA, Hideko NAGASAWA*, Yoshihiro UTO, Hitoshi HORI, Kenji NAGATA, Minoru SUZUKI, Yuko KINASHI and Koji ONO

BACKGROUND: Intratumor quiescent (Q) cells and p53-mutated tumor cells are more difficult to control than intratumor proliferating (P) cells and p53 wild-type tumor cells, respectively. The usefulness of 3 hypoxic cell radio-sensitizers was compared in terms of a radio-sensitizing effect under aerobic and hypoxic conditions and a repair-inhibiting effect following irradiation on both Q and total (P + Q) cell populations in solid tumors. RESULTS: Both the radio-sensitizing effects under aerobic and hypoxic conditions and the repair-inhibiting effects following gamma-ray irradiation increased in the following order: nimorazole < SR-2514 < misonidazole in both total and Q cells in these 3 tumors. Both effects were more marked in the Q cells and p53-mutated tumors than in the total cells and p53-wild tumors, respectively.

[*Int. J. Oncol.*, 1533-1539 (2006)] [Lab. of Pharm. Chemistry]

Hypoxia Accelerates Cancer Invasion of Hepatoma Cells by Upregulating MMP Expression in an HIF-1Alpha-Independent Manner.

Atsushi MIYOSHI, Yoshihiko KITAJIMA, Takao IDE, Kazuma OHTAKA, Hideko NAGASAWA*, Yoshihiro UTO, Hitoshi HORI and Kohji MIYAZAKI

We aimed to solve the molecular mechanism of tumor invasion under the hypoxic condition. We showed that tumor hypoxia accelerated cancer invasion in two hepatoma cell lines. Using Western blot and RT-PCR analyses we demonstrated striking evidence that the expression of HIF-1alpha, ETS-1, MMP-7 and MT1-MMP was strongly upregulated by hypoxic stimulation. To examine whether these invasion-related genes are regulated by HIF-1alpha, we treated hepatoma cells with TX-402, which was reported to repress HIF-1alpha expression. HIF-1alpha expression was strongly repressed by the TX-402 treatment. In contrast, the expression of ETS-1, MMP-7 and MT1-MMP mRNA was not affected by TX-402 treatment. In the pHIF-1alphaDN cells, the expression of ETS-1, MMP-7 and MT1-MMP was not repressed.

[*Anticancer. Res.*, **26**, 4073-4078 (2006)] [Lab. of Pharm. Chemistry]

The Role of Gc Protein Oligosaccharide Structure as a Risk- Factor for COPD.

Kazuto OHKURA, Hideko NAGASAWA*, Yoshihiro UTO, Natsuko OKAMURA, Aya MURAKAMI and Htoshi HORI

RESULTS: The MO parameter of the sugar moiety was different for each Gc protein model. The electrostatic potential (ESP) field of beta-1,4 type Gc2 protein was similarly distributed to beta-1,4 linked Gcl-type proteins (GclF, GclS). In the beta-1,3 type Gc protein models, the results of these parameters (i.e., dipole moment, dGW and ESP) were similar to those of beta-1,4 type models. Conclusion: The relationship between COPD risk and the features of the sugar structure in Gc proteins was examined, and it appeared that the active factors (i.e., dipole moment, dGW) might be risk factors for COPD, but passive factors (i.e., ESP) did not affect COPD risk. The bond type (beta-1,4 or beta-1,3) between galactose and N-acetylgalactosamine did not affect the molecular features.

[*J. Biol. Chem.*, **281(22)**, 15554-15563 (2006)] [Lab. of Pharm. Chemistry]

Expression of Vascular Endothelial Growth Factor Receptor 1 in Bone Marrow-Derived Mesenchymal Cells is Dependent on Hypoxia-Inducible Factor 1.

Hiroaki OKUYAMA, Balaji KRISHNAMACHARY, Yi Fu ZHOU, Hideko NAGASAWA*, Marta BOSCH-MARCE and Gregg L. SEMENZA

In this study, we found that exposure of cultured mouse bone marrow-derived mesenchymal stromal cells (MSC) to hypoxia or an adenovirus encoding a constitutively active form of hypoxia-inducible factor 1 (HIF-1) induced VEGFR1 mRNA and protein expression and promoted ex vivo migration in response to VEGF or PLGF. MSC in which HIF-1 activity was inhibited by a dominant negative or RNA interference approach expressed markedly reduced levels of VEGFR1 and failed to migrate or activate AKT in response to VEGF or PLGF. Thus, loss-of-function and gain-of-function approaches demonstrated that HIF-1 activity is necessary and sufficient for basal and hypoxia-induced VEGFR1 expression in bone marrow-derived MSC.

[*Adv. Exp. Med. Biol.*, **578**, 113-118 (2006)] [Lab. of Pharm. Chemistry]

Artepillin C Isoprenomics: Design and Synthesis of Artepillin C Analogues as Antiatherogenic Antioxidants.

Yoshihiro UTO, Shuzo AE, Azusa HOTTA, Junji TERAO, Hideko NAGASAWA* and Hitoshi HORI

In this study, we developed promising antiatherogenic antioxidant, TX-2012 based on isoprenomics. The inhibitory potency of our artepillin C analogues for LDL oxidation depends not only on the lipophilicity and free radical scavenging activity, but also on the topological properties such as linearity and compactness required for LDL interaction.

[*Bioorg . Med..Chem.*, **14**, 5721-5728 (2006)] [Lab. of Pharm. Chemistry]

Artepillin C Isoprenomics: Design and Synthesis of Artepillin C Isoprene Analogues as Lipid Peroxidation Inhibitor Having Low Mitochondrial Toxicity

Yoshihiro UTO, Shuzo AE, Daisuke KOYAMA, Mitsutoshi SAKAKIBARA, Naoki OTOMO, Mamoru OTSUKI, Hideko NAGASAWA*, Kenneth L. KIRK and Hitoshi HORI

We designed and synthesized isoprene analogues of artepillin C, a major component of Brazilian propolis, and investigated the inhibitory activity on lipid peroxidation of rat liver mitochondria (RLM) and RLM toxicity based on isoprenomics. We succeeded in the synthesis of artepillin C isoprene analogues using regioselective prenylation within the range from 22% to 53% total yield. Reactivity of artepillin C and its isoprene analogues with ABTS (2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonate)) radical cations showed only a slight difference among the molecules. From these results we conclude that artepillin C isoprene analogues could be potent lipid peroxidation inhibitors having low mitochondrial toxicity. We also conclude that elongation of the isoprene side chain of artepillin C to increase lipophilicity had little influence on the inhibitory activity toward RLM lipid peroxidation.

[*J. Cancer. Res. Clin. Oncol.*, **133**, 47-55 (2007)] [Lab. of Pharm. Chemistry]

Dependency of the Effect of a Vascular Disrupting Agent on Sensitivity to Tirapazamine and Gamma-Ray Irradiation Upon the Timing of its Administration and Tumor Size, with Reference to the Effect on Intratumor Quiescent Cells.

Shin-ichiro MASUNAGA, Hideko NAGASAWA*, Kenji NAGATA, Minoru SUZUKI, Yoshihiro UTO, Hitoshi HORI, Yuko KINASHI and Koji ONO

PURPOSE: The effect of vascular disrupting agent ZD6126 with time on the sensitivity to the hypoxic cytotoxin tirapazamine (TPZ) and gamma-rays was examined in large and small solid tumors. CONCLUSIONS: Following ZD6126 treatment, in terms of tumor control, especially large tumors and total tumor cell population, administering TPZ 1 h later and gamma-ray irradiation 24 h later were effective. Intratumor physiologic factors such as the size of the HF, depending on the time after ZD6126 injection, have to be taken into account when combining another treatment with ZD6126.

[*Radiother. Oncol.*, **78**, S83-S84 (2006)] [Lab. of Pharm. Chemistry]

Evaluation of Bioreductive Agent-Sodium Borocaptate-^{10}B Hybrid Compounds, as ^{10}B-Carriers in Boron Neutron Capture Therapy.

Shin-ichiro MASUNAGA, Hideko NAGASAWA*, Keiko GOTOH, Yoshihiro UTO, Yoshinori SAKURAI, Hitoshi HORI, Kenji NAGATA, Minoru SUZUKI, Akira MARUHASHI, Yuko KINASHI and Koji ONO

To evaluate the usefulness of 5 new 10Bcompounds (TX-2091, TX-2095, TX-2097, TX-2100 and TX-2110) as 10B-carriers in boron neutron capture therapy (BNCT). They were conjugates that had been synthesized from a hypoxia-specific cytotoxic bioreductive agent, quinoxaline oxide TX-402, and a clinically used 10B-carrier, sodium borocaptate-10B (BSH). A 10B-carrier with an effect as a hypoxic cytotoxin on tumor cells as well as the potential to keep 10B in tumors and sensitize tumor cells more markedly than conventional 10B-carriers, such as TX-2100, is a promising candidate for use in BNCT.

[*Chem. Eur. J.*, **12**, 3896-3904 (2006)] [Lab. of Pharm. Chemistry]

Asymmetric tandem Michael-aldol reactions between 3-cinnamoyloxazolidine-2-thiones and aldehydes.

Hiroshi KINOSHITA, Takashi OSAMURA, Kazumi MIZUNO, Sayaka KINOSHITA, Tatsunori IWAMURA, Shin-ichi WATANABE, Tadashi KATAOKA*, Osamu MURAOKA, and Genzo TANABE

Reactions between chiral 3-cinnamoyl-4-methyl-5-phenyl-1,3-oxazolidine-2-thiones and aromatic aldehydes in the presence of BF_3-Et_2O diastereoselectively produced tricyclic compounds incorporating a bridgehead carbon bound to four heteroatoms in high yields. Four stereocenters were induced during the reaction. The tricyclic products were transformed into propane-1,3-diols bearing three consecutive stereocenters by acid hydrolysis, *S*-methylation, and reductive removal of the chiral auxiliary.

[*Tetrahedron Lett.*, **48(5)**, 813-816 (2007)] [Lab. of Pharm. Chemistry]

The First Isolation of Allenylselenonium Salts: Their Synthesis and Properties as Electrophiles.

Shin-ichi WATANABE, Yukio MIURA, Tatsunori IWAMURA, Hideko NAGASAWA* and Tadashi KATAOKA

The first synthesis of allenylselenonium salts and their reactivities are described. The corresponding allenyl methyl selenides were alkylated with methyl trifluoromethanesulfonate to afford the desired compounds. Their reactions with active methylene carbanions produced furan, dihydrofuran or methylene cyclopropane derivatives via the Michael addition of nucleophiles to the selenonium salts.

[*Heterocycles* **67**, 399-405 (2006)] [Lab. of Medicinal Chemistry]

Formation of 4',5'-Didehydro-5'-deoxy-3'-*O*-methyluridine via Regioselective Nucleophilic Addition of Methoxide Anion to 2',3'-Anhydro-5'-dehydro-5'-iodouridine

Hironao SAJIKI,* Hideki TAKASU and Hirota, KOSAKU

Treatment of 2',3'-anhydro-5'-deoxy-5'-iodouracil with sodium methoxide regioselectively provided 4',5'-didehydro-5'deoxy-3'-*O*-methyluridine as the sole product via 4',5'-didehydro-5'-deoxy-2',3'-epoxyuridine as an intermediate.

[*Org. Lett.* **8**, 987-990 (2006)] [Lab. of Medicinal Chemistry]

Pd/C-Catalyzed Deoxygenation of Phenol Derivatives Using Mg Metal and MeOH in the Presence of NH_4OAc

Hironao SAJIKI*, Akinori MORI, Tomoteru MIZUSAKI, Takashi IKAWA, Tomohiro MAEGAWA and Kosaku HIROTA

A Pd/C-catalyzed deoxygenation method of phenolic hydroxyl groups via aryl triflates or mesylates using Mg metal in MeOH at room temperature was developed. The addition of NH_4OAc dramatically affects the reactivity and reaction rate. This method is particularly attractive to provide an environmentally benign and widely applicable removal method of phenolic alcs. under quite mild reaction conditions. Treatment of 2',3'-anhydro-5'-deoxy-5'-iodouracil with sodium methoxide regioselectively provided 4',5'-didehydro-5'deoxy-3'-*O*-methyluridine as the sole product via 4',5'-didehydro-5'-deoxy-2',3'-epoxyuridine as an intermediate.

[*Synlett* 1440-1442 (2006)] [Lab. of Medicinal Chemistry]

A Mild and Facile Method for Complete Hydrogenation of Aromatic Nuclei in Water

Tomohiro MAEGAWA, Akira AKASHI and Hironao SAJIKI*

A mild and complete hydrogenation of aromatic rings catalyzed by heterogeneous 10% Rh/C proceeds at 80 °C in water under 5 atm of H_2 pressure. This method is applicable to the hydrogenation of various carbon and heteroaromatic compounds such as alkylbenzenes, biphenyls, pyridines and furans.

[*Adv. Synth. Catal.* **348**, 1025-1028 (2006)] [Lab. of Medicinal Chemistry]

Synergistic Effect of a Palladium-on-carbon/Platinum-on-carbon Mixed Catalyst in Hydrogen/Deuterium Exchange Reactions of Alkyl-substituted Aromatic Compounds

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

We have found a synergistic effect in the H-D exchange reaction of alkyl-substituted aromatic compounds. using the Pd/C-Pt/C-D_2O-H_2 system. This system would lead to fully H-D exchange results even on the sterically hindered sites which were only low-deuterium incorporated by Pd/C or Pt/C independently. Since the reaction was general for a variety of aromatic compounds, it could be applied to the deuteration of dianiline derivatives as raw materials for polyimides.

[*Bioconjugate Chem.* **17**, 1545-1550 (2006)] [Lab. of Medicinal Chemistry]

Rigid Linkers for Bioactive Peptides

Josef VAGNER, Heather L. HANDL, Yasunari MONGUCHI,* Umasish JANA, Lucinda J. BEGAY, Eugene A. MASH, Victor J. HRUBY and Robert J. GILLIES

Rigid linkers of variable length were used to connect two high-affinity Nle4-D-Phe7-α-MSH (NDP-α-MSH) or two low-affinity MSH(4) ligands. The linked peptides were synthesized by solid-phase methods. Control experiments indicate there is little or no effect of these linkers on NDP-α-MSH or MSH(4) binding to the human melanocortin 4 receptor (hMC4R). Tethering two high-affinity ligands gave no binding enhancement, while tethering two low-affinity ligands resulted in binding enhancement that decreased with increased linker length. Furthermore, for the low-affinity ligands, the enhancement of affinity is inversely proportional to the estd. mol. moments of inertia. These results are consistent with a model wherein binding is enhanced when the rate of ligand reattachment to the receptor is fast relative to the rate of ligand diffusion.

[*Tetrahedron* **62**, 7926-7933 (2006)] [Lab. of Medicinal Chemistry]

Pd/C–Et_3N-mediated Catalytic Hydrodechlorination of Aromatic Chlorides under Mild Conditions

Yasunari MONGUCHI, Akira KUME, Kazuyuki HATTORI, Tomohiro MAEGAWA and Hironao SAJIKI *

A mild and efficient one-pot procedure for the hydrodechlorination of aromatic chlorides using a Pd/C–Et_3N system was developed. A variety of aromatic chlorides could be dechlorinated at room temperature and under ambient hydrogen pressure. Et_3N activates the catalysis and is likely to work as a single electron donor in this system.

[*Tetrahedron* **62**, 8384-8392 (2006)] [Lab. of Medicinal Chemistry]

Facile and Catalytic Degradation Method of DDT Using Pd/C–Et_3N System under Ambient Pressure and Temperature

Yasunari MONGUCHI, Akira KUME and Hironao SAJIKI *

The catalytic degradation method of *p,p'*-DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] and its regioisomer *o,p'*-DDT [1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane] using the Pd/C–Et_3N system under ambient hydrogen pressure and temperature was established. The presence of Et_3N was necessary for the quick and complete breakdown of DDT. The independent degradation study of two intermediates, *p,p'*-DDD [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane] and *p,p'*-DDE [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene] using GC–MS let us to speculate the degradation pathway of *p,p'*-DDT. In the initial phase of the reaction, *p,p'*-DDT degradation splits into two ways: a dehydrochlorination pathway and a hydrodechlorination pathway. In each pathway, reaction starts from an aliphatic moiety and subsequent hydrodechlorination from the benzene moieties takes place in a stepwise manner. The former pathway leads to the formation of 1,1-diphenylethane and the latter leads to the formation of 1,1-dichloro-2,2-diphenylethane. These diphenylethane analogs, which are less toxic compared with *p,p'*-DDT, are terminal degradation products in our system. The distinctive features of our catalytic degradation method of DDTs are reliability, simplicity, efficiency, and inexpensiveness.

[*Tetrahedron* **62**, 10954-10961 (2006)] [Lab. of Medicinal Chemistry]

General Method of Obtaining Deuterium-labeled Heterocyclic Compounds Using Neutral D_2O with Heterogeneous Pd/C

Hiroyoshi ESAKI, Nobuhiro ITO, Shino SAKAI, Tomohiro MAEGAWA, Yasunari MONGUCHI and Hironao SAJIKI*

A protocol of a versatile H-D exchange reaction of heterocyclic compds. catalyzed by heterogeneous Pd/C in D_2O is described. The reaction of various nitrogen-contg. heterocycles with 10% Pd/C (10 wt% of the substrate) under hydrogen atomosphere in D_2O as a deuterium source at 110-180 °C for 24 h afforded the deuterated compounds with satisfactory efficiency of deuteration in moderate to excellent isolated yields. Furthermore, the Pd/C-H_2-D_2O system can be extended to the direct deuteration of biol. active compounds. such as sulfamethazine, which is used as a synthetic antibacterial drug for fat stocks and would be applied as a general method for the preparation of the standard materials for the analysis of residual chemicals. in foods and so on.

[*Tetrahedron* **62**, 11925-11932 (2006)] [Lab. of Medicinal Chemistry]

Chemoselective Hydrogenation Method Catalyzed by Pd/C Using Diphenylsulfide as a Reasonable Catalyst Poison

Akinori MORI, Tomoteru MIZUSAKI, Yumi MIYAKAWA, Eri OHASHI, Tomoko HAGA, Tomohiro MAEGAWA, Yasunari MONGUCHI and Hironao SAJIKI*

While Pd/C is one of the most useful catalysts for hydrogenation, the high catalyst activity of Pd/C causes difficulty in its application to chemoselective hydrogenation between different types of reducible functionalities. In order to achieve chemoselective hydrogenation using Pd/C, we investigated catalyst poison as a controller of the catalyst activity. We found that the addn. of Ph_2S (diphenylsulfide) to the Pd/C-catalyzed hydrogenation reaction mixture led to reasonable deactivation of Pd/C. By the use of the Pd/C-Ph2S catalytic system, olefins, acetylenes, and azides can be selectively reduced in the coexistence of arom. carbonyls, aromatic halides, cyano groups, benzyl esters, and *N*-Cbz (benzyloxycarbonyl) protecting groups. The present method is promising as a general and practical chemoselective hydrogenation process in synthetic organic chemistry.

[*Org. Lett.* **8**, 3279-3281 (2006)] [Lab. of Medicinal Chemistry]

Pd/C-Catalyzed Chemoselective Hydrogenation in the Presence of Diphenylsulfide

Akinori MORI, Yumi MIYAKAWA, Eri OHASHI, Tomoko HAGA, Tomohiro MAEGAWA and Hironao SAJIKI*

A Pd/C-catalyzed chemoselective hydrogenation using diphenyl sulfide as a catalyst poison has been developed. This methodology selectively hydrogenates olefin and acetylene functionalities without hydrogenolysis of aromatic carbonyls and halogens, benzyl esters, and *N*-Cbz protective groups.

[*Heterocycles*, **68**, 1025—1030 (2006)] [Lab. of Pharm. Synthetic Chemistry]

Asymmetric Diels–Alder Reaction of 2-(4-Tolylsulfinyl)-1-indolyl α ,β -Unsaturated Enones.

Yoshitsugu Arai,* Takeshi Katori, Yukio Masaki

The asymmetric Diels—Alder reaction of chiral 1-[2-(4-tolylsulfinyl)indolyl] enones was examined. The cycloaddition of cinnamyl and crotonyl α,β-unsaturated enones with cyclopentadiene in the presence of a lanthanoid triflate as a Lewis acid proceeds smoothly to give the corresponding *endo* cycloadducts with high diastereoselectivity.

[*Synlett*, 288—290 (2006)] [Lab. of Pharm. Synthetic Chemistry]

Recyclable Polymeric π-Acid Catalyst Effective in Aqueous and Solvent-Free Inverse-Electron-Demand Aza-Diels–Alder Reactions

Yukio Masaki, Tomoyasu Yamada, Hyouei Kawai, Akichika Itoh,* Yoshitsugu Arai,* Hiroshi Furukawa

A polymer-supported π-acid dicyanoketene ethyleneacetal was found to be a highly efficient and recyclable catalyst in two- and three-component inverse-electron-demand aza-Diels–Alder reactions at room temperature not only in water but also under solvent-free conditions.

[*Synthesis*, 1949—1952 (2006)] [Lab. of Pharm. Synthetic Chemistry]

Facile Aerobic Photo-oxidation of Alcohols in the Presence of Catalytic *N*-Bromosuccinimide

Kiyoto Kuwabara, Akichika Itoh*

Alcohols were found to be oxidized to the corresponding carboxylic acid in the presence of catalytic *N*-bromosuccinimide in an oxygen atmosphere.

[*Synthesis*, 1757—1759 (2006)] [Lab. of Pharm. Synthetic Chemistry]

Synthesis of Benzoic Acids by Aerobic Photo-oxidation with Hydrobromic Acid

Shin-ichi Hirashima, Akichika Itoh*

A methyl group at an aromatic nucleus was found to be oxidized to the corresponding carboxylic acid directly in the presence of molecular oxygen and catalytic hydrobromic acid under photo-irradiation.

[*Chem. Pharm. Bull.* **54**, 591-593 (2006)] [Lab. of Pharm. Synthetic Chemistry]

Recyclable Polymeric π–Acid Catalyst Effective on Mannich-Type Reaction in Water

Yukio Masaki, Kouichi Yamazaki, Hyouei Kawai, Tomoyasu Yamada, Akichika Itoh,*
Yoshitsugu Arai,* Hiroshi Furukawa

Polymer-supported dicyano-ketene acetal (poly-DCKA-1), synthesized by copolymn. of a DCKA bearing a 4-vinylbenzyl group with ethyleneglycol dimethacrylate, was found to be an excellent recyclable catalyst for the three-component Mannich-type reaction of aldehydes, arom. amines, and TMS enolate of ethyl isobutyrate in water as the sole solvent.

[*Tetrahedron*, **62**, 7887-7891 (2006)] [Lab. of Pharm. Synthetic Chemistry]

Synthesis of Benzoic Acids by Aerobic Photo-oxidation with Hydrobromic Acid

Shin-ichi Hirashima, Shouei Hashimoto, Yukio Masaki, Akichika Itoh*

Alcohols were found to be oxidized to the corresponding carboxylic acid in the presence of a catalytic inorganic bromo source, for example, lithium bromide, bromine and hydrobromic acid, under photo-irradiation.

[*Chem. Pharm. Bull.*, **54**, 1457-1458 (2006)] [Lab. of Pharm. Synthetic Chemistry]

Facile Aerobic Photo-oxidation of Aldehydes in the Presence of Catalytic Lithium Bromide

Shin-ichi Hirashima, Shouei Hashimoto, Yukio Masaki, Akichika Itoh*

Aldehydes were found to be oxidized to the corresponding carboxylic acid in the presence of catalytic lithium bromide under photo-irradiation.

[*Chem. Pharm. Bull.*, **54**, 1571-1575 (2006)] [Lab. of Pharm.Synthetic Chemistry]

Oxidative Photo-decarboxylation in the Presence of Mesoporous Silicas

Akichika Itoh,* Tomohiro Kodama, Yukio Masaki, Shinji Inagaki

FSM-16, a mesoporous silica, was found to catalyze oxidative photo-decarboxylation of α-hydroxy carboxylic acid, phenyl acetic acid derivatives and *N*-acyl-protected α-amino acids to afford the corresponding carbonyl compounds. Furthermore, FSM-16 proved to be re-usable by re-calcination at 450°C after the reaction.

[*Chem. Pharm. Bull.*, **54**, 1620-1621 (2006)] [Lab. of Pharm. Synthetic Chemistry]

Aerobic Oxidation of Benzyl- and Allylic Alcohols under Visible Light Irradiation of a Fluorescent Lamp in the Presence of Catalytic Iodine

Hiroki Nakayama, Akichika Itoh*

Benzyl alcohols and allylic alcohols were found to be oxidized to the corresponding aldehydes in the presence of a catalytic amount of iodine under irradiation of a fluorescent lamp.

[*J. Photopolym. Sci. Technol.*, **19**, 265-268 (2006)] [Lab. of Pharm. Physical Chemistry]

Immobilization of Antithrombotic Biomolecules on LDPE Surface Functionalized by Plasma Techniques.

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

We have previously reported a novel method to introduce hydrophilic carboxyl groups on LDPE surface by plasma assisted-immobilization of vinylmethyether-maleic acid copolymer (VEMAC). In this study, we fabricated the well-defined polyglycidyl-methacrylate (polyGMA) brushes on the surface of VEMAC-immobilized LDPE sheet by surface-initiated atom transfer radical polymerization (ATRP) of GMA for the covalent immobilization of antithrombotic biomolecule, urokinase (UK). The immobilization of UK was achieved through a coupling reaction involving epoxy group of GMA and nucleophilic groups of UK under mild conditions (in pH 8.9 buffer solution at 4 °C). The relatively high enzyme activity was observed in the immobilized UK on the polyGMA brush prepared under the optimal ATRP condition.

[*Chem. Pharm. Bull.*, **54**, 514-518(2006)] [Lab. of Pharm. Physical Chemistry]

Preparation of Floating Drug Delivery System by Plasma Technique.

Tomoya NAKAGAWA, Shin-ichi KONDO, Yasushi SASAI, and Masayuki KUZUYA*

A novel intragastric floating drug delivery system (FDDS) has been prepared by pulsed plasma-irradiation on the double-compressed tablet of 5-Fluorouracil (5-FU) as a core material with outer layer composed of a 68/17/15 weight ratio of Povidone (PVP), Eudragit RL (E-RL) and NaHCO3. The plasma heat flux caused the thermal decomposition of NaHCO3 to generate carbon dioxide and the resultant gases were trapped in bulk phase of outer layer, so that the tablets turned to have a lower density than the gastric contents and remained buoyant in simulated gastric fluid for a prolonged period of time. In addition, the release of 5-FU from the tablet is sustained by occurrence of plasma-induced crosslink reaction on the outer layer of tablet and the release rate of 5-FU can be well controlled by plasma operational conditions.

[*Yakugakuzassi*, 126, 439-454 (2006)] [Lab. of Pharm. Physical Chemistry]

Novel Pharmaceutical and Biomedical Applications of Plasma Techniques.

Masayuki KUZUYA

In this review, applications using plasma-irradiated organic polymers are described, which include: 1) preparation of double-compressed tablets applicable for reservoir-type drug-delivery systems (DDS) for sustained and delayed release, intragastric floating DDS (FDDS) for oral controlled-release dosage forms with gastric retention capabilities, and fabrication of functionalized composite powders applicable for controlled drug release with the mechanical application of plasma-irradiated polymer powder; 2) an approach to "patient-tailored DDS," administered by taking into account that the environment (pH, transit time, etc.) in the gastrointestinal tract varies with each patient, based on the combination of the above-mentioned DDS devices; 3) plasma-assisted promotion of seed germination of herbal medicines with hard coats; and 4) fabrication of polymer surfaces with durable hydrophilicity and lubricity using plasma techniques and the immobilization of oligo-nucleotides on hydrophilic polymer surfaces.

[*Die Pharmazie*, **61**, 106-111 (2006)] [Lab. of Pharm. Engineering]

Properties of liposomes coated with hydrophobically modified chitosan in oral liposomal drug delivery.

Jringjai THONGBORISUTE, Hirofumi TAKEUCHI*, Hiromitsu YAMAMOTO and Yoshiaki KAWASHIMA

Dodecylated chitosan (DC), a hydrophobically modified chitosan, was synthesized to enhance the adhesive properties of chitosan (CS). BIACORE results showed that both CS and DC could interact with mucin, although they show the differences in binding kinetics. The zeta potential of DC-coated liposomes (DC-Lip) showed positive values in both liposomal formulations, negatively charged and neutral-charge liposomes. CS seemed to be less effective in the coating of a neutral-charge liposome because of the low positive values. Confocal Laser Scanning Microscopy (CLSM) images indicated that both chitosan-coated liposomes (CS-Lip) and DC-Lip could adhere to and penetrate through the small intestine of rats after oral administration. The pharmacological results showed that DC-Lip had a greater effect in decreasing blood calcium concentration during the first 12 h compared with CS-Lip. Therefore, dodecylated chitosan can be useful in designing oral liposomal drug delivery systems.

[*J. Liposome Res.*, **16**, 127-141 (2006)] [Lab. of Pharm. Engineering]

Visualization of the penetrative and mucoadhesive properties of chitosan and chitosan-coated liposomes through the rat intestine.

Jringjai THONGBORISUTE, Hirofumi TAKEUCHI*, Hiromitsu YAMAMOTO and Yoshiaki KAWASHIMA

To observe the penetrative and mucoadhesive behavior of polymer-coated liposomes into the intestinal mucosa of rats, chitosan (CS) and negatively charged liposomes were chosen as model polymer-coated liposomes. CS was labeled with Fluorescence Isothiocyanate (FITC) via chemical reaction and the liposomes (Lips) were marked by incorporation of DiI into the liposomal formulation. FITC-labeled chitosan (FITC-CS), Non-Lips, and FITC-labeled CS-coated Liposomes (FITC-CS-Lips) were intragastrically administered into male Wistar rats, and the behavior of the molecules was subsequently visualized by CLSM (Confocal Laser Scanning Microscopy). The results demonstrated that the chitosan molecules themselves, as well as the liposomes, could penetrate across the intestinal mucosa. Moreover, no separation effect between chitosan molecules and liposomes during penetrate through intestinal after the administration of chitosan-coated liposomes.

[*J. Drug Target.*, **14**, 147-154 (2006)] [Lab. of Pharm. Engineering]

The effect of particle structure of chitosan-coated liposomes and type of chitosan on oral delivery of calcitonin.

Jiringjai THONGBORISUTE, Ayumi TSURUTA, Yohei KAWABATA and Hirofumi TAKEUCHI*

To optimize the properties of chitosan-coated liposomes for oral administration of peptide drugs, the effect of type of chitosan and the structure of liposomal systems on the mucoadhesiveness of liposomes and pharmacological effects of the liposomal peptide drug were examined. A low-molecular weight chitosan (LCS) and a high-molecular weight chitosan (CS) were used as coating polymers of liposomes containing elcatonin (eCT). The results showed that both LCS-coated liposomes (LCS-Lips) and CS-coated liposomes (CS-Lips) could permeate the mucous layer in the small intestine. LCS-Lips containing eCT showed remarkably more prolonged effectiveness in decreasing the blood calcium concentration than did CS-Lips containing eCT. LCS had more efficiency to protect eCT from the enzymatic degradation than CS. In addition, eCT adsorbed on LCS-Lips (eCT-ad-LCS-Lips) and eCT encapsulated in LCS-Lips (eCT-encap-LCS-Lips) showed much higher effectiveness than in case of using CS. Thus, LCS is a good mucoadhesive polymer candidate for enhancing the bioavailability of orally administered peptide containing liposomes.

[*J. Soc. Powder Technol. Jpn.*, **43**, 640-647 (2006)] [Lab. of Pharm. Engineering]

Improvement in Dissolution Property of Poorly Water-solube Drugs by Using Mechanofusion System.

Yoshiro NAGAI, Hiromitsu YAMAMOTO and Hirofumi TAKEUCHI*

The dissolution property of poorly water-soluble drug, indomethacin , was improved by using a ball mill or a mechanofusion device. Co-grinded samples with indomethacin loading higher than 80% were prepared by mixing it with several additives. The highest dissolution rate of indomethacin was attained for the drug dispersion prepared with magnesium aluminomethasilicate (Neusilin) and additives such as PEG6000, sorbitol, mannitol.

[*J. Soc. Powder Technol. Jpn.*, **43**, 648-652 (2006)] [Lab. of Pharm. Engineering]

Direct Compaction of Poorly Compactable Pharmaceutical Powders with Spray-dried HPC-L.

Shinji TANIMURA, Hiroyoshi KAWAZOE, Hiromitsu YAMAMOTO and Hirofumi TAKEUCHI*

The binding effect of hydroxypropylcellulose (HPC-L) in direct compaction of pharmaceutical powders was evaluated. Several types of HPC-L particles having different properties were tested in direct compaction of poorly compactable powders, erythritol and ascorbic acid. When the particle size of HPC-L in the tablet formulation was decreased, the hardness of resultant tablet increased. In comparing the spray-dried HPC-L and the freeze-ground one, the spray-dried HPC-L showed higher tablet hardness although their particle sizes were comparable. The different binding effects of these powders were attributed to the more spherical shape and smoother surface of spray-dried particle. The hardness of tablet containing 2% of the fine spray-dried particles was significantly higher than that containing 10% of commercial HPC-L. Thus, the fine HPC-L particles prepared with spray-drying is one of the most suitable binders in direct compression.

[*J. Incl. Phenom. Macrocyc. Chem.*, **54**, 9-16 (2006)] [Lab. of Pharm. Engineering]

Equimolar complex Formation of Urea or Thiurea with 2-Alkoxy-Benzamides: Structual Factors Required for the Equimolar Complex Formation.

Kunikazu MORIBE, Masami TSUCHIYA, Yuichi TOZUKA*, Kentarou YAMAGUCHI, Toshio OGUCHI and Keiji YAMAMOTO

Equimolar complex formation of either urea or thiourea with 2-ethoxy-benzamide (2-EB) and 2-methoxybenzamide (2-MB) was investigated. Complex formation of urea and 2-MB was observed both by co-grinding method and by coprecipitation method. The crystal structure of the urea-2-MB complex, especially hydrogen bond networks, was quite different from that of thiourea-2-MB complex. Reduction of intramolecular hydrogen bond length of 2-MB and the conformational change to the flatter structure affected the equimolar complex formation.

[*Chem. Pharm. Bull.*, **54**, 1097-1101 (2006)] [Lab. of Pharm. Engineering]

Specific Inclusion Mode of Guest Compounds in the Amylose Complex Analyzed by Solid State NMR Spectroscopy.

Yuichi TOZUKA*, Aya TAKESHITA, Ayako NAGAE, Kunikazu MORIBE, Toshio OGUCHI and Keiji YAMAMOTO

The inclusion compound formation between linear amylose of molecular weight 102,500 (AS100) and *p*aminobenzoic acid (PA) during the sealed-heating process was investigated by powder X-ray diffractometry, infrared spectroscopy and solid state NMR spectroscopy. Solid state NMR spectroscopy suggested that PA molecules were included in the amylose helix core in the 7_1-helix inclusion compound, while in the case of 6_1-helix inclusion compound, PA molecules were accommodated in the interstices between amylose helices.

[*Die Pharmazie*, **61**, 97-101 (2006)] [Lab. of Pharm. Engineering]

Drug Nanoparticle Formation from Drug/HPMC/SDS Ternary Ground Mixtures.

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

Drug nanoparticle formation from a ternary ground mixture consisting of a poorly water-soluble drug, hydroxypropylmethylcellulose (HPMC), and sodium dodecyl sulfate (SDS) was investigated. The drug/HPMC/SDS ternary grinding method was applicable not only for flurbiprofen but also for other hydrophobic drugs, such as tolbutamide, probucol, phenytoin and griseofulvin. The drug nanoparticles were also obtained by using other cellulose derivatives, indicating that these pharmaceutical excipients were alternative to PVP for the grinding-induced drug nanoparticle formation.

[*J. Incl. Phenom. Macrocyc. Chem.*, **56**, 29-32 (2006)] [Lab. of Pharm. Engineering]

Investigation of Drug Nanoparticle Formation by Co-grinding with Cyclodextrins: Studies for Indomethacin, Furosemide and Naproxen.

ArpansireeWONGMEKIAT, Satoko YOSHIMATSU, Yuichi TOZUKA*, Kunikazu MORIBE and Keiji YAMAMOTO

Drugs with poor water solubility were co-ground with cyclodextrins (CDs) to create nanoparticles with improved solubility characteristics. Indomethacin, furosemide and naproxen (NAP) were coground with CDs at the molar ratio of 2:1 (CD:drug). Co-grinding of a drug with CD resulted in not only the formation of drug nanoparticles but also the solubilization of the drug by inclusion complex formation with CD in aqueous media.

[*Drug Dev. Ind. Pharm.*, **32**, 877-891 (2006)] [Lab. of Pharm. Engineering]

Micronization and Polymorphic Conversion of Tolbutamide and Barbital by Rapid Expansion of Supercritical Solutions.

Hiroshi SHINOZAKI, Toshio OGUCHI, Shoko MORISHITA, Yuichi TOZUKA*, Kunikazu MORIBE and Keiji YAMAMOTO

Rapid expansion of supercritical solutions (RESS) was applied to tolbutamide and barbital. The solubility in supercritical CO2 was determined to estimate the extraction efficiency by a direct spectrophotometric technique. Significant size reduction to micron or sub-micron level with narrow size distribution was achieved by RESS method. As for tolbutamide, three polymorphs (Forms I, II, and IV) could be produced by changing the extraction conditions, while in the case of barbital, one polymorph (Form II) was produced consistently.

[*J. Incl. Phenom. Macrocyc. Chem.*, **56**, 33-37 (2006)] [Lab. of Pharm. Engineering]

Ibuprofen-Cyclodextrin Inclusion Complex Formation using Supercritical Carbon Dioxide.

Yuichi TOZUKA*, Takayuki FUJITO, Kunikazu MORIBE and Keiji YAMAMOTO

Supercritical carbon dioxide (SC-CO_2) processing was performed with mixtures of cyclodextrins (CDs) and ibuprofen (IBP) to create inclusion complexes of ibuprofen and CD. The inclusion complex between IBP and trimethyl-β-CD was successfully prepared using SC-CO_2 technique. No inclusion formation was found when nitrogen was used as the supercritical fluid. Complexation of IBP and CD would not occur only on a high-pressure condition. The solubility of cyclodextrin into SC-CO_2 might play an important role in the formation of the inclusion complex.

[*Pharm. Res.*, **23**, 2566-2574 (2006)] [Lab. of Pharm. Engineering]

Molecular Interaction among Probucol/PVP/SDS Multicomponent System Investigated by Solid-State NMR.

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

Effects of polyvinylpyrrolidone (PVP) molecular weight on the solid-state intermolecular interactions among probucol/PVP/sodium dodecyl sulfate (SDS) ternary ground mixtures and the formation of nanoparticles were investigated by solid-state NMR spectroscopy. Grinding-induced solid-state interactions among drug, PVP and SDS could be detected using solid state ^{13}C NMR. The interactions in both probucol-PVP and PVP-SDS should occur simultaneously to generate nanometer-sized particles of probucol.

[*Anal. Chem.*, **78**, 8142-8149 (2006)] [Lab. of Pharm. Anal. Chem.]

Surfactant Gradient Methods Using Mixed Systems of Cethyltrimethylammonium Chloride and Nonionic Surfactants Possessing Polyethylene Chains for Electrokinetic Separation of Benzoate Anions as Model Analytes.

Yukihiro ESAKA,* Mika SAWAMURA, Hiroya MURAKAMI and Bunji UNO

Surfactant gradient methods for electrokinetic separation of ten benzoates as model organic anions were investigated using mixed micellar solutions of cetyltrimethylammonium chloride (CTAC) and non-ionic surfactants possessing polyoxyethylene chains, polyoxyethylene sorbitan monolaurate (Tween 20) or polyoxyethylene lauryl ether (Brij 35). In a pure CTAC system, the synergistic influences of attractive electrostatic and hydrophobic interactions gave rise to quite large retention factors of many of the benzoate anions, resulting in their co-elution. Addition of an adequate amount of Tween 20 to the pure CTAC system decreased the electrostatic interaction significantly to give remarkably improved separation of the analytes, but long analysis time was required. All the benzoates were separated completely within reasonable analysis times using both stepwise and continuous gradient programs for the concentrations of Tween 20 or Brij 35 in the presence of 100 mM CTAC.

[*Cancer Lett.*, **237**, 223-233 (2006)] [Lab. of Pharmaceutics]

Incadronate Inhibits Aminopeptidase N Expression in Prostate PC-3 Cells.

Kazuhiro IGUCHI, Toru NAKANO, Shigeyuki USUI, and Kazuyuki HIRANO*

Bisphosphonates are widely used for the treatment and prevention of osteoporosis, but recently have been observed to be effective in controlling prostate cancer metastasis. Incadronate induced inhibition of AP-N mRNA and protein expression in PC-3 cells. The decrease of AP-N mRNA expression induced by incadronate was inhibited by co-incubation with geranylgeranyl diphosphate. Moreover, GGTI-286 treatment also resulted in reduced AP-N mRNA expression. The translocation of small G protein Rap1 from the cytosol to the membrane was inhibited by incadronate and prqvastatin. These above results indicate that the decrease in AP-N expression elicited by bisphosphonate is related to the inhibition of the mevalonate pathway.

[*Anticancer Res.*, **26**, 2977-2982 (2006)] [Lab. of Pharmaceutics]

Inhibition of Caveolin-1 Expression by Incadronate in PC-3 Prostate Cells.

Kazuhiro IGUCHI, Shinji MATSUNAGA, Toru NAKANO, Shigeyuki USUI, and Kazuyuki HIRANO*

Caveolin-1 is essential component of caveolae and its expression is known to increase in human prostate cancer. The reduction caveolin-1 expression has been reported to decrease the tumorigenic and metastatic potential of prostate cancer. Incadronate was found to inhibit the caveolin-1 mRNA and protein expression in PC-3 prostate cells. This decrease was prevented by co-incubation with geranylgeranyl diphosphate, but not with farnesol. Moreover, treatment of GGTI-286, but not FTI-277, also resulted in the inhibition of caveolin-1 mRNA expression. These results indicate that the decrease in caveolin-1 expression elicited by incadronate is related to the inhibition of protein geranylgeranylation.

[*Biol. Pharm. Bull.*, **29**, 991-996 (2006)] [Lab. of Pharmaceutics]

Regulators for Blood Glucose Level Affect Gene Expression of Aquporin 3.

Mariko ASAI, Shouta HIGUCHI, Masafumi KUBOTA, Kazuhiro IGUCHI, Shigeyuki USUI, and Kazuyuki HIRANO*

Aquaporin 3 (AQP3), a membrane protein, is known to permeabilize water and other small molecules such as glycerol and urea and is localized in the bowel, skin, kidney, and erythrocytes. We first investigated whether insulin regulating the glycolytic pathway took part in glycerol transport through AQP3 in the gastrointestinal tract and found that insulin significantly suppressed mRNA and protein expressions of AQP3 in Caco-2 cells. The antidiabetic drugs troglitazone and tolbutamide were also observed to suppress significantly AQP3 expression, but the biguanides metformin and buformin did not induce such suppression. Epinephrine was found to increase expression of AQP3, although glucagon showed no change of expression. Wortmannin and rapamycin were demonstrated to deactivate suppression of AQP3 expression by insulin and troglitazone, suggesting that the signal transducers, phosphoinositide 3 kinase (PI3K) and the mammalian target of rapamycin (mTOR), are involved in the signal pathway for regulating transcription of AQP3.

[*Biol. Trace Elem. Res.*, **112**, 159-167 (2006)] [Lab. of Pharmaceutics]

Correlation between ZIP2 Messenger RNA Expression and Zinc Level in Rat Lateral Prostate.

Kazuhiro IGUCHI, Takashi OTSUKA, Shigeyuki USUI, Yoshiki SUGIYAMA and Kazuyuki HIRANO*

Zinc content in rat lateral prostate (LP) is higher compared with the other tissues, but the zinc retention system in the prostate remains unclear. In the present study, we examined the expression of ZRT, a IRT-like protein (ZIP) family transporter in rat prostate. The zinc level in rat LP was higher compared with the ventral (VP) and dorsal prostate (DP). The predicted Zip2 mRNA was really expressed in LP at a high level. The expression was decreased in LP from castrated rats, associated with a decrease in zinc level, and these changes were prevented by testosterone replacement. Moreover, ZIP2 expression levels in LP positively correlated with the zinc levels. These findings strongly suggest that ZIP2 is involved in zinc homeostasis of rat prostate.

[*Biol. Pharm. Bull.*, **29**, 1006-1009 (2006)] [Lab. of Pharmaceutics]

Buformin Suppresses the Expression of Glyceraldehyde 3-Phosphate Dehydrogenase.

Akiko YANO. Masafumi KUBOTA, Kazuhiro IGUCHI, Shigeyuki USUI and Kazuyuki HIRANO*

We have examined genes changing in its expression by the treatment of buformin to HepG2 cells in order to obtain a clue elucidating the mechanisms of the side effect. A subtraction cDNA library was constructed by the method of suppressive subtractive hybridization and the screening of the library was performed with the cDNA probes prepared from HepG2 cells treated with or without buformin for 12 h. We found that the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPD) gene was suppressed by treating HepG2 cells with 0.25 mM buformin for 12 h as a result of the library screening. The amount of GAPD protein also decreased simultaneously with the suppression of the gene expression by the treatment with buformin. The amount of ATP and NAD^+ in the HepG2 cells treated with buformin decreased to 10 and 20% of the control, respectively. These observations imply that the biguanide causes the deactivation of the glycolytic pathway and subsequently the accumulation of pyruvate and NADH, and the decrease of NAD^+. Therefore, the reaction equilibrium catalyzed by lactate dehydrogenase lean toward the lactate production and this may result in lactic acidosis.

[*Int. J. Oncol.*, **29**, 1469-1478 (2006)] [Lab. of Pharmaceutics]

The Up-Regulation of Type I Interferon Receptor Gene Plays a Key Role in Hepatocellular Carcinoma Cells in the Synergistic Antiproliferative Effect by 5-Fluorouracil and Intrferon-α.

Shinji OIE, Mayumi ONO, Yuichiro MARUYAMA, Tadafumi TERADA, Yuji YAMADA, Takato UENO, Masamichi KOJIRO, Kazuyuki HIRANO* and Michihiko KUWANO

We examined the molecular events underlaying the antiproliferative effects of IFN-a and 5-FU in combination using six human cell lines. When the antiproliferative effects of administering INF-a and 5-FU together were analyzed using isobolograms, we found that the cell lines could be divided into two groups: the S-group containing three cell lines, which showed synergestic effects, and the A-group, containing the remaining three, which showed additive effects. The 5-FU induced modulation of IFN receptor expression could playa pivotal role in the therapeutic efficacy of IFN receptors, and their ability to be up-regulated, may be a promising method for selecting HCC patients for this type of combination therapy.

[*Chem. Pharm. Bull.*, **54**, 1196-1199 (2006)] [Lab. of Pharmaceutics]

Apparent Increase of Insulin Peak Area in HPLC Analysis of a Preparation Consisting of a Mixture of Insulin and Total Parental Nutrition.

Etsuko ICHIKAWA, Michiko KIMURA, Hiromi MORI, Futoshi YAMAZAKI and Kazuyuki HIRANO*

The peak area of insulin in a mixture of K.C.L. injection and hyperalimentation fluid was found to increase in a time dependent manner up to 24h in measurement by a HPLC. The increase of peak area corresponding to the insulin was detected at wavelength of both 210 and 280 nm. This increase was only observed in the presence of the sugars, tryptophan, riboflavin, and insulin, and ascorbate was shown to counteract the increase. These results suggest the possibility that insulin forms a mixture caused by the oxidation reaction in a hyperalimentation fluid.

[*Mutat. Res.*, **609**, 68-73 (2006)] [Lab. of Hygienics]

In vitro Anti-mutagenic Effect of Magnolol against Direct and Indirect Mutagens.

Junichiro SAITO, Yoshimichi SAKAI and Hisamitsu NAGASE*

In this study, anti-mutagenic activity of magnolol against mutagenicity induced by direct mutagens [MNNG, ENNG] and indirect mutagens [IQ,Glu-P-2, B(a)P, 2-AA and DMBA] were investigated using Ames test. Results show that magnolol strongly inhibits mutagenicity induced by indirect mutagens, but does not affect direct mutagens. To elucidate the mechanism of this effect against indirect mutagens, effect of magnolol on CYP1A1- and CYP1A2-related enzyme activities of ethoxyresorufin-O-deethylase (EROD) and methoxyresorufin-*O*-demethylase (MROD) were investigated. Magnolol strongly and competitively suppressed these enzyme activities, suggesting it inhibited mutation induced by indirect mutagens through suppression of CYP1A1 and CYP1A2 activity.

[*Anal. Chim. Acta*, **555**, 225-232. (2006)] [Lab. of Hygienics]

The High Throughput Analysis of *N*-Methyl Carbamate Pesticides in Fruits and Vegetables by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry Using a Short Column.

Tomomi GOTO, Yuko ITO, Sadaji YAMADA, Hiroshi MATSUMOTO, Hisao OKA and Hisamitsu NAGASE*

The authors developed a new anal. method for the 9 N-Me carbamate pesticides in fruits and vegetables using ESI LC/MS/MS with direct sample injection into a short column. After extn. of the pesticides with Et acetate from sample, the ext. is evapd. to dryness and redissolved in ultra pure water before injection into LC/MS/MS. The method needs no cleanup steps. The av. recoveries from fruits and vegetables fortified at the level of 0.01 μg/g ranged from 56.0 to 119.1% with the coeffs. of variation ranging from 0.2 to 7.6% for intra-day (n = 5 × 3 days) and from 0.8 to 18.4% for inter-day (n = 15). The method is considered to be satisfactory for the monitoring of the carbamate pesticide residues in fruits and vegetables, suggesting that the present method is applicable to other pesticide residues in foods.

[*J. Liq. Chromatogr. Rel. Technol.*, **29**, 2651-2661(2006)] [Lab. of Hygienics]

High Throughput Analysis of *N*-Methyl Carbamate Pesticides in Cereals and Beans by Dual Countercurrent Chromatography and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry.

Tomomi GOTO, Yuko ITO, Sadaji YAMADA, Hiroshi MATSUMOTO, Hisao OKA, Hisamitsu NAGASE*and Yoichiro Ito

We developed a new anal. method for anal. of N-Me carbamate pesticides in cereals and beans using dual counter-current chromatog. (dual CCC) and liq. chromatog.-electrospray ionization tandem mass spectrometry (ESI LC/MS/MS). After pesticides were extd. from cereals and beans with Et acetate, each ext. was cleaned up by dual CCC using a non-aq. binary solvent system composed of n-hexane-acetonitrile and analyzed by ESI LC/MS/MS with a short column. The av. recoveries from cereals and beans fortified at the level of 0.01 ppm ranged from 73.9 to 119.6%, with the coeffs. of variation from 0.7 to 6.8%. At the fortified level of 0.5 ppm, the recoveries ranged from 72.1 to 117.1% with coeffs. of variation from 0.4 to 9.3%. The present anal. method of N-Me carbamate pesticides in cereals and beans is considered to be useful for monitoring the pesticide residues in cereals and beans.

[*J. Chromatogr. A*, **1108**, 20-25 (2006)] [Lab. of Hygienics]

Application of Dual Counter-current Chromatography for Rapid Sample Preparation of N-methylcarbamate Pesticides in Vegitable Oil and Citrus Fruit.

Yuko ITO, Tomomi GOTO, Sadaji YAMADA, Hiroshi MATSUMOTO, Hisao OKA, Hisamitsu NAGASE*, Nobuyuki TAKAHASHI, Hiroyuki NAKAZAWA and Yoichiro ITO

Dual counter-current chromatog. (dual CCC) was successfully applied to rapid sample prepn. for the simultaneous detn. of residual carbaryl, fenobucarb and methomyl in vegetable oil and citrus fruit. The citrus fruit samples were extd. with n-hexane soln. contg. stable isotopically labeled internal stds. (methomyl-d3, fenobucarb-d3 and carbaryl-d9), and applied to dual CCC using a 2 -phase solvent system of n-hexane-acetonitrile to purify the carbamate pesticides from aliph. sample matrix. Due to the high partition efficiency of dual CCC, the lower phase fraction collected from 2 to 5 min after injection could be subjected to flow-injection tandem mass spectrometry directly after concn. Repetitive sample injection can be performed at high reproducibility without a risk of contamination from the compds. retained in the column.

[*Leukemia lymphoma*, **47**, 2234-2243 (2006)] [Lab. of Hygienics]

AMF/G6PI Induces Differentiation of Leukemia Cells via an Unknown Receptor that Differs from gp78.

Arayo HAGA*, Sachiko KOMAZAKI, Tatsuyoshi FUNASAKA, Kazunori HASHIMOTO, Yuichi YOKOYAMA, Hideomi WATANABE, Avraham RAZ and Hisamitsu NAGASE

Autocrine Motility Factor (AMF)/maturation factor (MF)/neuroleukin (NLK) is a multifunctional protein, which acts as a glucose 6-phosphate isomerase (G6PI) intracellularly. Exto-G6PI stimulates invasion and metastasis of tumor cells, neurotropic growth and differentiation of leukemic cells. The cell motility and proliferation receptor is known to be gp78 (78 kilo-Dalton glycoprotein), which has seven transmembrane domains in its N-terminal region, but the maturation factor receptor remains unclear. The human acute monocytic leukemia line does not express gp78 and its motile activity is not enhanced by AMF though it is well differentiated by AMF exposure. The forced expression of gp78 in leukemic cells recovered acceptable motile stimulation, concomitant with reduced differentiation ability.

[*J. Mol. Biol.*, **356**, 312-324 (2006)] [Lab. of Hygienics]

Crystal Structures of Mouse Autocrine Motility Factor in Complex with Carbohydrate Phosphate Inhibitors Provide Insight into Structure-activity Relationship of the Inhibitors.

Nobutaka TANAKA, Arayo HAGA*, Noriko NABA, Katsura SHIRAKAWA, Yosio KUSAKABE, Kazunori HASHIMOTO, Tatsuyoshi FUNASAKA, Hisamitsu NAGASE, Avraham RAZ and Kazuo T. NAKAMURA.

We report the first comprehensive high-resoln. crystal structure analyses of the inhibitor-free form and the eight types of inhibitor (phosphate, erythrose 4-phosphate (E4P), arabinose 5-phosphate (A5P), sorbitol 6-phosphate (S6P), 6-phosphogluconic acid (6PGA), fructose 6-phosphate (F6P), glucose 6-phosphate (G6P), or mannose 6-phosphate (M6P)) complexes of mouse AMF (mAMF). We assayed the inhibitory activities of these inhibitors against the cytokine activity of mAMF. The inhibitory activities of the six-carbon sugars (G6P, F6P, M6P, and 6PGA) were found to be significantly higher than those of the four or five-carbon sugars (E4P or A5P). The present structure-activity relationship studies will be valuable not only for designing more effective AMF inhibitors but also for studying general protein-inhibitor interactions.

[*Amyotroph. Lateral Sc.*, **7**, 22-26 (2006)] [Lab.of Hygienics]

Association between Metallothionein Genes Polymorphisms and Sporadic Amyotrophic Lateral Sclerosis in a Japanese Population.

Yuichi HAYASHI, Tatsuma HASHIZUME, Kenji WAKIDA, Masahiko SATOH*, Yoko UCHIDA, Kazuhiko WATABE, Zenjiro MATSUYAMA, Akio KIMURA, Takashi INUZUKA and Isao HOZUMI.

Amyotrophic lateral sclerosis (ALS) is a progressive, lethal neurodegenerative disease that selectively affects motor neurons. Metallothioneins (MTs) are self-protective, multifunctional proteins that scavenge ROS. In particular, metallothionein-III (MT-III) has a strong scavenging effect on hydroxyl radicals. MTs have been suggested to have important roles in the pathophysiology of ALS. Therefore we investigated single nucleotide polymorphisms (SNPs) of the MT-III and the metallothionein-IIA (MT-IIA) promoter region in 37 Japanese SALS cases and 206 sex-matched healthy controls. We detected no SNPs of the MT-III gene in SALS cases and controls, and no detectable association between SALS phenotypes and a SNP of the MT-IIA promoter region.

[*Drug Chem. Toxicol.*, **29**, 379-396 (2006)] [Lab. of Hygienics]

Effect of Co-treatment of Aspirin Metabolites on Mitomycin C-induced Genotoxicity Using the Somatic Mutation and Recombination Test in *Drosophila meanogaster*.

Miki NIIKAWA, Takeshi NAKAMURA and Hisamitsu NAGASE *

To reveal the mechanism of the anti-genotoxicity of aspirin, we evaluated the suppressing ability of each aspirin metabolite, such as salicylic acid (SA), salicyluric acid (SUA), gentisic acid (GA), gentisuric acid (GUA), and 2,3-dihydroxybenzoic acid (DHBA), in SMART in Drosophila melanogaster using the cotreatment protocol in this report. SUA, GA, GUA, and DHBA reduced the no. of the three types of spot induced by MMC without decrease of survival. These aspirin metabolites decreased the genotoxicity frequency of MMC for total spots in a dose-dependent manner. It is suggested that these metabolites are the main substances of anti-genotoxicity in the aspirin metabolic pathway.

[*J. Health Sci.*, **52**, 292-299 (2006)] [Lab.of Hygienics]

Characterization of Gene Expression Profiles of Metallothionein-null Cadmium-Resistant Cells.

Hitomi FUJISHIRO, Satomi OKUGAKI, Sachi NAGAO, Masahiko SATOH* and Seiichiro HIMENO.

To understand metallothionein (MT)-independent mechanisms for Cd resistance in mammalian cells, we previously established MT-null Cd-resistant cells from embryonic fibroblast cells of MT knockout mice. To identify genes responsible for Cd resistance as well as Cd transport, we carried out several DNA microarray analyses using cDNAs obtained from two clones of Cd-resistant cells and parental cells. We found that the expression of 78 genes was enhanced and that of 48 genes was reduced in Cd-resistant cells compared with those in parental cells. Several genes for transporters including solute carrier family transporters and ATP-binding cassette transporters were up- or down-regulated. The examination of mRNA levels using quant. real-time PCR revealed that the expression of Slc39a14 encoding ZIP14, a member of the zinc transporter ZIP family, was markedly down-regulated in both clones of Cd-resistant cells.

[*FASEB J.*, **20**, 533-535 (2006)] [Lab.of Hygienics]

Role of Metallothionein in Coagulatory Disturbance and Systemic Inflammation Induced by Lipopolysaccharide in Mice.

Ken-Ichiro INOUE, Hirohisa TAKANO, Akinori SHIMADA, Emiko WADA, Rie YANAGISAWA, Miho SAKURAI, Masahiko SATOH* and Toshikazu YOSHIKAWA.

We detected whether metallothionein (MT) protects against coagulatory and fibrinolytic disturbance and systemic inflammation induced by i.p. administration of lipopolysaccharide (LPS) in MT null (-/-) and wild-type (WT) mice. As compared with WT mice, MT (-/-) mice revealed prolongation of prothrombin and activated partial thromboplastin time, an increase in the levels of fibrinogen and fibrinogen/fibrin degradation products, and a decrease in activated protein C, after LPS treatment. LPS induced inflammatory organ damage in the lung, kidney, and liver in both genotypes of mice. The damage, including neutrophil infiltration, was more prominent in MT (-/-) mice than in WT mice after LPS treatment.

[*Biol. Pharm. Bull.*, **29**, 1466-1469 (2006)] [Lab.of Hygienics]

Region-dependent Differences and Alterations of Protective Thiol Compound Levels in Cultured Astrocytes and Brain Tissues.

Tatsumi ADACHI, Masahiko SATOH*, Rocky PRAMANIK, Sakiko KURODA, Masami ISHIDO and Manabu KUNIMOTO.

We examined region-dependent differences and alterations in the levels of protective thiol compounds, glutathione (GSH) and metallothionein (MT), in cultured rat astrocytes under several culture conditions and in brain tissues of rats at postnatal and weaning periods. Regardless of culture conditions, both protein concentrations and mRNA expressions of MT were much higher in the cerebral hemisphere than in cerebellar astrocytes, whereas no difference was observed in GSH concentrations In both astrocytes, the GSH concentrations did not change within 12 h but significantly increased 24 h after being maintained in a serum-free defined medium. At 24 h, protein concentrations and mRNA expressions of MT also increased in the respective astrocytes. In the brain tissues, the MT protein concentrations were significantly higher in the cerebral cortex than in the cerebellum, whereas the GSH concentrations was similar at both postnatal day (P)1 and P35.

[*Toxicol. Lett.*, **161**, 210-218 (2006)] [Lab.of Hygienics]

Behavioral Changes in Metallothionein-null Mice after the Cessation of Long-term, Low-level Exposure to Mercury Vapor.

Minoru YOSHIDA, Chiho WATANABE, Mami KISHIMOTO, Akira YASUTAKE, Masahiko SATOH*, Masumi SAWADA and Yoshifumi AKAMA.

Metallothionein (MT)-null and wild-type females were continuously (24 h/day) exposed to vapor Hg0 at 0.055 mg/m^3 (range: 0.043-0.073 mg/m^3), which was similar to the current threshold limit value (TLV), for 29 wk. Immediately after the exposure had ceased, total locomotor activity in the open field (OPF) was decreased in the both strain of mice, although the MT-null mice appeared to show more distinct effect. In the passive avoidance response (PA) test, the exposed animals of both strains showed learning impairment as compared to un-exposed mice. Twelve weeks after the cessation of exposure, the locomotor activity in OPF was elevated in the exposed mice of both strains, while the learning ability in the PA test appeared normal in both strains. Spatial learning ability was not affected at all.

[*J. Mol. Biol.*, **358**, 741-753 (2006)] [Lab. of Hygienics]

The Autocrine Motility Factor (AMF) and AMF-receptor Combination Needs Sugar Chain Recognition Ability and Interaction Using the C-terminal Region of AMF.

Arayo HAGA*, Nobutaka TANAKA, Tatsuyoshi FUNASAKA, Kazunori HASHIMOTO, Kazuo T. NAKAMURA, Hideomi WATANABE, Avraham RAZ and. Hisamitsu NAGASE

Site-directed mutagenesis was used to investigate 18 recombinant human autocrine motility factor (hAMF) point mutants involving crit. amino acid residues for substrate or enzyme inhibitor recognition or binding. Mutation of residues that interact with the phosphate group of the PHI substrate significantly reduced the cell motility-stimulating activity. Their binding capacities for AMFR were also lower than wild-type hAMF. Mutants that retained the enzymic activity showed the motility-stimulating effect and receptor binding and had sensitivity to a PHI inhibitor. Mutant AMFR lacking the N-sugar chain was expressed on the cell membrane but did not respond to AMF-stimulation, and N-glycosidase-treated AMFR did not compete with receptor binding of AMF. Furthermore, the AMF domains that contain the substrate storage domain and C-terminal region stimulate cell locomotion.

[*Shoyakugaku Zasshi*, **60**, 39-50 (2006)] [Lab. of Herbal Garden]

Comparative Study on Testing Methods and Specification Values for Crude Drugs Used in Monographs Among For Western Pacific Regional Countries

Nobuo KAWAHARA,* Eiji SAKAI, Nanae ITOKAZU, Motoyoshi SATAKE and Yukihiro GODA

The Sub-committee I Meeting of the Western Pacific Regional Forum for the Harmonization of Herbal Medicine (FHH) nomenclature and standardization was held at the National Institute of Health Sciences, Tokyo, Japan. In the meeting, all the participants recognized the importance of comparing the descriptions for herbal medicines contained in member countries' pharmacopoeias or monograph standards as the first step in the harmonization of nomenclature and standardization and agreed to set up five expert working groups (EWG) to carry out the following specific tasks. The task of EWG2 is to list the testing methods in monographs. In this paper, we report on the preparation of a comparative table of testing methods and specification values for crude drugs used in monographs and obtain some knowledge from this comparative table.

[*Shoyakugaku Zasshi*, **60**, 73-85 (2006)] [Lab. of Herbal Garden]

Comparative Study on Testing Methods and Specification Values for Crude Drugs Used in Monographs Among For Western Pacific Regional Countries (Japan, China, Korea and Vietnam) (2) Comparative Study on TLC and Assay Conditions

Nobuo KAWAHARA,* Eiji SAKAI, Nanae ITOKAZU, Motoyoshi SATAKE and Yukihiro GODA

Five expert working groups (EWG1-5) were established in the Sub-committee I Meeting of the Western Pacific Regional Forum for the Harmonization of Herbal Medicine (FHH) nomenclature and standardization. The ask of EWG2 (Testing Methods used in Monographs) is to list the testing methods in each monograph. In this paper, we report on the further preparation of comparative tables on TLC conditions for identification and chemical assay conditions for component quantification used in monographs and to obtain some knowledge from these comparative tables.

[*Int. J. Oncol.*, **28**, 1193-1199(2006)] [Lab. of Med. Ther. & Mol. Ther.]

Acyclic retinoid, a novel synthetic retinoid, induces growth inhibition, apoptosis, and changes in mRNA expression of cell cycle- and differentiation-related molecules in human colon carcinoma cells.

Masumi SUZUI*, Nao SUNAGAWA, Itaru CHIBA, Hisataka MORIWAKI, and Naoki YOSHIMI

The purpose of this study was to examine the inhibitory effects of ACR in human colon carcinoma cells and to characterize the molecular mechanism of action of this agent. ACR inhibited the growth of the HCT116 and SW480 human colon carcinoma cell lines. ACR also induced G1-phase cell cycle arrest and apoptosis in these cell lines. When the HCT116 cells were treated with 5-25 μM ACR, there was a marked decrease in the cellular levels of cyclin D1 mRNA and an approximate 2.5- to 3-fold increase in those of $p21^{CIP1}$ mRNA, and this induction occurred via a p53-independent mechanism. These novel results suggest that cyclin D1 and $p21^{CIP1}$ play critical roles in the molecular mechanisms of growth inhibition and differentiation induced by ACR. Collectively, these findings provide further evidence that ACR may be a potential agent for the chemoprevention and therapy of human colon cancer.

[*Clin. Cancer Res.*, **1**, 3478-3484 (2006)] [Lab. of Med. Ther. & Mol. Ther.]

Sulindac sulfide and exisulind inhibit expression of the estrogen and progesterone receptors in human breast cancer cells.

Jin T.E. LIM, Andrew K. JOE, Masumi SUZUI*, Masahito SHIMIZU, Muneyuki MASUDA and I. Bernard WEINSTEIN

The present study focuses on the effects of sulindac sulfide and exisulind on hormone signaling components in breast cancer cells. Sulindac sulfide and exisulind also caused a decrease in expression of the ER in estrogen-responsive MCF-7 breast cancer cells. Therefore, although sulindac sulfide and exisulind can cause activation of PKG, the inhibitory effects of these two compounds on ER and PR expression do not seem to be mediated by PKG. Our findings suggest that the growth inhibition by sulindac sulfide and exisulind in ER-positive and PR-positive human breast cancer cells may be mediated, in part, by inhibition of ER and PR signaling. Thus, these and related compounds may provide a novel approach to the prevention and treatment of human breast cancers, especially those that are ER positive.

[*Ryukyu Med. J.*, **25**, 41-46 (2006)] [Lab. of Med. Ther. & Mol. Ther.]

Pulmonary hypoplasia and chondrocyte $p21^{CIP1}$ expression in patients with lethal short-limbed dwarfism.

Masumi SUZUI*, Takuji TANAKA, Hideki MORI, Takashi NAKAYAMA, Miyuki AONAHATA and Naoki YOSHIMI

Lethal short-limbed dwarfism is a rare disorder associated with chondrodysplasia, micromelia, and severe pulmonary hypoplasia. Thus, most of affected infants are stillborn or die shortly because of respiratory insufficiency. Two autopsy cases of lethal short-limbed dwarfism are presented in this article. Both stillborn infants had markedly short extremities and small thorax. Pulmonary alveolar cells were immature and air apace was diminished. Chondrocytes of a long bone showed hypertrophy and irregular arrangement. Growth plate chondrocytes displayed a significant expression of the $p21^{CIP1}$ protein. This is the systematic examination of the autopsy cases that highlight pulmonary hypoplasia and chondrocyte $p21^{CIP1}$ expression in patients with lethal short-limbed dwarfism.

[*Carcinogenesis*, **27**, 619-630 (2006)] [Lab. of Med. Ther. & Mol. Ther.]

An animal model for the rapid induction of tongue neoplasms in human c-Ha-ras proto-oncogene transgenic rats by 4-nitroquinoline 1-oxide: its potential use for preclinical chemoprevention studies.

Rikako SUZUKI, Hiroyuki KOHNO, Masumi SUZUI*, Naoki YOSHIMI, Hiroyuki TUSDA, Keiji WAKABAYASHI and Takuji TANAKA

We tried to establish an animal model using the human c-Ha-ras proto-oncogene-carrying transgenic (Tg) rats and the carcinogen 4-nitroquinoline 1-oxide (4-NQO). 4-NQO was administered to Tg and non-Tg rats for 8 weeks in their drinking water, and then the occurrence of tongue carcinogenesis was compared during the experimental period of 22 weeks. These results may thus indicate that our 4-NQO-induced Tg rat tongue carcinogenesis model simulates many aspects of human oral carcinogenesis and it can be applied for an analysis of oral cancer development while also helping to identify potentially effective cancer chemopreventive agents against oral cancer.

[*Anticancer Res.*, **26**, 2829-2832 (2006)] [Lab. of Med. Ther. & Mol. Ther.]

Reduced expression level of *Mgmt* mRNA and *β-catenin* gene mutation in rat colon tumors.

Tatsuya KINJO, Masumi SUZUI*, Takamitsu MORIOKA, Morihiko INAMINE, Tatsuya KANESHIRO, Junya ARAKAKI, Itaru CHIBA, Nao SUNAGAWA, Tadashi NISHIMAKI and Naoki YOSHIMI

BACKGROUND: O6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein and protects DNA from the biological effects of alkylating carcinogens. The purpose of this study was to investigate the association between the mRNA expression level of the *Mgmt* gene and mutation of the *β-catenin* gene in rat colon tumors induced by azoxymethane (AOM) plus dextran sulfate sodium (DSS). MATERIALS AND METHODS: Eleven tumor samples from rat colon treated by AOM plus DSS were examined. RESULTS: Four out of five adeno-carcinoma samples bearing *β-catenin* gene mutation (5 out of 11, 45%) displayed a decrease in expression levels of *Mgmt* mRNA ($P<0.02$). CONCLUSION: These results suggest that the reduced expression of *Mgmt* mRNA and *β-catenin* gene mutation may contribute to the development of rat colon tumors.

[*J. Exp. Clin. Cancer Res.*, **25**, 89-95 (2006)] [Lab. of Med. Ther. & Mol. Ther.]

Distribution of preneoplastic lesions and tumors, and β-catenin gene mutations in colon carcinomas induced by 1,2-dimethylhydrazine plus dextran sulfate sodium.

Tatsuya KINJO, Masumi SUZUI*, Takamitsu MORIOKA, Viengvansay NABANDITH, Morihiko INAMINE, Tatsuya KANESHIRO, Junya ARAKAKI, Tadashi NISHIMAKI and Naoki YOSHIMI

Mucin-depleted foci (MDF) are considered as useful biomarkers in rat colon carcinogenesis. The purpose of the present study was to examine the mechanism(s) underlying rat colon carcinogenesis induced by 1,2-dimethylhydrazine (DMH) plus 1% dextran sulfate sodium (DSS). We found that MDF and tumors were induced in the rat colon after treatment with DMH plus DSS and that the number of MDF in each segment of the colon was significantly correlated with that of tumors (P=0.006). In addition, we found that the β-catenin protein was accumulated in cytoplasm and nuclei of MDF and the frequent β-catenin gene mutations in the colon tumors. These results suggest that MDF is closely related to rat colon carcinogenesis induced by DMH plus DSS.

[*Eur. J. Pharmacol.*, **28**, 46-53 (2006)] [Lab. of Med. Ther. & Mol. Ther.]

Anthraquinone derivative emodin inhibits tumor-associated angiogenesis through inhibition of extracellular signal-regulated kinase 1/2 phosphorylation.

Tatsuya KANESHIRO, Takamitsu MORIOKA, Morihiko INAMINE, Tatsuya KINJO, Junya ARAKAKI, Itaru CHIBA, Nao SUNAGAWA, Masumi SUZUI*, Naoki YOSHIMI

An anthraquinone derivative, emodin, suppresses tumor development both in vitro and in vivo. In this study, we examined the anti-angiogenic activity of emodin and its modifying effect on the phosphorylation of extracellular signal-regulated kinase (ERK) 1/2. In cell cultures, emodin inhibited endothelial cell proliferation, migration, and tube formation in a dose-dependent manner. In addition, the mouse dorsal air sac assay revealed the vivo anti-angiogenic potential of emodin. The phosphorylation of ERK 1/2 decreased after exposure to emodin in a dose-dependent manner. These observations suggest that emodin has the potential to inhibit several angiogenic processes and that these effects may be related to suppression of the phosphorylation of ERK 1/2.

[*Asian Pac. J. Cancer Prev.*, **7**, 467-471 (2006)] [Lab. of Med. Ther. & Mol. Ther.]

Antioxidative and modifying effects of a tropical plant *Azadirachta indica* (Neem) on azoxymethane-induced preneoplastic lesions in the rat colon.

Junya ARAKAKI, Masumi SUZUI*, Takamitsu MORIOKA, Tatsuya KINJO, Tatsuya KANESHIRO, Morihiko INAMINE, Nao SUNAGAWA, Tadashi NISHIMAKi and Naoki YOSHIMI

The purpose of the present study was to examine whether Neem leaf (*Azadirachta indica*) has short-term chemopreventive effects on endpoint preneoplastic lesions involved in rat colon carcinogenesis. Dietary feeding of the Neem extract significantly inhibited the induction of aberrant crypt foci (ACF) (P<0.0002), when compared to the AOM-treated group. Treatment of rats with the Neem extract also significantly decreased the proliferating cell nuclear antigen (PCNA) labeling indices (P<0.0006) of colon epithelium and ACF. Moreover, the Neem extract also showed antioxidative activity. The finding that dietary Neem has possible chemopreventive effects in the present short-term colon carcinogenesis bioassay suggests that longer-term exposure may cause suppression of tumor development.

[*BMC Genet.*, **7**: 19 (2006)] [Lab. of Med. Ther. & Mol. Ther.]

A set of highly informative rat simple sequence length polymorphism (SSLP) markers and genetically defined rat strains.

Tomoji MASHIMO , Birger VOIGT, Toshiko TSURUMI,Kuniko NAOI*, Satoshi NAKANISHI, Ken-ichi YAMASAKI, Takashi KURAMOTO and Tadao SERIKAWA

NBRP-Rat is focusing on collecting, preserving and distributing various rat strains. To evaluate their value as models of human diseases, we are characterizing them using 109 phenotypic parameters. Here, we report on a set of 357 SSLP markers and 122 rat strains, which were genotyped by the marker set. The average number of informative markers between all possible pairs of strains was 259, showing their high degree of polymorphism. From the genetic profile of these rat inbred strains, we constructed a rat family tree to clarify their genetic background. These highly informative SSLP markers as well as genetically and phenotypically defined rat strains are useful for designing experiments for QTL analysis and to choose strategies for developing new genetic resources.

[*J. Immunol.*, **176**, 52-60 (2006)] [Lab. of Pharmacology]

Integrin $\alpha_{IIb}\beta_3$ induces the adhesion and activation of mast cells through interaction with fibrinogen.

Toshihiko OKI, Jiro KITAURA, Koji ETO, Yang LU, Mari MAEDA-YAMAMOTO, Naoki INAGAKI*, Hiroichi NAGAI, Yoshinori YAMANISHI, Hideaki NAKAJIMA, Hidetoshi KUMAGAI and Toshio KITAMURA

Integrin α_{IIb}, a well-known marker of megakaryocyte-platelet lineage, has been recently recognized on hemopoietic progenitors. We now demonstrate that integrin $\alpha_{IIb}\beta_3$ is highly expressed on mouse and human mast cells including mouse bone marrow-derived mast cells, peritoneal mast cells, and human cord blood-derived mast cells, and that its binding to extracellular matrix proteins leads to enhancement of biological functions of mast cells in concert with various stimuli. With exposure to various stimuli, mast cells adhered to extracellular matrix proteins such as fibrinogen and von Willebrand factor in an integrin $\alpha_{IIb}\beta_3$-dependent manner. In addition, the binding of mast cells to fibrinogen enhanced proliferation, cytokine production, and migration and induced uptake of soluble fibrinogen in response to stem cell factor stimulation, implicating integrin $\alpha_{IIb}\beta_3$ in a variety of mast cell functions.

[*Proc. Natl. Acad. Sci. U. S. A.*, **103**, 153-158 (2006)] [Lab. of Pharmacology]

Regulation of Dendritic Cell Maturation and Function by Bruton's Tyrosine Kinase via IL-10 and Stat3.

Yuko KAWAKAMI, Naoki INAGAKI*, Shahram SALEK-ARDAKANI, Jiro KITAURA, Hiroyuki TANAKA, Koichi NAGAO, Yu KAWAKAMI, Wenbin XIAO, Hiroichi NAGAI, Michael CROFT and Toshiaki KAWAKAMI

Btk plays crucial roles in the differentiation and activation of B and myeloid cells. Despite drastic reductions of other Ig isotypes, paradoxically high IgE responses have been known in btk mutant mice. Here we show that btk (-/-) dendritic cells exhibit a more mature phenotype and a stronger in vitro and in vivo T cell-stimulatory ability than wild-type cells. Increased IgE responses were induced by adoptive transfer of btk (-/-) dendritic cells into mice. Btk (-/-) mice exhibited enhanced inflammation in Th2-driven asthma and Th1-driven contact sensitivity experiments. These negative regulatory functions of Btk in dendritic cells appear to be mediated mainly through autocrine secretion of IL-10 and subsequent activation of Stat3.

[*Allergol. Int.*, **55**, 67-76 (2006)] [Lab. of Pharmacology]

Production of Matrix Metalloproteinases in Human Cultured Mast Cells: Involvement of Protein Kinase C-Mitogen Activated Protein Kinase Kinase-Extracellular Signal-Regulated Kinase Pathway.

Masahiro KIMATA, Masayuki ISHIZAKI, Hiroyuki TANAKA, Hiroichi NAGAI and Naoki INAGAKI*

To confirm the role of mast cells as a source of MMPs, we investigated the production of MMP and its pathway in human cultured mast cells (HCMC). We detected the de novo synthesis of MMP-9 in HCMC after stimulation with PMA through protein kinase C-mitogen activated protein kinase kinase-ERK pathway. The MMP-9 production was suppressed by simultaneous treatment with A23187, whereas GM-CSF production was potentiated. We also detected the expression of mRNA for membrane-type 1 (MT1)-MMP, TIMP-1 and TIMP-2 after stimulation with PMA. Glucocorticoids and flavonoids inhibited MMP-9 production, and TIMPs and MMP inhibitors inhibited the gelatinolytic activity of mast cell-derived MMP-9. Furthermore, phenylmethylsulfonyl fluoride, a protease inhibitor, inhibited the conversion from proMMP-9 to active MMP-9.

[*J. Allergy Clin. Immunol.*, **118**, 98-104 (2006)] [Lab. of Pharmacology]

Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals.

Go TAKAYAMA, Kazuhiko ARIMA, Taisuke KANAJI, Shuji TODA, Hiroyuki TANAKA*, Shunsuke SHOJI, Andrew N McKENZIE, Hiroichi NAGAI, Takao HOTOKEBUCHI T and Kenji IZUHARA.

Subepithelial fibrosis is a cardinal feature of bronchial asthma. Extensive evidence supports the pivotal role of IL-4 and IL-13 in subepithelial fibrosis; however, the precise mechanism remains unclear. We explore the possibility that periostin is involved in subepithelial fibrosis in bronchial asthma. Both IL-4 and IL-13 induced secretion of periostin in lung fibroblasts independently of TGF-beta. Periostin colocalized with other extracellular matrix proteins involved in subepithelial fibrosis in both asthma patients and ovalbumin-sensitized and ovalbumin-inhaled wild-type mice, but not in either IL-4 or IL-13 knockout mice. Periostin had an ability to bind to fibronectin, tenascin-C, collagen V, and periostin itself.

[*Eur. J. Pharmacol.*, **546**, 189-196 (2006)] [Lab. of Pharmacology]

Inhibition of Scratching Behavior Associated with Allergic Dermatitis in Mice by Tacrolimus, but not by Dexamethasone.

Naoki INAGAKI*, Noriko SHIRAISHI, Katsuhiro IGETA, Tomokazu ITOH, Takao CHIKUMOTO, Masafumi NAGAO, John Fan KIM and Hiroichi NAGAI.

We established a mouse allergic dermatitis model involving frequent scratching behavior by repeated painting with 2,4-dinitrofluorobenzene (DNFB) acetone solution onto the mouse skin. Repeated DNFB painting caused typical dermatitis accompanied by elevated serum immunoglobulin E (IgE) and frequent scratching behavior. The scratching behavior was inhibited by dibucaine and naloxone. Dexamethasone inhibited the accumulation of lymphocytes and eosinophils, although tacrolimus did not. Tacrolimus significantly inhibited the scratching behavior that was associated with the inhibition of nerve fiber extension into the epidermis, whereas dexamethasone failed to have any effect.

[*Int. Arch. Allergy Immunol.*, **141**, 172-180 (2006)] [Lab. of Pharmacology]

Effects of Alpha Tocopherol and Probucol Supplements on Allergen-Induced Airway Inflammation and Hyperresponsiveness in a Mouse Model of Allergic Asthma.

Nami OKAMOTO, Takuji MURATA, Hiroshi TAMAI, Hiroyuki TANAKA* and Hiroichi NAGAI.

We investigated the role of antioxidants in airway hyperresponsiveness (AHR) to acetylcholine (Ach) using young asthma model mice. Twenty-four hours after the last antigen challenge, both IL-4 and IL-5 in the bronchoalveolar lavage fluid of α-tocopherol-supplemented mice were significantly decreased. Differential cell rates of the fluid revealed a significant decrease in eosinophils due to antioxidant supplementation. AHR to Ach was also repressed in antioxidant-supplemented mice. In histological sections of lung tissue, inflammatory cells and mucus secretion were markedly reduced in antioxidant-supplemented mice. We investigated the antioxidant effect on our model mice by examining 8-isoprostane in BALF and lung tissue, and acrolein in BALF; however, our experiment gave us no evidence of the antioxidant properties of either α-tocopherol or probucol contributing to the reduction of airway inflammation.

[*Allergol. Int.*, **55**, 387-393 (2006)] [Lab. of Pharmacology]

Effects of Salmeterol Xinafoate and Fluticasone Propionate on Immunological Activation of Human Cultured Mast Cells.

Hirotomo AKABANE, Masayuki MURATA, Masafumi KUBOTA, Eiji TAKASHIMA, Hiroyuki TANAKA, Naoki INAGAKI*, Michiaki HORIBA and Hiroichi NAGAI.

Here we examine the effect of salmeterol xinafoate (SX) and fluticasone propionate (FP) alone and in combination on the immunological activation of human cultured mast cells (HCMC) in vitro. The release of each chemical mediator was inhibited by 10^{-9}-10^{-8}M SX but not by 10^{-10}-10^{-7}M FP. The production of granulocyte macrophage colony stimulating factor (GM-CSF) was inhibited by a concentration of 10^{-8}M in both drugs and the inhibition was augmented by combined treatment with 10^{-11}M of each drug. The immunological release of chemical mediators from HCMC was inhibited by SX but not by FP. SX and FP inhibited the production of GM-CSF by HCMC and both drug showed synergistic inhibition in the production of GM-CSF.

[*J. Trad. Med.*, **23**, 196-202 (2006)] [Lab. of Pharmacology]

Immunopharmacological Studies on the Effects of Juzentaihoto and Hochuekkito on Experimental Autoimmune Encephalomyelitis in Rats.

Xinkun GAO, Hiroyuki TANAKA, Naoki INAGAKI*, Hitomi TERAMACHI, Teruo TSUCHIYA and Hiroichi NAGAI

Experimental autoimmune encephalomyelitis (EAE) in a prototype experimental model for human multiple sclerosis (MS). This study was conducted to research the effects of Juzentaihoto and Hochuekkito on the onset and development of EAE. Both Juzentaihoto and Hochuekkito suppressed the onset and development of EAE. Suppressive mechanisms of both Kampo medicines are mainly based on the immunosuppressive and anti-inflammatory activity. In addition, Hochuekkito showed immunomodulating activity in the central nervous system. These findings will contribute to consider a new therapy of human MS.

[*J. Biochem. (Tokyo)*, **139**, 1053-1063 (2006)] [Lab. of Biochemistry]

Molecular Cloning of a Novel Type of Rat Cytoplasmic 17ß-Hydroxysteroid Dehydrogenase Distinct from the Type 5 Isozyme.

Shuhei ISHIKURA, Kengo MATSUMOTO, Masaharu SANAI, Kenji HORIE, Toshiyuki MATSUNAGA, Kazuo TAJIMA, Ossama EL-KABBANI and Akira HARA*

Rat liver contains two cytosolic enzymes (TBER1 and TBER2) that reduce 6-*tert*-butyl-2,3-epoxy-5-cyclohexene-1,4-dione into its 4*R*- and 4*S*-hydroxy metabolites. The recombinant TBER1 efficiently oxidized 17β-hydroxysteroids and xenobiotic alicyclic alcohols using NAD^+ as the preferred coenzyme at pH 7.4, and showed low activity towards 20α- and 3α-hydroxysteroids, and 9-hydroxyprostaglandins. The enzyme was potently inhibited by diethylstilbestrol, hexestrol and zearalenone. The mRNA for TBER1 was highly expressed in rat liver, gastrointestinal tract and ovary. Thus, TBER1 represents a novel type of 17β-hydroxysteroid dehydrogenase with unique catalytic properties and tissue distribution. In addition, TBER2 was identified as 3α-hydroxysteroid dehydrogenase on chromatographic analysis of the enzyme activities in rat liver cytosol.

[*Biol. Pharm. Bull.*, **29**, 2488-2492 (2006)] [Lab. of Biochemistry]

Substrate Specificity of a Mouse Aldo-Keto Reductase (AKR1C12).

Satoshi ENDO, Kengo MATSUMOTO, Toshiyuki MATSUNAGA, Shuhei ISHIKURA, Kazuo TAJIMA, Ossama EL-KABBANI and Akira HARA *

AKR1C12, a mouse member of the aldo-keto reductase (AKR) superfamily, is highly expressed in the stomach, and is identical to a protein encoded in an interleukin-3-regulated gene in mouse myeloid cells. The recombinant enzyme reduced various α-dicarbonyl compounds, several ketosteroids, aldehydes and some ketones using NADH as the preferred coenzyme. In the reverse reaction, the enzyme showed coenzyme preference for NAD^+, and oxidized 3α-, 17β- and 20α-hydroxysteroids, and non-steroidal aliphatic and alicyclic alcohols, of which many hydroxysteroids and geranylgeraniol were good substrates. The results suggest that AKR1C12 functions as a dehydrogenase for the endogenous hydroxysteroids and geranylgeraniol in mouse stomach and myeloid cells.

[*Biol. Pharm. Bull.*, **29**, 539-542 (2006)] [Lab. of Biochemistry]

Enzymatic Properties of a Member (AKR1C20) of the Aldo-Keto Reductase Family.

Kengo MATSUMOTO, Satoshi ENDO, Shuhei ISHIKURA, Toshiyuki MATSUNAGA, Kazuo TAJIMA, Ossama EL-KABBANI and Akira HARA *

AKR1C20, a member of the aldo-keto reductase (AKR) superfamily, was expressed and purified to homogeneity. The enzyme was a 36-kDa monomer, and showed both 17β- and 3α-hydroxysteroid dehydrogenase (HSD) activities using NADP(H) as the coenzymes. While the Km values for 17β-hydroxysteroids were high, those for 3α-hydroxy- and 3-keto-steroids were low. The enzyme also highly reduced α-dicarbonyl compounds. The pH optima of the dehydrogenase and reductase activities were 10.5 and 6.5-7.5, respectively. The enzyme was inhibited by sulfobromophthalein, hexestrol, indomethacin and flufenamic acid. The properties of AKR1C20 are distinct from those of previously known other members of the AKR1C subfamily. Thus, AKR1C20 is a novel 3α(17β)-HSD, which may also function as a reductase for xenobiotic α-dicarbonyl compounds.

[*Free Radic. Biol. Med.*, **40**, 1034-1044 (2006)] [Lab. of Biochemistry]

Upregulation of Immunoproteasomes by Nitric Oxide: Potential Antioxidative Mechanism in Endothelial Cells.

Srigiridhar KOTAMRAJU, Sadis MATALON, Toshiyuki MATSUNAGA*, Tiesong SHANG, J.M. HICKMAN-DAVIS, and B. KALYANARAMAN

We report that NO/cGMP/cAMP-induced immunoproteasome subunit expression is responsible for the increased proteasomal activities. Results show that the NO/cGMP/cAMP signaling mechanism enhanced the phosphorylation of cAMP-response element-binding protein, elevated the cAMP-response element-promoter activity and induced the expression of immunoproteasomal subunits (LMP2 and LMP7). Lower levels of LMP2 and LMP7 were detected in aorta of iNOS(-/-) mice compared to wild-type controls, suggesting that endogenous NO production is important in the basal regulation of immunoproteasome. The NO/cGMP/cAMP signaling pathway mitigates transferrin-iron-mediated oxidative stress and apoptosis via induction of immunoproteasomes.

[*Acta. Cryst. F*, **62**, 1037-1040 (2006)] [Lab. of Biochemistry]

Crystallization and Preliminary X-ray Crystallographic Studies of Pig Heart Carbonyl Reductase.

Kennichi AOKI, Nobutada TANAKA, Shuhei ISHIKURA, Naoko ARAKI, Yorishige IMAMURA, Akira HARA * and Kazuo NAKAMURA

Pig heart carbonyl reductase (PHCR), which belongs to the short-chain dehydrogenase/reductase family, has been crystallized by the hanging-drop vapour-diffusion method. Two crystal forms (I and II) have been obtained in the presence of NADPH. Form I crystals belong to the tetragonal space group P4(2), with unit-cell parameters a = b = 109.61, c = 94.31 A, and diffract to 1.5 A resolution. Form II crystals belong to the tetragonal space group P4(1)2(1)2, with unit-cell parameters a = b = 120.10, c = 147.00 A, and diffract to 2.2 A resolution. Both crystal forms are suitable for X-ray structure analysis at high resolution.

[*Jpn. J. Pharm. Health Care Sci.*, **32**, 985-996 (2006)] [Lab. of Clinical Pharmacy]

Evaluation of TDM System for Setting of Initial Anti-MRSA Drug Dosages by Pharmacists at Two Hospitals.

Hitomi TERAMACHI*, Minako YASUDA, Michiyo OKADA, Eiji TAKASHIMA, Masafumi KUBOTA, Yukio IMAI and Teruo TSUCHIYA

We developed a system for setting initial dosages called "TDM system for the setting of initial anti-MRSA drug dosages by pharmacists". We also informed doctors and nurses about the basics of the system and TDM and created a TDM manual. As a result, the rates for attaining target vancomycin (VCM) concentrations, and setting of initial VCM dosage by pharmacists rose significantly. In addition renal dysfunction could be prevented and high clinical efficacy achieved. Our system also enabled TDM to be conducted for all anti-MRSA drugs at Nishimino Kosei hospital, where TDM had previously not been practiced, and it produced the same clinical and economic benefits as at Chu-nou Kousei hospital. Our experience of introducing the system showed that it was very necessary to actively inform doctors and nurses concerning its use and TDM.

[*Jpn. J. Pharm. Health Care Sci.*, **32**, 997-1008 (2006)] [Lab. of Clinical Pharmacy]

Evaluation of Practical Training in a Hospital Pharmacy and Community Pharmacy by Students and Pharmacists.

Hitomi TERAMACHI*, Eiji TAKASHIMA, Masafumi KUBOTA and Teruo TSUCHIYA

All students in the 4th year at our university undergo 4 weeks of training in pharmacy practice. Of these students, 121 were asked to make a self-evaluation regarding their understanding of the pharmacy practice and degree of difficulty of the lectures and pharmacy practice. Since the experience of pharmacy practice was given on a one-students-to-one pharmacist basis, the pharmacists (preceptors) were also asked to evaluate the students whom they had been responsible for. Overall, for hospital pharmacy practice, students gave themselves higher scores than their preceptors did. However, for community pharmacy practice, the preceptors gave higher scores than the students. Thus, in order for the new Japanese model curriculum to be as effective as possible, it is important that lecturers cooperate closely with preceptors regarding the content of lectures at university and items of pharmacy practice.

[*Jpn. J. Ther. Drug. Monit.*, **23**, 233-237 (2006)] [Lab. of Drug Informatics]

Comparison of Cyclosporine A Concentrations by FPIA Method in Whole Blood with Those from Other Methods.

Mitsuhiro NAKAMURA*, Yoshinori IMANISHI, Kana FUKAWA, Kouseki HIRADE, Tadashi SUGIYAMA and Yoshihiro KATAGIRI

The most common methods for routine cyclosporine A (CYA) monitoring are monoclonal antibody-based immunoassay (fluorescence polarization immunoassay (FPIA), the affinity column mediated immunoassay (ACMIA), the enzyme-multiplied immunoassay technique (EMIT), the radio immunoassay (RIA)) and HPLC. Immunoassay using anti-CYA monoclonal antibodies have variable cross-reactivities on CYA metabolites. Therefore, a comprehensive assessment of these methods in clinical samples is needed. We compared CYA concentrations by FPIA with those from other methods. Coefficient of variation of reproducibility obtained by FPIA, ACMIA, EMIT and RIA showed below 6.7% in between-run. $AUC_{0\text{-}6hr}$ with ACMIA was low compared with other methods' value. CYA concentrations in TDM should be carefully interpreted because of the differences in the cross-reactivity.

[*Nat. Prod. Comm.*, **1**, 949-952 (2006)] [Lab. of Pharmacognosy]

Two Novel Natural Flavonoids from *Primula palinuri.*

Munekazu IINUMA,* Toshiyuki TANAKA, Masayoshi OYAMA,
and Eckhard WOLLENWEBER

The farinose exudate produced by *Primula palinuri* was found to consist of 12 flavones at least. Two of them are novel natural compounds, 2'-hydroxy-2-methoxychalcone and 8,2',5'-trihydroxyflavone 5'-benzoate. The structures were determined by analysis of MS and 2D NMR spectral data and spectral comparison with the known compounds.

[*Heterocycles*, **68**, 1617-1630 (2006)] [Lab. of Pharmacognosy]

Stilbenoids with One Epoxy Group from *Cotylelobium lanceolatum.*

Tetsuro ITO, Zulfiqar ALI, Toshiyuki TANAKA, Ken-ichi NAKAYA, Jin MURATA,
Dedy DARNAEDI and Munekazu IINUMA*

A phytochemical investigation of an acetone extract of *Cotylelobium lanceolatum* stem resulted in the isolation of two orange pigments – two stilbene trimer derivatives, cotylelophenols D and E and an artifact derivative, cotylelophenol F. The structure and relative configuration were confirmed by 1D and 2D NMR spectral data. Cotylelophenols D and E are the first examples of stilbene oligomers bearing an epoxy group. The biogenetic relationships between the isolates are also discussed.

[*J. Nat. Prod.*, **69**, 1215-1217 (2006)] [Lab. of Pharmacognosy]

Allergy-preventive Phenolic Glycosides from *Populus sieboldii.*

Yuko OGAWA, Emiko IWAOKA, Munekazu IINUMA* and Kyoko ISHIGURO

Allergy-preventive activity was demonstrated for an extract of the bark of *Populus sieboldii* in a continuing search for allergy-preventive substances from natural sources. By bioassay directed fractionation of this plant bark, two phenolic glycosides, siebolside A {2-hydroxy-5-[(benzoyloxy)methyl]phenyl (6'-*O*-acetyl) β-D-glucopyranoside} and siebolside B [2-hydroxy-5-{(benzoyloxy)methyl]phenyl β-D-glucopyranoside} were isolated, together with three known compounds, salicine, sakuranetin, and neosakuranetin. The structures of new glucosides were elucidated by spectroscopic method. The isolated five compounds all showed allergy-preventive effects.

[*J. Health Sci.*, **52**, 578-584 (2006)] [Lab. of Pharmacognosy]

Cell Growth Inhibition by Membrane-Active Components in Brownish Scale of Onion.

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Masayoshi OYAMA, Tetsuro ITO, Munekazu IINUMA* and Hiroshi TAKEUCHI

The growth-inhibitory effects of the brownish scale components of onion on tumor cells were studied with relating to their membrane activity. Quercetin, quercetin-4'-*O*-glucoside and two isomeric quercetin dimers (10 μM for each) isolated from the scale reduced the fluidity of tumor cell model membranes consisting of phospholipids and cholesterol more significantly than that of normal cell model membranes. In flavonoidal components, the membrane activity was greatest in the order of dimers, aglycone and glucoside. Quercetin and its dimers intensively acted on the membrane center rather than the membrane surface, while quercetin-4'-*O*-glucoside was relatively effective on the hydrophilic regions of membranes. Membrane-active flavonoids inhibited the growth of mouse myeloma cells at 10–100 μM with the same rank of order of potency as they rigidified liposomal membranes. Quercetin and its dimers rigidified cell membranes by acting on the hydrophobic inner regions simultaneously with inhibiting the cell growth, but not quercetin-4'-*O*-glucoside. Flavonoidal components in the brownish scale of onion have the potent anti-proliferative activity associated with the structure-specific rigidification of cell membranes, which is induced by the interaction with membrane lipid bilayers.

[*Biol. Pharm. Bull.*, **29**, 834-837 (2006)] [Lab. of Pharmacognosy]

Melanogenesis Stimulation in Murine B16 Melanoma Cells by Kava (*Piper methysticum*) Rhizome Extract and Kavalactones.

Hideaki MATSUDA, Noriko Hirata, Yoshiko KAWAGUCHI, Shunsuke NARUTO, Takanobu TAKATA, Masayoshi OYAMA, Munekazu IINUMA* and Michinori KUBO

Melanogenesis stimulation activity of aqueous ethanolic extracts obtained from several different parts of five *Piper* species, namely *Piper longum*, *P. kadsura*, *P. methysticum*, *P. betle*, and *P. cubeba*, were examined by using cultured murine B16 melanoma cells. Among them, the extract of *P. methysticum* rhizome (Lava) shoed potent stimulatory effect on melanogenesis as well as *P. nigrum* leaf extract. Activity-guided fractionation of Kava extract led to the isolation of two active kavalactones, yangonin (**2**) and 7,8-epoxyyangonin (**5**), along with three inactive kavalactones, 5,6-dehydrokawain (**1**), (+)-kawain (**3**) and (+)-methysticin (**4**), and a glucosylsterol, daucosterin (**6**). 7,8-Epoxyyangonin (**5**) showed a significant stimulatory effect on melanogenesis in B16 melanoma cells. Yangonin (**2**) exhibited a weak melanogenesis stimulation activity.

[*Biol. Pharm. Bull.*, **29**, 1490-1492 (2006)] [Lab. of Pharmacognosy]

Growth Inhibition of Stilbenoids in Welwitschiaceae and Gnetaceae through Induction of Apoptosis in Human Leukemia HL60 Cells.

Ibrahim ILIYA, Yukihiro AKAO, Kenji MATSUMOTO, Yoshihito NAKAGAWA, Zulfiqar ALI, Tetsuro ITO, Masayoshi OYAMA, Hiroko MURATA, Toshiyuki TANAKA, Yoshinori NOZAWA and Munekazu IINUMA*

Fifty-six stilbenoids isolated from the families of Welwitschiaceae and Gnetaceae were screened for growth inhibitory activity against HL60 cells, and two compounds (gnemonol G and gnetin I) among them exhibited a strong activity with IC_{50} of 10.0 μM and 12.2 μM at 48 h incubation, respectively. The growth suppression by gnemonol G and gnetin I was found to be in part due to apoptosis which was assessed by morphological findings such as nuclear condensation and fragmentation, and DNA ladder formation in human leukemia HL60 cells.

[*Biol. Pharm. Bull.*, **29**, 1504-1507 (2006)] [Lab. of Pharmacognosy]

Inhibitory Activity of Plant Stilbene Oligomers against DNA Topoisomerase II.

Masashi YAMADA, Ken-ichi HAYASHI, Shogo IKEDA, Ken TSUTSUI, Kimiko TSUTSUI, Tetsuro ITO, Munekazu IINUMA* and Hiroshi NOZAKI

The inhibitory activity of 40 stilbene oligomers isolated from six plant species against topoisomerase II were evaluated, of which nine compounds showed a potent inhibitory effect, stronger than daunorubicin, a topoisomerase II inhibitor used as an anti-cancer drug. The specificity of active stilbene oligomers on topoisomerase II was assessed by their effect on DNA restriction enzyme. In particular, specific inhibitory activity was observed in α-viniferin 13-*O*-β-glucopyranoside and hemsleyanol C.

[*FEBS J.*, **273**, 948-959 (2006)] [Lab. of Pharmacognosy]

Identification of β-Amyrin and Sophoradiol 24-Hydroxylase by Expressed Sequence Tag Mining and Functional Expression Assay.

Masaaki SHIBUYA, Masaki HOSHINO, Yuji KATSUBE, Hiroaki HAYASHI,* Tetsuo KUSHIRO and Yutaka EBIZUKA

Cyclization of oxidosqualene is the initial origin of structural diversity of skeletons in their biosynthesis, and subsequent regio- and stereospecific hydroxylation of the triterpene skeleton produces further structural diversity. The enzymes responsible for this hydroxylation were thought to be cytochrome P450-dependent monooxygenase, although their cloning has not been reported. To mine these hydroxylases from five cytochrome P450 genes were amplified by PCR, and screened for their ability to hydroxylate β-amyrin or sophoradiol by heterologous expression in the yeast. Among them, *CYP93E1* transformant showed hydroxylating activity on both substrates. This is the first identification of triterpene hydroxylase cDNA from any plant species.

[*Plant Cell Physiol.*, **47**, 565-571 (2006)] [Lab. of Pharmacognosy]

Lanosterol Synthase in Dicotyledonous Plants.

Masashi SUZUKI, Ting XIANG, Kiyoshi OHYAMA, Hikaru SEKI, Kazuki SAITO, Toshiya MURANAKA, Hiroaki HAYASHI,* Yuji KATSUBE, Tetsuo KUSHIRO, Masaaki SHIBUYA and Yutaka EBIZUKA

Sterols are important as structural components of plasma membranes and precursors of steroidal hormones in both animals and plants. The differences in the biosynthesis of sterols between plants and animals begin at the step of cyclization of 2,3-oxidosqualene, which is cyclized to lanosterol in animals and to cycloartenol in plants. However, here we show that plants also have the ability to synthesize lanosterol directly from 2,3-oxidosqualene, which may lead to a new pathway to plant sterols.

[*Phytochemistry*, **67**, 307-313 (2006)] [Lab. of Pharmacognosy]

Stilbennoids of *Kobresia nepalensis* (Cyperaceae) Exhibiting DNA Topoisomerase II Inhibition.

Masashi YAMADA, Ken-ichiro HAYASHI, Hiroshi HAYASHI, Shogo IKEDA, Takuji HASHINO, Ken TSUTSUI, Kimiko TSUTSUI, Munekazu IINUMA* and Hiroshi NOZAKI

Resveratrol oligomers, nepalensinols A, B and C, were isolated from the stem of Kobresia nepalensis (Cyperaceae). The structures were established on the basis of chemical properties and spectroscopic evidence including 2D NMR spectroscopic analysis. Nepalensinols A, B and C showed a potent inhibitory effect on topoisomerase II, stronger than etoposide (VP-16), a topoisomerase II inhibitor used as an anti-cancer drug. Nepalensinol B, in particular, exhibited the most potent activity with an IC50 of 0.02 μg/ml.

[*J Neurosci* **26**, 3335-3344 (2006)] [Lab. of Mol. Biology]

An analog of a dipeptide-like structure of FK506 increases GDNF expression through cAMP response element-binding protein activated by heat shock protein 90/Akt signaling pathway.

Xiaobo CEN, Atsumi NITTA, Shin OHYA, Yinglan ZHAO, Naoya OZAYA, Akihiro MOURI, Daisuke IBI, Li WANG, Makiko SUZUKI, Kuniaki SAITO, Yasutomo ITO, Tetsuya KAWAGOE, Yukihiro NODA, Yoshihisa ITO, Shoei FURUKAWA*, Toshitaka NABESHIMA

Leu-Ile induced GDNF expression. Leu-Ile transport was investigated in cultured neurons, and the results showed the transmembrane mobility of this dipeptide. We identified heat shock cognate protein 70 as a protein binding to Leu-Ile, and molecular modeling showed that the ATPase domain is the predicted binding site. Leu-Ile stimulated Akt phosphorylation. Moreover, enhanced interaction between phosphorylated Akt and Hsp90 was detected. Leu-Ile elevated GDNF mRNA and protein expression, whereas inhibition of CREB blocked such effects. Leu-Ile promoted the binding activity of phosphorylated CREB with cAMP response element. These show that CREB plays a key role in transcriptional regulation of GDNF expression induced by Leu-Ile.

[*J Neurosci* **26**, 13218-13230 (2006)] [Lab. of Mol. Biology]

Brain-derived neurotrophic factor participates in determination of neuronal laminar fate in the developing mouse cerebral cortex.

Hidefumi FUKUMITSU, Masanari OHTSUKA, Rina MURAI, Hiroyuki NAKAMURA, Kazuo ITOH, Shoei FURKKAWA*

Lamina formation in the developing cerebral cortex requires precisely regulated generation and migration of the cortical progenitor cells. To test the possible involvement of BDNF in the lamina formation, we investigated the effects of BDNF and anti-BDNF antibody administered into the ventricular space of 13.5-d-old mouse embryos. BDNF altered the position, gene-expression, and projections of neurons, whereas anti-BDNF antibody changed some of those of neurons of upper layers II/III. BDNF altered the laminar fate of neurons only if their parent progenitor cells were exposed to it at approximately S-phase and that it hastened the timing of the withdrawal of their daughter neurons from the ventricular proliferating pool by accelerating the completion of S-phase, downregulation of the Pax6 expression and interkinetic nuclear migration of cortical progenitors in the ventricular zone. These observations suggest that BDNF participates in the processes forming the neuronal laminas in the developing cerebral cortex.

[*Biochem. Biophys. Res. Commun.*, **344**, 941-947 (2006)] [Lab. of Mol. Biology]

Autocrine activation of cultured macrophages by brain-derived neurotrophic factor.

Toshio ASAMI, Takuya ITO, Hidefumi FUKUMITSU, Hiroshi NOMOTO, Yoshiko FURUKAWA, Shoei FURUKAWA*

We examined BDNF actions on or BDNF synthesis by cultured macrophages. They synthesized BDNF and NT-3 in addition to expressing high-affinity neurotrophin receptors, full-length TrkB (FL), truncated TrkB (TK(-), and TrkC, thus suggesting an autocrine influence of BDNF and NT-3. BDNF, but not NT-3, enhanced phagocytic activity and stimulated synthesis of IL-1β. There was a correlation of the phagocytic activity with the expression of BDNF or TrkB (FL). Namely, the phagocytic activity of macrophages depends on BDNF synthesis and/or TrkB (FL) expression, suggesting that BDNF participates in the activation processes of macrophages by acting in an autocrine manner.

[*Biosci. Biotechnol. Biochem.*, **70**, 897-906 (2006)] [Lab. of Mol. Biology]

Identification of AMP N_1-oxide in royal jelly as a neurotrophic component for cultured rat pheochromocytoma PC12 cells.

Noriko HATTORI, Hiroshi NOMOTO, Satoshi MISHIMA, Shinsuke INAGAKI, Masashi GOTO, Magoichi SAKO, Shoei FURUKAWA*

An extract of royal jelly (RJ) induced processes from cultured PC12 cells. Active components were identified as AMP and AMP N1-oxide. AMP N1-oxide was more than 20 times as active as AMP. Chemically-synthesized AMP N1-oxide was active similarly to the molecule purified from RJ, confirming AMP N1-oxide as the active entity. AMP N1-oxide also suppressed proliferation of PC12 cells and stimulated expression of neurofilament M, demonstrating AMP N1-oxide to induce neuronal differentiation of PC 12 cells. Pharmacological experiments suggested that AMP N1-oxide actions are mediated by adenyl cyclase-coupled adenosine receptors, including A2A. Thus AMP N1-oxide is a key molecule that characterizes RJ, and is not found in natural products other than RJ.

[*J. Lipid Res.*, **47**, 1434-1443 (2006)] [Lab. of Mol. Biology]

Lysophosphatidylethanolamine in *Grifola frondosa* as a neurotrophic activator via activation of MAP kinase.

Akiyoshi NISHINA, Hirokazu KIMURA, Akihiro SEKIGUCHI, Ryo-hei FUKUMOTO, Satoshi NAKAJIMA, Shoei FURUKAWA*

Grifola frondosa extracts activated MAPK in cultured PC12 cells. The active substance was lysophosphatidylethanolamine (LPE). LPE from G. frondosa (GLPE) was confirmed to induce the activation of MAPK and was found to suppress cell condensation and DNA ladder generation evoked by serum deprivation. Moreover, GLPE caused morphological changes in and upregulation of neurofilament M expression of PC12 cells, demonstrating that the GLPE could induce neuronal differentiation of these cells. The activation of MAPK by GLPE was suppressed by AG1478, an antagonist of EGFR, and by U0126, an inhibitor of MEK1/2, but not by K252a, an inhibitor of TrkA, or by pertussis toxin. These results demonstrate that GLPE induced the MAPK cascade, the activation of which induced neuronal differentiation and suppressed serum deprivation-induced apoptosis.

[*J. Histochem. Cytochem.*, **54**, 1061-1071 (2006)] [Lab. of Mol. Biology]

Localization of fibroblast growth factor-1 in cholinergic neurons innervating the rat larynx.

Hiroyuki OKANO, Ken-ichiro TOYODA, Hitoshi BAMBA, Yasuo HISA, Yutaka OOMURA, Toru IMAMURA, Shoei FURUKAWA*, Hiroshi KIMURA, Ikuo TOOYAMA

Cholinergic neurons in the dorsal motor nucleus of the vagus (DMNV) are particularly vulnerable to laryngeal nerve damage, possibly because they lack fibroblast growth factor-1 (FGF1). To test this hypothesis, we investigated the localization of FGF1 in cholinergic neurons innervating the rat larynx by immunohistochemistry using central-type antibodies to choline acetyltransferase (cChAT) and peripheral type (pChAT) antibodies, as well as tracer experiments. In the DMNV, only 9% of cChAT-positive neurons contained FGF1, and 71% of FGF1-positive neurons colocalized with cChAT. In the nucleus ambiguus, 100% of cChAT-positive neurons were FGF1 positive. In the intralaryngeal ganglia, all neurons contained both pChAT and FGF1. In the nodose ganglia, 66% of pChAT-positive neurons were also positive for FGF1, and 90% of FGF1-positive cells displayed pChAT immunoreactivity.

[*J. Endotoxin Res.*, **12**, 346-351 (2006)] [Lab. of Microbiology]

Defective responsiveness of CD5$^+$ B1 cells to lipopolysaccharide in cytokine production.

Naoki KOIDE, Akiko MORIKAWA, Hiroyasu ITO, Tsuyoshi SUGIYAMA*, Ferdaus HASSAN, Shamima ISLAM, Shamima TUMURKHUU, Isamu MORI, Tomoaki YOSHIDA, and Takashi YOKOCHI.

The effect of LPS on cytokine production by TH2.52 B1 cells was studied. TH2.52 cells constitutively produced a small amount of TNF-α and IL-6, and TNF-α and IL-6 production was markedly enhanced by LPS stimulation. Although IFN-γ caused the production of various cytokines, such as IL-2, IL-4, IL-6 and TNF-α in TH2.52 cells, LPS did not cause the production of such cytokines. TH2.52 cells lacked the expression of IFN-β and other several molecules participating in the MyD88-independent pathway in LPS signaling. Defective responsiveness of TH2.52 B1 cells to LPS in cytokine production might be responsible for the failure of IFN-β production due to the lack of molecules participating in the MyD88-independent pathway.

[*Cancer Sci.*, **97**, 896-904 (2006)] [Lab. of Radiochemistry]

Modification by curcumin of mutagenic activation of carcinogenic *N*-nitrosamines by extrahepatic cytochromes P-450 2B1 and 2E1 in rats.

Yukiko MORI*, Kenjiro TATEMATSU, Akihiro KOIDE, Shigeyuki SUGIE, Takuji TANAKA and Hideki MORI

A mechanism underlying suppression by curcumin of esophageal carcinogenesis induced by *N*-nitrosomethylbenzylamine (NMBA) has been elucidated in F344 rats. No significant alterations in hepatic cytochrome P450 (CYP) levels, mutagenic activation by liver S9 or hepatic UDP-glucuronyltransferase activities were produced by curcumin and/or NMBA. In contrast, curcumin decreased esophageal and gastric CYP2B1 and 2E1, and mutagenicities of *N*-nitrosamines, including NMBA, with these tissue S9, while curcumin increased large intestinal CYP2B1 and those with large intestine S9. Consequently, modifying effects of curcumin can be attributed to a decrease in metabolic activation of NMBA by esophageal CYP2B1 without the contribution of hepatic (in)activation. Further, the potential of curcumin for modification of gastric and intestinal carcinogenesis is suggested.

[*Food Chem.Toxicol.*, **44**, 952-963 (2006)] [Lab. of Radiochemistry]

Exploration for unknown substances in rapeseed oil that shorten survival time of stroke-prone spontaneously hypertensive rats. Effects of super critical gas extraction fractions.

Naoki OHARA, Yukiko NAITO, Tomoko NAGATA, Kenjiro TATEMATSU*, Shin-ya FUMA, Shigehiro TACHIBANA and Harumi OKUYAMA

To identify the causative substances for the shortening of survival time in stroke-prone spontaneously hypertensive rats (SHRSP) by rapeseed oil (RO), SHRSP were fed on the diet supplemented with soybean oil (control), RO, or one of the fractions of RO obtained by super critical gas extraction (SCE). Survival times in the RO and two SCE fraction groups were shorter than the soybean oil and another fraction groups. In addition, chronic nephropathy, heart weights gain and cerebral necrosis were found in RO group and/or a few SCE fraction groups, but these symptoms and fatty acid and sterol levels in the diets were not associated with the life-shortening. SCE appeared to produce a safe fraction of RO, though it failed to separate clearly the causative substances.

[*J. Neurosci. Res.*, **84**, 860-866 (2006)] [Lab. of Clinical Pharmaceutics]

Apomorphine protects against 6-hydroxydopamine-induced neuronal cell death through activation of the Nrf2-ARE pathway.

Hirokazu HARA*, Mitsuhiro OHTA, Tetsuo ADACHI

NF-E2-related factor-2 (Nrf2), a basic leucine zipper transcription factor, is involved in the expression of numerous detoxifying and antioxidant genes via the antioxidant response element (ARE). In this study, we investigated whether the Nrf2-ARE system is involved in the protection by apomorphine (Apo). Pretreatment of SH-SY5Y cells with Apo suppressed 6-hydroxydopamine-induced cell death in a dose-dependent manner. Apo stimulated the translocation of Nrf2 into the nucleus and the transactivation of the ARE. The expression of heme oxygenase-1 (HO-1) was dose dependently induced by Apo. Moreover, we found that the activation of the ARE and the induction of HO-1 mRNA caused by Apo were suppressed in the presence of the antioxidant N-acetylcysteine and also that Apo produced intracellular reactive oxygen species (ROS), indicating that the low level of ROS produced by Apo may play a critical role in this phenomenon.

[*Biol. Pharm. Bull.*, **29**, 2095-2098 (2006)] [Lab. of Clinical Pharmaceutics]

Infliximab neutralizes the suppressive effect of TNF-α on expression of extracellular-superoxide dismutase *in vitro*.

Tetsuo ADACHI*, Taisuke TOISHI, Eiji TAKASHIMA, Hirokazu HARA

We investigated the effect of TNF-α on the expression of EC-SOD in cultured cells and the cooperating effect of infliximab. In the *in vitro* assays examined, expression of EC-SOD, but not other SOD isozymes, in smooth muscle and fibroblast cells were suppressed by the addition of TNF-α. Simultaneous addition of infliximab dose-dependently and significantly prevented the suppressive effects of TNF-α. p38 Mitogen-activated protein kinase (MAPK) inhibitor, SB203580, prevented significantly the suppressive effect of TNF-α suggesting that p38 MAPK is an important signaling molecule downstream of TNF-α to inhibit the EC-SOD expression. This reveals a potential usefulness of infliximab on TNF-α related pathological conditions such as arthritis and insulin resistance.

[*Atherosclerosis*, **187**, 131-138 (2006)] [Lab. of Clinical Pharmaceutics]

Heparin-released extracellular superoxide dismutase is reduced in patients with coronary artery atherosclerosis.

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We studied whether the amount of heparin-released extracellular-superoxide dismutase (EC-SOD), which is an antioxidative enzyme, is associated with coronary artery disease (CAD). EC-SOD was measured in plasma at basal and at post-heparin injection in patients. Although the basal values were similar among the groups, heparin-released EC-SOD levels were significantly lower in the atherosclerosis group than in the normal group. Moreover, logistic analysis revealed that heparin-released EC-SOD independently contributed to CAD. The coronary score showed a significant correlation with heparin-released EC-SOD. As for factors affecting the level of heparin-released EC-SOD, the level of high-density lipoprotein cholesterol and age showed a positive correlation.

[*Clin. Chim. Acta*, **374**, 129-134 (2006)] [Lab. of Clinical Pharmaceutics]

Relationship between insulin resistance and inflammatory markers and anti-inflammatory effect of losartan in patients with type 2 diabetes and hypertension.

Hyohun PARK, Goji HASEGAWA, Hiroshi OBAYASHI, Aya FUJINAMI, Mitsuhiro OHTA, Hirokazu HARA, Tetsuo ADACHI*, Shoko TAMAKI, Yoshiki NAKAJIMA, Fumiaki KIMURA, Masakazu OGATA, Michiaki FUKUI, Toshikazu YOSHIKAWA, Naoto NAKAMURA

We investigated the relationship between the homeostasis model assessment-insulin resistance index (HOMA-R) and various serum inflammatory markers and the effect of losartan on serum concentration of these markers in patients with type 2 diabetes and hypertension. The HOMA-R values were positively related to TNF-α and inversely related to adiponectin and EC-SOD. Serum EC-SOD may be a sensitive biochemical marker of insulin resistance in patients with type 2 diabetes and hypertension and that losartan improves insulin sensitivity by increasing EC-SOD and adiponectin production and decreasing TNF-α production.

[*J. Biol. Chem.*, **281**, 11397-11404(2006)] [Lab. of Biofunctional Molecules]

Glucocorticoid modulatory element-binding protein 1 binds to initiator procaspases and inhibits ischemia-induced apoptosis and neuronal injury.

Kazuhiro TSURUMA, Tadashi NAKAGAWA, Nobutaka MORIMOTO, Masabumi MINAMI, Hideaki HARA*, Takashi UEHARA and Yasuyuki NOMURA

We demonstrated that glucocorticoid modulatory element-binding protein 1 (GMEB1) interacts with the prodomain of procaspase-2, thereby disrupting its autoactivation and the induction of apoptosis. Here we show that GMEB1 is also capable of binding to procaspase-8 and -9. GMEB1 attenuated the Fas-mediated activation of these caspases and the subsequent apoptosis. The knockdown of endogenous GMEB1 using RNA interference revealed that cells with decreased GMEB1 expression are more sensitive to stress and undergo accelerated apoptosis. Transgenic mice expressing a neurospecific GMEB1 had smaller cerebral infarcts and less brain swelling than wild-type mice in response to transient focal ischemia. These results suggest that GMEB1 is an endogenous regulator that selectively binds to initiator procaspases and inhibits caspase-induced apoptosis.

[*J. Cereb. Blood Flow Metab.*,**26,** 402-413(2006)] [Lab. of Biofunctional Molecules]

Neuroprotective effect of recombinant human granulocyte colony-stimulating factor in transient focal ischemia of mice.

Miki KOMINE-KOBAYASHI, Ning ZHANG, Meizi LIU, Ryota TANAKA, Hideaki HARA*, Akimichi OSAKA, Hideki MOCHIZUKI, Yoshikuni MIZUNO and Takao URABE

The current study was designed to assess the neuroprotective mechanisms of G-CSF in ischemia/reperfusion injury using bone marrow chimera mice known to express enhanced green fluorescent protein (EGFP). Mice were subjected to ischemia/reperfusion and divided into two groups: those treated with G-CSF (G-CSF group) and vehicle (control group). G-CSF upregulated Stat3, pStat3, and Bcl-2, and suppressed iNOS and nitrotyrosine expression. In EGFP chimera mice, G-CSF decreased the migration of Iba-1/EGFP-positive bone marrow-derived monocytes/macrophages and increased intrinsic microglia/macrophages at ischemic penumbra, suggesting that bone marrow-derived monocytes/macrophages are not involved in G-CSF-induced reduction of ischemic injury size. Our study indicated that G-CSF exerts a neuroprotective effect through the direct activation of antiapoptotic pathway, and suggested that G-CSF is important for expansion of the therapeutic time window in patients with cerebral ischemia.

[*Exp. Eye Res.*, **82**, 427-440(2006)] [Lab. of Biofunctional Molecules]

Morphometric evaluation of changes with time in optic disc structure and thickness of retinal nerve fiber layer in chronic ocular hypertensive monkeys.

Masamitsu SHIMAZAWA, Goji TOMITA, Takazumi TANIGUCHI, Masaaki SASAOKA, Hideaki HARA*, Yoshiaki KITAZAWA, and Makoto ARAIE

We examined the time course of changes in optic disc structure by means of a scanning laser ophthalmoscope (Heidelberg Retina Tomograph: HRT) in ocular hypertensive monkeys, and clarified the relationships between the histological RNFL thickness and the HRT parameters. Further, the time course of changes in retinal nerve fiver layer (RNFL) thickness in individual eyes were measured using a scanning laser polarimeter with fixed corneal polarization compensator (GDx FCC). In conclusion, alongside the IOP elevation, time-related changes in optic disc topography and RNFL thickness were demonstrated in monkey eyes using HRT and GDx. HRT (rim and cup) parameters showed good correlations with histological RNFL thickness, and significant interrelations.

[*Neurosci. Lett.*, **409**, 192-195(2006)] [Lab. of Biofunctional Molecules]

Inhibitor of double stranded RNA-dependent protein kinase activity protects against cell damage induced by ER stress.

Masamitsu SHIMAZAWA and Hideaki HARA*

Phosphorylation of double stranded RNA-dependent protein kinase (PKR) has been demonstrated in brain tissues in patients with Alzheimer's and Huntington's diseases. Here, we examined the effect of a PKR inhibitor on the neuronal cell death induced by ER-stress in cultured human neuroblastoma cells (SH-SY5Y). Treatment with tunicamycin at 2 μg/ml for 24 h induced apoptotic cell death accompanied by nuclear condensation and/or fragmentation, and these cells were positive for YO-PRO-1. Treatment with the PKR inhibitor at 0.1 or 0.3 μM led to a decrease in the number of apoptotic cells induced by tunicamycin. In the resazurin-reduction test, the PKR inhibitor (at 0.1 and 0.3 μM) concentration-dependently inhibited the tunicamycin-induced decrease in metabolic activity. These results indicate that inhibition of PKR activation may be neuroprotective against ER stress-induced cell damage.

[*Invest. Ophthalmol. Vis. Sci.*, **47**, 3975-3982(2006)] [Lab. of Biofunctional Molecules]

Metallothionein, an endogenous antioxidant, protects against retinal neuron damage in mice.

Shinsuke SUEMORI, Masamitsu SHIMAZAWA, Kazuhide KAWASE, Masahiko SATOH, Hisamitsu NAGASE, Tetsuya YAMAMOTO and Hideaki HARA*

To clarify the functional role of metallothionein (MT) in retinal damage using mice deficient in both MT-I/-II-deficient and wild-type (C57BL/6J) mice, and MT inducers [$ZnSO_4$ and 1α, 25-dihydroxyvitamin D_3 (Vit. D_3)]. In wild-type mice, MT-II mRNA expression was time-dependently elevated by NMDA, with the normal level being regained within 24 h. In contrast, MT-I and -III showed persistent decreases from 4 to 24 h. At 7 days after NMDA injection in MT-I/-II-deficient mice, GCL cell loss was increased, but IPL thickness was not different. Pretreatment with $ZnSO_4$ or Vit. D_3 increased inner retinal MT-like immunoreactivity 24 h after NMDA injection and significantly attenuated NMDA-induced GCL cell loss in wild-type mice, but $ZnSO_4$ pretreatment did not protect against such cell loss in MT-I/-II-deficient mice. These findings suggest that MT, especially MT-II, may protect against retinal neurone damage by acting as an endogenous antioxidant.

[*Exp. Eye Res.*, **82**, 427-440(2006)] [Lab. of Biofunctional Molecules]

Intravitreal injection of endothelin-1 caused optic nerve damage following to ocular hypoperfusion in rabbits

Masaaki SASAOKA, Takazumi TANIGUCHI, Masamitsu SHIMAZAWA, Naruhiro ISHIDA, Atsushi SHIMAZAKI, Hideaki HARA*

The purpose of this study was to investigate the time course of the ocular hypoperfusion, retinal damage, and optic nerve damage induced by intravitreal injection of endothelin-1 (ET-1) in rabbits. ET-1, at 5 pmol (20 μl, twice a week for 2 or 4 weeks), was injected from the pars plana into the posterior vitreous of the right eye. Optic nerve head (ONH) blood flow and retinal artery diameter, together with the neurofilament light chain (NF-L) content, retinal morphology, and axon density of the optic nerve, were evaluated at 2, 4, and 8 weeks after the first injection of ET-1 (n=7 or 8). In conclusion, intravitreal injection of ET-1 induced chronic hypoperfusion in the ONH and retina, which presumably caused decreases in NF-L content and axon number in the optic nerve noted in the later part of the observation period.

[*Mol Vision*, **12**, 977-982 (2006)] [Lab. of Biofunctional Molecules]

Elevated neprilysin activity in vitreous of patients with proliferative diabetic retinopathy.

Hideaki HARA*, Kentaro OH-HASHI, Shinji YONEDA, Masamitsu SHIMAZAWA, Masaru INATANI, Hidenobu TANIHARA and Kazutoshi KIUCHI

In this study, we measured both the enzymatic activity of neprilysin and the concentration of Aβ in patients with proliferative Diabetic retinopathy (DR) (as compared to their levels in patients with macular hole), and we analyzed their association. In vitreous samples collected from patients who underwent vitrectomy, an HPLC-fluorometric system and sensitive and specific enzyme-linked immunosorbent assays were used to determine the enzymatic activity of neprilysin and the concentration of Aβ, respectively. By comparison with the levels in the control (macular-hole) patients, there was a significant increase in neprilysin activity level and a significant decrease in Aβ level in proliferative DR patients. There was a significant inverse correlation between neprilysin and Aβ among all subjects. Neprilysin activity and Aβ concentrations displayed converse changes in patients with proliferative DR.

[*J. Pharmacol. Sci.*, **102**, 196-204(2006)] [Lab. of Biofunctional Molecules]

Lig-8, a bioactive lignophenol derivative from bamboo lignin, protects against neuronal damage *in vitro* and *in vivo*.

Yasushi ITO, Masamitsu SHIMAZAWA, Yukihiro AKAO, Yoshimi NAKAJIMA, Norio SEKI, Yoshinori NOZAWA, and Hideaki HARA*

Lig-8, a lignophenol derivative from bamboo lignin, potently suppresses oxidative stress-induced apoptosis. Here, we first examined *in vitro* whether lig-8 protects against neuronal damage induced by oxygen-glucose deprivation (OGD) followed by reoxygenation, tunicamycin, or PSI (proteasome inhibitor). In PC12 cell cultures, lig-8 (1 to 30 μM) concentration-dependently inhibited OGD- and tunicamycin-induced cell deaths. In human neuroblastoma (SH-SY5Y) cell culture, the PSI-induced apoptotic cell death and fusion protein accumulation was inhibited by lig-8. *In vivo*, lig-8 reduced intravitreal *N*-methyl-D-aspartate-induced retinal damage. Hence, lig-8 protects, partly by inhibiting excessive ER-stress, against neuronal damage *in vitro* and *in vivo*.

[*eCAM.*, **3**, 71-77(2006)] [Lab. of Biofunctional Molecules]

Brazilian Green Propolis Protects against Retinal Damage *In vitro* and *In vivo*.

Yuta INOKUCHI, Masamitsu SHIMAZAWA, Yoshimi NAKAJIMA, Shinsuke Suemori, Satoshi MISHIMA and Hideaki HARA*

We investigated whether Brazilian green propolis exerts neuroprotective effects in the retina *in vitro* and/or *in vivo*. *In vitro*, retinal damage was induced by 24-h hydrogen peroxide exposure. Propolis inhibited the neurotoxicity and apoptosis induced in cultured retinal ganglion cells (RGC-5) by 24-h hydrogen peroxide exposure. Regarding the possible underlying mechanism, in pig retina homogenates propolis protected against oxidative stress (lipid peroxidation), as also did trolox. In mice *in vivo*, propolis (100 mg/kg; intraperitoneally administered four times) reduced the retinal damage induced by intravitreal NMDA injection. These findings indicate that Brazilian green propolis has neuroprotective effects against retinal damage both *in vitro* and *in vivo*, and that a propolis-induced inhibition of oxidative stress may be partly responsible for these neuroprotective effects.

[*Curr. Neurovasc. Res.*, **3**, 81-88(2006)] [Lab. of Biofunctional Molecules]

Endothelin-1 Impairs Retrograde Axonal Transport and Leads to Axonal Injury in Rat Optic Nerve.

Takazumi TANIGUCHI, Masamitsu SHIMAZAWA, Masaaki SASAOKA, Atsushi SHIMAZAKI and Hideaki HARA*

The purpose of this study was to examine the effects of endothelin-1 (ET-1) on retrograde axonal transport in the rat optic nerve. ET-1 at 5 pmol/eye caused a significant constriction of retinal vessels at 10 min after the injection. Intravitreous injection of ET-1 caused a dose-related decrease in the number of retrogradely labeled RGCs. Injection of 5 pmol/eye ET-1 led to a statistically significant decrease in the number of retrogradely labeled RGCs. ET-1 at 1 and 5 pmol/eye caused histological optic nerve damage. The histological optic nerve damage correlated with the number of retrogradely labeled RGCs. In conclusion, a single intravitreous injection of ET-1 impaired retrograde axonal transport in the rat optic nerve and this impairment correlated with the histological optic nerve damage.

[*Ophthalmic.Res.*, **38**, 1-7(2006)] [Lab. of Biofunctional Molecules]

Nerve fiber layer measurement using scanning laser polarimetry with fixed corneal compensator in normal cynomolgus monkey eyes.

Masamitsu SHIMAZAWA, Takazumi Taniguchi, Masaaki SASAOKA and Hideaki HARA*

The purpose of this study was to examine retinal nerve fiber layer (RNFL) thickness in normal cynomolgus monkeys using a scanning laser polarimeter with fixed corneal compensator (GDx FCC), and to clarify the reproducibility and symmetries between both eyes in the GDx parameters. All parameters showed small right-left differences. The CV (SD/mean×100) for all parameters were less than 10%. Highly significant correlations were seen between bilateral eyes in the macular retardation or the baseline values. Significant correlation was also seen between macular retardation and baseline values. Considering individual difference in corneal birefringence, GDx parameters obtained from GDx FCC may be useful for objective evaluation in time-related changes of individual eye or for binocular comparisons in cynomolgus monkeys.

[*Brain Res.*, *1116*, 187-193(2006)] [Lab. of Biofunctional Molecules]

Cilostazol reduces ischemic brain damage partly by inducing metallothionein-1 and -2.

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The neuroprotective effect of cilostazol, an antiplatelet drug, was examined after 24 h permanent middle cerebral artery (MCA) occlusion in mice, and explored the possible underlying mechanism by examining metallothionein (MT)-1 and -2 induction *in vivo*. Cilostazol (30 mg/kg) was intraperitoneally administered at 12 h before, 1 h before, and just after MCA occlusion. Cilostazol significantly reduced the infarct area and volume, especially in the cortex. Real-time RT-PCR revealed increased mRNA expressions for MT-1 and -2 in the cortex of normal brains at 6 h after cilostazol treatment without MCA occlusion. MT-1 and -2 immunoreactivity was also increased in the cortex of such mice, and this immunoreactivity was observed in the ischemic hemisphere at 24 h after MCA occlusion. The strongest MT-1 and -2 immunoreactivity was detected in MCA-occlused mice treated with cilostazol. These findings indicate that cilostazol has neuroprotective effects *in vivo* against permanent focal cerebral ischemia, especially in the penumbral zone in the cortex, and that MT-1 and -2 may be partly responsible for these neuroprotective effects.

[*Brain Res.*, **1082**, 196-204(2006)] [Lab. of Biofunctional Molecules]

Rifampicin attenuates the MPTP-induced neurotoxicity in mouse brain.

Yasuhisa OIDA, Kiyoyuki KITAICHI, Hironao NAKAYAMA, Yukiko ITO, Yohei FUJIMOTO, Masamitsu SHIMAZAWA, Hiroichi NAGAI and Hideaki HARA*

This study was designed to elucidate its neuroprotective effects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity. Rifampicin at 20 mg/kg (i.p., twice) had protective effects against MPTP-induced neuronal damage in both the substantia nigra and striatum. Rifampicin also protected against the MPTP-induced depletions of dopamine, 3,4-dihydroxy- phenylacetic acid, and homovanillic acid in the striatum. At 1 μM or more, rifampicin significantly inhibited both lipid peroxidation in the striatum and free radical production. These findings suggest that in mice, rifampicin can reach brain tissues at concentrations sufficient to attenuate MPTP-induced neurodegeneration in the nigrostriatal dopaminergic neuronal pathway, and that an inhibitory effect against oxidative stress may be partly responsible for its observed neuroprotective effects.

[*Proc. Natl. Acad. Sci. USA.*, **103**(1), 153-158 (2006)] [Lab. of Clinical Pharmacology]

Regulation of denderitic cell maturation and function by Bruton's tyrosine kinase via IL-10 and Stat3.

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Y. KAWAKAMI, W. XIAO, H. NAGAI, * M. CROFT and T. KAWAKAMI

Btk plays crucial roles in the differentiation and activation of B and myeloid cells. Despite drastic reductions of other Ig isotypes, paradoxically high IgE responses have been known in btk mutant mice. Here we show that btk(-/-) dendritic cells exhibit a more mature phenotype and a stronger in vitro and in vivo T cell-stimulatory ability than wild-type cells. Increased IgE responses were induced by adoptive transfer of btk(-/-) dendritic cells into mice. Consistent with the stronger T cell-stimulatory ability of btk(-/-) dendritic cells, btk(-/-) mice exhibited enhanced inflammation in Th2-driven asthma and Th1-driven contact sensitivity experiments. These negative regulatory functions of Btk in dendritic cells appear to be mediated mainly through autocrine secretion of IL-10 and subsequent activation of Stat3.

[*J. Immunol.*, **176**, 52-60 (2006)] [Lab. of Clinical Pharmacology]

Integrin $\alpha_{IIb}\beta_3$ induces the adhesion and activation of mast cells through interaction with fibrinogen.

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* Yoshinori YAMANISHI, Hideaki NAKAJIMA, Hidetoshi KUMAGAI and Toshio KITAMURA

We now demonstrate that integrin alphaIIbbeta3 is highly expressed on mouse and human mast cells including mouse bone marrow-derived mast cells, peritoneal mast cells, and human cord blood-derived mast cells, and that its binding to extracellular matrix proteins leads to enhancement of biological functions of mast cells in concert with various stimuli. With exposure to various stimuli, including cross-linking of FcepsilonRI and stem cell factor, mast cells adhered to extracellular matrix proteins such as fibrinogen and von Willebrand factor in an integrin alphaIIbbeta3-dependent manner. In conclusion, mouse and human mast cells express functional integrin alphaIIbbeta3.

[*Allergol. Int.*, **55**(1), 67-76 (2006)] [Lab. of Clinical Pharmacology]

Production of matrix metalloproteinases in human culutured mast cells: involvement of protein kinase c-mitogen activated protein kinase kinase-extracellular signalregulated kinase pathway.

Masahiro KIMATA, Masayuki ISHIZAKI, Hiroyuki TANAKA, Hiroichi NAGAI* and Naoki INAGAKI

We detected the de novo synthesis of MMP-9 in HCMC after stimulation with PMA and found that the synthesis was mediated through protein kinase C-mitogen activated protein kinase kinase (MEK)-ERK pathway. The MMP-9 production induced by PMA was suppressed by simultaneous treatment with A23187, whereas GM-CSF production was potentiated. We also detected the expression of mRNA for membrane-type 1 (MT1)-MMP, TIMP-1 and TIMP-2 after stimulation with PMA. Glucocorticoids and flavonoids inhibited MMP-9 production, and TIMPs and MMP inhibitors inhibited the gelatinolytic activity of mast cell-derived MMP-9. Furthermore, phenylmethylsulfonyl fluoride, a protease inhibitor, inhibited the conversion from proMMP-9 to active MMP-9. These results suggest that the human mast cell is a leading member of MMP production, and the production, activation and activity are controllable by pharmacological agents.

[*Brain Res.*, **1082**, 196-204 (2006)] [Lab. of Clinical Pharmacology]

Rifampicin sttenuates the MPTP-induced neurotoxicity in mouse brain.

Y. OIDA, K. KITAICHI, H. NAKAYAMA, Y. ITO, Y. FUJIMOTO, M. SHIMAZAWA, H. NAGAI* and H. HARA

Rifampicin, an antibacterial drug, is highly effective in the treatment of tuberculosis and leprosy. Recently, it has been reported to have neuroprotective effects in in vitro and in vivo models. This study was designed to elucidate its neuroprotective effects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity (known as an in vivo mouse model of Parkinson's disease). These findings from this experiment; suggest that in mice, rifampicin can reach brain tissues at concentrations sufficient to attenuate MPTP-induced neurodegeneration in the nigrostriatal dopaminergic neuronal pathway, and that at inhibitory effect against oxidative stress may be partly responsible for its observed neuroprotective effects.

[*J. Allergy Clin. Immunol.*, **118** (1), 98-104 (2006)] [Lab. of Clinical Pharmacology]

Periostin: A novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals.

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Both IL-4 and IL-13 induced secretion of periostin in lung fibroblasts independently of TGF-beta. Periostin colocalized with other extracellular matrix proteins involved in subepithelial fibrosis in both asthma patients and ovalbumin-sensitized and ovalbumin-inhaled wild-type mice, but not in either IL-4 or IL-13 knockout mice. Periostin had an ability to bind to fibronectin, tenascin-C, collagen V, and periostin itself. CONCLUSION: Periostin secreted by lung fibroblasts in response to IL-4 and/or IL-13 is a novel component of subepithelial fibrosis in bronchial asthma. Periostin may contribute to this process by binding to other extracellular matrix proteins. These results suggest that periostin induced by IL-4/IL-13 shows promise in inhibiting subepithelial fibrosis in bronchial asthma.

[*Eur. J. Pharmacol.*, **546**, 189-196 (2006)] [Lab. of Clinical Pharmacology]

Inhibition of scratching behavior associated with allergic dermatitis in mice by tacrolimus, but not by dexamethasone.

Naoki INAGAKI, Noriko SHIRAISHI, Katsuhiro IGETA, Tomokazu ITOH, Takao CHIKUMOTO, Masafumi NAGAO, John Fan KIM and Hiroichi NAGAI*

Itching is the most important problem in many allergic and inflammatory skin diseases especially in atopic dermatitis. We established a mouse allergic dermatitis model involving frequent scratching behavior by repeated painting with 2,4-dinitrofluorobenzene (DNFB) acetone solution onto the mouse skin, and comparatively examined the effects of tacrolimus and dexamethasone on the dermatitis and associated scratching behavior. Tacrolimus significantly inhibited the scratching behavior that was associated with the inhibition of nerve fiber extension into the epidermis, whereas dexamethasone failed to have any effect.

[*Allergol. Int.*, **55**(4), 387-393 (2006)] [Lab. of Clinical Pharmacology]

Effects of salmeterol xinafoate and fluticasone propionate on immunological activation of human cultured mast cells.

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The clinical efficacy of combination therapy comprising a long acting beta(2)-agonist (LABA) and corticosteroid is widely recognized for the treatment of adult asthma. Here we examine the effect of salmeterol xinafoate (SX) and fluticasone propionate (FP) alone and in combination on the immunological activation of human cultured mast cells (HCMC) in vitro. The immunological release of chemical mediators (histamine, LT, PGD(2)) from HCMC was inhibited by SX but not by FP. SX and FP inhibited the production of GM-CSF by HCMC and both drug showed synergistic inhibition in the production of GM-CSF.

[*J. Trad. Med.*, **23**, 196-202 (2006)] [Lab. of Clinical Pharmacology]

Immunopharmacological studies on the effects of Juzentaihoto and Hochuekkito on experimental autoimmune encephalomyelitis in rats.

Xinkun GAO, Hiroyuki TANAKA, Naoki INAGAKI, Hitomi TERAMACHI, Teruo TSUCHIYA and Hiroichi NAGAI*

Experimental autoimmune encephalomyelitis (EAE) is a prototype experimental model for human multiple sclerosis (MS). This study was conducted to research the effects of Juzentaihoto and Hochuekkito on the onset and development of EAE. Both Juzentaihoto and Hochuekkito suppressed the onset and development of EAE. Suppressive mechanisms of both Kampo medicines are mainly based on the immunosuppressive and anti-inflammatory activity. In addition, Hochuekkito showed immunomodulating activity in the central nervous system. These findings will contribute to consider a new therapy of human MS.

[*J.Educ.Health Sci.*, **51**, 253-262 (2006)] [Lab. of Health and Physical Education]

A Strategy and an Effect of Health Education to Increase the Maximum Bone Mass in High School Boys

Hiroyuki NISHIDA*, Kaei WASHINO, Haruo SUGIURA, Atsushi MAGUSA,
Hiroki YAMAMOTO and Sanae KUZE

The effect of the health education by pamphlet distribution was not clear but that by a lecture was obvious from the result, the rate of bone mass increase was observed in an education group. The scholars whose measured values of bone mass were low at 1st grade tended to have the higher rate of bone mass increase and there are many boys who think themselves into increase the bone mass. The rate of bone mass increase for 2 years of the education group (7.47%) was significantly higher than that of the control group (5.28%). We concluded that the health education regarding exercise and nutrition by a lecture was effective in improving the bone mass and enhancing the knowledge of better lifestyle for bone in high school boys who were at low risk of for osteoporosis. In addition, this education had a good influence on their subsequent improvement of lifestyle.

[*J.Educ.Health Sci.*, **51**, 274-281 (2006)] [Lab. of Health and Physical Education]

Effect Evaluation of Smell by the Chaos Index of Acceleration Plethysmograms

Kaei WASHINO, Hiroyuki NISHIDA* and Sanae KUZE

In this study, we evaluated the effect of aroma by using the chaos analysis of acceleration plethysmogram. We tried to apply the trajectory parallel measure (TPM) and the recurrences plot method (RP) as a chaotic analysis and used the values of TPM ave. (average of TPM) and the value of RP-dw (the ratio of white to the whole figure expressed by RP) for analysis. The value of RP-dw tended to decrease by the inhalation of peppermint and sweet orange in persons who liked the smell. And the value of TPM ave. was significantly decreased by the inhalation of sweet orange in persons who disliked the smell. The results suggest that the inhalation of aroma make the circulatory homeostasis more stable in persons who like the smell. On the other hand, it is considered that an unpleasant smell has simplified the blood stream by making the functional stiffness of the vessel wall decrease.

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