[*Phosphorus, Sulfur, and Silicon*, **180**, 989-992 (2005)] [Lab. of Pharm. Chemistry]

Chalcogeno-Morita-Baylis-Hillman Reaction of Chalcogenide-Enones with Carbonyl Compounds

Tadashi KATAOKA* and Hironori KINOSHITA

The chalcogeno-Morita-Baylis-Hillman reaction was achieved by the reactions of 2-(methylchalcogeno)phenyl vinyl ketones with carbonyl compounds or acetals in the presence of $BF_3 \cdot Et_2O$. This reaction proceeds via the intramolecular Michael addition of the chalcogenide group to an enone moiety followed by the aldol reaction of the resulting chalcogenonio-enolate with an aldehyde. The reactions were worked up with triethylamine or saturated aqueous $NaHCO_3$ to give the α-methylene aldols (the Morita-Baylis-Hillman adducts).

[*Tetrahedron Lett.*, **46**, 7155-7158 (2005)] [Lab. of Pharm. Chemistry]

Synthesis of 3-Sulfanylpropanols Containing Three Consecutive Stereocenters via Tandem Michael-Aldol Reaction of Enoylthioamides with Acetals as Key Reaction

Hironori KINOSHITA, Natsuko TAKAHASHI, Tatsunori IWAMURA, Shin-ichi WATANABE, Tadashi KATAOKA,* Osamu MURAOKA and Genzoh TANABE

(*2S,3S,1'R*)-2-(α-Methoxybenzyl)-3-phenyl-3-sulfanylpropionimides were diastereoselectively prepared by the reactions of *N*-cinnamoyl-4*S*-isopropyl-5,5-dimethyloxazolidinethione with acetals in the presence of $SnCl_4$. The absolute configuration of three contiguous stereocenters newly created was determined by the X-ray analysis of the disulfide. The imides were transformed into propanols by the reductive removal of the oxazolidinone moiety.

[*J. Health Sci.*, **51**, 325-332 (2005)] [Lab. of Pharm. Chemistry]

Substrate Specificity of Opioid Compounds to UDP-Glucuronosyltransferase (UGT), *h*UGT2B7 and Bovine Microsomal UGT.

Tatsunori IWAMURA* Yuko ITO, Nayumi KUNO and Takaharu MIZUTANI

We studied the substrate specificity of some opioid derivatives of 5,9-dimethyl-2'-hydroxybenzomorphan (**1**) for human UDP-glucuronosyltransferase 2B7 (*h*UGT2B7), a typical glucuronidation enzyme to morphine and for bovine microsomal UGT. *h*UGT2B7 reacted with those having longer alkyl substituents of more than 3 carbon chains. Substances with alkenyl and isobutyl substituents are the best substrates (the Km value, 15 μM and 25 μM, respectively). Opioids with alkynyl and aralkyl hydrocarbon substituents are of low affinity (the Km value, 119 μM and 542 μM, respectively). Meanwhile, bovine UGT reacted well with opioid substances with alkenyl and alkynyl substituents on the same level as alkyl substituents. Thus, a clear difference between human UGT2B7 and bovine microsomal UGT was found in the reactivity of alkynyl group and this comes from species specificity.

[*Eur. J. Org. Chem.*, 1493-1496 (2005)] [Lab. of Pharm. Chemistry]

The Formation of Cyclopropane Derivatives Bearing 1,2-Dicarbonyl Groups through Tandem Michael-Favorskii-Type Reactions with (*E*)-β-Styrylselenonium Triflate.

Shin-ichi WATANABE, Ippei NAKAYAMA and Tadashi KATAOKA*

A novel tandem Michael-Favorskii-type reaction is described. Treatment of active methylene carbanions, prepared by the reaction of sodium hydride and active methylene compounds, with (*E*)-β-styrylselenonium triflate in DMF at 70°C for 3 hours gave cyclopropane derivatives bearing 1,2-dicarbonyl groups in moderate to good yields through tandem Michael-Favorskii rearrangement. On the other hand, the carbanions derived from malonates reacted with the selenonium salt to afford 1,1-dicarbonyl cyclopropane compounds in good yields.

[*Tetrahedron*, **61**, 2217-2231 (2005)] [Lab. of Medicinal Chemistry]

Highly chemoselective hydrogenation method using novel finely dispersed palladium catalyst on silk-fibroin: its preparation and activity

Takashi IKAWA, Hironao SAJIKI* and Kosaku HIROTA

A palladium-fibroin complex (Pd/Fib) was prepared by soaking silk-fibroin in MeOH solution of $Pd(OAc)_2$ for 2 days (under Ar atmosphere)-4 days (under air). $Pd(OAc)_2$ was gradually absorbed by fibroin and the rapid reduction of fibroin conjugated $Pd(OAc)_2$ proceeded with MeOH as a reductant at room temperature to be the Pd(0) complex. Pd/Fib catalyzed chemoselective hydrogenation of acetylenes, olefins and azides in the presence of aromatic ketones and aldehydes, halides, *N*-Cbz protective groups and benzyl esters which are readily hydrogenated using Pd/C or Pd/C(en) as a catalyst.

[*Org. Process Res. Dev.*, **9**, 219-220 (2005)] [Lab. of Medicinal Chemistry]

A practical and one-pot procedure for the synthesis of 3-amino-2-cyclohexen-1-one from 3-aminophenol

Hironao SAJIKI,* Takashi IKAWA and Kosaku HIROTA

A simple and totally catalytic process for the synthesis of 3-amino-2-cyclohexen-1-one (**1**) has been demonstrated. The mild and neutral conditions, quantitative yield of **1**, operational simplicity, easy availability, and low cost of the reagents make this process a more useful and practical alternative to the existing methods for the preparation of 3-amino-2-cyclohexen-1-one. The waste disposal of the process was greatly diminished compared with conventional methods.

[*Synlett*, 1046 (errata for 619-622) (2005)] [Lab. of Medicinal Chemistry]

Easy copper-, ligand- and amine-free Sonogashira coupling reaction catalyzed by palladium on carbon at low catalyst loading and by exposure to air

Hironao SAJIKI,* Guolin ZHANG, Yoshiaki KITAMURA Tomohiro MAEGAWA and Kosaku HIROTA

A copper-, ligand- and amine-free Sonogashira coupling reaction catalyzed by commercially available palladium on carbon with low loading (0.2 mol% Pd) has been developed. Aryl iodides were coupled with aromatic alkynes to give good to excellent yields and with aliphatic alkyne to give moderate to good yields. The reaction system is easy to handle (stability to air and moisture, no inert atmosphere and no ligands are necessary).

[*J. Chem. Res.*, 344 (errata for 593-595, 2004) (2005)] [Lab. of Medicinal Chemistry]

Ligand-free Suzuki–Miyaura reaction catalysed by Pd/C at room temperature

Hironao SAJIKI,* Takanori KURITA, Atsushi KOZAKI, Guolin ZHANG, Yoshiaki KITAMURA, Tomohiro MAEGAWA and Kosaku HIROTA

A ligand-free Suzuki-Miyaura coupling reaction has been developed which utilises a commercially available Pd/C in 2-propanol-H_2O (1/1) and Na_3PO_4 at room temperature. The reaction is mild and generates excellent yields of the coupled products. A substrate with a base sensitive group was tolerated. The catalyst is heterogeneous, ligand-free and can be recycled using a simple filtration. The product isolation is simple.

[*Synthesis*, 852 (errata for 537-542) (2005)] [Lab. of Medicinal Chemistry]

Efficient protocol for the phosphine-free Suzuki–Miyaura reaction catalyzed by palladium on carbon at room temperature

Hironao SAJIKI,* Takanori KURITA, Atsushi KOZAKI, Guolin ZHANG, Yoshiaki KITAMURA, Tomohiro MAEGAWA and Kosaku HIROTA

A mild and efficient protocol for the phosphine-free Suzuki–Miyaura coupling reaction of aryl bromides with arylboronic acids has been developed which utilizes the commercially available 10% Pd/C (3.5 mol% Pd) in ethanol–water (1:1) and Na_2CO_3 at room temperature. The reaction is convenient, environmentally benign and generates excellent yields of the coupled products (94–100%). The catalyst can be recycled using simple filtration and washing sequences without significant decrease in the yield of coupling product up to the fourth run.

[*Synlett*, 1385-1388 (2005)] [Lab. of Medicinal Chemistry]

Palladium-catalyzed base-selective H-D exchange reaction into nucleosides in deuterium oxide

Hironao SAJIKI,* Hiroyoshi ESAKI, Fumiyo AOKI, Tomohiro MAEGAWA and Kosaku HIROTA

We have developed an efficient and extensive deuterium incorporation method using a heterogeneous Pd/C-D_2O-H_2 system into the base moiety of nucleosides. The results presented here provide a deuterium gas-free, totally catalytic and post-synthetic deuterium labeling method in D_2O media. The present D_2 gas-free and selective H-D exchange reaction retains sufficient usefulness in nucleic acid chemistry. It discloses a convenient route to the post-synthetic introduction of deuterium atoms into the base moiety of nucleosides with high deuterium efficiency under neutral reaction conditions. Studies to further elucidate the scope of this incorporation method are currently underway.

[*Tetrahedron*, **61**, 8499-8504 (2005)] [Lab. of Medicinal Chemistry]

3'-Selective modification of 4',5'-didehydro-5'-deoxy-2',3'-epoxyuridine using nucleophiles

Hideki TAKASU, Yoshie TSUJI, Hironao SAJIKI* and Kosaku HIROTA

1-(2,3-Anhydro-5-deoxy-4,5-didehydro-α-L-*erythro*-pent-4-enofuranosyl)uracil **4** was obtained by the treatment of 5'-iodo-2',3'-epoxyuridine **5** with LiHMDS in excellent yield. The pyrimidine nucleoside **4** possesses quite unique vinyl epoxide moiety within the molecules. The reactions of **4** with a variety of nucleophiles gave 3'-substituted pyrimidine nucleosides without the formation of the corresponding 2'-substituted isomers. In the case of NaN_3 or PhSH, the corresponding 5'-adduct was obtained as a minor product together with the expected 3'-adduct.

[*Tetrahedron*, **61**, 11027-11031 (2005)] [Lab. of Medicinal Chemistry]

Rearrangement of allylic azide and phenylthio group of 3'-azido- or 3'-phenylthio-4',5'-didehydro-4',5'-dideoxyarabinofuranosyluridines

Hideki TAKASU, Yoshie TSUJI, Hironao SAJIKI* and Kosaku HIROTA

The reversible intramolecular [3,3]-sigmatropic rearrangement between 1-(3-azido-3,5-dideoxy-β-D-*threo*-pent-4-enofuranosyl)uracil (**3**) and 1-(5-azido-3,5-dideoxy-β-D-*glycero*-pent-4-enofuranosyl)uracil (**4**) and irreversible radical rearrangement of 1-(3,5-dideoxy-3-phenylthio-β-D-*threo*-pent-4-enofuranosyl)uracil (**5**) and 1-[3,5-dideoxy-3-(4-tolyl)thio-β-D-*threo*-pent-4-enofuranosyl]uracil (**7**) into 1-(3,5-dideoxy-5-phenylthio-β-D-*glycero*-pent-4-enofuranosyl)uracil (**6**) and 1-[3,5-dideoxy-5-(4-tolyl)thio-β-L-*glycero*-pent-3-enofuranosyl]uracil (**8**) were attained at room temperature.

[*Tetrahedron Lett.*, **46**, 6995-6998 (2005)] [Lab. of Medicinal Chemistry]

Aromatic ring favorable and efficient H-D exchange reaction catalyzed by Pt/C

Hironao SAJIKI,* Nobuhiro ITO, Hiroyoshi ESAKI, Tsuneaki MAESAWA,
Tomohiro MAEGAWA and Kosaku HIROTA

An effective and applicable Pt/C-catalyzed deuteration method of aromatic rings using D_2O as a deuterium source under hydrogen atmosphere was developed. 5% Pt/C would lead to quite effective H-D exchange results on the aromatic ring systems. The reaction is general for a variety of aromatic compounds including biologically active compounds.

[*Heterocycles*, **65**, 2991-2999 (2005)] [Lab. of Medicinal Chemistry]

Chemincal modification of the sugar moiety of pyrimidine nucleosides *via* a 4',5'-epoxyuridine intermediate

Hideki TAKASU, Hironao SAJIKI* and Kosaku HIROTA

The reaction of 4',5'-unsaturated nucleoside (**5**) with *m*-CPBA provided different products depending on the solvent. 4',5'-Epoxynucleoside (**6**) was generated as a key intermediate although **6** was not stable enough to isolate and further reaction progressed. When the reaction was performed in CH_2Cl_2, 2,4'-cyclonucleoside (**7**) and 4'-ketonucleoside (**8**) were obtained. On the other hand, 4'-alkoxy derivatives (**9** and **11**) together with their epimers (**10** and **12**) were aquired as a mixture by the nucleophilic attack of alcohol.

[*Heterocycles*, **66**, 361-369 (2005)] [Lab. of Medicinal Chemistry]

Synthesis of base-selectively deuterium-labelled nucleosides by the Pd/C-catalyzed H-D exchange reaction in deuterium oxide

Hiroyoshi ESAKI, Fumiyo AOKI, Tomohiro MAEGAWA , Kosaku HIROTA and Hironao SAJIKI*

An efficient base selective H-D exchange reaction can be achieved with the Pd/C-H_2-D_2O system under heating conditions. This deuteration method does not require the use of D_2 gas and special apparatus, and is applicable to a variety of nucleic acids under neutral reaction conditions. The reactions are very clean and the products were obtained in excellent yields without chromatographic purification. This chemoselective deuterating method should contribute to extensive studies of nucleic acids chemistry.

[*J. Org, Chem.*, **70**, 10581-10583 (2005)] [Lab. of Medicinal Chemistry]

Facile and efficient post-synthetic tritium labelling method catalyzed by Pd/C in HTO

Tomohiro MAEGAWA, Kosaku HIROTA, Kenjiro TATEMATSU,
Yukio MORI and Hironao SAJIKI*

We have developed a facile and efficient tritiation method catalyzed by Pd/C or Pt/C using HTO as a tritium source in the presence of a catalytic amount of H_2 gas. The method presented here provides a T_2 gas-free, and totally post-synthetic tritium labelling method under neutral conditions without requiring the chromatographic purification. The reaction is applicable to various substrates and tolerates a wide range of different functional groups, such as amine, carboxylic acid, amide and so on. The multi-tritium incorporation provides tritium-labelled compounds with high specific activities using HTO possessing low specific activities. Accordingly, the presented reaction possesses potential to be an efficient, applicable and environmentally benign tritium labelling method.

[*Synlett*, 845-847 (2005)] [Lab. of Medicinal Chemistry]

Efficient and selective deuteration of phenylalanine derivatives catalyzed by Pd/C

Tomohiro MAEGAWA, Akira AKASHI, Hiroyoshi ESAKI, Fumiyo AOKI,
Hironao SAJIKI* and Kosaku HIROTA

We have developed a facile and efficient method for deuteration of phenylalanine derivatives using the Pd/C-H_2-D_2O system under neutral conditions. The deuteration sites can be controlled by the reaction temperature, and the specifically deuterium-labeled phenylalanine derivatives such as L-phenylalanine-β,β-d_2, L-phenylalanine-$4,\beta,\beta$-d_3 and phenylalanine-α,β,β-d_3 were provided in quantitative yields. Furthermore, the β-selective deuteration method does not cause racemization. Since D_2O is the cheapest deuterium-labeled compound, the present method provides inexpensive and readily available deuterium-labeled aromatic amino acids.

[*Synlett*, 2107—2109 (2005)] [Lab. of Pharm. Synthetic Chemistry]

Aerobic Photooxidation of Methyl Group at Aromatic Nucleus with LiBr

Akichika Itoh,* Shouei Hashimoto, Tomohiro Kodama,
Yukio Masaki

A methyl group at the aromatic nucleus was found to be oxidized to the corresponding carboxylic acid directly in the presence of lithium bromide under aerobic photoirradiation.

[*Synlett*, 2639—2640 (2005)] [Lab. of Pharm.Synthetic Chemistry]

Facile Aerobic Photooxidation of Alcohols in the Presence of Catalytic Lithium Bromide

Akichika Itoh,* Shouei Hashimoto, Yukio Masaki

Alcohols were found to be oxidized to the corresponding carboxylic acids in the presence of catalytic lithium bromide under photoirradiation.

[*Green Chem.*, **7**, 830—832 (2005)] [Lab. of Pharm. Synthetic Chemistry]

Facile Solar Oxidation of Alcohols with Molecular Oxygen

Akichika Itoh,* Shouei Hashimoto, Kiyoto Kuwabara,
Tomohiro Kodama, Yukio Masaki

A useful method for the aerobic oxidation of alcohols directly to the corresponding carboxylic acid or ketone with combination of sodium bromide and Amberlyst 15 (safe and inexpensive reagents) under solar radiation is reported.

[*J. Photopolym. Sci. Technol.*, **18**, 281-284 (2005)] [Lab. of Pharm. Physical Chemistry]

Development of Drug Delivery System by Atmospheric Pressure Glow Plasma.

Yasushi SASAI, Shin-ichi KONDO, Masaki Nagato, and Masayuki KUZUYA*

In this communication, we report an extended work of the development of the DDS by the atmospheric pressure glow (APG) plasma. This plasma techniques is a new plasma-irradiation method and it is different from the cold plasma which is homogeneous glow discharge. These results indicate that APG plasma irradiation to the surface of double-compressed tablet having a mixture of Eudragit L100-55 and Eudragit RS as outer layer leads to the formation of time-controlled release systems with the lag-time. The lag-time can readily be controlled by plasma operational conditions, similarly in the case of low pressure-cold plasma as reported previously.

[*Yakugakuzasshi*, **125**, 389-396 (2005)] [Lab. of Pharm. Physical Chemistry]

Radical Formation by Grinding of Commercial Tablets according to Hospital and Pharmacy Prescription.

Masayuki KUZUYA,* Shin-ichi KONDO, Takaaki ISHIKAWA, Youji FURUTA, Hideki ARAMAKI, Yasushi SASAI, and Yukinori YAMAUCHI

We examined mechanoradical formation in the grinding process of commercial tablets using electron spin resonance (ESR). Mechanoradicals were detected in all tested samples (23 types of commercial tablets) when the ball-milling of tablets was conducted under anaerobic conditions and some were fairly stable even in air. Thus the grinding may cause changes in the physicochemical properties of ingredients included in commercial tablets. Because high quality is demanded in pharmaceuticals, these results suggest more caution should be taken in the grinding of commercial tablets in hospitals and pharmacies.

[*Chem. Pharm. Bull.*, **53**, 863-865 (2005)] [Lab. of Pharm. Physical Chemistry]

Synthesis of DNA Conjugate by Mechanochemical Solid-State Polymerization and Its Affinity Separation of Oligonucleotides Having Single-Base Difference by Capillary Electrophoresis.

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

In this communication, we discuss the characterization of DNA conjugate synthesized by mechanochemical polymerization, Con-M, on the separation of model oligo-DNA and its single nucleotide polymorphisms (SNPs) by affinity capillary electrophoresis, compared with that prepared by radical-initiated solution polymerization, Con-RL. The average molecular weight of Con-M was similar to that of Con-RL, although the molecular weight distribution of Con-M was narrower than that of Con-RL. Capillary electrophoresis of oligo-DNA was performed using the capillary filled with DNA conjugate. The resolution of the capillary filled with Con-M was apparently higher than with Con-RL. It is considered that higher resolution using the capillary filled with Con-M could be ascribed not only to the narrow molecular weight distribution but also to the difference of copolymer structure.

[*Chem. Pharm. Bull.*, **53**(5), 487-491 (2005)] [Lab. of Pharm. Engineering]

Tabletting of Solid Dispersion Particles Consisting of Indomethacin and Porous Silica Particles.

Hirofumi TAKEUCHI*, Shinsuke NAGIRA, Shinji TANIMURA, Hiromitsu YAMAMOTO and Yoshiaki KAWASHIMA

We attempted to make the rapidly dissolving tablet (Tab) containing solid dispersion particles (SD) with indomethacin (IMC) and porous silica (Sylysia350) as carrier prepared by using spray-drying technique. Rapidly dissolving tablet was formulated with mannitol as a diluent and low substituted hydroxypropylcellulose (L-HPC) or partly pre-gelatinized starch (PCS) as a disintegrant. The percent dissolved from Tab (SD) was higher than that of tablet containing physical mixture (PM) at 20 min. Nearly 100% of drug in Tab (SD) was dissolved within 60 min, while the drug dissolution of Tab (PM) was not completed at the same time period. In addition, the tensile strength of Tab (SD) was much higher than that of Tab (PM).

[*Int. J. Pharm.*, **293**, 155-164 (2005)] [Lab. of Pharm. Engineering]

Solid Dispersion Particles of Amorphous Indomethacin with Fine Porous Silica Particles by Using Spray-drying Method.

Hirofumi TAKEUCHI*, Shinsuke NAGIRA, Hiromitsu YAMAMOTO and Yoshiaki KAWASHIMA

The solid dispersion particles of indomethacin (IMC) were prepared with different types of silica, non-porous (Aerosil 200) or porous silica (Sylysia 350) by using spray-drying method. Powder X-ray diffraction analysis showed that IMC in solid dispersion particles is in amorphous state irrespective of the type of silica formulated. In DSC analysis, the melting peak of IMC in solid dispersion particles with Sylysia 350 shifted to lower temperature than that in solid dispersion particles with Aerosil 200 although the peak of each solid dispersion particles was much smaller than that of original IMC crystals. Dissolution property of IMC was remarkably improved by formulating the silica particles to the solid dispersion particles. In comparing the effect of the type of the silica particles, the dissolution rate of solid dispersion particles with Sylysia 350 was faster than that with Aerosil 200.

[*J. Drug. Del. Sci. Tech.*, **15**(2), 177-182 (2005)] [Lab. of Pharm. Engineering]

Effect of Lubrication on the Compaction Properties of Pharmaceutical Excipients as Measured by Die Wall Pressure.

Hirofumi TAKEUCHI*, Shinsuke NAGIRA, Motokazu AIKAWA,
Hiromitsu YAMAMOTO and Yoshiaki KAWASHIMA.

The effects of lubrication on the compaction properties of pharmaceutical powders were evaluated by measuring several compaction parameters, including maximum die wall pressure (MDP), residual die wall pressure (RDP), and RDP/MDP, which is a novel parameter for evaluating the compaction properties of materials. In tableting model powders lactose and mannitol, a higher RDP/MDP and a tendency toward capping were observed. The addition of a small amount of internal lubricant to either powder decreased RDP/MDP and inhibited the capping tendency. When external lubrication was applied to the powders, similar improvements in these compaction properties were observed. However, the tensile strength of the mannitol tablet prepared under external lubrication conditions was much greater than that of the tablet prepared with 0.5%internal lubricant.

[*Adv.Drug Deliv. Rev.*, **57**, 1583-1594 (2005)] [Lab. of Pharm. Engineering]

Novel mucoadhesion tests for polymers and polymer-coated particles to design optimal mucoadhesive drug delivery systems.

Hirofumi TAKEUCHI*, Jringjai THONGBORISUTE, Yuji MATSUI, Hikaru SUGIHARA,
Hiromitsu YAMAMOTO and Yoshiaki KAWASHIMA

To design an effective particulate drug delivery system having mucoadhesive function, several mucoadhesion tests for polymers and the resultant particulate systems were developed. Mucin particle method is a simple mucoadhesion test for polymers, in which the commercial mucin particles are used. By measuring the change in particle size or zeta potential of the mucin particle in a certain concentration of polymer solution, we could estimate the extent of their mucoadhesive property. BIACORE method is also a novel mucoadhesion test for polymers. On passing through the mucin suspension on the polymer-immobilized chip of BIACORE instrument, the interaction was quantitatively evaluated with the change in its response diagram.

[*Int.J.Pharm.*, **303**, 160-170 (2005)] [Lab. of Pharm. Engineering]

Effectiveness of submicron-sized, chitosan-coated liposomes in oral administration of peptide drugs.

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

The mucoadhesive behavior of chitosan-coated liposomes in the intestinal tract of the rat was examined to elucidate their particle size effects on the absorption of an entrapped drug, calcitonin. The intestine was removed from rats after oral administration of liposomes containing a fluorescent dye, and its various parts were observed with confocal laser scanning microscopy. Penetration of submicron-sized liposomes (ssLip) or chitosan-coated ssLip (ssCS-Lip) into the mucosa was observed, while such behavior was not observed for the multilamellar liposomes, even when coated with chitosan (CS-Lip). The retentive property of ssCS-Lip was confirmed by measuring the amount of dye in each part of the intestine. The pharmacologic effects of calcitonin-loaded liposomes of different particle size were measured after oral administration in rats. The pharmacologic effect of oral administration of ssLip coated with chitosan was detected up to 120 h after administration.

[*J. Pharmacol. Exp. Ther.*, **315**, 196-202 (2005)] [Lab. of Pharm. Engineering]

Nanoparticles enhance therapeutic efficiency by selectively increased local drug dose in experimental colitis in rats.

Alf LAMPRECHT, Hiromitsu YAMAMOTO, Hirofumi TAKEUCHI* and Yoshiaki KAWASHIMA

Nanoparticles (NP) are proposed for targeted drug delivery to the inflammation site in severe cases of inflammatory bowel disease where state-of-the-art delivery devices fail. FK506 (tacrolimus) entrapped into NP was administered either orally or rectally to male Wistar rats suffering from a preexisting experimental colitis. Clinical activity score, colon/body weight index, and myeloperoxidase activity were determined to assess the inflammation. The myeloperoxidase activity and colon/body weight ratio decreased significantly ($P < 0.05$) only after the rectal administration of FK506-NP. The relative drug penetration into the inflamed tissue is about 3-fold higher compared with healthy tissue when using NP as drug carriers.

[*J. Control. Release.*, **18**, 337-346 (2005)] [Lab. of Pharm. Engineering]

A pH-sensitive microsphere system for the colon delivery of tacrolimus containing nanoparticles.

Alf LAMPRECHT, Hiromitsu YAMAMOTO, Hirofumi TAKEUCHI* and Yoshiaki KAWASHIMA

Nanoparticles (NP) are known to accumulate at the site of inflammation in inflammatory bowel disease. In order to avoid premature uptake or degradation of NP during their passage through the small intestine, it appeared necessary to devise a form of local delivery system for NP. Tacrolimus (FK506) loaded poly(lactic-co-glycolic acid) NP entrapped into pH-sensitive microspheres (NPMS) were designed to achieve greater selectivity to their site of action when administered orally. The in vitro characterization showed a successful incorporation of FK506-NP and strongly pH-sensitive release kinetics of both NP and drug. When observing colon/body weight index and myeloperoxidase activity, only the NPMS group reached statistically significant differences ($P<0.05$) compared to the colitis control group while other groups did not. The results suggest that the NPMS system can provide selective delivery of NP in the colon and develop a significant mitigating effect, while the control group treatments appeared to be insufficient.

[*Pharm. Research.*, **22**, 193-199 (2005)] [Lab. of Pharm. Engineering]

FK506 microparticles mitigate experimental colitis with minor renal calcineurin suppression.

Alf LAMPRECHT, Hiromitsu YAMAMOTO, Nathalie UBRICH, Hirofumi TAKEUCHI*, Philippe MAINCENT and Yoshiaki KAWASHIMA

FK506 microparticles providing selective colonic drug delivery were tested for their efficiency in a local treatment to the inflamed gut tissue in inflammatory bowel disease (IBD). FK506 was entrapped into microspheres (MS) prepared with the pH-sensitive polymer Eudragit P-4135F in order to allow drug delivery to the colon. The clinical activity score and myeloperoxidase activity decreased after the administration of all FK506-containing formulations. The MS formulations proved to be as efficient in mitigating the experimental colitis as the subcutaneous drug solution and to be superior to drug solution given by oral route. The development of this selective delivery system for FK506 should be given particular consideration in the treatment of IBD, as it allows therapy that profits from FK506's high immune suppressive effect with a simultaneously reduced nephrotoxicity.

[*Eur. J. Pharm. Biopharm.*, **59**, 367-371 (2005)] [Lab. of Pharm. Engineering]

Observations in simultaneous microencapsulation of 5-fluorouracil and leucovorin for combined pH-dependent release.

Alf LAMPRECHT, Hiromitsu YAMAMOTO, Hirofumi TAKEUCHI* and Yoshiaki KAWASHIMA

5-Fluorouracil (5-FU) in combination with leucovorin (LV) is nowadays the standard treatment in colon cancer. Eudragit P-4135F or Eudragit RS100 were used separately to prepare microspheres by an oil/oil emulsification process. Generally, higher encapsulation rates were found with RS100 compared to P-4135F. Microparticles made from Eudragit RS100 released the incorporated drug combination within 8 h not exhibiting general differences between the kinetics of both drugs. P-4135F was found to maintain the undesired 5-FU release at pH 6.8 lower than 25% within 4 h while at pH 7.4. These observations were concluded to be related to the high lipophilicity of P-4135F provoking a separation between P-4135F and LV during the preparation process.

[*J. Control. Release.*, **102**, 373-381 (2005)] [Lab. of Pharm. Engineering]

Surface-modified PLGA nanosphere with chitosan improved pulmonary delivery of calcitonin by mucoadhesion and opening of the intercellular tight junctions.

Hiromitsu YAMAMOTO*, Yoshio KUNO, Shohei SUGIMOTO, Hirofumi TAKEUCHI and Yoshiaki KAWASHIMA

Surface-modified DL-lactide/glycolide copolymer (PLGA) nanospheres with chitosan (CS) were prepared by the emulsion solvent diffusion method for pulmonary delivery of peptide. After pulmonary administration, CS-modified PLGA nanospheres were more slowly eliminated from the lungs than unmodified PLGA nanospheres. CS-modified PLGA nanospheres loaded with elcatonin reduced blood calcium levels to 80% of the initial calcium concentration and prolonged the pharmacological action to 24 h, which was a significantly longer duration of action than that by CS-unmodified nanospheres. CS and CS on the surface of the nanospheres enhanced the absorption of drug.

[*Biol. Pharm. Bull.*, **28**, 1148-1153 (2005)] [Lab. of Pharmaceutics]

Identification of Differentially Expressed Genes in Hepatic HepG2 cells Treated with Acetaminophen Using Suppression Subtractive Hybridization.

Kazuhiro IGUCHI, Yukari TAKAHASHI, Yoko KANETO, Masafumi KUBOTA, Shigeyuki USUI, and Kazuyuki HIRANO

To clarify the mechanism of Acetaminophen (APAP)-related liver damage, we attempted the identification of the differential gene expression in response to APAP treatment in hepatic HepG2 cells. In the present study, we used the technique of suppression subtractive hybridization for the identification of the differentially expressed genes between untreated and treated cells and identified 14 candidate genes showing increased expression in response to APAP treatment. RT-PCR and real-time RT-PCR analysis confirmed that the expression of two genes was increased within 24 h following APAP treatment. Among them, only lysyl hydroxylase 2 expression was increased in a time- and dose-dependent manner. Since lysyl hydroxylase 2 is known to be a key enzyme of liver fibrosis, the increased expression of lysyl hydroxylase 2 may be involved in hepatotoxins-related liver fibrosis.

[*J. Jpn. Pharm. Associ.*, **57**, 823-826 (2005)] [Lab. of Pharmaceutics]

Prevention of Bacterial Contamination on Preservative-free Multi-Dose Eye Drops: Methods and Validation.

Masabumi KUBOTA, Yuko HIRANO, Eiji TAKASHIMA, Kazuhiro IGUCHI, Shigeyuki USUI, Teruo TSUCHIYA, Tetsuo ADACHI, and Kazuyuki HIRANO*

In order to encourage the proper use of preservative-free multi-dose eye drops, patients need to pay attention to the handling of the medicines for preventing the bacterial contamination in the instruction on the usage of the medicines. We examined how to avoid the bacterial contamination in use of these medicines. The disposal operation that the first and second drops were discarded before dropping to the affected eye was confirmed to be very effective as a means for the alleviation of the bacterial contamination if the tip of the dropper might be contaminated by touching with the affected area. This method was also effective even if the contamination reached to the inner membrane filter.

[*Clin. Chim. Acta*, **358**, 139-145 (2005)] [Lab. of Pharmaceutics]

Effect of Buformin and Metformin on Formation of Advanced Glycation End Products by Methylglyoxal.

Tadashi KIHO, Motohiro KATO, Shigeyuki USUI, and Kazuyuki HIRANO*

The formation and accumulation of advanced glycation end products (AGE) in various tissues are known to be involved in the aging process and complications of long-term diabetes. Aminoguanidine as AGE inhibitors was first studied, and metformin as biguanide compounds have been reported to react with reactive dicarbonyl precursors such as methylglyoxal. Buformin is a more potent inhibitor of AGE formation than metformin, and suggets that the amino group of buformin trap the carbonyl group of methylglyoxal to suppress formation of AGE. In addition to that of metformin, buformin may clinically useful to prevent diabetic complications.

[*Analytica Chimica Acta*, **531**, 79-86 (2005)] [Lab. of Hygienics]

The High Throughput Analysis of N-methyl Carbamate Pesticides in Wine and Juice by Elrctrospray Ionization Liquid Chromatography Tandem Mass Spectrometry with Direct Sample Injection into a Short Column.

Tomomi GOTO, Yuko ITO, Hisao OKA, Isao SAITO, Hiroshi MATUMOTO, Hideo SUGIYAMA, Chiyoji OHKUBO, Hiroyuki NAKAZAWA and Hisamitsu NAGASE *

We developed a new analysis method for the N-methy] carbamate pesticides in juice and wine. The juice and wine were diluted with ultra pure water, and determined by electrospray ionization tandem mass spectrometry (ESI LC/MS/MS) with direct sample injection into a short column. The new method, including sample preparation and determination, is simple and rapid, and allows simultaneous determination of nine N-methyl carbamate pesticides in juice and wine within analysis time that is much shorter as compared with the traditional method. The method is considered to be satisfactory for the monitoring of the carbamate pesticides residues in juice and wine, suggesting that the present method is applicable to other pesticide residues in foods.

[*Exp. Biol. Med. (Maywood)*, **230**, 75-81 (2005)] [Lab. of Hygienics]

Role of Metallothionein in Antigen-related Airway Inflammation.

Ken-ichiro INOUE, Hirohisa TAKANO, Rie YANAGISAWA, Miho SAKURAI, Takamichi ICHINOSE, Kaori SADAKANE, Kyoko HIYOSHI, Masahiko SATO*, Akinori SHIMADA, Mamoru INOUE and Toshikazu YOSHIKAWA

We determined whether metallothionein (MT) protects against antigen-related airway inflammation induced by ovalbumin (OVA). Significant increases were shown in the numbers of total cells, eosinophils, and neutrophils in bronchoalveolar lavage fluid from MT-I/II null (MT [-/-]) mice than in those from wild-type (WT) mice after OVA challenge. The protein level of IL-1beta was significantly greater in MT (-/-) mice than in WT mice after OVA challenge. Immunohistochemical analysis showed that the formations of 8-oxy-deoxyguanosine and nitrotyrosine in the lung were more intense in MT (-/-) mice than in WT mice after OVA challenge. These results indicate that endogenous MT is a protective molecule against antigen-related airway inflammation induced by OVA, at least partly, via the suppression of enhanced lung expression of IL-1beta and via the antioxidative properties.

[*Int. J. Mol. Med.*, **15**, 221-224 (2005)] [Lab. of Hygienics]

Cytoprotection by Interleukin-6 Against Liver Injury Induced by Lipopolysaccharide.

Ken-ichiro INOUE, Hirohisa TAKANO, Akinori SHIMADA, Takehito MORITA, Rie YANAGISAWA, Miho SAKURAI, Masahiko SATO*, Shin YOSHINO and Toshikazu YOSHIKAWA

The present study elucidated the role of IL-6 in liver damage during severe inflammation induced by intraperitoneal administration of lipopolysaccharide (LPS; 1 mg/kg) using IL-6 null (-/-) mice. Histological study showed that LPS treatment caused more severe liver injury with centrilobular vacuolation of hepatocytes and neutrophilic infiltration in the liver of IL-6 (-/-) mice; in contrast, neutrophilic infiltration and mild vacuolar change of hepatocytes were found in the liver of LPS-treated wild-type (WT) mice. Protein levels of proinflammatory molecules, such as IL-1beta, MIP-1alpha, and MCP-1, in the livers were significantly greater in IL-6 (-/-) mice than in WT mice after LPS challenge. These results directly indicate that IL-6 is protective against liver injury induced by bacterial endotoxin, at least partly, via the modulation of proinflammatory cytokines and chemokines.

[*Toxicol. Lett.*, **155**, 361-368 (2005)] [Lab. of Hygienics]

Neurobehavioral Changes in Metallothionein-null Mice Prenatally Exposed to Mercury Vapor.

Minoru YOSHIDA, Chiho WATANABE, Kazuteru HORIE, Masahiko SATOH*, Masumi SAWADA and Akinori SHIMADA

We studied the neurobehavioral effects of prenatal exposure of MT-null and wild-type mice to elemental mercury vapor (Hg^0). Pregnant mice of both strains were repeatedly exposed to Hg^0 vapor at 0.50 and 0.56 mg/m^3 for 6 h/day until the 18th day of gestation. Hg^0-exposed MT-null mice (at 12 weeks of age) showed a significant decrease in total locomotor activity in males, and a learning disability in the passive avoidance response and a retarded acquisition in the Morris water maze in females as compared with the control. In contrast, Hg^0-exposed wild-type mice (at 12 weeks of age) did not differ from controls in the three behavioral measurements. The results indicate that MT-null mice would be more susceptible than wild-type mice to the behavioral neurotoxicity of prenatal Hg^0 exposure.

[*Biol. Pharm. Bull.*, **28**, 1859-1863 (2005)] [Lab. of Hygienics]

Induction of Hepatic Metallothionein by Trivalent Cerium: Role of Interleukin 6.

Kazuo KOBAYASHI, Rumi SHIDA, Tatsuya HASEGAWA, Masahiko SATOH*, Yoshiyuki SEKO, Chiharu TOHYAMA, Junji KURODA, Nobuo SHIBATA, Nobumasa IMURA ans Seiichiro HIMENO

We examined the induction of metallothionein (MT) synthesis by cerium, a trivalent lanthanoid metal. Administration of cerium chloride ($CeCl_3$) to mice resulted in accumulation of cerium and induction of MT in the liver in a dose-dependent manner. Distribution profiles of metals in the soluble fraction of the liver of $CeCl_3$-treated mice analyzed by HPLC/ICP-MS demonstrated that the metal bound to MT-I and MT-II was zinc, but not cerium. In order to evaluate the involvement of IL-6 in the induction of MT by cerium, we examined MT induction by $CeCl_3$ in IL-6 null mice. Both the induction of hepatic MT and the increases in serum amyloid A (SAA) levels were markedly suppressed in IL-6 null mice. These results suggest that IL-6 plays an important role in the induction of hepatic MT by cerium.

[*Exp. Toxicol. Pathol.*, **57**, 117-125 (2005)] [Lab. of Hygienics]

Localization and Role of Metallothioneins in the Olfactory Pathway After Exposure to Mercury Vapor.

Akinori SHIMADA, Yoko NAGAYAMA, Takehito MORITA, Minoru YOSHIDA, Junko S. SUZUKI, Masahiko SATOH* and Chiharu TOHYAMA

We have investigated the localization and physiological roles of metallothioneins (MTs) in the olfactory pathway after exposure to mercury (Hg^0) vapor using male MT-null mice. Light and electron microscopy of the samples stained with autometallography demonstrated chronological transfer of exposed mercury granules to the olfactory bulb by way of the olfactory tract. Basal expression of MT-I and -II immunoreactivity was observed in supporting cells, basal cells and acinar cells of the Bowman's gland of the olfactory mucosa in wild-type mice even without mercury exposure. In situ hybridization showed that signals for MT-III mRNA dominated in the olfactory cells of the olfactory mucosa, neurons in the olfactory bulb and those of brain in MT-null and wild-type mice. No difference in these findings was observed between samples taken at any interval after mercury exposure.

[*Proteins*, **60**, 424-432 (2005)] [Lab. of Biochemistry]

Structure of the Tetrameric Form of Human L-Xylulose Reductase: Probing the Inhibitor-Binding Site with Molecular Modeling and Site-Directed Mutagenesis.

Ossama EL-KABBANI, Vincenzo CARBONE, Connie DARMANIN, Syuhei ISHIKURA, and Akira HARA*

In this study we report the structure of the biological tetramer of human L-xylulose reductase (XR) in complex with $NADP^+$ and a competitive inhibitor solved at 2.3 Å resolution. The tetrameric structure of XR, which is held together *via* salt bridges formed by the guanidino group of Arg203 from one monomer and the carboxylate group of the C-terminal residue Cys244 from the neighboring monomer, explains the ability of XR in preventing the cold inactivation seen in the rodent forms of the enzyme. The orientations of Arg203 and Cys244 are maintained by a network of hydrogen bonds and main chain interactions of Gln137, Glu238, Phe241 and Trp242. Molecular modeling and site-directed mutagenesis identified the active site residues His146 and Trp191 as forming essential contacts with inhibitors of XR. These results could provide structural basis in the design of potent and specific inhibitors for human XR.

[*Bioorg. Med. Chem.*, **13**, 301-312 (2005)] [Lab. of Biochemistry]

Structure-Based Discovery of Human L- Xylulose Reductase Inhibitors from Database Screening and Molecular Docking.

Vincenzo CARBONE, Syuhei ISHIKURA, Akira HARA*, and Ossama EL-KABBANI

L-Xylulose Reductase (XR) is an enzyme of the glucuronic acid/uronate cycle of glucose metabolism and is a possible target for treatment of the long-term complications of diabetes. We utilised the molecular modelling program DOCK to analyse the 249,071 compounds of the National Cancer Institute Database and retrieved those compounds with high predicted affinity for XR. XR was inhibited by nicotinic acid (IC_{50} = 100 μM), benzoic acid (IC_{50} = 29 μM) and their derivatives. To optimize the interactions between the inhibitor and the holoenzyme, the program GRID was used to design *de novo* compounds based on the inhibitor benzoic acid. The inclusion of a hydroxy-phenyl group and a phosphate to the benzoic acid molecule increased the net binding energy 1.3- and 2.4-fold respectively.

[*J. Biol. Chem.*, **280**, 16319-16324 (2005)] [Lab. of Biochemistry]

Implication of Phospholipase D_2 in Oxidant-induced Phosphoinositide 3-Kinase Signaling via Pyk2 Activation in PC12 Cells.

Yoshiko BANNO, Kenji OHGUCHI, Naoki MATSUMOTO, Masahiro KODA, Masashi UEDA, Akira HARA*, Ivan DIKIC and Yoshinori NOZAWA

The role of phospholipase D (PLD) activation in H_2O_2-induced signal transduction and cellular responses is not completely understood. Here we present evidence that Ca^{2+}-dependent tyrosine kinase, Pyk2, requires PLD activation to mediate survival pathways in rat pheochromocytoma PC12 cells under oxidative stress. The H_2O_2-induced phosphorylation of two Pyk2 sites was suppressed by 1-butanol, an inhibitor of transphosphatidylation by PLD, and also by transfection of catalytically negative mouse PLD2K758R (PLD2KR). PLD2 was associated with Pyk2 and Src, and the activation of PLD2 was required for H_2O_2-enhanced association of Src with Pyk2 leading to full activation of Pyk2. H_2O_2-induced phosphorylation of Akt and p70S6K was dependent on phosphatidylinositol 3-kinase (PI3K) activity and was abolished by 1-butanol but not t-butanol. The PI3K/Akt activation in response to H_2O_2 was reduced by transfection of either PLD2KR or the dominant negative Pyk2DN.

[*Biol. Pharm. Bull.*, **28**, 1075-1078 (2005)] [Lab. of Biochemistry]

Enzymatic Properties of a Member (AKR1C19) of the Aldo-Keto Reductase Family.

Syuhei ISHIKURA, Kenji HORIE, Masaharu SANAI, Kengo MATSUMOTO, and Akira HARA*

The recombinant AKR1C19, a member of the aldo-keto reductase (AKR) superfamily, was expressed and purified to homogeneity. The enzyme was a 36-kDa monomer, and reduced α-dicarbonyl compounds using both NADH and NADPH as the coenzymes. Although apparent kinetic constants for the two coenzymes were similar, the NADPH-linked activity was potently inhibited by NAD^+, but the inhibition of the NADH-linked activity was not significant. AKR1C19 slowly oxidized some xenobiotic alcohols, but was inactive towards steroids, prostaglandins, monosaccharides. In addition, the enzyme was inhibited only by dicumarol, lithocholic acid and genistein of various compounds tested. Thus, AKR1C19 possesses properties distinct from other members of the AKR superfamily.

[*J. Biochem. (Tokyo)*, **137**, 303-314 (2005)] [Lab. of Biochemistry]

Structural and Functional Characterization of Rabbit and Human L-Gulonate 3-Dehydrogenase.

Syuhei ISHIKURA, Noriyuki USAMI, Mayuko ARAKI, and Akira HARA*

L-Gulonate 3-dehydrogenase (GDH) catalyzes the NAD^+-linked dehydrogenation of L-gulonate into dehydro-L-gulonate in the uronate cycle. We isolated the enzyme and its cDNA from rabbit liver, and found that the cDNA is identical to that for rabbit lens λ-crystallin. In addition, recombinant human λ-crystallin displays enzymatic properties similar to rabbit GDH. These data indicate that GDH is recruited as λ-crystallin without gene duplication. An outstanding feature of GDH is modulation of its activity by low concentrations of Pi, which decreases the catalytic efficiency in a dose dependent manner. Pi also protects the enzyme against both thermal and urea denaturation. Kinetic analysis suggests that Pi binds to both the free enzyme and its NAD(H)-complex in the sequential ordered mechanism. Furthermore, we suggested a role of Asp36 in the coenzyme specificity of GDH and those of Ser124, His145 and Asn196 in the catalytic function of the enzyme by site-directed mutagenesis of these residues.

[*Acta. Cryst.* F**61**, 688-690 (2005)] [Lab. of Biochemistry]

Crystallization and Preliminary X-ray Diffraction Analysis of Mouse 3(17)α-Hydroxysteroid Dehydrogenase

Ossama EL-KABBANI, Syuhei ISHIKURA, Armin WAGNER, Clemens SCHULZE-BRIESE, Akira HARA*

The 3(17)α-hydroxysteroid dehydrogenase from mouse is involved in the metabolism of estrogens, androgens, neurosteroids and xenobiotic compounds. The enzyme was crystallized by the hanging-drop vapour-diffusion method in space group $P222_1$, with unit-cell parameters a = 84.91, b = 84.90, c = 95.83 Å. The Matthews coefficient (V_M) and the solvent content were 2.21 $Å^3$ Da^{-1} and 44.6%, respectively, assuming the presence of two molecules in the asymmetric unit. Diffraction data were collected to a resolution of 1.8 Å at the Swiss Light Source beamline X06SA using a MAR CCD area detector and gave a data set with an overall R_{merge} of 6.8% and a completeness of 91.1%.

[*FEBS J.*, **272**, 2477-2486 (2005)] [Lab. of Biochemistry]

Characterization of Structural and Catalytic Differences in Rat Intestinal Alkaline Phosphatase Isozymes.

Tsuyoshi HARADA, Iwao KOYAMA, Toshiyuki MATSUNAGA*, Akira KIKUNO, Toshihiko KASAHARA, Masatoshi HASSIMOTO, David H. ALPERS, and Tsugikazu KOMODA

The crystal structures for rat intestinal alkaline phosphatase isozymes, rIAP-I and rIAP-II, were compared. These structural models displayed a typical alpha/beta topology, but the crown domain of rIAP-I contained an additional beta-sheet, while the embracing arm region of rIAP-II lacked the alpha-helix. The coordinated metal at the active site was predicted to be a zinc triad in rIAP-I, whereas the typical combination of two zinc atoms and one magnesium atom was proposed for rIAP-II. Between the rIAPs, a difference was observed at amino acid position 317 that is indirectly related to the coordination of the metal at metal-binding site 3 and water molecules, suggesting that the alignment of Q317 might be the major determinants for activation of the zinc triad in rIAP-I.

[*J. Atheroscler. Thromb.*, **12**, 169-174 (2005)] [Lab. of Biochemistry]

CLOCK/BMAL1 is Involved in Lipid Metabolism via Transactivation of the Peroxisome Proliferator-activated Receptor (PPAR) Response Element.

Ikuo INOUE, Yuichi SHINODA, Masaaki IKEDA, Kenji HAYASHI, Kenta KANAZAWA, Masahiko NOMURA, Toshiyuki MATSUNAGA*, Haiyuan XU, Shinichiro KAWAI, Takuya AWATA, Tsugikazu KOMODA and Shigehiro KATAYAMA

The aim of this study was to examine the contribution of clock system to lipid metabolism. Here we show the evidence that heterodimer of circadian genes, CLOCK/BMAL1, has transcriptional activity for genes via the peroxisome proliferator-activated receptor response element (PPRE). Male mice 8-12 weeks old were maintained under a 12:12 hour light-dark cycle for at least two weeks before the day of the experiment. The mRNA expressions of BMAL1 and of the PPAR target genes, acyl-CoA oxidase, 3-hydroxy-3-methylglutaryl coenzyme A synthase, and cellular retinol binding protein II (CRBPII), in intestine have a robust circadian rhythm. The promoter activities of the three enzymes were increased by CLOCK/BMAL1 expression as assessed in vitro by luciferase assay. Deletion of the PPRE from the construct abrogated CLOCK/BMAL1-induced CRBPII transactivation, demonstrating that CLOCK/BMAL1 transactivates PPAR target genes via the PPRE.

[*eCAM*, **2**, 191-199 (2005)] [Lab. of Pharmacology]

Kampo Medicines for Mite Antigen-Induced Allergic Dermatitis in MC/Nga Mice.

Xiu Kun GAO, Kazutoshi FUSEDA, Tomonori SHIBATA, Hiroyuki TANAKA, Naoki INAGAKI* and Hiroichi NAGAI

We have established an allergic dermatitis model in NC/Nga mice by repeated local exposure of mite antigen for analyzing atopic dermatitis. We examined the effects of four Kampo medicines, Juzen-taiho-to, Hochu-ekki-to, Shofu-san and Oren-gedoku-to, on the dermatitis model. Mite antigen solution was painted on the ear of NC/Nga mice after tape stripping. The procedure was repeated five times at 7 day intervals. Oral administration of all four Kampo medicines inhibited the formation of ear swelling and inflammatory cell accumulation. Juzen-taiho-to and Hochu-ekki-to apparently prevented the elevation of serum IgE level. Furthermore, the four Kampo medicines showed a tendency to prevent not only the increase in interleukin-4 mRNA expression but also the decrease in interferon-γ mRNA expression.

[*eCAM*, **2**, 369-374 (2005)] [Lab. of Pharmacology]

Inhibition of IgE-dependent mouse triphasic cutaneous reaction by a boiling water fraction separated from mycelium of Phellinus linteus.

Naoki INAGAKI*, Tomonori SHIBATA, Tomokazu ITOH, Tomohiro SUZUKI, Hiroyuki TANAKA, Tomoyuki NAKAMURA, Yukihito AKIYAMA, Hirokazu KAWAGISHI and Hiroichi NAGAI

In the present study, we separated the constituents of mycelium of Phellinus linteus into five fractions-chloroform-soluble (CF), ethyl acetate-soluble (EA), methanol-soluble (AE), water-soluble (WA) and boiling water-soluble (BW) fractions-and examined their suppressive effects on the IgE-dependent mouse triphasic cutaneous reaction. ME, WA and BW given orally significantly inhibited the first and second phase ear swelling, and BW also inhibited the third phase response. CF only inhibited the second phase. BW also inhibited vascular permeability increase caused by passive cutaneous anaphylaxis and histamine, and ear swelling caused by tumor necrosis factor-α. These results indicate that BW contains some constituents with anti-allergic properties.

[*Nature Immunol.*, **6**, 524-531 (2005)] [Lab. of Pharmacology]

Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3

Tomonori KUNIKATA, Hana YAMANE, Eri SEGI, Toshiyuki MATSUOKA, Yukihiko SUGIMOTO, Satoshi TANAKA, Hiroyuki TANAKA*, Hiroichi NAGAI, Atsushi ICHIKAWA and Shuh NARUMIYA

Prostaglandins, including PGD_2 and PGE_2, are produced during allergic reactions. Although PGD_2 is an important mediator of allergic responses, aspirin-like drugs that inhibit prostaglandin synthesis are generally ineffective in allergic disorders, suggesting that another prostaglandin-mediated pathway prevents the development of allergic reactions. Here we show that such a pathway may be mediated by PGE_2 acting at the prostaglandin E receptor EP3. Mice lacking EP3 developed allergic inflammation that was more pronounced than that in wild-type mice or mice deficient in other prostaglandin E receptor subtypes. Conversely, an EP3-selective agonist suppressed the inflammation. Thus, the PGE_2-EP3 pathway is an important negative modulator of allergic reactions.

[*Heterocycles*, **65**, 173-179 (2005)] [Lab. of Pharmacognosy]

Stilbenoids from *Upuna borneensis*.

Tetsuro ITO, Ibrahim ILIYA, Toshiyuki TANAKA, Ken-ichi NAKAYA, Yukihiro AKAO, Yoshinori NOZAWA, Jin MURATA, Dedy DARNAEDI and Munekazu IINUMA*

Dipterocarpacecous plants have been shown to be rich in resveratrol oligomers. In continuation of our study to search the chemical constituents with bioactive potency from Dipterocarpaceous plants, the chemical components (resveratrol oligomers) in some plants of *Vatica, Shorea, Vateria* and *Dipterocarpus* were characterized. An acetone extract of the leaves of *Upuna borneensis* afforded two stilbene tetramer derivatives (upunaphenols F and G) together with four stilbenoids (vaticanols B and C, piceid and *cis*-piceid). The structure and their relative stereochemistry were determined by spectroscopic techniques, in particular by using 2D NMR method. Upunaphenol G was found to suppress cell growth in HL60 cells with IC_{50} at 15.6 μM.

[*Chem. Pharm. Bull.*, **53**, 219-224 (2005)] [Lab. of Pharmacognosy]

Resveratrol Derivatives from *Upuna borneensis*.

Tetsuro ITO, Miyuki FURUSAWA, Toshiyuki TANAKA, Zulifiqar ALI, Ibrahim ILIYA, Ken-ichi NAKAYA, Jin MURATA, Dedy DARNAEDI and Munekazu IINUMA*

Upuna borneensis (Dipterocarcaceae) is a monotypic genus distributed in Malaysia. In previous papers, we reported the isolation and structure determination of new compounds of a resveratrol hexamer (upunaphenol A), resveratrol *O*-glucosides and acetophenone *C*-glucosides together with four known resveratrol oligomers. In the present experiment, four new resveratrol derivatives, upunaphenols B and C (resveratrol tetramer) and E (resveratrol dimer with a C_6-C_1 unit), together with nine known resveratrol oligomers were isolated from an acetone soluble part of stem of *U. borneensis*. The structures of new compounds were determined by spectral analysis including 1D and 2D NMR experiments.

[*Chem. Pharm. Bull.*, **53**, 229-231 (2005)] [Lab. of Pharmacognosy]

A New and Known Cytotoxic Aryltetralin-Type Lignans from Stems of *Bursera graveolens*.

Tsutomu NAKANISHI, Yuka INATOMI, Hiroko MURATA, Kaori SHIGETA, Naoki IIDA, Akira INADA, Jin MURATA, Miguel A. P. FARRERA, Munekazu IINUMA*, Toshiyuki TANAKA, Shogo TAJIMA and Naoto OKU

A new 4α-aryltetralin-type lignan called burseranin and a known analogous lignan picropolygamain were isolated along with known triterpene, lupeol and epi-lupeol from the methanol extract of stems of *Bursera graveolens*, which showed a remarkable inhibitory activity against human HT1080 fibrosarcoma cells. The whole structure of burseranin was established based on combined spectral studies and the absolute structure for picroplygamain was first confirmed by CD spectral evidence. In addition, cytotoxic activities of the stem (methanol) extract and its components were evaluated.

[*J. Pharmacol. Sci.*, **97**, 242-252 (2005)] [Lab. of Pharmacognosy]

A Potent Apoptosis-Inducing Activity of a Sesquiterpene Lactone, Arucanolide, in HL-60 Cells: a Crucial Role of Apoptosis-Inducing Factor.

Yoshihito NAKAGAWA, Munekazu IINUMA*, Nobuyasu MATSUURA, Kong YI, Makoto NAOI, Toshihiro NAKAYAMA, Yoshinori NOZAWA and Yukihiro AKAO

Six main sesquiterpene lactones (germacranolides) from *Calea urticifolia* were evaluated for in vitro cytotoxicity against human tumor cell lines HL60 and SW480 cells. Among them, arucanolide and parthenolide displayed marked cytotoxicity against both cell lines. The cytotoxic activity of arucanolide was observed at lower concentrations compared to that of parthenolide, which has been reported to be a typical and simple germacranolide. The activity was found to be mainly due to apoptosis that was assessed by morphological findings, DNA ladder formation (24-36h), and flow cytometric analysis in HL60 cells. Western blotting and an apoptosis inhibition assay using caspase inhibitors did not demonstrate the activation of any caspases tested. However, the mitochondorial membrane potential HL-60 cells was lost after 24h treatment with aruanolide, and concurrently apoptosis-inducing factor released from mitochondria was detected by Western blot analysis.

[*Helv. Chim. Acta*, **88**, 23-34 (2005)] [Lab. of Pharmacognosy]

Occurrence of Stilbene Glucosides in *Upuna borneensis*.

Tetsuro ITO, Zulfiqar ALI, Ibrahim ILIYA, Miyuki FURUSAWA, Toshiyuki TANAKA, Ken-ich NAKAYA, Yoshikazu TAKAHASHI, Ryuichi SAWA, Jin MURATA, Dedy DARNAEDI and Munekazu IINUMA*

The family Dipterocarpaceae is well known to its abundance of resveratrol oligomers. Since the monomer as well as its oligomers display multifunctional bioactivities. Then in the present experiment the occurrence of stilbenoids in *Upuna borneensis* was examined. Four new stilbene glucosides, upunosides A, B, C and D, were isolated from the stem of this plant together with the three known glucosides. Upunoside A is the first natural instance of a glucoside pentamer, and its aglycone has a dibenzo-fused bicyclo [5.3.0]octadiene and two dihydrobenzofuran moieties. The relative structure of the aglycone was determined by spectral analysis including 1D and 2D NMR experiments.

[*Planta Medica*, **71**, 90-92 (2005)] [Lab. of Pharmacognosy]

Inhibitory Effects of Xanthones from Guttiferae Plants on PAF-Induced Hypotension in Mice.

Hisae OKU, Yoshimi UEDA, Munekazu IINUMA* and Kyoko ISHIGURO

The inhibitory effects of 22 xanthones from three Guttiferae plants (*Hypericum patulum, Calophyllum inophyllum* and *C. aursroindicum*) on exogenous platelet activation factor (PAF)-induced hypotension were examined using a blood pressure monitoring *in vivo* assay method. Guanandin, caloxanthone E, 1,3,5,6-tetrahydroxy-2-isoprenylxanthone, 6-deoxyjacareubin and patulone showed strong inhibition of PAF-induced hypotension, with inhibitory effects of more than 60%. Their ID_{50} values were greater than that of ginkgolide B (BN-52021), a natural PAF-antagonist from *Ginkgo biloba*.

[*Tetrahedron Lett.*, **46**, 3111-3114 (2005)] [Lab. of Pharmacognosy]

Rotational Isomerism of a Resveratrol Tetramer, Shoreaketone, in *Shorea uliginosa*.

Tetsuro ITO, Miyuki FURUSAWA, Ibrahim ILIYA, Toshiyuki TANAKA, Ken-ichi NAKAYA, Ryuichi SAWA, Yumiko KUBOTA, Yoshikazu TAKAHASHI, Soedarsono RISWAN and Munekazu IINUMA*

A new resveratrol tetramer, shoreaketone, was isolated from the stem bark of *Shorea uliginosa* (Dipterocarpaceae). The structure and the relative configuration were confirmed on the basis of 1D- and 2D-NMR spectral data. The structure has a novel framework of fused heptacyclic ring system including an α,β-unsaturated carbonyl group. In NMR spectra, shoreaketone was observed as two different conformers due to rotational isomerism.

[*Chem. Pharm. Bull.*, **53**, 591-593 (2005)] [Lab. of Pharmacognosy]

Flavonol Glycosides in Leaves of Two *Diospyros* Species.

Miyuki FURUSAWA, Toshiyuki TANAKA, Tetsuro ITO, Ken-ichi NAKAYA, Ibrahim ILIYA,Masayoshi OHYAMA, Munekazu IINUMA*, Hiroko MURATA, Yuka INATOMI, Akira INADA, Tsutomu NAKANISHI, Shigeru MATSUSHITA, Yumiko KUBOTA, Ryuichi SAWA and Yoshikazu TAKAHASHI

The genus *Diospyros* belongs to the family Ebenaceae and comprises about 500 species distributed in the tropical and temperate zone. Although many studies about quinone compounds in the *Diospyros* plants have been reported, there are few reports about constituents in leaves in spite of the medicinal use. Fourteen flavonol glycosides including two new compounds (kaempferol 3-*O*-β-(2"-*O*-α-rhamnnopyranosyl-3"-*O*-β-glucopyranosyl)-β-glucuronopyranoside and kaempferol 3-*O*-[2"-*O*-α-rhamnopyranosyl-3"-*O*-(6""-*O*-α-rhamnopyranosyl-β-glucopyranosyl)]-β-glucopyranoside) were isolated from the leaves of two *Diospyros* plants (*D. cathayensis* and *D. rhombifolia*). The structures of isolated compounds were determined by spectroscopic analysis. The scavenging activity of 1,1-dipheny-2-picrylhydrazyl radical of the isolated compounds was investigated.

[*J. Health Sci.*, **51**, 376-378 (2005)] [Lab. of Pharmacognosy]

Antioxidant Activity of Hydroxyflavonoids.

Miyuki FURUSAWA, Toshiyuki TANAKA, Tetsuro ITO, Asami NISHIKAWA, Naomi YAMAZAKI, Ken-ichi NAKAYA, Nobuyasu MATSUURA, Hironori TSUCHIYA, Motohiko NAGAYAMA and Munekzu IINUMA*

The antioxidant activity of 28 natural and synthetic hydroxyflavonoids was estimated through 1,1-diphenyl-2-picrylhyrazyl (DPPH) radical scavenging and superoxide dismutase (NBT method) activities and inhibition of lipid peroxidation (TBA method). The results showed the hydroxylation pattern has close relationship with the appearance of activities and that the patterns were clarified to four fundamental patterns as 3',4'-dihydroxyflavone, 3,4'-dihydroxyflavone, 3,5,7-trihydroxyflavone and 3,3',4'-trihydroxyflavone.

[*Helv. Chim. Acta*, **88**, 1048-1058 (2005)] [Lab. of Pharmacognosy]

Novel, Complex Flavonoids from *Mallotus philippiensis* (Kamala Tree).

Miyuki FURUSAWA, Yoshimi IDO, Toshiyuki TANAKA, Tetsuro ITO, Ken-ichi NAKAYA, Ibrahim ILIYA, Masayoshi OHYAMA, Munekazu IINUMA*, Yoshiaki SHIRATAKI and Yoshikazu TAKAHASHI

One new flavanone, 4'-hydroxyisorottelerin, and two new chalcone derivatives, kamalachalcones C and D, were isolated from *Mallotus philippiensis* (Kamala tree). Kamalachalcone D with a molecular weight (1,098) possessed a unique, fused-ring system made of two hydroxy-chalcone units, giving rise to eight fused benzene/pyran units. From the same plant, the following known six compounds were also isolated; kamalachalcones A and B, isoallorottlerin, isorottelerin, 5,7-dihydroxy-8-methyl-6-prenylflavanone; 6,6-dimethylpyrano(2",3":7,6)-5-hydroxy-8-methylflavanone, and rottlerin. The structures of new compounds were confirmed by in-depth spectral analysis, including 2D-NMR techniques. The full ^{13}C-NMR assignment of the known flavanones is published for the first time.

[*Chem. Biodiversity*, **2**, 773-779 (2005)] [Lab. of Pharmacognosy]

Four New Trimeric Stilbene Glucosides from *Welwitschia mirabilis*.

Hiroko MURATA, Ibrahim ILIYA, Toshiyuki TANAKA, Miyuki FURUSAWA, Tetsuro ITO, Ken-ichi NAKAYA, Masayoshi OYAMA and Munekazu IINUMA*

The genus *Welwitschia* is a monotypic member of the gymnospermous family of Welwitschiaceae. Since its discovery, ca 140 years ago, very little is known about its phytochemistry, and taxonomists have not been able to agree on its classification in the plant kingdom. For comparison to morphological classification, the phytochemicals in *W. mirabilis* were examined and four new trimeric stilbene glucosides named mirabilosides C-F were isolated from MeOH extract of the stem and root along with three known stilbenoids, resveratrol, gnemonoside B and gnetin G. The structures of these compounds were elucidated by spectroscopic methods.

[*Chem. Pharm. Bull.*, **53**, 783-787 (2005)] [Lab. of Pharmacognosy]

A Monoterpene Glucoside and Three Megastigmane Glycosides from *Juniperus communis* var. *depressa*.

Tsutomu NAKANISHI, Naoki IIDA, Yuka INATOMI, Hiroko MURATA, Akira INADA, Jin MURATA, Frank A. LANG, Munekazu IINUMA*, Toshiyuki TANAKA and Yoshikazu SAKAGAMI

In a survey of chemical components from useful plants grown in western North America, we have identified a number of various types of phenolic compounds (nine phenylpropanoids, six neolignan, and seven flavonoids) in their glycoside form from the aerial parts of *Juniperus communis* var. *depressa*. In succession to the previous experiment, a new monoterpene glucoside and three new natural megastigmane glycoside were isolated along with a known megastigmane glucoside from twigs with leaves of this plant collected in Oregon, U.S.A. Their structures were determined on the basis of spectral and chemical evidence. In addition, the antibacterial activities of the isolated compounds against *Helicobacter pylori* were investigated. The megastigmane glucosides showed potent inhibition (MIC value 50 μg/ml) comparable to those of natural and synthetic hinokitiol as positive control.

[*Tetrahedron Lett.*, **46**, 6533-6535 (2005)] [Lab. of Pharmacognosy]

A Pair of New Atropisomeric Cupressusflavone Glucosides Isolated from *Juniperus communis* var. *depressa*.

Yuka INATOMI, Naoki IIDA, Hiroko MURATA, Akira INADA, Jin MURATA, Frank A. LANG, Munekazu IINUMA*, Toshiyuki TANAKA and Tsutomu NAKANISHI

A pair of new atropisomers, (*M*)- and (*P*)-cupressusflavone 4-*O*-β-glucoside, were isolated from *Juniperus communis* var. *depressa*, and their absolute structures and axial configuration were determined using 2D NMR and circular dichroism. These were the first report of the stable presence of (*M*)- and (*P*)-isomers of cupressusflavone glucosides isolated from natural source. The bulky glucosyl moiety attached at 4'-OH restricts free rotation around the 8-8" linkage, resulting in the formation of stable (*M*)- and (*P*)-isomers. These isomers were also stable in DMSO-d_6 solution during ^{1}H NMR measurement and were not converted from each other.

[*Biol. Pharm. Bull.*, **28**, 1786-1790 (2005)] [Lab. of Pharmacognosy]

Antianaphylactic and Antipruritic Effects of the Flowers of *Impatiens textori* Miq.

Yoshimi UEDA, Hisae OKU, Munekazu IINUMA* and Kyoko ISHIGURO

The anti-anaphylactic and anti-pruritic activities of a 35% EtOH extract (IT) of the flowers of *Impatiens textori* (Balsaminaceae) were investigated by *in vivo* assay. IT and apigenin, apigenin 7-glucoside and luteolin, which are principal compounds in IT, inhibited compound 48/80 (COM)-induced by blood pressure (BP) decrease, which was an immunoglobulin (Ig)E-independent anagphylaxis-like response. These flavonoid compounds all inhibited BP decrease induced by IgE-dependent anaphylaxis. Furthermore, IT inhibited the blood flow (BF) decrease induced by antigen-induced anaphylaxis in actively sensitized mice. IT also significantly inhibited platelet activating factor ad serotonin-induced scratching behavior and mitigated protease-induced scratching behavior.

[*Chem. Biodiversity*, **2**, 1200-1216 (2005)] [Lab. of Pharmacognosy]

Two Novel Resveratrol Derivatives from *Cotylelobium lanceolatum*.

Tetsuro ITO, Zulfiqar ALI, Miyuki FURUSAWA, Ibrahim ILIYA, Toshiyuki TANAKA, Ken-ichi NAKAYA, Jin MURATA, Dedy DARNAEDI, Masayoshi OYAMA and Munekazu IINUMA*

Two new resveratrol (= 5-[(*E*)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol) trimers, named cotylelophenols A and B, were isolated from the stem of *Cotylelobium lanceolatum* (Dipterocarpaceae) together with ten known resveratrol oligomers. The structures of the isolates were established on the basis of spectroscopic analyses, including a detailed NMR spectroscopic investigation of cotylelophenol A under different conditions. Cotylelophenol A is the first resveratrol trimer with an arranged 4-hydroxyphenyl group. Four possible biogenetic pathways towards resveratrol oligomers are proposed.

[*Bioorg. Med. Chem.*, **13**, 6064-6069 (2005)] [Lab. of Pharmacognosy]

Xanthones Induce Cell-Cycle Arrest and Apoptosis in Human Colon Cancer DLD-1 Cells.

Kenji MATSUMOTO, Yukihiro AKAO, Kenji OHGUCHI, Tetsuro ITO, Toshiyuki TANAKA, Munekazu IINUMA* and Yoshinori NOZAWA

We investigated the antiprofirative effects of four structurally similar prenylated xanthones, α-mangostin, β-mangostin, γ-mangostin and β-mangostin monomethyl ether, in human colon cancer DLD-1 cells. These xanthones differ in the number of hydroxyl and methoxyl groups. Except for the monomethyl ether, the other three xanthones strongly inhibited cell growth at 20 μM and their antitumor efficacy was correlated with the number of hydroxyl groups. Hoechst 33342 nuclear staining and nucleosomal DNA-gel electrophoresis revealed that the antiprofirative effects of α- and γ-mangostin, but not that of β-mangostin, were associated with apoptosis. It was also shown that their antiprofirative effects were associated with cell-arrest by affecting the expression of cyclins, cdc2, and p27; G1 arrest was by α-mangostin and β-mangostin, and S arrest by γ-mangostin. These findings provide a relevant basis for the development of xanthones as an agent for cancer prevention and combination therapy with anti-cancer drugs.

[*Chem. Biodiversity*, **2**, 1673-1684 (2005)] [Lab. of Pharmacognosy]

New Resveratrol Tetramers from the Stem Bark of *Upuna borneensis*.

Tetsuro ITO, Zulfiqar ALI, Miyuki FURUSAWA, Ibrahim ILIYA, Toshiyuki TANAKA, Ken-ichi NAKAYA, Jin MURATA, Dedy DARNAEDI, Masayoshi OYAMA and Munekazu IINUMA*

The monotypic genus *Upuna borneensis* belongs to the largest subfamily Dipterocarpoideae in the Dipterocarpaceae. We previously reported the characterization of the resveratrol oligomers upunaphenols A-G and of the *O*-glucosides of upunosides A-D from the stem bark and of this species. As part of our chemical research of other minor components of this plant, we added five new resveratrol tetramers; upunaphenols H-J, *trans*- and *cis*-upunaphenol K. Their structures were elucidated on the basis of 1D- and 2D-NMR as well as FAB-MS data. Upunaphenols H-J bear a rare biphenyl bond in their frameworks. Upunaphenols H and J have an unprecedented nonacylic fused ring system, and upunaphenols I and J have symmetrical structures, respectively.

[*Phytomedicine*, **12**, 203-208 (2005)] [Lab. of Pharmacognosy]

Antibacterial Activity of α-Mangostin against Vancomycin Resistant *Enterococci* (VRE) and Synergism with Antibiotics.

Y. SAKAGAMI, M. IINUMA*, K.G.N.P. PIYASEBA and H.R.W. DHARNARTNE

α-Mangostin, isolated from the stem bark of *Garcinia mangostana* L., was found to be active against vancomycin resistant *Enterococci* (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA), with MIC values of 6.25 and 6.25 to 12.5μg/ml, respectively. Our studies showed synergism between α-mangostin and gentamicin (GM) against VRE and between α-mangostin and vancomycin hydrochloride (VCM) against MRSA. Further studies exhibited partial synergism between α-mangostin and commercially available antibiotics such as ampicillin and minocycline. These findings suggested that α-mangostin alone or in combination with GM against VRE and in combination with VCM against MRSA might be useful in controlling VRE and MRSA infections.

[*Biol. Pharm. Bull.*, **28**, 161-164 (2005)] [Lab. of Pharmacognosy]

Phylogenetic Relationship of *Glycyrrhiza lepidota*, American Licorice, in Genus *Glycyrrhiza* Based on *rbcL* Sequences and Chemical Constituents.

Hiroaki HAYASHI*, Etsuko MIWA and Kenichiro INOUE

Two known saponins, licorice-saponin H2 and macedonoside A, were isolated from the stolons of *Glycyrrhiza lepidota* (American licorice) as major saponins. Since licorice-saponin H2 and macedonoside A are minor saponins isolated from the three glycyrrhizin-producing species (i.e. *G. glabra*, *G. uralensis*, *G. inflata*) and the three macedonoside C-producing species (i.e. *G. macedonica*, *G. echinata*, *G. pallidiflora*), respectively, the present study suggests that *G. lepidota* is an intermediate of both glycyrrhizin-producing and macedonoside C-producing species. The phylogenetic tree constructed from the nucleotide sequences of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (*rbcL*) of these seven *Glycyrrhiza* plants indicated that G. lepidota was separated from the other six *Glycyrrhiza* species, and this phylogenetic relationship was in accordance with their saponin compositions.

[*Biol. Pharm. Bull.*, **28**, 1113-1116 (2005)] [Lab. of Pharmacognosy]

Comparative Analysis of Ten Strains of *Glycyrrhiza uralensis* Cultivated in Japan.

Hiroaki HAYASHI*, Kenichiro INOUE, Kazuo OZAKI and Hitoshi WATANABE

Comparative analysis of 10 strains of *Glycyrrhiza uralensis* cultivated in Kyoto, Japan, was undertaken to characterize their variations. Based on the chemical characteristics of their leaves and underground parts, the 10 strains were divided into two chemotypes, the China type and Kazakhstan type. The content of licoleafol in the leaves of the China type (0-0.03% of dry weight) were lower than those of the Kazakhstan type (0.05-1.16% of dry weight). In addition, a China type-specific unidentified compound was also detected in the leaves of China-type plants. Glycyrrhizin contents in the underground parts of the China type (2.08-5.12% of dry weight) were relatively higher than those of Kazakhstan type (0.75-2.55% of dry weight). These 10 strains were also divided into two genotypes, the GA type and AT type, based on their chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (*rbcL*) sequences, although there was no correlation between the chemotype and the *rbcL* genotype.

[*Plant Biotechnology*, **22**, 241-244 (2005)] [Lab. of Pharmacognosy]

Differential Regulation of Soyasaponin and Betulinic Acid Production by Yeast Extract in Cultured Licorice Cells.

Hiroaki HAYASHI*, Noboru HIRAOKA and Yasumasa IKESHIRO

Mode of soyasaponins and betulinic acid accumulation was examined in cultured cells of *Glycyrrhiza glabra* L. (licorice). The time course of their accumulation was different in the cultured cells. Yeast extract promoted betulinic acid accumulation, whereas soyasaponin accumulation was suppressed. These results indicate that soyasaponin and betulinic acid production are differently regulated in cultured cells of *G. glabra*.

[*J. Neurosci. Res.*, **79**, 476-487 (2005)] [Lab. of Mol. Biology]

Involvement of glial cell line-derived neurotrophic factor in activation processes of rodent macrophages.

Mamoru HASHIMOTO, Atsumi NITTA, Hidefumi FUKUMITSU, Hiroshi NOMOTO, Liya SHEN and Shoei FURUKAWA*

mRNA expression of chemokines, including monocyte chemoattractant protein (MCP)-1, was evoked within 1 hr after transection of the spinal cord, and glial cell line-derived neurotrophic factor (GDNF) mRNA expression was similarly up-regulated. GDNF was coexpressed with MCP-1 in the CD11b-positive cells. GDNF enhanced the phagocytic activity of the macrophages via GFRalpha-1, GPI-anchored specific binding site of GDNF, in a c-Ret-independent manner. The influence of autocrine and/or paracrine GDNF synthesis was evaluated by performing activation experiments using macrophages cultured from heterozygous (+/-) GDNF gene-deficient mice or wild-type (+/+) mice. The GDNF mRNA level, but not the MCP-1 or GFRalpha-1 mRNA level, was substantially lower in the mutant macrophages than in the +/+ cells irrespective of stimulation with MCP-1 or lipopolysaccharide (LPS). The phagocytic activity enhanced by MCP-1 or LPS was significantly lower in the mutant cells (+/-) than in the +/+ ones, demonstrating the involvement of endogenous GDNF in the activation processes of macrophages in vitro and suggesting that not only neuroprotective function but also activation of macrophages is effected by the GDNF produced after a spinal cord injury.

[*NeuroReport*, **16**, 99-102 (2005)] [Lab. of Mol. Biology]

Inflammation-induced GDNF improves locomotor function after spinal cord injury.

Manabu HASHIMOTO, Atsumi NITTA., Hidefumi FUKUMITSU, Hiroshi NOMOTO, Liya SHEN and Shoei FURUKAWA*

Activation of microglia/macrophages after injury occurs limitedly in the CNS, which finding may explain unsuccessful axonal regeneration. Therefore, the relationship between lipopolysaccharide (LPS)-induced inflammation and recovery of locomotor function of rats after spinal cord injury was examined. High-dose LPS improved locomotor function greater than low-dose LPS, being consistent with the expression of neurotrophic factor (GDNF) in microglia/macrophages. Experiments using GDNF gene mutant mice confirmed that the increase in the GDNF mRNA level, rather than the reduction in the mRNA level of inducible NO synthase, could be correlated with the restoration activity of locomotor function. These results suggest that a higher degree of inflammation leads to a higher degree of repair of CNS injuries through GDNF produced by activated microglia/macrophages.

[*Biosci Biotechnol Biochem*, **69**, 800-805 (2005)] [Lab. of Mol. Biology]

Oral Administration of Royal Jelly Facilitates mRNA Expression of Glial Cell Line-Derived Neurotrophic Factor and Neurofilament H in the Hippocampus of the Adult Mouse Brain.

Manabu HASHIMOTO, Masafumi KANDA, Kumiko IKENO, Yoshirou HAYASHI, Tadashi NAKAMURA, Yoshinobu OGAWA, Hidefumi FUKUMITSU, Hiroshi NOMOTO and Shoei FURUKAWA*

Royal jelly (RJ) is known to have a variety of biological activities toward various types of cells and tissues of animal models, but nothing is known about its effect on brain functions. Hence, we examined the effect of oral administration of RJ on the mRNA expression of various neurotrophic factors, their receptors, and neural cell markers in the mouse brain. Our results revealed that RJ selectively facilitates the mRNA expression of glial cell line-derived neurotrophic factor (GDNF), a potent neurotrophic factor acting in the brain, and neurofilament H, a specific marker predominantly found in neuronal axons, in the adult mouse hippocampus. These observations suggest that RJ shows neurotrophic effects on the mature brain via stimulation of GDNF production, and that enhanced expression of neurofilament H mRNA is involved in events subsequently caused by GDNF. RJ may play neurotrophic and/or neuroprotective roles in the adult brain through GDNF.

[*Biomed Res*, **26**, 223-229 (2005)] [Lab. of Mol. Biology]

Stimulation of production of glial cell line-derived neurotrophic factor and nitric oxide by lipopolysaccharide with different dose-responsiveness in cultured rat macrophages.

Mamabu HASHIMOTO, Takuya ITO, Hidefumi FUKUMITSU, Hiroshi NOMOTO, Yoshiko FURUKAWA and Shoei FURUKAWA*

We investigated the effects of lipopolysaccharide (LPS) on production of glial cell line-derived neurotrophic factor (GDNF) in the injured rat spinal cord or in cultured rat macrophages in comparison with the effects on synthesis/secretion of inducible nitric oxide synthase (iNOS) and nitric oxide (NO). We found that GDNF mRNA expression lasted longer than that of iNOS mRNA in the injured spinal cord after injection of the high-dose LPS that had improved locomotor function, suggesting that the GDNF expression and its balance with NO generation were critical for injury regeneration. Therefore, we next investigated the effects of LPS on cultured macrophages. Levels of iNOS mRNA and secreted NO were enhanced by LPS at lower concentrations (10 ng/mL and above), whereas mRNA expression and secretion of GDNF were elevated only at higher concentrations (100 ng/mL and above). The culture medium of macrophages treated with 10 ng/mL of LPS was actually neurotoxic against cultured cortical neurons, whereas that conditioned at 1000 ng/mL was not. These observations suggest that neurotoxicity partly based on NO is induced by a lower degree of inflammation, whereas neurotrophic effects based on GDNF are manifested at a higher degree of inflammatory activity.

[*J Infect Chemother*, **11**, 93-96, (2005)] [Lab. of Microbiology]

Escherichia coli O157 infection mimicking acute appendicitis: usefulness of computed tomography for differential diagnosis.

Yoichiro ITO, Katsuhisa TODA, Hiroo HATAKEYAMA, Toshiyuki NAKAMURA, Shigeru KIYAMA, Yoshifumi KATAGIRI, Hiromichi MIMOTO, Atsuyoshi ONITSUKA and Hiroshi MORI*

A 19-year-old man was admitted to our hospital with acute abdominal pain in the right lower quadrant and mild diarrhea. Although physical findings were consistent with a diagnosis of acute appendicitis, computed tomography showed marked wall thickening from the ascending colon to the cecum, similarly to those in patients with hemorrhagic colitis due to *E. coli* O157. Instead of emergency laparotomy, the patient was treated with antibiotics, which led to rapid recovery. Diagnosis of infection with *E. coli* O157 was established later by serum and immuno-histochemical tests. *E. coli* O157 infection should be included in differential diagnosis of diseases that exhibit marked wall thickening of the colon on CT in patients with acute abdominal pain with diarrhea.

[*FEMS Immunol Med Microbiol*, **45**, 213-219, (2005)] [Lab. of Microbiology]

Inhibition of extracellular signal-regulated kinase 1/2 augments nitric oxide production in lipopolysaccharide-stimulated RAW264.7 macrophage cells.

Naoki KOIDE, Hiroyasu ITO, Mya Mya MU, Tsuyoshi SUGIYAMA*, Ferdaus HASSAN, Shamima ISLAM, Isamu MORI, Tomoaki YOSHIDA and Takashi YOKOCHI

The present study was conducted to determine effects of U0126, a specific inhibitor of ERK1/2, on production of NO in RAW264.7 cells. U0126 significantly enhanced NO production in LPS but not CpG DNA or IFN-γ-stimulated RAW264.7 cells. In contrast, a series of inhibitors of p38, PI3-K and JAK rather caused suppression in LPS-stimulated RAW264.7 cells. U0126 was found to definitely inhibit phosphorylation of ERK1/2 and augment the levels of iNOS. Antisense oligonucleotides of ERK1/2 also augmented LPS-induced NO production. Inactivation of ERK1/2 by U0126 furthermore inhibited LPS-induced AP-1 activation, but not NF-κB activation. The results suggest that ERK1/2 might negatively regulate NO production in LPS-stimulated RAW264.7 cells.

[*Microbiol Immunol*, **49**, 529-534 (2005)] [Lab. of Microbiology]

A quantitative analysis of cedar pollen-specific immunoglobulins in nasal lavagesupported the local production of specific IgE, not of specific IgG.

Tomoaki YOSHIDA, Ayako USUI, Taeko KUSUMI, Shigeru INAFUKU, Tsuyoshi SUGIYAMA*, Naoki KOIDE and Takashi YOKOCHI

In this study, we explored a quantitative comparison of the local concentration of allergen-specific IgE with the systemic concentration. Among seasonal rhinitis patients, total and Japanese cedar pollen (JCP)-specific IgE, IgA and IgG antibodies were quantified in nasal lavage fluid (NLF) and serum. The proportions of specific IgE in the NLF were remarkably higher than in serum, which strongly supported the predominant in situ production of the specific IgE. In contrast, the specific proportions of IgG in the NLF were consistent with serum, suggesting that the specific IgG was mostly produced in the downstream lymphoid organs. The local productions of specific IgE would encourage the topical therapies and the usage of the NLF for the diagnosis of allergic rhinitis.

[*FEMS Immunol Med Microbiol*, **43**, 277-286 (2005)] [Lab. of Microbiology]

A role of mitogen and stress-activated protein kinase 1/2 in survival of lipopolysaccharide-stimulated RAW 264.7 macrophages.

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

The effect of inhibition of MSK1/2 on LPS-stimulated RAW 264.7 cells was investigated. Pretreatment with Ro 31-8220, an inhibitor of MSK1/2, induced cell death in LPS-stimulated RAW 264.7 cells. Cell death was accompanied by DNA fragmentation and annexin V binding. Nuclear translocation of apoptosis-inducing factor (AIF) was detected in Ro 31-8220-pretreated cells after LPS stimulation. Ro 31-8220 exclusively inhibited the phosphorylation of CREB, a substrate of MSK1/2. RAW 264.7 cells transfected with the dominant-negative MSK1 clones underwent cell death in response to LPS. Hence, it was suggested that MSK1/2 might play a critical role in the survival of LPS-stimulated RAW 264.7 cells.

[*Clin Rheumatol*, **24**, 11-13 (2005)] [Lab. of Microbiology]

Polymorphism of interleukin-10 promoter and tumor necrosis factor receptor II in Vietnamese patients with systemic lupus erythematosus.

Pham Dan KHOA, Tsuyoshi SUGIYAMA* and Takashi YOKOCHI

The polymorphism of the interleukin-10 (IL-10) promoter and tumor necrosis factor receptor II (TNFRII) in Vietnamese patients with systemic lupus erythematosus (SLE) was examined by using the polymerase chain reaction (PCR) method with genomic DNA and allele-specific primers. In the frequency of IL-10 promoter 1082 genotypes consisting of AA, A/G and GG, the allele frequency of G in the SLE patients was significantly higher than that in the healthy controls. On the other hand, there was no statistical difference in the frequency of TNF receptor (TNFR) II 196 genotypes between the SLE patients and healthy controls. It was therefore suggested that the polymorphism of the IL-10 promoter, but not TNFRII, might participate in the pathogenesis of SLE in Vietnamese.

[*Int. J. Cancer*, **115**, 346-350 (2005)] [Lab. of Radiochemistry]

Effect of α-naphthyl isothiocyanate on 2-amino-3-methylimidazo[4,5-*b*]pyridine (PhIP)-induced mammary carcinogenesis in rats.

Shigeyuki SUGIE, Masami OHNISHI, Jun USHIDA, Tomohiro YAMAMOTO, Akira HARA, Akihiro KOIDE, Yukio MORI*, Hiroyuki KOHNO, Rikako SUZUKI, Takuji TANAKA, Keiji WAKABAYASHI and Hideki MORI

The modifying effect of α-naphthyl isothiocyanate (ANIT) on PhIP-induced mammary carcinogenesis was investigated in female Sprague-Dawley (SD) rats fed a high fat diet. Treatment with a combination of PhIP and ANIT markedly decreased the incidences of mammary tumors compared with treatment with PhIP alone and enhanced hepatic activities of glutathione *S*-transferase (GST) and quinone reductase (QR). These results imply that ANIT shows potent inhibitory effects on mammary carcinogenesis induced by PhIP in female SD rats when administered during the initiation stage, possibly due to enhancing metabolic inactivation of PhIP by GST and QR.

[*Mutagenesis*, **20**, 15-22 (2005)] [Lab. of Radiochemistry]

Effects of α-naphthyl isothiocyanate and a heterocyclic amine, PhIP, on cytochrome P450, mutagenic activation of various carcinogens and glucuronidation in rat liver.

Yukio MORI*, Akihiro KOIDE, Kenjiro TATEMATSU,
Shigeyuki SUGIE and Hideki MORI

A mechanism underlying suppression by α-naphthyl isothiocyanate (ANIT) of mammary carcinogenesis induced by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) was elucidated in female Sprague-Dawley rats fed a high fat diet. Treatment with a combination of PhIP and ANIT markedly decreased hepatic cytochrome P450 (CYP) 1A1, 1A2 and newly found 51 and 53 kDa proteins, CYP2B2 and mutagenicities of PhIP, four other heterocyclic amines and benzo[*a*]pyrene in the presence of liver S9 which were highly induced by PhIP. Either PhIP, ANIT or PhIP + ANIT enhanced 4-nitrophenol UDP-glucuronyltransferase activity, indicating that the chemoprevention by ANIT can be explained by a dual action mechanism, i.e. a reduction in metabolic activation of PhIP by CYP1A2 and an enhancement of detoxification by UDPGT.

[*Cancer Sci.*, **96**, 637-644 (2005)] [Lab. of Radiochemistry]

Differences in susceptibility to *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine-induced urinary bladder carcinogenesis between SD/gShi rats with spontaneous hypospermatogenesis and SD/cShi rats with spontaneous hydronephrosis.

Takashi MURAI, Yukio MORI*, Kenjiro TATEMATSU, Akihiro KOIDE, Akihiro HAGIWARA,
Susumu MAKINO, Satoru MORI, Hideki WANIBUCHI and Shoji FUKUSHIMA

Differences in susceptibility to *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN)-induced urinary bladder carcinogenesis between two substrains of SD rats were examined. SD/gShi rats had a lower incidence of urinary bladder tumors, 5-bromo-2'-deoxyuridine labeling indices and urinary concentrations of *N*-butyl-*N*-(3-carboxypropyl)nitrosamine (BCPN) than SD/cShi rats. *In vitro* analysis also showed significantly less BCPN formation in SD/gShi rats than in SD/cShi rats and this formation was inhibited by non-selective cytochrome P450 (CYP) inhibitors. However, there were no significant differences in hepatic or renal CYP 1A1/2, 2B1/2, 2E1 and 3A2 levels between both substrains, indicating that SD/gShi rats are less susceptible to BBN, possibly because less BCPN is produced by CYP isoform other than those investigated.

[*Int. J. Cardiol.*, **104**, 163-169 (2005)] [Lab. of Clinical Pharmaceutics]

Experimental Hyperhomocysteinemia Impairs Coronary Flow Velocity Reserve.

Kazuhito YAMASHITA, Hiromi TASAKI, Yoshitaka NAGAI, Hiroshi SUZUKI, Shun-ichi NIHEI, Kengo KOBAYASHI,
Masataka HORIUCHI, Yasuhide NAKASHIMA and Tetsuo ADACHI*

Hyperhomocysteinemia has been identified as an independent risk factor for coronary artery disease. One mechanism is considered to be deteriorated endothelial function that is recovered by vitamin C. However, its direct action on coronary circulation has yet to be examined. This study was designed to test the hypothesis that experimental acute hyperhomocysteinemia would impair coronary flow velocity reserve (CFR) by increasing oxidative stress. Experimentally induced acute hyperhomocysteinemia significantly decreased CFR, and this decrease was significantly reserved by vitamin C administration. Oxidative stress is suggested to play a major role in the deleterious effects of homocysteine on the coronary microcirculation.

[*Atherosclerosis*, **181**, 55-62 (2005)] [Lab. of Clinical Pharmaceutics]

Extracellular Superoxide Dismutase Overexpression Reduces Cuff-induced Arterial Neointimal Formation.

Kiyoshi OZUMI, Hiromi TASAKI, Hiroyuki TAKATSU, Sei NAKATA, Tsuyoshi MORISHITA, Shinichiro KOIDE,
Kazuhito YAMASHITA, Masato TSUTSUI, Masahiro OKAZAKI, Yasuyuki SASAGURI,
Tetsuo ADACHI* and Yasuhide NAKASHIMA

The mechanism of neointimal formation in cuff-injury models are still uncertain. To examine whether extracellular superoxide dismutase (EC-SOD) can reduce neointimal formation in cuff-injury model, adenoviruses expressing EC-SOD (AxCAEC-SOD) or *Escherichia coli* β-galactosidase (AxCALaxZ) was injected between the cuff and the adventitia of rat femoral arteries. In comparison with cuff-treated control arteries and AxCALacZ-trasfected arteries, neointimal formation was significantly reduced in AxCAEC-SOD-transfected arteries. Furthermore, proliferating smooth muscle cells in neointima and media were reduced by EC-SOD treatment. Reactive oxygen species (ROS) generation in tissue was reduced by EC-SOD expression, as assessed by dihydroethidium staining and coelenterazine chemiluminescence. These results suggest that ROS, especially superoxide anion at an adventitia, are responsible for neointimal formation in cuff-injury model.

[*Jpn. J. Pharm. Health Care Sci.*, **31**, 822-826 (2005)] [Lab. of Clinical Pharmaceutics]

Understanding of the Use of Insulin Preparations by Patients Visiting Pharmacies Covered by Health Insurance Scheme. —Survey with a View to Improving Guidance—

Eiji TAKASHIMA, Masafumi KUBOTA, Hirokazu HARA, Kazuyuki HIRANO and Tetsuo ADACHI*

We conducted a questionnaire survey of patient attitudes, degree of compliance and knowledge of warnings concerning insulin therapy since these are important factors in the proper administration of insulin preparations. Patients understanding of the method of use and storage requirements of insulin preparations was generally satisfactory. However, understanding of some aspects of the use of insulin preparations was significantly low among elderly patients and understanding of particular warning for the use of insulin was significantly low among patients who had not participated in diabetic seminars or other educational meetings. We also found that understanding of sick days was low even for patients who had participated in educational meeting, presumably because symptoms vary from person to person and the way they deal with them also varies.

[*Jpn. J. Pharm. Health Care Sci.*, **31**, 845-850 (2005)] [Lab. of Clinical Pharmaceutics]

Survey of the Use of Supplements by Persons Visiting Gifu Pharmaceutical University Pharmacy.

Tetsuo ADACHI*, Shinji MATSUNAGA, Masafumi KUBOTA, Eiji TAKASHIMA, Teruo TSUCHIYA and Kazuyuki HIRANO

Amid the increasing use of supplements due to people's growing interest in their health and deregulation, there have been cases of supplements adversely affecting people's health and interacting with drugs. For this reason, pharmacists are often required to give guidance on the use of supplements. We conducted a questionnaire survey concerning the use of supplements by persons visiting the Gifu Pharmaceutical University Pharmacy, which had the following findings. Of those surveyed, 41.8% had used supplements. A large number of younger people used vitamin and mineral preparations for health maintenance and dieting purposes, and many older people used various supplements for health maintenance and as an additional treatment for diseases. Eighty-five percent of those surveyed said that they read the precautions for use, but only 55% checked warning and adverse effects and just 59% said they were aware of interactions with drugs, indicating a lack of awareness of the risks of supplements.

[*Jpn. J. Nephrol*, **47**, 32-37 (2005)] [Lab. of Clinical Pharmaceutics]

Extracellular-Superoxide Dismutase Production in Mesangial Cell Growing in Extracellular Matrix.

Harutaka YAMADA, Tetsuo ADACHI*, Yasukazu YAMADA, Sachiko MISAO, Keisuke SUZUKI, Hitoshi WATANABE, Wataru KITAGAWA, Naoto MIURA, Cyouryu YOU, Masahito SAKUMA, Kazuhiro NISHIKAWA, Arao FUTENMA and Hirokazu IMAI

To study the protective function against oxygen radicals in the mesangial area, we assessed extracellular superoxide dismutase (EC-SOD) production in mesangial cells (MCs) *in vitro*. These cells have a major protective function against oxygen radicals in the extracellular space. In two different kinds of culture conditions: "growth medium" with fetal cow serum, and "differentiation medium" with reduced growth factor, and four extracellular matrixes: type I collagen, type IV collagen, laminin and fibronectin, were added to the MC culture. With the difference in the culture media, differentiation medium induced EC-SOD hyper-production associated with the both of the slowing down of cell proliferation and the suppression of IL-6 and IL-8 production. With difference in the extracellular matrix, the presence of type VI collagen and laminin promoted higher production of EC-SOD.

[*J. Clin. Endocrinol. Metab.*, **90**, 529-537 (2005)] [Lab. of Clinical Pharmaceutics]

Expression of Allograft Inflammatory Factor-1 in Human Eutopic Endometrium and Endometriosis: Possible Association with Progression of Endometriosis.

Hisato KOSHIBA, Jo KITAWAKI, Mariko TERAMOTO, Yui KITAOKA, Hiroaki ISHIHARA, Hiroshi OBAYASHI, Mitsuhiro OHTA, Hirokazu HARA*, Tetsuo ADACHI and Hideo HONJO

In the current work we examined the expression of AIF-1 in human eutopic endometrium and endometriosis, and measured AIF-1 in peritoneal fluid samples from women with and without endometriosis. AIF-1 mRNA and protein were expressed both in eutopic endometrium and in endometriotic tissue. In eutopic endometrium, expression was greater in the late secretory and menstrual phases than in other phases of the menstrual cycle. AIF-1 protein was present in greater amounts in peritoneal fluid from patients with endometriosis than in women without it, and its concentration correlated with the Revised American Society for Reproductive Medicine score. These results demonstrate for the first time that AIF-1 is expressed in eutopic endometrium and endometriotic tissue, suggesting that AIF-1 is one cytokine in the local network involved in the onset of menstruation.

[*Br. J. Ophthalmol.*, **89**, 1058-1062(2005)] [Lab. of Biofunctional Molecules]

Optic Disc Topographic Parameters Measured in the Normal Cynomolgus Monkey by Confocal Scanning Laser Tomography.

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

The purpose was to study optic disc topographic parameters in normal cynomolgus monkeys by Heidelberg Retina Tomograph (HRT). Disc area, rim area and height variation contour showed smaller right-left differences than other parameters. The coefficients of variation for rim area, height variation contour, rim volume, mean cup depth, maximum cup depth, mean retinal nerve fiber layer (RNFL) thickness and RNFL cross section area were less than 10%. Rim area and height variation contour showed relatively weak interrelations and neither showed a correlation with disc area. For evaluating time-related changes in the optic disc by HRT in monkeys, rim area and height variation contour might be useful parameters because coefficients of variation and right-left differences were lower than for other parameters and because these parameters showed weak interrelations and no correlation with disc area.

[*Brain Res.*, **1044**, 8-15(2005)] [Lab. of Biofunctional Molecules]

Minocycline Inhibits Oxidative Stress and Decreases *in vitro* and *in vivo* Ischemic Neuronal Damage.

Nobutaka MORIMOTO, Masamitsu SHIMAZAWA, Tetsumori YAMASHIMA, Hiroichi NAGAI and Hideaki HARA*

The neuroprotective effects of minocycline were investigated both in vitro and in vivo. Focal cerebral ischemia was induced by permanent middle cerebral artery occlusion in mice. Minocycline at 90 mg/kg intraperitoneally administered 60 min before or 30 min after (but not 4 h after) the occlusion reduced infarction, brain swelling, and neurological deficits at 24 h after the occlusion. Minocycline significantly inhibited glutamate-induced cell death at 2 μM in cortical-neuron cultures from rat fetuses, and lipid peroxidation and free radical scavenging at 0.2 and 2 μM, respectively. These findings indicate that minocycline has neuroprotective effects in vivo against permanent focal cerebral ischemia and in vitro against glutamate-induced cell death, and that an inhibition of oxidative stress by minocycline may be partly responsible for these effects.

[*Eur. J. Pharmacol.*, **508**, 223-229(2005)] [Lab. of Biofunctional Molecules]

Comparison of the Therapeutic Indexes of Different Molecular Forms of Botulinum Toxin Type A.

Shinji YONEDA, Masamitsu SHIMAZAWA, Masanori KATO, Akira NONOYAMA, Yasushi TORII, Hajime NISHINO, Nakaba SUGIMOTO and Hideaki HARA*

Botulinum toxin is produced by Clostridium botulinum in three different molecular-weight forms: LL toxin, 900 kDa; L toxin, 500 kDa; and M toxin, 300 kDa. We isolated the M toxin, then compared its muscle-weakening efficacy with those of L+LL toxin and BOTOX both in vitro and in vivo. The twitch tension of the mouse isolated phrenic nerve-hemidiaphragm was used for the in vitro study. For the in vivo study, grip strength was measured in the toxin-injected legs. Undesirable muscle weakening was evaluated by grip-strength measurement in the contralateral leg. Concentration-response curves for effects on the phrenic nerve-hemidiaphragm showed that M toxin was 10 times more potent than L+LL toxin. The therapeutic index in vivo was 3- to 5-times higher for M toxin than for L+LL toxin or BOTOX, indicating a greater separation for M toxin between doses with local efficacy and systemic toxicity. These findings indicate that the M toxin preparation may have a better pharmacological profile than the conventional preparation.

[*Evid. Based. Complement. Alternat. Med.*, **2**, 201-207(2005)] [Lab. of Biofunctional Molecules]

Neuroprotective Effects of Brazilian Green Propolis against *in vitro* and *in vivo* Ischemic Neuronal Damage.

Masamitsu SHIMAZAWA, Satomi CHIKAMATSU, Nobutaka MORIMOTO, Satoshi MISHIMA, Hiroichi NAGAI and Hideaki HARA*

We examined whether Brazilian green propolis, a widely used folk medicine, exerts neuroprotective effects in vitro and/or in vivo. In vitro, propolis significantly inhibited neurotoxicity induced in neuronally differentiated PC12 cell cultures by either 24h hydrogen peroxide (H_2O_2) exposure or 48h serum-deprivation. Regarding the possible underlying mechanism, in mouse forebrain homogenates propolis protected against oxidative stress and scavenged free radicals [induced by diphenyl-p-picrylhydrazyl (DPPH)]. In mice in vivo, propolis reduced brain infarction at 24 h after the occlusion. Thus, a propolis-induced inhibition of oxidative stress may be partly responsible for its observed neuroprotective effects against in vitro cell death and in vivo focal cerebral ischemia.

[*Behav. Brain Res.*, **161**, 18-30(2005)] [Lab. of Biofunctional Molecules]

Comparison between Monkey and Human Visual Fields using a Personal Computer System.

Masaaki SASAOKA, Hideaki HARA* and Katsuki NAKAMURA

We established a new system with personal computers for precise measurement of the monkey visual field. Four monkeys and three humans served as subjects. The luminance-contrast sensitivity of the central 24 degrees field was measured while the subject was fixating a small spot. Reliability indices demonstrated high and stable behavioral performance by both monkeys and humans. The luminance-contrast sensitivity was highest around the fovea, and declined as eccentricity increased. The overall sensitivity was higher in humans than in monkeys and the sensitivity dropped more sharply in the periphery in monkeys than in humans. We recommend this system as a convenient and reliable way to measure visual functions in monkeys in basic ophthalmologic research or in assessment of the drug effects on the visual field.

[*J. Ocul. Pharmacol. Ther.*, **21**, 436-444(2005)] [Lab. of Biofunctional Molecules]

Continuous Monitoring of Circadian Variations in Intraocular Pressure by Telemetry System Throughout a 12-Week Treatment with Timolol Maleate in Rabbits.

Takahiro AKAISHI, Naruhiro ISHIDA, Atsushi SHIMAZAKI, Hideaki HARA* and Yasuaki KUWAYAMA

The aim of this study was to examine the effect of a 12-week treatment with two formulations of timolol maleate on the amplitude of the circadian fluctuation in intraocular pressure (IOP). Using conscious Japanese White rabbits, IOP was measured by a telemetry system. Each animal was treated topically for 12 weeks with 0.5% timolol solution (TM) twice-daily, 0.5% timolol gel-forming solution (TM-gel) once-daily, or saline twice-daily, and the circadian variation in IOP was measured every week. TM-gel administered once-daily was as effective at lowering IOP as TM administrated twice-daily over the 12-week experimental period. This study reveals that, in rabbits, both formulations of the timolol maleate induced significant IOP reductions in the dark phase and decreased the amplitudes of the circadian fluctuations in IOP for 12 successive weeks.

[*Jpn. J. Ophthalmol.*, **49**, 106-108(2005)] [Lab. of Biofunctional Molecules]

Vitreous Fluid Levels of ß-Amyloid$_{(1-42)}$ and Tau in Patients with Retinal Diseases.

Shinji YONEDA, Hideaki HARA*, Akira HIRATA, Mikiko FUKUSHIMA, Yasuya INOMATA and Hidenobu TANIHARA

To test the idea that ß-amyloid$_{(1-42)}$ (Aß$_{42}$) and tau contribute to the development of retinal diseases, we measured Aß$_{42}$ and tau concentrations in the vitreous fluid from patients with macular hole, diabetic retinopathy, or glaucoma concurrent with other ocular diseases. Vitreous samples were collected from patients who underwent vitrectomy, and sensitive and specific enzyme-linked immunosorbent assays were used to determine the concentrations of Aß$_{42}$ and tau. By comparison with the levels in the control macular-hole patients, there was a significant decrease in the Aß$_{42}$ level and a significant increase in the tau level in patients with diabetic retinopathy or glaucoma concurrent with other ocular diseases. Our findings indicate the possibility of a role for Aß$_{42}$ and tau in the pathogenesis of some retinal diseases.

[*Eur. J. Pharmacol.*, **520**, 118-126(2005)] [Lab. of Biofunctional Molecules]

Sulfatides, L- and P-Selectin Ligands, Exacerbate the Intimal Hyperplasia Occurring after Endothelial Injury.

Masamitsu SHIMAZAWA , Kazunao KONDO, Hideaki HARA*, Mitsuyoshi NAKASHIMA, Kazuo UMEMURA

This study was designed to determine whether sulfatides (3-sulfated galactosyl ceramides) which are native ligands of L- and P-selectin affect the development of intimal hyperplasia. Endothelial damage was inflicted on the femoral artery via the photochemical reaction between rose bengal and green light. Scanning electron and light microscopic observations 3 days after the injury indicated that sulfatides-treated animals had more neutrophils adhering to the injury site than vehicle-treated controls. At 21 days, sulfatides-treated animals had a greater neointimal area than controls. In in vitro studies, sulfatides (i) increased cytosolic free calcium in mouse neutrophils, (ii) caused increases in expression of Mac-1 (CD 11 b/CD 18) on the neutrophil membrane surface in mouse whole blood. These findings suggest that neutrophil accumulation on the subendothelial matrix or adherence of platelets mediated by adhesive interactions between L- or P-selectin and sulfatides may contribute to the development of intimal hyperplasia.

[*Eur. J. Pharmacol.*, **520**, 156 – 163 (2005)] [Lab. of Biofunctional Molecules]

Neutrophil Accumulation Promotes Intimal Hyperplasia after Photochemically Induced Arterial Injury in Mice.

Masamitsu SHIMAZAWA, Shinji WATANABE, Kazunao KONDO, Hideaki HARA*,
Mitsuyoshi NAKASHIMA, Kazuo UMEMURA

The aim of this study was to determine whether neutrophil accumulation would participate in the development of intimal hyperplasia after endothelial injury in mice, and whether d-myo-inositol hexakisphosphate (phytic acid) which inhibits the binding of L- and P-selectin to sialyl Lewis(X) could inhibit the development of intimal hyperplasia. Endothelial injury was inflicted in one femoral artery via the photochemical reaction between systemically injected rose bengal and transillumination with green light (wavelength: 540 nm). Scanning electron microscopic observation at 3 days after the injury showed an increase in the number of leukocytes adhering to the injury site. Histological observation at 21 days showed that in the neutropenia group administered anti-neutrophil antibody and in the phytic acid-treated group the progression of intimal hyperplasia was significantly attenuated by comparison with the corresponding control groups. These results suggest that neutrophil accumulation contributes to the initiation and/or development of intimal hyperplasia and L- and/or P-selectin may participate in their mechanisms.

[*Jpn. J. Thromb. Hemost.*, **16**, 212 – 221(2005)] [Lab. of Biofunctional Molecules]

Effect of Pamicogrel, a New Antiplatelet Drug, on the Progression of Sodium Laurate-induced Arterial Occlusion Disease in Guinea Pigs.

Masamitsu SHIMAZAWA, Yuki MIYAKE, Takafumi YAMAGUCHI, Koichi YOKOTA,
Takayuki SUKAMOTO and Hideaki HARA*

We examined the protective effect of pamicogrel, a new antiplatelet drug, on sodium laurate-induced peripheral arterial occlusive disease of posterior limb in the guinea-pig. In control animals, the sodium laurate injection at 1mg/leg into the femoral artery caused an ischemic change of peripheral tissues in the posterior limb followed by violet color around whole paw, edema, gangrene, mummification and fell off of fingers, whole paw and lower leg after 3 to 14 days. Skin temperature of thigh at the site of the sodium laurate injection significantly indicated lower values than that of sham-operated group at 1 and 3 days, and returned to sham-operated level at 7 days. Pamicogrel and ticlopidine dose-dependently inhibited the development of ischemic lesion in the posterior limb and lowering skin temperature of the ipsilateral thigh at site of the sodium laurate injection. Cilostazol had little effect in terms of preventing the progression of ischemic lesion and the lowering skin temperature of the ipsilateral thigh at the site of injury. These observations suggest that pamicogrel may be clinically effective against chronic arterial occlusive disease.

[*Brain Res.*, **1053**, 185-194 (2005)] [Lab. of Biofunctional Molecules]

Neuroprotective Effects of Minocycline against *in vitro* and *in vivo* Rretinal Ganglion Cell Damage.

Masamitsu SHIMAZAWA, Tetsumori YAMASHIMA, Neeraj Agarwal and Hideaki HARA*

The purpose of this study was to determine whether minocycline, a semi-synthetic tetracycline derivative, reduces (a) the *in vitro* neuronal damage occurring after serum-deprivation in cultured retinal ganglion cells (RGC-5, a rat ganglion cell line transformed using E1A virus) and/or (b) the *in vivo* retinal damage induced by *N*-methyl-D-aspartate (NMDA) intravitreal injection in mice. *In vitro*, retinal damage was induced by 24 h serum-deprivation, and cell viability was measured by Hoechst 33342 staining or resazurin-reduction assay. In cultures of RGC-5 cells maintained in serum-free medium for up to 24 h, the number of cells undergoing cell death was reduced by minocycline (0.2-20 μM). Serum-deprivation resulted in increased oxidative stress, as revealed by an increase in the fluorescence intensity for 5-(and-6)-chloromethyl-2', 7'-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA), a reactive oxygen species (ROS) indicator. Minocycline at 2 and 20 μM inhibited this ROS production. Furthermore, in mice *in vivo* minocycline at 90 mg/kg intraperitoneally administered 60 min before an NMDA intravitreal injection reduced the NMDA-induced retinal damage. These findings indicate that minocycline has neuroprotective effects against *in vitro* and *in vivo* retinal damage, and that an inhibitory effect on ROS production may contribute to the underlying mechanisms.

[*J. Jpn. Health Med. Associ.*, **14**, 20-27 (2005)] [Lab. of Health and Physical Education]

Effects of chronic exercise on the cellular immune functions in aged mice

Haruo SUGIURA, * Hiroko SUGIURA, Naotaka ISHIDA, Etsuo UEYA and Minoru OHNUKI

We determined the effects of voluntary running exercise on cellular immune functions in aged mice (male BALB/c mice, 16-month-old). The applied exercise consisted of spontaneous running in wheels for 3 days per week over 8 weeks. In the exercise group, stimulation indices by Con A and PHA were significantly higher than they were in the control group. When compared with the control group, the exercise group showed a significant increase in the splenic lymphocyte production of IL-2 stimulated by Con A. The production of IFN-γ stimulated by Con A of the two groups showed no significant differences. These results suggest that 8-week voluntary running exercise effectively enhanced cellular immune functions in aged mice, and that it contributes to the prevention of the immunosenescence.

[*J. Edu. Health Sci.*, **51**, 168-176 (2005)] [Lab. of Health and Physical Education]

Effects of voluntary running exercise on macrophage functions in middle aged mice

Haruo SUGIURA,* Hiroyuki NISHIDA and Seyed Mohammad MIRBOD

We determined the effects of voluntary running exercise on resident peritoneal macrophage functions in middle aged mice by measuring production of nitric oxide (NO) and interleukin1β (IL-1β) to stimulation by lipopolysaccharide (LPS). Male BALB/c mice (12 months old) were divided into two groups: a group given voluntary running exercise (exercise group) and a non-exercise group (control group). The applied exercise consisted of spontaneous running in wheels for 3 days per week over 8 weeks. Compared with the control group, the exercise group had a significant increase in macrophage production of NO stimulated by LPS. A significant increase in macrophage production of IL-1β stimulated by LPS was noticed in the exercise group. We concluded that voluntary running exercise enhanced macrophage functions in the middle aged mice.

[*BMC Compl. Alter. Med.*, **5**, 1-10 (2005)] [Lab. of Health and Physical Education]

Effects of Maharishi Amrit Kalash 5 as an Ayurvedic herbal food supplement on immune functions in aged mice

Ryoichi INABA,* Seyed Mohammad MIRBOD and Haruo SUGIURA

We determined the effects of administration of Maharishi Amrit Kalash 5 (MAK5) on immune functions in old mice (male C3H/He N, 22-month-old). MAK5 was given p.o. at 50 mg/kg, 100 mg/kg or 200 mg/kg per day for 2 months. We found that splenocytes proliferative responses and IL-2 production capacity in old mice gavaged with MAK5 at all doses were significantly higher than that in the old control group. Production of IFN-γ and IL-4 in old mice given MAK5 at doses of 100 mg/kg and 200 mg/kg were significantly higher than that in the old control group. The results suggest that MAK5 suppressed the age associated cellular immune function reduction, and that it contributes to the prevention of the immunosenescence.