

2006

The Jikei University School of Medicine

Research Activities 2006

The Jikei University School of Medicine

Published by The Jikei University School of Medicine 3-25-8 Nishi-shimbashi, Minato-ku Tokyo 105-8461, Japan

March 1, 2008

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Introduction

I am pleased to publish *Research Activities 2006*, which is a report on the scientific and educational activities at The Jikei University School of Medicine in 2006. In parallel with this English-language version, a Japanese version is published annually. This report contains the research activities in departments, institutes, and laboratories of the Medical Science Center at The Jikei University School of Medicine in 2006. In *Research Activities*, selected papers published by each department, institute, and laboratory are listed at the end of each report due to the limited space of the report. Similarly, the names of department staff are limited to those above lecturer.

Research Activities summarizes the annual research work at The Jikei University School of Medicine. I hope that *Research Activities* is widely used by people outside our university as well by as our staff. In addition, this report will be used to evaluate The Jikei University School of Medicine from the viewpoint of research activity. As President of The Jikei University School of Medicine, I have been encouraging traditional and unique research studies, in particular, research studies that support and are linked with the clinical activities of our university hospital. Evidence obtained from these traditional basic-research studies will contribute to medicine in the future. I would like to emphasize the importance of basic research from the long-term view. Ι also have been promoting research projects focusing on topics in various areas, including molecular biology, and research using highly advanced technology. Some results will contribute to the diagnosis and treatment of patients with refractory diseases. These projects have been partly supported by generous grants from the Japanese government (Ministry of Education, Culture, Sports, Science and Technology, and Ministry of Health, Labour, and Welfare) and various private foundations. Advanced clinical research studies of clinical departments are mostly performed in the university's Medical Science Center, because it is well equipped with various advanced instruments. We support the Medical Science Center financially and provide grants for young scientists to encourage their research activities. I hope that the research studies supported by these grants will be continued. The results of some of these projects are described in this report.

I greatly appreciate the cooperation of Professor Senya Matsufuji, Editor of the Jikeikai Medical Journal, and Associate Professor Masao Okazaki in editing this issue.

I am also grateful to the members of the Medical Information Center for their help in preparing this issue.

Satoshi Kurihara President The Jikei University School of Medicine

February 2008

Continuing Medical Education Center The Continuing Medical Education Committee

Kunihiro Kurihara, Director Yutaka Suzuki Katsuyoshi Tojo Keizo Takagi Akihiko Ohno

General Summary

The Continuing Medical Education (CME) Center was established in 1982 to commemorate the centennial of The Jikei University and to support the education of doctors outside the university hospital. Registered members consist of alumni throughout Japan, members of the local medical association, and physicians who have been approved by the Jikei CME Center. Members are allowed to use the facilities (video, library) of the Center and other facilities (medical library, medical museum) of the university. A telephone service is available at all times. Members may also attend or participate in summer and monthly seminars sponsored by the Center and in scientific meetings and conferences held by the department.

Activities

1. Registered members: 259 (as of April 1, 2007)

Members using the Center: 189 per year

Telephone service: 117 cases

2. The 27th summer seminar was held on August 5, 2006. 81 persons participated.

3. Monthly seminars were held on the second Saturday afternoons of the month in April, May, June, July, September, November, February, and March. 25 to 30 persons attended each seminar.

4. The CME Center News is mailed monthly to registered members.

Center for Medical Education

Masahiro Kawamura, Professor and Director Naofumi Kimura, Professor Tetsuya Kawamura, Associate Professor Hideaki Kashiwagi, Associate Professor Kazunori Utsunomiya, Associate Professor Sugino Oishi, Associate Professor Nobuyuki Furutani, Lecturer Hiroyuki Takahashi, Lecturer Hideki Sasaki, Lecturer Osamu Fukushima, Professor and Vice-Director Mariko Itsubo, Professor Hisashi Onoue, Associate Professor Masato Matsushima, Associate Professor Machiko Hirao, Associate Professor Yasuki Unemura, Lecturer Yoshio Ishibashi, Lecturer Toshikazu Sakuyama, Lecturer

General Summary

The Office of Medical Development was established in 1999. Staff members were recruited from the School of Medicine. Its main interests were analysis of medical education reports published by the Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labor and Welfare; and medical education associations; technical support of faculty; management of faculty development and educational seminars; and the implementation of tutorials, objective structured clinical examinations (OSCEs), and community-service programs in undergraduate medical education. However, many improvements have been required in our undergraduate medical and nursing education, postgraduate clinical training programs, and continuing professional development health-care workers. In 2005, the Office of Educational Development was reorganized as the Center for Medical Education. The Center consists of the Office of Medical Education (Director, Professor Kimura), the Office of Nursing Education (Director, Associate Professor Hirao), the Office of Postgraduate Clinical Training (Director Associate Utsunomiya), and the Office of Educational Development (Director, Professor Fukushima). Furthermore, the secretariat was set up in the Center in April. The Office of Medical Education contributed to revisions of the undergraduate curriculum, to implementation of OSCEs in years 4 and 5, and to faculty development programs held in June (writing multiple-choice questions) and December (rater training for OSCE).

The Office of Postgraduate Clinical Training helped evaluate the residency program and conducted faculty development programs in November and February. The description of our residency program for medical students has been updated on the university's Internet home page. We established a system for reviewing the mental and physical problems of trainees to allow them to carry out the program effectively.

Research Activities

1. Establishing an e-Learning system for undergraduate students and health-care professions in the community: Our proposal was selected as a "Distinctive university educational program according to social needs" by the Ministry of Education, Culture, Sports, Science and Technology in 2006. As the first stage of this project, we checked educational materials offered by intranet from the view point of authors' copyright.

2. Interprofessional Education: We presented an international session, "Problem-Based Learning in Interprofessional Education" at the 6th Asia-Pacific Conference on Problem-Based Learning held at the Tokyo Women's Medical University in May. We visited King's College School of Medicine in London to observe their interprofessional education program, especially in general practitioner offices, and hold discussions with the faculty in June and October. Furthermore, we invited Professor Roger Jones, chair of the Department of General Practice and Primary Care at King's College School of Medicine, to present a seminar (November). Risk-Management and Ethics Workshops at attached hospitals were also held for interprofessional education. Professionals of many occupations attended the workshop. We organized workshops held in April (Nishi-shimbashi), May (Aoto), June (Daisan), July (Kashiwa), August and September (Nishi-shimbashi), October (Aoto), November (Daisan), December (Kashiwa), and January (Nishi-shimbashi).

3. Abuse of medical students: Our survey found that 68.5% of medical students experienced abuse during clinical practice. Several students reported that they had frequently been neglected by teaching physicians during clinical clerkships and that such attitudes discouraged them and decreased their motivation.

4. Advanced OSCE: We observed OSCEs at the graduate level held at Sapporo Medical College (September) and the School of Medicine, Okayama University (October), to investigate requirements in OSCE at medical licensure. Our research group held a symposium on Advanced OSCE in November.

5. Contribution to other institutions of higher education (faculty development lecture and workshop): Tokai University Hospital (April, September, and October); Kitasato University Hospital (April); Matsumoto Dental College (May); School of Medicine, Yamagata University (August); Tokyo Medical and Dental University (September and December); Kyouritu Pharmacy College (October and March); Japanese Association of Medical Education (November); School of Dentistry, Niigata University (December); Kanazawa Medical College (December), and the Occupational Therapy/Physical Therapy Association (January).

Publications

Nagata-Kobayashi S, Sekimoto M, Koyama H, Yamamoto W, Goto E, Fukushima O, Ino T, Shimada T, Shimbo T. Medical students abuse during clinical clerkship in Japan. J Gen Intern Med 2006; 66: 212-8.

Fukushima O. Common achievement test (in Japanese). *Nihon Ishikai Zasshi* 2006; **135:** 560-2.

Fukushima O. CBT (in Japanese). Showa Igakkai Zasshi 2006; 66: 63-7.

Itsubo M. Diagnosis and treatment of polycystic liver disease (in Japanese). *Sogo Rinsho* 2006; **55:** 1339–40.

Reviews and Books

Nagata-Kobayashi S, Sekimoto M, Koyama H, Yamamoto W, Goto E, Fukushima O, Ino T, Shimada T, Shimbo T, Fukui T. Medical students abuse during clinical clerkship. *Igakukvouiku* 2007: **38**: 29-35.

Fukushima O. New stream in undergraduate medical education 3: Common achievement test CBT. In: Japanese Association of Medical Education, editor. White Paper of Medical Education in Japan. Tokyo: Shinohara Shuppanshinnsha; 2006. p. 65-9.

Department of Anatomy (I)

Yoshinori Kawai, Professor

General Summary

Our department's research activities have focused on neuroanatomy and gross anatomy. In neuroanatomical research, organization and development of neuronal networks were investigated to elucidate brain function and diseases using immunocytochemistry, electron microscopy, *in-situ* hybridization histochemistry, single-cell tracer injection, and patch-clamp electrophysiology. Our primary interests are the architecture and dynamics of neuronal networks. In gross anatomical research, the functional importance of variations of organ systems was explored using human cadavers and animals.

Research Activities

Pattern differentiation of excitatory and inhibitory synaptic inputs on distinct neuronal types in the rat caudal nucleus of the tractus solitarius

Region- and size-specific neuronal organizations of the caudal nucleus of the tractus solitarius (cNTS) were investigated, followed by analyses of excitatory and inhibitory synaptic input patterns onto specific cell types by patch clamp recordings and immunoelectron microscopy. The cell-size distribution and numerical density of cNTS neurons were examined in subregions at the level of the area postrema. In the subpostremal and dorsomedial subnuclei, characterized by the presence of dense glutamatergic and sparse GABAergic somata, small calbindin neurons constituted 42% of all cells. The medial subnucleus contained large numbers of glutamatergic, GABAergic, and catecholaminergic somata, and large tyrosine-hydroxylase-containing cells constituted 13% of cells in this region. In total, small neurons ($<150 \ \mu m^2$) represented about 80% of the cell population in the cNTS. Predominant excitatory postsynaptic currents were observed in the adult small neurons, while inhibitory postsynaptic currents were more evident in larger neurons, regardless of subnuclear location. This distinct differentiation of postsynaptic current patterns was not evident in neonates. GABAergic synapses were more frequently associated with dendrites of large catecholaminergic cells (73%) than with those of small calbindin-containing cells (10%) in adults. These results indicate that differential synaptic input patterns were developmentally established in distinct small and large neurons.

Local axonal arborization patterns of distinct neuronal types in the cNTS

Neurons in the cNTS differ in size (50 to 450 μ m² in somal area) and other morphologic characteristics. For a more objective classification of cNTS neurons, their morphologic features were analyzed quantitatively on the basis of reconstructed biocytin-filled cells after whole-cell patch-clamp recordings. According to the patterns of axonal branching behaviors, cNTS cells could be classified into 2 groups: smaller cells (94.1 μ m² in mean somal area; range, 62-120 μ m²; n=22) and larger cells (245 μ m² in mean somal area;

range, 142–411 μ m²; n=23). Extensive axonal arborization with numerous possible synaptic boutons was specifically associated with smaller neurons, whereas larger cells possessed no or few axon collaterals, suggesting their distinct roles as local-circuit neurons (or interneurons) and projection neurons, respectively. With regard to somatodendritic characteristics, the following correlations with cell size were found: smaller cells had larger form factors than did larger cells (P<0.05). Larger neurons had more extensive dendritic arborization, expressed by total dendritic length (P<0.01) and number of dendritic branching points (P<0.01), than did smaller cells. These results suggest that small cNTS neurons contribute specifically to the integration of input information generated in local circuits, whereas large neurons convey the integrated information to other autonomic brain regions.

Postnatal development of GABAergic axon terminals in the rat NTS

The proper function of the brain depends on a precise arrangement of excitatory and inhibitory synapses. Although the cNTS plays a pivotal role in cardiorespiratory reflexes, we know little about the formation of the local neural network in the cNTS. In the present study, we focused on GABAergic axon terminals and investigated postnatal changes in GABAergic synaptic organization in the rat cNTS immunocytochemically at both the light and electron microscopic levels. Counting of synaptic and nonsynaptic GABAergic axon terminals revealed that the number of GABAergic axon terminal in the cNTS was constant until the second postnatal week and that GABAergic axon terminals were reorganized at approximately postnatal day (P)10. Electron microscopic observation revealed that most GABAergic axon terminals formed axosomatic synapses on neurons with smaller soma (smaller neurons) at P2 to P4 but that the number of axosomatic synapses decreased considerably after P8. Orphan GABAergic boutons were present specifically near somata of smaller neurons at P10, and the number of axodendritic synapses on thicker dendrites decreased gradually during postnatal development. These results show that GABAergic axon terminals detach from somata of smaller neurons during the second postnatal week. Such morphologic changes in axon terminals could cause changes in electrophysiological activity and might contribute to the reorganization of the local network within the cNTS from the neonatal to the adult type. These postnatal changes in the cNTS local network might be required for the cardiorespiratory reflexes of the adult type.

Activity-dependent reorganization of local circuitry in the developing visceral sensory system

Neural activity during critical periods could fine-tune functional synaptic connections. *N*-methyl-D-aspartate receptor activation is critically implicated in this process, and blockade leads to disruption of normal circuit formation. This phenomenon has been thoroughly investigated in several neural systems, including the somatosensory system, but has not yet been evidenced in the visceral sensory system. Ultrastructural analysis of GABAergic synapses and electrophysiological analysis of inhibitory and excitatory postsynaptic currents of cNTS cells revealed that developmental changes in the synaptic organizations were blocked by MK-801, an antagonist of *N*-methyl-D-aspartate receptor,

when administered on P5 to P8, a presumed critical period for the visceral sensory system. Normal synapse reorganization during postnatal development dictates undifferentiated neonatal cNTS neurons, in terms of synaptic input patterns measured with electron microscopy and electrophysiology, into 2 cell groups: small and large cells under far stronger excitatory and inhibitory influence, respectively. Blockade by MK-801 during the critical period might leave adult neurons wired in the undifferentiated synaptic networks, possibly preventing synapse elimination and subsequent stabilization of the proper wiring.

Publications

Okada T, Yoshioka Y, Inoue K, Kawai Y. Local axonal arborization patterns of distinct neuronal types in the caudal nucleus of the tractus solitarius. *Brain Res* 2006; **1083:** 134-44.

Yoshioka M, Tashiro Y, Inoue K, Kawai Y. Postnatal development of GABAergic axon terminals in the rat nucleus of tractus solitarius. Brain Res 2006; 1107: 111-20.

Yoshioka M, Okada T, Inoue K, Kawai Y. Pattern differentiation of excitatory and inhibitory synaptic inputs on distinct neuronal types in the rat caudal nucleus of the tractus solitarius. *Neurosci Res* 2006; **55:** 300-15.

Department of Anatomy (II)

Hiroshi Ishikawa, Professor Toshiaki Tachibana, Lecturer Hisashi Hashimoto, Associate Professor Takaki Shimada, Lecturer (Concurrent Post)

General Summary

We are engaged in teaching histology, embryology, endocrinology, and neuroanatomy to medical students. Our research projects have 5 focuses: histologic endocrinology, molecular biology of embryogenesis from embryonic stem (ES) cells, cell differentiation, the role of extracellular matrices in morphogenesis, and measurements with oxygen electrodes of respiration of cultured cells as a metabolic indicator for drug screening.

Research Activities

Establishment and characterization of a spontaneously cisplatin-resistant human gastric adenocarcinoma cell line

We established a cell line (IGSK-I) derived from a poorly differentiated adenocarcinoma of stomach. IGSK-1 cells grew as adhesive or floating cultures in the culture dishes. The population doubling time was about 34 hr. The cells were round shaped and had one nucleus. Electron micrographs of the cells showed many secretory granules and well-developed mitochondria. IGSK-1 cells secreted gastrin and somatostatin. Although IGSK-1 cells showed immunoreactivity against serotonin, high performance liquid chromatography revealed the conditioned medium by IGSK-1 cells contained 3.7 ng/ml of serotonin, while cell-free medium contained 203.0 ng/ml of serotonin. These results suggested the IGSK-1 cells may reuptake serotonin in GM. The anti-cancer susceptibility test with an oxygen electrode-apparatus (DAIKIN, Dox-10, JPN) revealed the cells derived from the same adenocarcinoma were sensitive to TXL and not sensitive to CDDP, CPT-11 and 5-FU.

Establishment and characterization of a cisplatin-resistant cell line derived from ascitic fluid of recurrent mucinous and poorly differentiated adenocarcinoma of stomach We successfully established a cisplatin-resistant adenocarcinoma cell line (IGSK-2) derived from ascitic fluid of a recurrent gastric cancer whose histopathological diagnosis was mucinous and poorly differentiated adenocarcinoma (mucinous>poorly) of stomach. IGSK-2 cells grew as adhesive cultures in the culture dishes. The population doubling time was about 83 hr. In xenotransplantation, no tumor mass was produced in host mice. Electron micrographs revealed the cells had one or two nuclei, well-developed Golgi apparati and numerous mitochondria. No mucous droplets were contained in the cytoplasm. The anti-cancer susceptibility test revealed the cells derived from the ascitic fluid were sensitive to TXL and not sensitive to CDDP, CPT-11 and 5-FU.

Establishment and characterization of human rhabdomyosarcoma cell line, HUMEMS, derived from primary embryonal rhabdomyosarcoma of the breast The HUMEMS cell line was established from the pleural fluid of a 13-year-old girl who had embryonal rhabdomyosarcoma (alveolar type) of the breast. The HUMEMS grew as monolayer and consisted of two cytologic types of cells resembling those of the original tumor, spindle cells and large multinucleated cells. The multinucleated cells contained immunoreactive myoglobin. This cell line is very useful for studies on the susceptibility of anti-cancer drugs and analysis of the mechanism of metastasis.

Establishment and characterization of two human malignant mesothelioma cell lines (HMMME and HMMMF) from the same patient

Two cell lines designated HMMME (epithelial) and HMMMF (fibroblastic) were established from a pleural fluid from a Japanese male who suffered from cough, fever, dyspnea and fatigue. These cell lines were separated by the colonial techniques from the primary cultures, grew well without interruption for 5 years and characterized as producing hyaluronic acid. The HMMME cells were epithelial cell like and transplantable into the subcutis of nude mice, while the HMMMF line were fibroblast-like and transplantable into submucosa of Hamster's cheek pouches.

Establishment and biological characteristics of stem cell strains derived from human amniotic membrane

The amniotic membranes of full-term placentas obtained with the informed consent of patients were cultured and followed by cloning the small round cells that proliferated in multiple layers to obtain human amniotic membrane (HAM) stem cells. Forty amniotic membrane stem cell lines were established and these cell lines had common cellular biological properties. More specifically, the cells maintained a normal human karyotype, an undifferentiated state, and were alkaline phosphatase activity. They also expressed transcription factors Oct-4, Rex-1 and Ecat-4.

Induction of differentiation of human amniotic membrane stem cells in vitro

The multi-differentiation ability of human amniotic membrane stem (HAM-1) cells were assessed. The addition of 1) EGF, FGF2 and FGF9, 2) VEGF, or 3) FGF4 and HGF to the culture medium induced the differentiation of HAM-1 cells to 1) astrocytes and nerve cells, 2) endothelial cells and blood cells or 3) hepatocytes, respectively. Embryoid bodies formed by the hanging drop culture of EGFP gene-transfected HAM-1 cells mixed with ddY mouse EES-7 cells (ES cells) were, then, cultured stationary in a culture medium containing embryotrophic factors (ETFs). The bodies formed tridermal germ layer primordial organs (including skin, blood cells, neural tubes and digestive organs), and fluorescence of EGFP was confirmed in each of the organs. The transplanted HAM-1 cells were stem cells that have the ability to multi-differentiate into tridermal germ layer primordial organs, and had considerable expectations for application to regenerative medicine.

Publications

Nojiri H, Shimizu T, Funakoshi M, Yamaguchi O, Zhou H, Kawakami S, Ohta Y, Sami M, Tachibana T, Ishikawa H, Kurosawa H, Kahn RC, Otsu K, Shirasawa T. Oxidative stress causes heart failure with impaired mitochondrial respiration. J Biol Chem 2006; **281**: 33789-801.

Tamagawa T, Ishiwata I, Nakamura Y, Ohoi S, Ishikawa H. Human amnion mesenchyme cells possess hepatocyte-like characteristics *in vitro*. *Hum Cell* 2007; **20**: 77-84.

Ohi S, Kyoda S, Tabei I, Ninomiya K, Sugiyama K, Hashimoto H, Tachibana T, Ishikawa H. Establishment and characterization of a cell line (NABCA) derived from metastatic lymph nodes of breast scirrhous carcinoma. *Hum Cell* 2006; **19:** 126-32.

Ohi S, Takahashi H, Ninomiya K, Nakajima M,

Hashimoto H, Tachibana T, Yanaga K, Ishikawa H. Establishment and characterization of a cisplatinresistant cell line (IGSK-1) from a poorly differentiated gastric ademocartinoma. *Hum Cell* 2007; **20:** 15–22.

Ohi S, Takahashi N, Hashimoto H, Tachibana T, Hirabayashi T, Sugiyama K, Yanaga K, Ishikawa H. Establishment and characterization of an IGSK-2 cell line derived from ascitic fluid of recurrent hCG and somatostatin secreted adenocarcinoma of the stomach. Hum Cell 2007; 20: 52-61.

Ohi S. Characterization, anti-cancer drug susceptibility, and atRA-induced growth inhibition of a novel cell line (HUMEMS) established from pleural effusion of alveolar rhabdomyosarcoma of breast tissue. *Hum Cell* 2007; **20:** 39-51.

Department of Physiology (I)

Yoshiki Umazume, Professor

Shigeru Takemori, Lecturer

General Summary

Our efforts have been concentrated on clarifying the mechanism of skeletal muscle contraction.

Research Activities

Dynamics of the sarcomere lattice structure of striated muscles

Regulated contractile interaction of striated muscle is performed within the liquid crystalline structure consists of thin actin filaments and thick myosin filaments. To understand the physiological contraction mechanism of structurally organized striated muscles, it is essential to understand the dynamics of its lattice spacing upon sarcomerelength perturbation. Using gelsolin to selectively remove thin actin filaments from skinned sarcomeres, we prepared the specimen which consists of thick myosin filament lattice without thin actin filaments. On the 45XU beam line at the Large-scale Synchrotron Radiation Facility (Spring 8, Hyogo Prefecture), we performed an x-ray diffraction study of the gelsolin-treated skinned striated muscles. The gelsolin-treated muscle was found to preserve physiological fine structures except for those related to thin filaments. Monitoring sarcomere spacing with the laser diffraction technique and passive tension with a force transducer, we observed the dynamic response of the gelsolin-treated lattice against sarcomere-length perturbation. The obtained results indicate that the lattice spacing was not determined by any of the mechanisms so far proposed, including the Y-shaped elastic connectin filament model and the Derjaguin, Landau, Verwey, and Overbeek model. We are now attempting to develop a novel model that accounts for the dynamics of lattice spacing and our previous results obtained with nuclear magnetic resonance experiments on the dynamics of myowater. This work was performed in collaboration with Drs. Okuyama and Toyota of Kawasaki Medical University and Dr. Yagi of the Large-scale Synchrotron Radiation Facility.

Structural change of mutant troponin related to hypertrophic cardiomyopathy

Many troponin mutants causing familial cardiomyopathy have been reported. To clarify the molecular mechanism of cardiomyopathy related to troponin, we performed a molecular dynamics study of the structure of troponin mutants related to familial hypertrophic cardiomyopathy (HCM). Two different troponin-T mutants, Glu244Asp and Lys247Arg, which are related to HCM were studied.

Dynamics was calculated with the Amber software package (version 9). Iteration was done in a TIP3P water sphere with 0.5- or 1-femtosecond time-step in periodic boundary condition at constant temperature (310 K) and pressure. Model structures of troponin mutants were constructed by introducing the mutation to the crystal structure of human

cardiac troponin (core region of the TIC complex) obtained from Protein Data Bank (ID number 1J1E). More than 8 trajectories of 1 nanosecond were obtained for wild and mutant structures.

We found that that electrostatic interaction between troponin I and troponin T which linked alpha helices of troponin T and troponin I in the wild type was lost in the mutant. Furthermore, when a terminal residue of troponin I was pulled toward an actin molecule mimicking the intramolecular force on relaxation, the structural change observed differed between the mutant and the wild type. This difference is likely involved in the enhanced tension development in mutant troponin.

The effect of trehalose on muscle contractility

Trehalose is a disaccharide present in various organisms which protects proteins or cellular membranes from inactivation and denaturation due to dehydration, heat, and cold, which can cause drastic changes in the water state. On the other hand, trehalose is reported to reduce the dynamics of water molecules through its hydrogen-bond network which is stronger than those of other disaccharides. Under the hypothesis that trehalose influences muscle contractility through changes in the water state, we examined the effect of the disaccharides trehalose and sucrose on the contractility of skeletal muscle.

Mechanically skinned fibers were prepared from sartorius muscles of bullfrogs, and pCa-tension curves were obtained with or without 0.5 M disaccharides. Before the experiment, the internal membrane systems of the fibers were destroyed and the sarcomere e length was adjusted to 2.4 μ m. The Ca sensitivity was decreased by 0.4 units with both disaccharides, whereas maximum tension was increased by 10% with trehalose alone. The differing effects of the disaccharides on the maximum tension would not be due to the increase in viscosity, which did not differ between the disaccharides. These results suggest that the change in water state induced by trehalose affects muscle contractility stereochemically.

Specific volume of myofibril suspension with polyethyleneglycol

Polyethylenglycol narrows the lattice spacing of skinned skeletal muscle sarcomeres. Because the polyethylenglycol molecule (molecular weight, 3,350) is several nanometers in size, a lattice spacing of 40 nm seems to be large enough to allow polyethylenglycol to penetrate. To determine whether polyethylenglycol penetrates the sarcomere, the specific gravity of myofibril suspensions from rabbit psoas muscle was measured in the presence or absence of polyethylenglycol. If polyethylenglycol does not penetrate the sarcomere, the specific gravity of the supernatant after centrifugation of the myofibril suspension would be larger than the specific gravity of the myofibril suspension. Our results suggest that polyethylenglycol does not penetrate the sarcomere.

Department of Physiology (II)

Satoshi Kurihara, Professor Masato Konishi, Visiting Professor Yoichiro Kusakari, Lecturer Iwao Ohtsuki, Visiting Professor Norio Suda, Lecturer

General Summary

The main research topics of our department are the physiology of cardiac and skeletal muscle contraction and other related subjects.

Research Activities

Physiology of cardiac muscles

Alpha₁ adrenoceptor (α_1 -AR) signaling plays crucial roles in the regulation of physiological and pathophysiological cardiac cellular responses. We next investigated the subcellular localization of α_1 -AR subtypes (α_{1A} and α_{1B}) and distinct signaling pathways in native cardiac cells. We compared the functional effects of subtype-selective α_1 -AR stimulation on L-type Ca²⁺ current (I_{ca,L}). We provided the direct evidence that cardiac α_1 -AR signaling diverges at the level of α_1 -AR subtype and G-protein. The α_{1A} -AR and α_{1B} -AR signaling pathways couple with different G-proteins, $G_{q/11}$ and G_{o} , respectively and produce different functional outcomes (an increase and decrease of I_{CaL}). We investigated the relations among the uptake rate, content, release (Ca2+-induced Ca2+ release [CICR]), and the leakage of Ca²⁺ in cardiac sarcoplasmic reticulum (SR). To modulate the Ca^{2+} uptake rate of SR, we used transgenic mice hearts that over-express sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA2a; SERCA-TG mice) or sarcolipin (SLN-TG mice), a SR-coupled protein to suppress Ca²⁺ uptake. We employed saponin-treated thin trabeculae to directly apply various solutions to SR. The Ca²⁺ remaining in the SR after various maneuvers modulating SR functions was released by caffeine (50 mM), and the released Ca²⁺ was measured with fluo-3. The Ca²⁺ uptake rate was estimated by measuring Ca²⁺ content in the SR after Ca²⁺ was loaded into the SR in the presence of ATP (4 mM) and various concentrations of Ca²⁺ for different periods. The Ca^{2+} leakage was estimated by measuring the Ca^{2+} remaining in the SR after it was washed with a solution containing EGTA for various durations (5 to 300 seconds) after Ca²⁺ loading. The CICR was estimated by measuring the Ca²⁺ remaining in the SR after CICR was induced by the application of solutions containing various concentrations of Ca^{2+} (pCa, 7.6-4.3) without ATP after Ca^{2+} loading. With a short Ca^{2+} -loading time, the Ca^{2+} content was larger in SERCA-TG mice (113.8%) and smaller in SLN-TG mice (71.6%) than in control non-transgenic mice. At pCa 6.6, the Ca²⁺ content was significantly larger in SERCA-TG mice (199.0%) and smaller in SLN-TG mice (69.8%) than that in non-transgenic mice. The maximal Ca^{2+} content, CIC, and Ca²⁺ leakage in SERCA-TG and SLN-TG mice did not differ from those in non-transgenic mice. Thus, Ca^{2+} leakage is independent of the rate of Ca^{2+} uptake in the SR, and the altered Ca^{2+} leakage in heart failure is mainly due to the dysfunction

of ryandine receptors.

Skinned cardiac fibers exhibit spontaneous oscillatory contraction (SPOC) over a broad range of intermediate activating conditions (Ca-SPOC) or in the coexistence of ADP and Pi under relaxing conditions (ADP-SPOC). We recently reported that the period of sarcomeric oscillations during SPOC correlates with that of the heartbeat in various animal species. These findings suggest that the intrinsic auto-oscillatory property of sarcomeres (myofibrils) may contribute to myocardial beating *in vivo*.

We investigated the possible involvement of troponin in the length effect (leftward shift of pCa-tension relation at longer muscle length) in cardiac muscle. We used skinned porcine left ventricular muscle, and cardiac troponin was replaced with fast skeletal troponin (sTn; prepared from rabbit psoas muscle). The replacement of cardiac Tn with sTn markedly attenuated length-dependent activation. We also measured the rate constant of force re-development (k_{tr}) and found that k_{tr} increased at submaximal activation upon sTn reconstitution, suggesting a reduction in the fraction of detached cross-bridges that can potentially produce active force upon attachment to actin.

Mechanism of lower Ca²⁺ sensitivity observed in immobilized skeletal muscle

Immobilization of rat hind limb causes a marked reduction in the wet weight of soleus muscle. We found that in immobilized fibers, Ca^{2+} sensitivity was reduced with a reduction in maximal Ca^{2+} -activated force. To clarify the molecular mechanism of these mechanical changes, we measured interfilament lattice spacing with the small-angle X-ray diffraction method. The interfilament lattice spacing was expanded in immobilized fibers, which could explain the lower maximal tension and the lower Ca^{2+} sensitivity of immobilized skeletal muscle fibers.

L-type Ca²⁺channel in secondary hyperparathyroidism

We investigated the effect of the extracellular Ca^{2+} concentration of the cell-culture medium on the depolarization-induced Ca^{2+} transients (with 150 mM K⁺) in parathyroid cells isolated from patients with secondary hyperparathyroidism. The peak of the Ca^{2+} transients was dependent upon the Ca^{2+} concentration in the culture medium. Thus, the expression of the voltage-dependent Ca^{2+} channel is influenced by the Ca^{2+} concentration around the parathyroid cells.

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Department of Biochemistry (I)

Kiyoshi Ohkawa, Professor Tadashi Asakura, Lecturer Koji Takada, Associate Professor

Research Activities

Cancer research

1. To establish methods for early cancer diagnosis and therapy, we evaluated several molecular properties of CD147 for cancer-cell-targeting. CD147, known as an extracellular matrix metalloprotease inducer (EMMRPIN) or basigin, is a transmembrane glycoprotein with two immunoglobulin-like domains. A murine monoclonal antibody against CD147 (MAb12C3) was generated by Ohkawa, et al. in 1995. Several studies of clinical tumor specimens have demonstrated significant correlations between patents' prognoses and expression levels of the CD147 protein in the tumors. To confirm these results, we performed immunohistopathologic studies in patients with endometrial carcinoma (112 cases) or early hepatocellular carcinoma (22 cases). Expression levels of CD147 on the surfaces of tumor cells were significantly correlated with both prognosis and malignant behavior, such as metastasis and invasion of tumor cells. In particular, CD147 expression in early hepatoma was useful pathodiagnostic information, even if small specimens had been obtained with a fine-needle aspiration biopsy. The tumortargeting ability via CD147 molecules localized on the surfaces of tumor cells was examined with tumor cells expressing CD147 at high levels and their CD147-knockdown sublines. An anticancer drug directed against CD147, MAb12C3-antibody-conjugated doxorubicin, showed specific cytotoxicity against CD147-expressing tumor cells both in *in vitro* and *in vivo* experiments. For early detection, diagnosis, and treatment of cancer by means of ultrasound technology, the MAb12C3 antibody and its active Fab' fragments were coupled to ultrasonographic contrast agents containing nanobubbles or microbubbles. Methods for effectively detecting accumulated bubbles in vitro and in vivo are now being investigated.

2. Resistance of tumor cells to chemotherapeutic agents is a serious obstacle in cancer therapy. A conjugate of doxorubicin and glutathione via glutaraldehyde (GSH-DXR) strongly inhibited the glutathione S-transferase (GST) activity of rat hepatoma AH66 cells. Treatment of the cells with GSH-DXR induced apoptosis, including caspase-3 activation, DNA fragmentation, and activation of c-Jun N-terminal kinase (JNK). The stress-induced apoptosis pathway *via* JNK activation was further evaluated by means of a JNK inhibitor, SP600125, and a dominant negative expression of mutant molecules with site-directed mutagenesis (K55R) in the ATP-binding domain of JNK in cells over-expressing GSTP1-1. Phosphorylation of JNK induced by GSH-DXR also resulted in apoptosis with translocation of cytoplasmic Bax to mitochondrial membranes, which was suppressed by GSTP1-1. Additionally, treatment of AH66 cells with GSH-DXR caused deamidation of BcL-xL (N52D and N66D), which was suppressed by GSTP1-1, followed by the induction of apoptosis. Further studies are necessary to

clarify the mechanism.

3. Six cell lines resistant to epoxomicin were established. The epoxomicin-resistant cell lines are reliable tools for therapeutic evaluation of proteasome inhibitors in preclinical trials. Moreover, these cell lines may also be useful for clarifying mechanisms of resistance to proteasome inhibitors and examining a wide variety of proteasomal functions.

Other Research

1. Using methods to purify and identify ubiquitinated proteins in biological materials, we analyzed several ubiquitin-protein conjugates in Tris-saline soluble and Tris-saline-insoluble but 2% sodium dodecylsulfate (SDS)-soluble fractions in cadmium-exposed human proximal tubular HK-2 cells and brains from a model mouse of Niemann-Pick type C (NPC) disease (lipid storage disease with progressive neuronal death). Immunofluorescent-antibody studies demonstrated that HK-2 cells exposed to cadmium at a concentration of 70 μ M (median lethal dose) showed a marked but diffuse increase in ubiquitin-protein conjugates without aggresome formation. Mean levels of the SDS-soluble ubiquitin-protein conjugates in the cerebrums of NPC(-/-) mice (4 and 9 weeks old) were significantly higher (up to two fold) than those in wild-type or heterozygous mice.

The regulatory mechanisms of transcriptional co-activator with PDZ-binding motif 2. (TAZ)-linked fibroblast growth factor (FGF)/receptor signaling, which plays an essential role in ossification, were investigated with MC3T3-E1 cells. Although TAZ functions as a transcriptional coactivator for RUNX2 and a co-repressor of peroxisome proliferator-activated receptor (PPAR) γ , the regulatory mechanisms of TAZ protein expression are largely unknown. Our findings suggest that FGF2 regulates osteoblast differentiation through TAZ protein expression via several signal-transduction cascades. 3. The radial flow bioreactor (RFB) is a highly functioned 3-dimensional culture system that can be used for high-density culture maintaining the original cellular functions and mimicking the architecture of human tissues. Several human cancer cell lines, which were also cultured in this system, were prepared for a well-organized artificial tumor tissue model in vitro, rather than for tumor tissue xenotransplantation These results suggest that the RFB culture method is a useful and into nude mice. powerful system for improving and maintaining conditions during acute liver failure in clinical situations and for studying the safety and efficacy of newly synthesized drugs or biomaterials before clinical use. To evaluate the efficacy of anticancer agents, a detection and evaluation system for reliable cell number in RFB in situ was established using a ¹³C-glucose/¹³CO₂ assay system.

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Department of Biochemistry (II)

Kiyoshi Ohkawa, Professor

Senya Matsufuji, Professor

General Summary

Antizyme (AZ) is a negative regulator of polyamines. It is conserved in a wide range of eukaryotes from yeasts to humans, and 3 AZ subtypes (AZ1, AZ2, AZ3) have developed in mammals. We have been studying the mechanisms of polyamine-dependent translational frameshifting required for the expression of AZ and the physiological function of AZ.

Research Activities

Mechanism of antizyme translational frameshifting

The messenger (m)RNAs of AZ1 and AZ2 have a pseudoknot structure as a signal for translational frameshifting. We used the ultraviolet-crosslinking method to survey RNA-binding proteins that bind to the pseudoknot structure and affect frameshift efficiency. Some of the candidate proteins of 20 to 50 kDa identified in the previous year turned out to be nonspecific. However, a new candidate protein of 34 kDa that specifically binds to a mutant version of the pseudoknot was identified. We are testing the hypothesis that the 34-kDa protein interacts with the partially melted pseudoknot structure by elongating ribosomes. We have also purified the 34-kDa protein using affinity to the mutant pseudoknot for identification with mass spectrometry.

The mammalian AZ frameshift signal sequence directs backward hopping of ribosomes or -2, -5, and -8 frameshifting in *Escherichia coli*. To facilitate the analysis of these phenomena, we constructed a vector with upstream glutathione-S-transferase and downstream Protein A-His tag reporter genes. The frameshift sequence was inserted between the two reporters. After purification with the tags, the product was cleaved with a specific protease at the border with the upstream reporter and subjected to mass spectrometry. The backward hopping was confirmed to occur in this system. When the transfer (t)RNA-binding codon for -8 frameshifting was mutated, the corresponding product disappeared, suggesting that the tRNA-binding site is a signal for the backward hopping.

We continued screening for novel recoding (reprogrammed genetic decoding) genes among the target genes of nonsense-mediated mRNA decay (NMD) in the fission yeast *Schizosaccharomyces pombe*. DNA microarray and computer analyses revealed that mRNAs of 98 genes increased in an NMD-knockout strain and that 33 of the genes contained potential recoding sites.

Study with AZ1 knockout mice

We demonstrated that homozygous AZ1 knockout mice die from impairments of embryonic hematopoiesis. Our earlier experiments using α -difluoromethylornithine (DFMO), a specific inhibitor of ornithine decarboxylase, suggested that early

hematopoietic cells already have high levels of putrescine caused by the absence of AZ1 before they migrate to the liver from the aorta-gonad-mesonephros (AGM) region. In another experiment, oral administration of 2% DFMO in drinking water to maternal mice during the AGM period (embryonic day 9.5–11.5), but not during the period of hepatic hematopoiesis (embryonic day 11.5–13.5), rescued the phenotype of AZ1 knockout mice. In addition, colony-forming assays using cells derived from the AGM region and bone marrow showed decreases in the burst-forming unit of erythroid cells and cells of earlier stages. These results indicate that early hematopoietic cells that have not yet migrated to the liver are particularly sensitive to high concentrations of putrescine.

Analysis of AZ2-interacting proteins

We previously found that cerebellar-degeneration-related protein 2 (CDR2) binds to AZ2, but not to AZ1 or AZ3, by means of the two-hybrid system, and determined the AZ2-binding region on the CDR2 molecule. Further study using AZ1/AZ2 chimera proteins indicated that the CDR2-binding region of AZ2 is located between amino acid residues 135 and 181. We then substituted 7 conserved residues in this region with alanine and found that 2 residues, arginine 140 and valine 174, are indispensable for binding to CDR2. Interestingly, AZ1 acquired interaction with CDR2 when the corresponding 2 residues were mutated to the AZ2 type, suggesting that the 2 residues are determinants of specific binding with CDR2.

We performed yeast two-hybrid screening of a mouse kidney complimentary DNA library to identify AZ2-interacting proteins. From 1.35×10^5 independent clones, about 300 positive clones were obtained, and 75 clones with stronger signals were sequenced. These contained known AZ2-binding proteins, such as ornithine decarboxylase, antizyme inhibitor, and CDR2. We selected several candidates and are confirming the specificity of binding.

Publications

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Department of Pharmacology (I)

Masahiro Kawamura, Professor Noboru Nakamichi, Lecturer Seiji Hori, Professor Yuji Ohno, Lecturer

General Summary

Our main interests are the physiological roles of nucleotide receptors and the mechanisms of regulation of intracellular Ca²⁺ concentrations in several kinds of cells, including bovine adrenocortical fasciculate cells (BAFCs), 3T3-L1 preadipocytes, Madin-Darby canine kidney (MDCK) cells, and brain astrocytes. We are also interested in the convulsant effect of theophylline, the nonantibacterial effects of quinolones, and the effect of the urocortin family on the cardiovascular system.

Research Activities

Cross-talk between ACTH receptors and ATP receptors in BAFCs

Extracellular ATP and uridine triphosphate (UTP) bind to P2 receptors to regulate several cell functions in many kinds of cell. P2 receptors are divided into 2 families: the ligand-gated P2X and the G-protein coupled P2Y. The P2Y family has 14 subfamilies. We have previously reported that BAFCs contain Gq-protein-coupled P2Y2. Both ATP and UTP bind to P2Y2 to stimulate Ca^{2+} influx via IP3 production from the extracellular space. ACTH is a physiological stimulator of adrenocortical steroidogenesis via cAMP production. We found that ATP and UTP potentiated both ACTH-induced steroidogenesis and cAMP production. Our findings suggested that the Ca^{2+} influx pathway or the B γ subunit of Gq-protein (which links to P2Y2) or both are involved in the cross-talk between the ACTH receptor and P2Y2. Under our experimental conditions, the Ca^{2+} influx pathway was not involved in the event. Furthermore, agents that disturb the actin cytoskeleton did not affect the cross-talk. Therefore, movement of the B γ subunit of Gq-protein might not participate in this cross-talk between the ACTH receptor and P2Y2.

Study of the intracellular Ca²⁺ dynamics

 Ca^{2+} is an important regulator in many cellular functions. Therefore, we studied intracellular Ca^{2+} dynamics and its physiological functions in BAFCs, 3T3-L1 preadipocytes, MDCK cells, and brain astrocytes through the use of samples loaded with a fluorescent calcium indicator.

1. Store-operated Ca²⁺ entry in BAFCs

Store-operated Ca^{2+} entry (SOCE), i.e., Ca^{2+} entry triggered by the depletion of intracellular Ca^{2+} stores, plays a physiological role in nonexcitable cells. Gq-protein-coupled receptor agonists stimulate IP3 production followed by Ca^{2+} release from the endoplasmic reticulum and depletion of luminal Ca^{2+} . Then Ca^{2+} enters from the extracellular space through the activation of SOCE. However, the precise mechanism of SOCE remains obscure. Three hypotheses for SOCE activation have been proposed: the conformational coupling model, the secretion-like model, and the diffusible messenger model. However, in all cases, an SOCE channel in the plasma membrane is necessary for Ca^{2+} to enter from the extracellular space. One candidate for the SOCE channel is the transient receptor potential protein (TRP), especially, the TRPC subtype. The plasma membrane of BAFCs reportedly contains TRPC. Therefore we studied the possible involvement of TRPC in SOCE in BAFCs. Although SOCE is high- Ca^{2+} selective, TRPC is not. Under our experimental conditions, the extracellular Ca^{2+} enters the cells after treatment with cyclopiazonic acid, a sarcoplasmic/endoplasmic reticulum Ca^{2+} (SERCA) pump inhibitor, to deplete its luminal Ca^{2+} but not Sr^{2+} or Ba^{2+} . The results suggest that TRPC is not the SOCE channel in BAFCs.

2. SOCE in 3T3-L1 preadipocytes

3T3-L1 preadipocytes differentiate to adipose cells under controlled culture conditions. Intracellular Ca²⁺ mobilization is believed to be an important factor in this process. Therefore, we studied the mechanism of Ca²⁺ influx in 3T3-L1 preadipocytes. We found that these cells have SOCE that is activated by prostaglandin (PG) F2 α via PG receptors and thapsigargin, a SERCA pump inhibitor. The 3T3-L1 preadipocytes biosynthesize and secrete PGF2 α . Thus, these results suggest the possible involvement of this SOCE-activation factor in the adipose differentiation of 3T3-L1 preadipocytes in an autocrine/paracrine fashion.

3. Intracellular Ca²⁺ dynamics in MDCK cells

MDCK cells are often used to study the mechanism of polycystic kidney formation, a process in which Ca^{2+} has been proposed to play an important role. However, intracellular Ca^{2+} dynamics in MDCK cells is not fully understood. We found that a calcium oscillation phenomenon occurs in MDCK cells either in the absence or presence of extracellular Ca^{2+} . The results suggest that the intracellularly stored Ca^{2+} plays a pivotal role in this phenomenon. The oscillation was inhibited by probenecid, but the mechanism of inhibition has not been identified.

4. Extracellular ATP-induced modulation of astrocytic calcium oscillations in rat hippocampal slice cultures

Activation of various P2 receptors increases the intracellular Ca^{2+} concentration $([Ca^{2+}]_i)$ of astrocytes in primary cultures. To analyze the role of extracellular ATP in astrocytic Ca^{2+} wave oscillations *in situ*, fluorometric measurements were made from rat hippocampal slice cultures. ATP induced a transient increase in $[Ca^{2+}]_i$, which was followed by an increase in the frequency of spontaneous Ca^{2+} oscillations. The increase in the frequency of spontaneous Ca^{2+} oscillations was mediated by the activation of adenosine receptors by adenosine, which was produced via extracellular breakdown of ATP. The transient increase in $[Ca^{2+}]_i$ was induced by the activation of either adenosine receptors or P2 receptors. These results suggest that extracellular ATP plays a dual role in astrocytic Ca^{2+} wave propagation through the activation of P2 receptors and adenosine receptors in the rat hippocampus.

Study of the non-antibacterial effects of qunolones

1. Convulsant activity of quinolones and their interaction with anti-inflammatory

drugs

Quinolones have potent convulsant activity, which is enhanced by concurrent administration of anti-inflammatory drugs. We studied the convulsant activity of respiratory quinolones, which have excellent antibacterial activities against organisms causing respiratory tract infections, and examined whether the convulsant activity is affected by anti-inflammatory drugs. Each respiratory quinolone had different convulsant potency. The order of potency was gatifloxacin>levofloxacin>moxifloxacin=sparfloxacin> tosufloxacin. The convulsant activity of these quinolones was not enhanced by concurrent administration of anti-inflammatory drugs.

2. Evaluation of intestinal absorption and drug interaction of quinolones *in vitro* (In collaboration with the Department of Practical Pharmacy, Kyouritsu University of Pharmacy)

In vivo studies have shown that the intestinal absorption of some quinolones is inhibited by the co-administration of divalent metal ions. We established the everted intestinal sac method to study in greater detail the mechanism of absorption of quinolones *in vitro*. Everted intestinal sacs were prepared from male Wistar rats. Norfloxacin and levofloxacin accumulated in the sac in a dose-dependent manner. Aluminum ion inhibited the absorption of the quinolones, whereas calcium and magnesium ions had no effect. These results suggest that each quinolone has a different manner of intestinal absorption and a different manner of drug interaction with metal ions.

3. Convulsant activity of theophylline and its metabolites

Subcutaneous injection of theophylline induces convulsions in a dose-dependent manner in mice. To examine the convulsant effects of thephylline and its metabolites, we administered these agents to the right lateral ventricles of mice. 1-Methylxanthine, a physiological metabolite of theophylline, showed strong convulsant activity, but theophylline itself had weak convulsant activity. These results suggest that 1-methylxanthine is the compound responsible for thophylline-induced convulsions.

4. Anti-inflammatory activity of theophylline (In collaboration with the Department of Practical Pharmacy, Kyoritsu University of Pharmacy)

Theophylline is considered to have anti-inflammatory activity as well as bronchodialatory activity. However, few reports have demonstrated the anti-inflammatory activity in animal models. We studied the effects of theophylline and its metabolites on carrageenan-induced edema in rat foot pad. Theophylline, but not its metabolites, reduced edema. The effect of theophylline was inhibited by pretreatment with mifepristone, a glucocorticoid receptor antagonist. These results suggest that the glucocorticoid receptor system is involved in the anti-inflammatory effects of theophylline.

Studies of the effects of cardiovascular regulatory substances on rat cardiomyocyte function

We have studied the effects of cardiovascular regulatory factors and agents on primary cultured neonatal rat cardiomyocytes. However, some factors and agents have little effect on isolated cardiomyocytes but have more prominent effects on cardiomyocytes in the diseased human heart or in in vivo animal models. In pathological conditions of the heart, the ratio of non-cardiac myocytes to cardiomyocytes increases. Therefore, the

cross-talk between cardiomyocytes and non-cardiac myocytes should be clarified. We prepared a cardiomyocyte/non-cardiac myocyte co-culture system as an experimental model of pathological conditions of the heart and evaluated the physiology of cardiomyocytes under such conditions. We showed that the secretory pattern of atrial and brain natriuretic peptides changed as the ratio of non-cardiac myocytes to cardiomyocytes increased. The results suggest that non-cardiac myocytes play an important role in heart disease. We also studied the involvement in cardiac disease of urocortin, a peptide related to cortiocotropin-releasing hormone, and other peptides of its family in HL-1 cardiomyocytes, a cardiomyocyte cell line from the mouse atrium.

Clinical studies of human blood rheology with the falling needle rheometer

The onset of ischemic cardiac disease is closely related to the viscosity of blood. A new type of rheometer, the falling needle rheometer, was developed jointly by the Department of Chemical Energy and Environmental Engineering, Kansai University, and the Department of Legal Medicine, Dokkyo Medical University. This new type of rheometer can quickly measure multiple apparent viscosity without the addition of anticoagulants. However, its clinical usefulness must be evaluated. Therefore, we studied the performance of the rheometer in adult volunteers. We demonstrated the blood fluidity analysis by multiple apparent viscosity and, in a few volunteers, the difference in fluidity with or without anticoagulant. The results show that blood coagulation ability is a factor in changes in blood fluidity.

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Department of Pharmacology (II)

Masahiro Kawamura, Professor Kazuo Takano, Lecturer Naofumi Kimura, Professor

General Summary

We have been focusing on the following areas: 1) verification of the hypothesis that coupled pairs of oscillators may generate the respiratory rhythm in vertebrates, 2) visualization of the spatiotemporal pattern of respiratory neural activities in the isolated frog brainstem, and 3) neural mechanisms of the vagal respiratory reflex.

Research Activities

Verification of the hypothesis that coupled pairs of oscillators generate the respiratory rhythm in vertebrates

The frog, with two distinct ventilatory acts, provides a useful model for investigating the prospective interaction of paired oscillators in generating the respiratory rhythm. Building on evidence supporting the existence of separate oscillators generating buccal ventilation and lung ventilation, we have attempted to uncouple the two rhythms in the isolated brainstem preparation. Opioids preferentially inhibited the lung rhythm, suggesting an uncoupling of the lung oscillator from the buccal oscillator. Reduction of the superfusate chloride concentration altered both the buccal rhythm and the lung rhythm. Joint application of opioids and reduced-chloride superfusate increased the variability of the buccal-burst-to-lung-burst intervals. This increase in variability suggested that chloride-mediated mechanisms are involved in coupling the buccal oscillator to the lung oscillator. Given the results of these interventions, we proposed a simple model of the frog respiratory rhythm generator, which consists of the coupling of the lung and buccal oscillators. This model can be used to explain various patterns in the breathing of all lunged vertebrates, although the mechanics of breathing differ fundamentally between amniotes and lower vertebrates (Collaboration with the Respiratory Research Group, University of Calgary).

Visualization of the spatiotemporal pattern of respiratory neural activities in the isolated frog brainstem

To identify the neural structures essential for the formation of respiratory motor-activity patterns (buccal and lung bursts) in frogs, we visualized the spatiotemporal pattern of respiratory neuronal activities in the isolated brainstem, using voltage-imaging techniques. We recorded optical signals from the ventral surface of the medulla using a voltage-sensitive dye and calculated cross-correlations between the integrated respiratory activity of the trigeminal nerve and each pixel. Lung-burst-related activities were visualized bilaterally as longitudinal columns in the ventrolateral medulla between the levels of the trigeminal and hypoglossal nerve rootlets. In contrast to that in neonatal

rats, the optically active area in frogs spreads in a caudal-to-rostral column during the lung burst. The optically identified area was verified with extracellular recordings of respiratory-related neurons. (Collaboration with the Department of Physiology, Hyogo College of Medicine, Hyogo, and the Department of Medicine, Keio University Tsu-kigase Rehabilitation Center).

Neural mechanisms of the vagal inspiration-promoting reflex and involvement of P2X receptors in nucleus of the solitary tract

To understand how respiratory movements are optimized in response to changes in internal and external environments, the research group for mammalian respiratory function has been studying neural mechanisms underlying various types of respiratory reflexes in mammals (in collaboration with the Laboratory of Neurophysiology, Department of Neuroscience). We have demonstrated that a local microinjection of pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS), a P2X receptor antagonist, into the caudal nucleus of the solitary tract (NTS) of the anesthetized rabbit preferentially attenuates the "inspiratory promotion reflex," a response driven by the pulmonary stretch receptor and activated at a small lung volume. This year, we analyzed the site of action of PPADS by histological investigation and found that the marked reduction of the vagal inspiratory-promotion reflex occurred mostly when the site of PPADS injection, identified with staining, contained the caudal part of the lateral This result argues clearly for a pivotal role for the P2X receptors in the caudal NTS. NTS in the inspiratory-promoting responses activated by decreased inputs from the pulmonary stretch receptor.

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Department of Pathology

Hiroshi Hano, Professor Masaharu Fukunaga, Associate Professor Masafumi Suzuki, Associate Professor Satoru Chiba, Lecturer Takako Kiyokawa, Lecturer Hiroyuki Kato, Lecturer Koichi Nomura, Lecturer Yutaka Yamaguchi, Professor Akihiko Sakata, Associate Professor Masahiro Ikegami, Associate Professor Yasushi Kikuchi, Lecturer Takashi Nikaido, Lecturer Hiroyuki Takahashi, Lecturer Yukiko Kanetsuna, Lecturer

General Summary

The research projects of our department have focused on studies of the pathogenesis, histogenesis, morphogenesis, and clinical pathology of nonneoplastic and neoplastic human diseases by means of light and electron microscopy, morphometry, immunohistochemistry, gene analysis, and other techniques.

Research Activities

Hepatology

We studied pathological changes of blood vessels in chronic viral hepatitis. To elucidate the pathogenesis of changes to the portal vein, such as stenosis and disappearance, we examined the immunohistochemical expression of E-selectin, intercellular adhesion molecule (ICAM), and endothelial nitric oxide synthase (eNOS) of endothelial cells of the portal veins in biopsy specimens of the liver in cases of chronic viral hepatitis. E-selectin and ICAM are adhesion molecules that are involved in leucocyte recruitment and inflammation. eNOS is involved in vasorelaxation, platelet aggregation, and cardiovascular homeostasis. Whereas ICAM was positive in few cases, E-selectin was expressed by endothelial tissue in all cases. eNOS expression was observed in the endothelial cells of the portal and central veins. Expression of E-selectin and ICAM and the level of activity of eNOS are considered to be involved in the development of portal vein damage.

We studied ductular reactions and progenitor cells of the liver in cases of obstructive jaundice by means of histologic reconstruction and immunohistochemistry. Histologic reconstruction focusing on ductular reactions showed bile ductular proliferation with a conspicuous complicated appearance, mainly located around the original portal tract. Biliary-type cells, hepatocytes, cytokeratin-7-positive hepatocytes, and intermediate cells were identified immunohistochemically. The results are being analyzed.

Damage and repair of the DNA of hepatocytes caused by oxidative stress in regenerative nodules of liver cirrhosis were investigated immunohistochemically. The results of immunohistochemcal studies showed that 8-hydroxydeoxyguanosine as a marker of oxidative stress was expressed frequently and extensively and that proliferating cell nuclear antigen was also expressed frequently. Hepatocytes positive for proliferating cell nuclear antigen were inferred to be under repair.

Renal pathology

Nephritis related to myeloperoxidase antineutrophil cytoplasmic autoantibodies (MPO-ANCA) was analyzed clinicopathologically. Angitis tended to develop in elderly patients and was related to the serum value of C-reactive protein. Histologic examination revealed numerous hemosiderin deposits in the tubules and interstitial tissue. Hemosiderosis was correlated with active glomerular and tubular lesions and the severity of hematuria.

Histologic examination in cases of chronic rejection of renal transplants revealed that endothelial cells of the glomerular tuft were replaced by cells with many caveolae, i.e., invaginations of the plasma membrane. This replacement was thought to cause proteinuria.

Specimens of kidneys with IgA nephropathy collected from universities and national hospitals were examined clinicopathologically to establish new criteria for the prognosis of IgA nephropathy. As a result of this examination, some points to be revised were discussed from the view point of the histopathology of IgA nephropathy.

We continued our histological re-evaluation of specimens of renal cell carcinoma in our department with revised general rules for clinical and pathological studies of renal cell carcinoma. Cases of renal cell carcinoma accompanied by various degrees of cystic lesions were studied clinicopathologically. We found that cases with cystic lesions occupying more than 50% of the whole kidney had no metastasis and a good prognosis.

Gastrointestinal pathology

The characteristics of mucin produced by cancer of the gastric cardia were examined. We found that differentiated adenocarcinoma that produces gastric-type mucus tended to invade and change to a poorly differentiated type. We are comparing these findings to those of gastric cancer developing in other regions.

The relation between the sprout-like growth pattern at the forward edge of cancer and lymphatic invasion was examined in colorectal cancer with submucosal invasion.

Gynecologic pathology

The reproducibility of the histologic diagnosis of endometrial carcinoma was examined. Histologic findings of endometrial carcinoma treated with hormone therapy were examined to establish criteria for the efficacy of medroxyprogesterone acetate treatment. Using a mouse model of antiphospholipid antibody syndrome, we examined morphological changes of the microcirculation of the placenta and kidney. Whereas no particular changes were found with light microscopy, electron microscopy disclosed the irregular arrangement of endothelial pores of the glomerular capillary and splitting of the basement membrane of blood vessels of the placenta. These findings strongly suggest the initial histologic changes of antiphospholipid antibody syndrome.

Urogenital pathology

Clinicopathological studies of prostatic carcinoma continued. Specimens of prostatic carcinoma in Japanese patients were evaluated with the revised Gleason grading system in 2005. The results suggest that separate scoring is clinicopathologically useful.

Lung pathology

The analysis of clinicopathologic data obtained from 787 autopsy cases of primary lung cancer was completed.

Oncology

Analysis of loss of heterozygosity in 33 surgically resected specimens of liver cell carcinoma was performed with microsatellite markers at chromosome 8. The results suggest that candidate susceptibility genes for liver cell carcinoma reside at 8p23-22. The inhibitory effects of the histone deacetylase inhibitor trichostatin A on cell lines of renal cell carcinoma were examined. The results showed that trichostatin A has a strong inhibitory effect on the proliferation of cultured carcinoma cells. Candidate susceptibility genes for this effect were investigated with DNA chip analysis and other methods.

Other

Hemorrhagic cyst of the right atrium was studied clinicopathologically. Histologic examination of spindle cell tumor of the soft tissue was performed to evaluate the grade of malignancy.

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Department of Microbiology (I)

Kazuhiro Kondo, Professor Takaaki Ohashi, Lecturer Mariko Nakamura, Associate Professor

General Summary

The human beta-herpesvirus subfamily consists of human cytomegalovirus (HCMV), human herpesvirus (HHV)-6, and HHV-7. Both HHV-6 and HHV-7 belong to the Roseolovirus genus of the β -herpesviruses, and the HHV-6 species are divided into two variants: HHV-6A and HHV-6B. The β -herpesviruses can establish a lifelong latent infection of the host and are frequently reactivated; some evidence suggests that the molecular mechanisms of viral latency and reactivation are shared by these viruses. We are studying the molecular mechanism of latency and pathogenesis of β -herpesviruses. Additionally, we are studying these viruses to investigate the mechanisms of fatigue and to develop viral vectors for gene therapy.

Research Activities

Chronic fatigue syndrome and herpesvirus

Chronic fatigue syndrome (CFS) is a disease of unknown etiology in which the main complaint is severe fatigue. The prominence of the acute onset of illness, the persistent symptoms consistent with a viral infection, and the increased titers of viral antibodies and enhanced activity of the interferon-induced enzyme suggest viruses play a role in CFS. In other words, CFS may be a kind of "postinfectious fatigue syndrome" following any viral infection. A variety of viruses have been evaluated, including enteroviruses, retroviruses, and human herpesviruses.

CFS is a condition that lasts far longer than postinfectious fatigue. For this reason, the infection causing CFS is believed to be a latent infection with a herpesvirus. The most promising candidate as a CFS-associated virus is HHV-6. Because an unusual latent infection with HHV-6 which may be a cause of CFS, studies of latent infection are considered important for elucidating CFS.

Several lines of evidence suggest that latent HHV-6 infection in the brain may be involved in development of some neurological conditions, such as recurrent febrile convulsion, multiple sclerosis, and encephalitis. However, the site of HHV-6 latency in the brain has not been identified.

To determine how CFS develops, we attempted to identify a special latent HHV-6 infection state termed the "intermediate phase." In this phase, several kinds of HHV-6 latency-associated proteins corresponding to Epstein-Barr virus nuclear antigens might be expressed. This intermediate stage is observed in the first phase in which HHV-6 commences reactivation but differs from reactivation in that no virus is produced. To examine the relationship between latent-infection proteins, whose expression is enhanced in the intermediate stage, and disease, serum titers of antibodies to cells that express

latent-infection proteins at high levels were examined in patients with CFS. This examination revealed that 40% of patients with CFS showed antibody reactions to intermediate-phase latent HHV-6 infection, whereas healthy subjects showed virtually no reaction.

Beta-herpesvirus latency and reactivation

Fatigue is a common problem of modern life. Many people who are under considerable stress have various kinds of fatigue. However, few scientific and medical studies have examined fatigue, and even fewer studies have involved definitive means or quantitative standards for quantitatively and objectively measuring fatigue, which is subjective.

Muscle fatigue (exercise fatigue) has mainly been studied as a representative example of fatigue. The indicator examined in most studies is the increase in the production of lactic acid in muscles. However, lactic acid is an important energy source for the central nervous system, and the theory that it inhibits muscle activity has been questioned. In addition, during muscle fatigue pyruvic acid levels increase and pH decreases in body fluids. These phenomena are indeed observed in response to the stress of a muscle load (exercise load); however, fatigue is distinct from local muscle exhaustion and is considered to be a broader and larger physiological phenomenon.

Both HHV-6 and HHV-7 are almost universally acquired by 2 to 3 years of age. These viruses belong to the beta-herpesvirus subfamily, and biological and molecular analyses show they are closely related to each other. Both viruses establish life-long latency, a hallmark of herpesviruses, reactivate frequently, and are shed in saliva.

To investigate viral reactivation, we have identified the latency-associated transcripts of HHV-6 and have clarified part of the mechanism of HHV-6 reactivation. HHV-6 establishes latency in macrophages, remains in a stable intermediate stage between latency and reactivation, and is reactivated by two or more factors.

HHV-6 can reactivate in immunosuppressed patients; however, the relationship between immunosuppression and the induction of reactivation is unclear. To identify the factor or factors involved in HHV-6 reactivation, we studied the association of HHV-6 reactivation and work-induced fatigue in healthy adults. Immune strength is thought to deteriorate when humans are fatigued, and virus infection is a possible manifestation of this deterioration. However, the relationship between fatigue and virus infection in humans remains unclear.

HHV-6 DNA was detected in 88% of subjects engaged in moderately excessive work due to long hours and other factors (the first test day). In contrast, HHV-6 DNA was detected in 23.8% of subjects immediately after a holiday (the second test day). These results show that HHV-6 is significantly reactivated on exertion. These results led to the discovery that HHV-6 DNA expressed in saliva through the reactivation of HHV-6 is a biomarker of the severity of fatigue. Accordingly, an simple, objective method for assessing the degree of fatigue by detecting HHV-6 DNA released into saliva as a result of the reactivation of HHV-6 was developed.

The amount of HHV-7 DNA was semiquantitatively measured with the double-nested polymerase chain reaction method in serially diluted saliva. HHV-7 DNA was detected
in 92% of patients with CFS. In contrast, HHV-7 DNA was detected in 50% of healthy subjects during work and in only 30% of healthy subjects at rest. The amount of HHV-7 DNA in half of patients with CFS was 10- to 100-fold higher than the average amount in healthy subjects. These results show that HHV-7 is significantly reactivated in the chronic fatigue state that accompanies disease. These results also led to the discovery that HHV-7 DNA expressed in saliva owing to reactivation of HHV-7 is a biomarker of fatigue which varies with the cause of chronic fatigue. Accordingly, an objective method for assessing the degree of fatigue by detecting HHV-7 DNA released into saliva owing to reactivation of HHV-7 was developed, enabling the simple assessment of the decline in physical strength caused by chronic fatigue.

Use of HHV-6 and HHV-7 as gene therapy vectors

Accumulation of knowledge and various technological advances in molecular biology and molecular genetics have contributed greatly to the recent progress in life science, providing abundant information about various biological phenomena. Active research and development is under way in various fields of life science, with particular interest in the analysis of gene functions. These activities have led to the development of techniques and vectors for introducing isolated genes into cells and individual living organisms.

Viral vectors have advantages over other known vectors in introducing a foreign gene into a cell for protein expression. The central idea underlying gene transfer with viral vectors is to introduce a foreign gene into an infected cell and transform the cell under the control of promoter sequences, taking advantage of the infectious capacity of the virus (productive infection, latent infection, abortive infection).

In particular, HHV-6 and HHV-7 have attracted considerable interest as candidate viral vectors for gene therapy, because infections with these viruses have mild symptoms. The use of herpesviruses, particularly HHV-6 and HHV-7, as recombinant viruses and as recombinant viral vectors has certain advantages, which include low pathogenicity, ease of gene introduction into blood cells, such as the T cells and macrophages, and introduction of relatively large genes. However, producing a recombinant virus or a recombinant virus vector that originates from HHV-6 or HHV-7 is difficult, and no method is available for producing such viruses and vectors. In addition to technical factors, the characteristics of the genes of HHV-6 and HHV-7 make recombination of HHV-6 and HHV-7 difficult.

We have identified the dispensable genes of HHV-6 and HHV-7 and have reported the establishment of recombinant HHV-6 and HHV-7. The dispensable locus of HHV-6 is 8.4 kbp, and that of HHV-7 is 7.3 kbp; both are useful sites for inserting a large gene. An exogenous nucleotide sequence may encode various substances, such as bacterial artificial chromosomes, cytokine genes, ribozyme, interference RNAs, immunological co-stimulator molecules, signal transduction molecules, enzymes, and chemical attractants. Furthermore, exogenous nucleotide sequences may be used for the gene therapy of mammals. Gene therapy might be used to prevent human immunodeficiency virus infection of compromised cells and for the immunotherapy of cancer.

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Department of Microbiology (II)

Shogo Masuda, Professor Hitomi Shinji, Lecturer Keiko Seki, Associate Professor

General Summary

Research projects of our department have focused on 1) the mechanism of inhibition of *Staphylococcus aureus* colonization by commensal *Staphylococcus epidermidis*; 2) the role of beta-hemolysin in the inhibition of interleukin (IL)-8 production of human umbilical endothelial cells; 3) fibronectin-mediated colonization via fibronectin-binding protein (FnBP) A in *S. aureus* infection; 4) induction of apoptosis in fibroblasts by intracellular *S. aureus*; and 5) the mechanism of bacterial biofilm formation.

Research Activities

Inhibition of S. aureus colonization by S. epidermidis

S. aureus has a marked affinity for human tissue. However, compared with indigenous staphylococci, *S. aureus* has a low detection rate in humans. Although this low detection rate is thought to be the result of some defense mechanism, the details remain poorly understood. We hypothesized that this phenomenon is attributable to inhibition of *S. aureus* colonization by *S. epidermidis*, the dominant indigenous staphylococci in the nasal cavities. To test this hypothesis, we performed an epidemiological survey. In addition, we performed *in vitro* experiments to explain the epidemiological findings. Two types of *S. epidermidis* were identified: 1 type inhibits *S. aureus* colonization and the other type does not. The detection rate of *S. aureus* was lower when the inhibitory *S. epidermidis* strain was present in the nasal cavities. *In vitro* studies showed that the *S. epidermidis* strain effectively inhibited *S. aureus* colonization and that the effect was directly proportional to the number of *S. epidermidis* organisms. Moreover, nasal administration of inhibitory *S. epidermidis* to volunteers significantly reduced *S. aureus* colonization. These findings suggest that the low detection rate of *S. aureus* colonization.

Beta-hemolysin from S. aureus inhibits the production of IL-8 and the transmigration of neutrophils through activated endothelium

S. aureus can cause a broad range of infections from superficial infections to severe invasive infections, such as endocarditis. Treatment of infections with antibiotics has become more difficult owing to the recent increase in *S. aureus* resistant to multiple antibiotics. The innate immune system plays a crucial role in the host response to infection with *S. aureus*, and leukocyte migration is a key event in host defense against bacterial infection. The endothelium plays an important role in neutrophil recruitment through modulation of the expression of cell adhesion molecules and cytokines, such as IL-8. Previously, we reported that culture supernatant from *S. aureus* inhibits IL-8

production by human umbilical endothelial cells (HUVECs). The inhibitory factor was isolated and identified as β -hemolysin (β -toxin, sphingomyelinase C). This protein is an enzyme that specifically cleaves sphingomyelin, the major sphingolipid in membranes, and is highly hemolytic for sheep erythrocytes. However, little is known about the action of β -hemolysin on host cells. We showed that β -hemolysin inhibited IL-8 production in HUVECs and decreased neutrophil transendothelial migration. Furthermore, VCAM-1 expression was inhibited by β -hemolysin. The electrophoretic mobility-shift assay revealed that β -hemolysin did not inhibit activation of NF- κ B, indicating that the other pathway is involved.

Apoptosis of fibroblasts induced by intracellular S. aureus

Two clinical isolates of *S. aureus*, OK1 and OK11, grown in brain-heart infusion broth at 37°C for 2 hours (exponential phase) or 18 hours (stationary phase) were used. To examine the interaction between *S. aureus* cells and L929 fibroblasts, each bacterial suspension was added to the fibroblasts adhering to the culture dish and incubated for 30 minutes. After washing, incubation was continued for an additional 3 hours in freshly prepared medium containing lysostaphin. The L929 fibroblasts ingested more OK11 cells during the exponential phase than in other phases and ingested many bacterial cells in spite of being a nonphagocytic cell. Intracellular bacteria of the exponential phase markedly induced caspase 3 activity. Cells infected with OK1 showed condensed chromatin with large clumps, which is characteristic of apoptosis. In contrast, intracellular organelles of some OK11-infected cells were severely damaged and showed small, condensed chromatin clumps beneath the nucleus membrane. More OK11 than OK1 cells could attach to the mouse kidneys during the exponential phase.

Fibronectin-mediated colonization via FnBPA is important in the infection of S. aureus S. aureus has a variety of adhesins that bind to extracellular matrix proteins or plasma proteins and facilitate the colonization of host tissues and organs. Among these adhesins, FnBPs are thought to be the most important for interacting with host cells, such as endothelial cells, epithelial cells, fibroblasts, and macrophages. To determine whether FnBPs are responsible for the in vivo infection of S. aureus, the fnbA mutant strain was compared with the parental SH1000 strain. Female BALB/c mice (6 to 8 weeks old) were intravenously infected with SH1000 or the mutant strain, after which their survival was studied. Most of the mice infected with the parental strain died within 1 week after infection, whereas mice infected with the mutant strain survived for more than 20 days. Furthermore, many abscesses were found in the kidneys of mice infected with the parental strain, but the kidneys of mice infected with the mutant strain appeared normal. These results indicate the importance of FnBPA in effective colonization of host tissues in S. aureus infection.

Curli fibers are required for development of biofilm architecture in K-12 Escherichia coli and enhance bacterial adherence to human uroepithelial cells

Sessile bacteria show phenotypical, biochemical, and morphological differences from their planktonic counterparts. Curli, extracellular structures important for biofilm formation, are only produced at temperatures less than 30° C in *E. coli* K-12 strains. We show that *E. coli* K-12 can produce curli at 37° C when grown as a biofilm community. Curli are required for the formation of a 3-dimensional mature biofilm, with characteristic water channels and pillars of bacteria. A wild-type curli-expressing *E. coli* strain adhered to several lines of human uroepithelial cells more readily than did an isogenic curli-deficient strain. The finding that curli are expressed at 37° C in biofilm and enhance bacterial adherence to mammalian host cells suggests an important role for curli in pathogenesis.

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Department of Public Health and Environmental Medicine

Hidesuke Shimizu, Professor Toshihiko Agata, Associate Professor Yuji Suzuki, Associate Professor Yuichi Miyakoshi, Lecturer

General Summary

Our major research project in the 2006 academic year focused on 1) the comet assay for detection of DNA damage; 2) genotoxic effects of micronucleus induction of vitamin E and its 2 derivatives, and magnetic fields; 3) induction of micronuclei by ketamine, 3, 4-methylenedioxymethamphetamine (MDMA), and its nitroso compounds; 4) metabolites of opium and heroin in human urine; 5) the epidemiology of studies of type 1 diabetes in Japan; 6) methods of medical informatics education and evidence-based medicine (EBM); 7) the health effects of electromagnetic fields (EMFs); 8) the urinary concentration of 8-hydroxydeoxyguanine (8-OHdG) as an indicator of oxidative DNA damage in workers exposed to intense EMFs.

Research Activities

In vitro micronucleus test

1. A possible mechanism for the enhancement by co-exposure to static magnetic fields of micronucleus formation by mutagens

We reported that micronuclei induced by chemicals that produce active oxygen radicals increased after co-exposure to strong static magnetic fields (SMFs) but that these increases were blocked by pretreatment with ascorbic acid. These results suggest that exposure to SMFs triggers the induction of mutagen-related radicals. The present study examined the optimal timing of ascorbic acid pretreatment to block this increase and the duration of the effect. The frequency of micronuclei induced by co-exposure to mitomycin C (MMC) and SMFs decreased strongly when the interval of ascorbic acid pretreatment was 20 minutes. The effects of ascorbic acid on the micronucleus frequency were maintained for at least 72 hours. These results suggest that active oxygen radicals may be related to micronucleus induction by SMFs.

2. Chromosomal aberration of astrocytes exposed to EMFs

We studied the effect of EMF exposure on chromosomal aberrations by means of an *in vivo* micronucleus assay of newborn rat astrocytes. Micronuclei were not increased by exposure to EMFs (50 Hz, 10 mT, 72 hours). On the other hand, micronuclei induced by vincristine were increased by co-exposure to EMFs (50 Hz, 10 mT, 48 hours).

3. DNA damage by exposure to EMFs

We studied the effect of EMF exposure on DNA damage using the comet assay. We found that damage to DNA was not increased by exposure to EMFs (50 Hz, 10 mT, 1, 3, and 6 hours).

4. Urine 8-OHdG in researchers exposed to intense magnetic fields

The urinary concentration of 8-OHdG, which is an indicator of oxidative DNA damage, was measured in 38 volunteers who worked in intense magnetic fields. The mean concentrations of urinary 8-OHdG did not differ significantly between the beginning and end of the workers' shift.

5. In vitro genotoxicity study

A genotoxicity study of 2 derivatives of vitamin E that react as antioxidants (a collaborative study with Kyoritsu Pharmaceutical University)

A genotoxicity study of 2 derivatives (6-hydroxy-2,2-dimethylchroman and 2,2,5,7,8pentamethyl-6-chromanol) of vitamin E was performed. Chinese hamster lung CHL/ IU cells were exposed separately to both derivatives and MMC. The number of chromosomal aberrations and the micronucleus frequencies were lower with exposure to both the derivatives and MMC than with exposure to MMC alone.

Physiological affects of drug abuse

1. Genotoxicity of MDMA as an illegal drug of abuse

Illegal use of MDMA has recently become a social problem. MDMA ingested orally reacts with nitrites in the stomach and is synthesized into *N*-nitroso-MDMA. We synthesized *N*-nitroso-MDMA and studied its genotoxicity using the micronucleus and chromosomal aberration tests. The genotoxicity of *N*-nitroso-MDMA was observed in both the micronucleus and chromosomal aberration tests.

2. Genetoxic effects of ketamine

Ketamine have recently begun to be abused in Japan. The Japanese government has proposed new studies of the toxicity of abused drugs as part of a plan to eliminate drug abuse. Orally administered ketamine may be metabolized to nitrites; therefore, we have synthesized their nitroso compounds and studied them with the micronucleus and chromosome aberration tests using Chinese hamster lung cells. N-nitrosoketamine showed positive results on both tests.

3. Inference of opium or heroin from analysis of metabolites in urine samples

Metabolites of opium and heroin were analyzed in human urine. After drug administration, the metabolites of both morphine and codeine were detected in urine by using the chromatography pattern analysis of gas chromatography/mass spectrometry.

Epidemiology, EBM, community health and medical informatics 1. EBM

A systematized body of epidemiologic principles with which studies can be designed and judged has been established only in the last two decades. These principles have evolved in tandem with an explosion of epidemiologic activity covering a wide range of health problems. Our greatest concern is to clarify risk factors for adult disease and intractable diseases. We also studied the methodology of medical informatics education and EBM. 2. Is changing from neutral protamine hagedon insulin to insulin glargine as the basal insulin in a basal bolus regimen effective in Japanese patients with diabetes?

We studied the clinical efficacy of changing from neutral protamine hagedon (NPH) insulin to insulin glargine as the basal insulin in a basal bolus regimen. The subjects

of this study were patients with type 1 and type 2 diabetes who had started to use glargine for a basal bolus regimen from 2004 through 2006. Twelve months after changing from NPH to glargine, the patients were able to decrease their required daily dosages and the numbers of insulin injections per day without changes in hemoglobin A1c or weight gain.

3. Epidemiological study of the effects of EMFs on health

To clarify the effects of EMFs on health, we performed an epidemiological study of workers exposed to strong EMFs and of control subjects. We also examined the effects of cellular telephones on health. The full epidemiological study, included health examinations, biochemical tests of blood, monitoring of personal exposure to EMFs, and measurement of the magnetic environment.

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Department of Forensic Medicine

Akihiro Takatsu, Professor

Kenji Fukui, Lecturer

General Summary

Our main research projects in 2006 have focused on the fields of forensic pathology, forensic application of DNA analysis, and forensic toxicology. Topics within forensic pathology include sudden, unexpected infant death, particularly infant mechanical suffocation death during sleep; the analysis of the pattern and severity of traffic injuries; and forensic autopsy cases related to medical practice.

Research Activities

Forensic pathology

1. Importance of infant mechanical suffocation during sleep

The cause of death in cases of sudden unexpected infant death during sleep is often diagnosed as sudden infant death syndrome (SIDS) with or without autopsy. Such a diagnosis can lead to medicolegal problems when a judge attempts to determine whether the cause of death is accidental, intentional, or SIDS. The purpose of this study was to identify the prevalence and importance of mechanical suffocation deaths during sleep in infants younger than 11 months and to propose risk factors for infant suffocation death during sleep. Autopsy cases in which the cause of death was diagnosed as mechanical suffocation were reviewed and analyzed according to the autopsy protocols, the clinical history, and the death scene investigation. A total of 47 cases of suffocation were statistically analyzed and compared with 184 control cases. We found that age younger than 6 months, cosleeping, a soft bed surface, oronasal obstruction, and the prone facedown position were important risk factors for infant suffocation death during sleep. Furthermore, 3 or more of these risk factors were present in each case of infant suffocation death during sleep. To avoid preventable deaths in infancy, the importance of infants sleeping alone on firm bed surfaces should be publicized.

- 2. Traffic injury
- 1) Analysis of injuries in bicycle accidents

We are analyzing the severity and patterns of injury in bicycle accident autopsy cases by using the Injury Severity Score and the 1998 revision of the Abbreviated Injury Scale. The head and neck are the most common sites of severe injury in motorcyclists and bicyclists. We are analyzing injuries of the head or neck in motorcyclists and bicyclists dying in traffic accidents and investigating the effects of helmet use on these injuries. 3. Autopsy cases related to medical malpractice

Public concerns about medical safety have recently increased in Japan, reflecting the increase in medical malpractice litigation and extensive media coverage. This study focused on characteristics of autopsy cases related to medical practice based on examinations at The Jikei University School of Medicine. The association between medical

practice and death was reviewed in 3,000 cases. A total of 257 cases related to medical practice were analyzed in terms of sex, age, cause of death, involved clinical subject, location of patient at serious exacerbation, and suspected type of malpractice. In these 257 cases, the percentage of female patients (41.6%) was greater than that in all 3,000 cases, and patients in their 70s accounted for the highest percentage of patients (16.3%). Deaths were natural in 70.8% of cases but were attributed to external causes in 28.8% of cases. The most commonly involved clinical specialty was internal medicine, followed by surgery and psychiatry. Serious exacerbations occurred when the patient was outside medical institutions in 37.7% of cases. The most common cause of medical malpractice (52.1% of cases) was incorrect diagnosis. The analysis of the autopsy cases related to medical practice might provide useful information for increasing medical safety.

Forensic application of DNA analysis

1. Personal identification of war remains by DNA analysis

We performed personal identification of war-dead remains buried in the former Soviet Union by means of DNA analysis as part of the war-dead remains return project of the Ministry of Health, Labour and Welfare. We used single nucleotide polymorphisms of hypervariable regions of mitochondrial DNA and short tandem repeats of nuclear DNA as genetic markers.

Forensic toxicology

1. Quantitative analyses of medicines and poisonous substances

Medicines and poisonous substances suspected to have caused deaths were quantitatively analyzed with gas chromatography and with gas chromatography/mass spectrometry of samples obtained at autopsy.

2. Relation of cardiovascular disease and prostanoids

We analyzed plasma levels of prostanoids in cases of sudden death examined at forensic autopsy and investigated their relation to the cause of death. In cases of death due to cardiovascular disease, the mean ratio of 6-keto-prostaglandin F_1 alpha to thromboxane B_2 was significantly lower than in cases of death not due to cardiovascular disease.

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Department of Tropical Medicine

Naohiro Watanabe, Professor Masahiro Kumagai, Lecturer Asao Makioka, Associate Professor Kenji Ishiwata, Lecturer

General Summary

Our research is concerned with mast cells in malaria, immune responses to helminth infection, and the growth and differentiation of *Entamoeba*.

Research Activities

Malaria and mast cells

Malaria is one of the most serious protozoan diseases. The pathogenesis of malaria is believed to depend on immune responses to *Plasmodium* proliferating in the blood. We have proposed a hypothesis that pericapillary mast cells are a major factor in the pathogenesis of malaria. We examined the molecules activating mast cells. Mast cells have two receptors, namely the IgE receptor in acquired immunity and toll-like receptor (TLR) in innate immunity. For the IgE receptor, anti-*Plasmodium* IgE antibody was detected in mice infected with the parasite, and peroxiredoxin induced tumor necrosis factor from mast cells through TLR4. The stimulation of TLR4 by peroxiredoxin and TLR4 was confirmed by coprecipitation of these molecules. These findings suggest a new concept that TLR4 can induce innate immunity through proteins instead of lipopolysaccharides.

Immune responses against gastrointestinal parasites

Gastrointestinal parasites require energy for active establishment in the gut against intestinal flow and peristaltic motion. We determined the ATP value of individual adult *Nippostrongylus brasiliensis* during the course of immune-mediated expulsion from the small intestine in rats. The ATP values of adult worms taken from the mucosa of the proximal small intestine, the preferred site of adult *N. brasiliensis*, were higher than those of worms from other sites of the small intestine. The reduction of ATP values in worms from unfavorable sites was observed not only at expulsion phase, but also at established phase(s) of the infection suggesting that the energy metabolism of the parasites is independent of the host immune response. When adult worms with low ATP values were surgically implanted into the small intestine of naïve rats, the worms re-established themselves in the recipients and completely restored the ATP values. Short-term *in vitro* culture of adult worms under low oxygen tension resulted in low ATP values in the worms. These results suggest that adult *N. brasiliensis* are dislodged from their preferred site by intact energy metabolism activity.

Protective immunity against re-infection by Hymenolepis nana onchospheres develops

within 24 hours after primary infection; however, the mechanism is unknown except for the requirement for CD4-positive cells. Histological observation revealed that when the interval between primary and secondary infections was as short as several days, protection took place on the mucosal surface; secondaily infected onchospheres could not invade host intestinal tissue. When the interval was longer than 3 weeks, the onchospheres could penetrate the mucosal tissue once but were then driven out. Antibodies are not involved in these protection mechanisms. These results suggest a novel protective immune mechanism in the gut.

Effect of artificial gastrointestinal fluids on the excystation and metacystic development of Entamoeba invadens

The effect of artificial gastric fluid containing 0.5% pepsin and 0.6% hydrochloric acid (pH 1.8) on the excystation and metacystic development of *E. invadens* was examined. Excystation was enhanced by pretreatment of cysts with artificial gastric fluid for 30 to 60 minutes at 37°C but not at 26°C. In addition, 0.6% hydrochloric acid had an enhancing effect on excystation comparable to that of artificial gastric fluid. Metacystic development was slightly enhanced by pretreatment with artificial gastric fluid. An artificial intestinal fluid containing 1% pancreatin, 1% sodium bicarbonate, and 5% ox bile (pH 8.0) had a significant toxic effect on cysts. These results suggest that gastric fluid but not intestinal fluid at 37°C enhances excystation in *Entamoeba* infection.

Differences in protein profiles of the isolates of Entamoeba histolytica and Entamoeba dispar by surface-enhanced laser desorption ionization time-of-flight mass spectrometry ProteinChip assays

Surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI-TOF MS) ProteinChip assays were used for protein profiling of different isolates of *E. histolytica* and *E. dispar*. When SELDI-TOF MS spectra of *E. histolytica* strain HM-1: IMSS were compared with those from 4 other laboratory strains grown under the same culture conditions, different peak patterns were observed among these strains independent of their zymodeme types. Similarly, 5 Japanese isolates of *E. histolytica* grown under the same culture conditions revealed different peak patterns among themselves. The spectra of 2 isolates of *E. dispar* showed the presence of peaks specific for *E. dispar* isolates and the absence of peaks common to *E. histolytica* isolates. Thus, the SELDI-TOF MS spectra accurately reflect proteins of *E. histolytica* and *E. dispar* isolates, showing their phenotypic differences and providing a unique means of distinguishing them.

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Department of Laboratory Medicine

Masato Suzuki, Professor Sadayori Hoshina, Associate Professor Hironari Sue, Associate Professor Kenichi Sugimoto, Associate Professor Hashizume Toshihiko, Lecturer Akihiro Ohnishi, Associate Professor Ken Kaito, Associate Professor Hiroshi Yoshida, Associate Professor Toshikazu Matsuura, Lecturer

General Summary

Research projects in our department in 2006 were concerned with clinical physiology, clinical microbiology, clinical chemistry, hematology, cardiology, clinical cell biology, clinical psychiatry, laboratory ethics, and cellular physiology. Research achievements in each division are described below.

Research Activities

Clinical physiology

Eight male volunteers performed an exhausting 400-m run twice with a 60-minute interval. After running, the subjects showed markedly decreased urinary Cl⁻ levels. The marked reduction in Cl⁻ might play a role in compensating for the increase in the anion gap in urine produced by running. Organic acids produced by running were excreted into tubular (lumen), and then Cl⁻ was reabsorbed to regulate the ion balance. The decreased Cl⁻ in the distal tubule might reduce the contraction of the mesangial cells via the macula densa and possibly results in an increase in the glomerular filtration rate and the urine flow rate.

Clinical microbiology

Research on plasma transfusion to extend the expiration date contamination control was performed using *Propionibacterium acnes* (more than 80% of transfusions are for cases of trauma). The result obtained role of *P. acnes* is minor groove estimation by histological and bacteriological study using a mouse intravenous injection model with contaminated human plasma and *Staphylococcus aureus* as a positive control. Chemo-kine production was induced by contaminated plasma, but this result did not agree with the results of studies in humans.

Clinical chemistry

Structural and glycation site changes of albumin in patients with diabetes and very high levels of glycated albumin

A patient with poor glycemic control had an extremely high level of plasma glycated albumin (94%). Treatment with insulin markedly decreased the blood glucose level. The glycated albumin level decreased almost linearly and reflected the improved glycemic state, whereas hemoglobin A1c was not altered initially.

The fluorometric spectrum of the advanced glycation end-product increased depending

on the glycated albumin level. The glycation sites were extended to 10 lysine (Lys) sites, including Lys-525, as shown by liquid chromatography/mass spectrometry. These results suggest that glycated albumin is a useful indicator for monitoring glycemic control during initial insulin treatment and that other sites of the albumin molecule in addition to Lys-525 are glycated when the blood glycated albumin level is elevated.

Clinical biochemistry

Effects of aerobic exercise on serum lipids were examined with a method of highperformance liquid chromatography we developed; high-density lipoprotein cholesterol was not increased, but very low density lipoprotein (VLDL) cholesterol was significantly decreased. This lowering of VLDL was pronounced in subjects with increased adiponectin (reported at the 14th International Symposium on Atherosclerosis, Rome, June 2006). The agreement between high-performance liquid chromatography and the homogenous method was excellent, but levels of low-density lipoprotein cholesterol, measured with the homogenous assay, increased when samples with increased levels of VLDL cholesterol were measured.

Hematology

Study of a new gene amplification system: the Transcription-Reverse Transcription Concerted Reaction System

We evaluated the efficacy of a new gene amplification method called the Transcription-Reverse Transcription Concerted Reaction (TRC) System. Using this system, we found the first case of multiple myeloma producing carcinoembryonic antigen and reported the system's satisfactory sensitivity and specificity in detecting *Mycobacterium tuberculosis*. The TRC system, which is a simple and rapid test, is a powerful, easy, and rapid method of gene amplification.

Cardiology

Pulmonary veins play an important role in both the initiation and maintenance of atrial fibrillation. Segmental ostial pulmonary vein isolation (PVI) is a new method of catheter ablation of atrial fibrillation. Our clinical studies are intended to assess the effects of PVI in patients with atrial fibrillation. This year we found that elevated plasma concentrations of brain natriuretic peptide decrease after PVI in patients with atrial fibrillation. We also investigated the effect of PVI on P-wave morphology.

Clinical cell biology

Development of implanted bioartificial liver

Immortalized mouse hepatocytes, sinusoidal endothelial cells, and Ito cells were cocultured, and liver organoids were constructed in a radial-flow bioreactor. The expressions of mRNAs of connexin26, and 32, hepatocyte nuclear factor 4, and glucose-6phosphatase were increased in transplanted tissues. The mRNAs of albumin and tyrosine aminotransferase were strongly expressed in the group transplanted in omentum.

Clinical psychiatry

1. We reported on the effects of atypical antipsychotics on electroencephalograms (EEGs) from the viewpoint of the glutamate hypothesis.

A retrospective study is under way to clarify the clinical significance of the 6-Hz spike and wave on EEG. We continued our investigation of epileptic seizures and chronological EEG changes in mentally handicapped patients. We reported on the clinical characteristics of epilepsy in the elderly.

2. We studied the correlation between the severity of Alzheimer disease, as determined with the Clinical Dementia Rating, and results of various subscales for assessing cognitive function. We found a strong correlation between visuospatial cognition function and the clinical severity of Alzheimer disease. Alzheimer disease can be diagnosed at an early stage by testing visuospatial cognition function.

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Department of Internal Medicine Division of Gastroenterology and Hepatology

Hisao Tajiri, Professor Yoshio Aizawa, Associate Professor Ichiro Takagi, Associate Professor Hisato Nakajima, Lecturer Fumitoki Watanabe, Lecturer Michiko Negishi, Lecturer Tomohisa Ishikawa, Lecturer Kiyotaka Fujise, Professor Hirokazu Nishino, Associate Professor Akira Torii, Associate Professor Tateki Yamane, Lecturer Shigeto Murakami, Lecturer Shigeo Koido, Lecturer Atsushi Hokari, Lecturer

Research Activities

Alimentary tract

1. The role of toll-like receptor 4 in the intestinal immune response: The roles of toll-like receptor 4-induced signal include the removal of invasive bacteria and mucosal repair.

2. The role of CCL25/CCR9 chemokine ligand/receptor pair in Crohn's disease small bowel: CCR9⁺ T cells in the small bowel in Crohn's disease are proinflammatory and support the use of CCR9 antagonists in Crohn's disease.

3. The phenomenon of CD4⁺CD25⁺ regulatory T cells in ulcerative colitis: CD4⁺ CD25⁺ T cells lose their original function in ulcerative colitis and may be unable to counterbalance the chronic mucosal inflammation in ulcerative colitis.

4. How do dendritic cell subsetsdetermine the development of tolerance of or immunity to commensal and pathogenic antigens?: Mucosal dendritic cell subsets populate the entire lamina propria of the small and large intestines and are constantly engaged in sampling and processing of food antigens, commensal bacteria, and intestinal pathogens.

5. The effect of urocortin I on mucosal cells in the colon: Increased expression of urocortin 1 results in inflammation of mucosal cells in the colon.

6. The correlation with the efficacy of leukocytapheresis (CAP) therapy with special attention to CD16+ subset: Profound depression of monocytes by CAPcould predict favorable response, while late over-shooting of CD16+ monocytes could be a hallmark of unfavorable response.

7. The efficacy of bridging therapy for ulcerative colitis: Bridging therapy consisting of azathioprine/6-mercaptopurine and granulocyte apheresis may become a new treatment strategy for cases of intractable ulcerative colitis in which endoscopic remission induction is problematic.

8. Cancer vaccine: Dendritic cells fused with human tumor cells and stimulated with OK-432 may be applicable to antitumor immunotherapy.

Liver

1. The study of intrahepatic immunological reaction in a model of portal vein injection of activated CD8+ T cells: The liver is a specific site for trapping activated CD8+ T cells.

2. Intrahepatic expression of the co-stimulatory molecules programmed death-1 and its ligands in autoimmune liver disease: The interaction of programmed death-1 on T cells with increased expression of B7-H1 and B7-DC on Kupffer cells and liver sinusoidal endothelial cells might be involved in the down-regulation of autoreactive lymphocytes and result in the regulation of pathogenesis in autoimmune liver disease.

3. A study of nonalcoholic fatty liver disease: Nonalcoholic fatty liver disease includes both nonalcoholic fatty liver and nonalcoholic steatohepatits. The serum ICAM-1 concentration is increased in patients with nonalcoholic steatohepatits. The serum level of ICAM-1 in patients with nonalcoholic fatty liver disease may be a useful marker for the diagnosis of nonalcoholic steatohepatits.

4. The possible cause of non-virus-related hepatocellular carcinoma: Patients with alcoholic liver disease or nonalcoholic steatohepatits must be followed up with special attention to the development of hepatocellular carcinoma.

5. Development of an implanted bioartificial liver: Immortalized mouse hepatocytes, sinusoidal endothelial cells, and Ito cells, were co-cultured, and liver organoids were constructed in a radial-flow bioreactor.

6. Immunohistochemical examination of lecithin: retinol acyltransferase, desmin and cellular retinol binding protein 1 in rodent liver: In mouse and rat livers, Ito cells were positively stained by antibodies against lecithin: retinol acyltransferase, desmin, and cellular retinol binding protein 1.

7. Antiviral effect on hepatitis B virus in the early stages of co-administration of adefovir: We investigated the therapeutic effects that can be expected after the start of adefovir administration. In our investigation, no significant factors related to antiviral effects for hepatitis B virus were observed in the early period (4 weeks) following the start of adefovir administration.

8. *In vitro* hepatotoxic examination using a mini-bioartificial liver (mini-liver) and ¹³C stability isotope labeling glucose: A mini-liver was constructed with human functional hepatocellular carcinoma cells cultured in 3 dimensions in a radial-flow bioreactor.

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Department of Internal Medicine Division of Neurology

Kiyoharu Inoue, Professor Hidehiko Honda, Associate Professor Akira Kurita, Associate Professor Hironori Sato, Lecturer Soichiro Mochio, Professor Hisayoshi Oka, Associate Professor Kazutaka Matsui, Lecturer Masahiko Suzuki, Lecturer

General Summary

Clinical research in 2006 was performed in the following areas: 1) spinocerebellar degeneration (SCD), 2) autonomic nervous system, 3) neurophysiological studies in dementia and diabetic neuropathy, 4) neuroradiological studies with nuclear medicine, 5) neuroimmunological disorders, 6) cerebrovascular disorders, and 7) neuropathological studies.

Research Activities

SCD

Taltirelin hydrate has recently been used to treat patients with SCD, but a better understanding of its effects is needed. The effects of taltirelin hydrate on cerebellar limb ataxia were evaluated quantitatively over the course of 4 years in 20 patients with SCD by means of a finger-tapping device containing a pressure sensor. We found neither improvement nor aggravation of symptoms over the course of 4 years. However, because the signs and symptoms of SCD gradually worsen in most patients, our results suggest that taltirelin hydrate is useful for the management of SCD.

Autonomic nervous system

Cardiovascular autonomic dysautonomia was investigated in patients with Lewy body disease (LBD), such as Parkinson's disease (PD) and dementia with Lewy bodies (DLB). Autonomic function was evaluated with cardiac ¹²³I-metaiodobenzylguanidine (MIBG) scintigraphy, hemodynamic function testing with the Valsalva maneuver, and orthostatic tolerance testing. We investigated whether PD can be distinguished from multiple system atrophy (MSA) on the basis of the results of cardiac ¹²³I-MIBG scintigraphy and testing of cardiovascular autonomic function, including baroreceptor reflex sensitivity. Latent sympathetic nervous dysfunction without parasympathetic dysfunction, especially that involving the sinus node, is already present in early de novo PD. However, whether the responsible lesion is central or peripheral remains unclear. Our results suggest that assessment of baroreceptor reflex sensitivity may be useful for differentiating between PD and MSA, 2 conditions in which ¹²³I-MIBG scintigraphy yields similar results.

Neurophysiological studies of dementia and diabetic neuropathy

Neurophysiological studies of visual information processing functions were evaluated in patients with DLB, PD, or Alzheimer's disease (AD) by means of visual event-related potential analyses. The author of these studies was invited as a guest speaker at a symposium at the 14th International Pharmaco-EEG Society symposium.

The clinical utility of nerve conduction studies and of neurological examination of the feet with newly established techniques was assessed in patients with diabetic neuropathy, in collaboration with the Department of Diabetes, Metabolism, and Endocrinology. The findings of the study suggest that neurological examinations and nerve conduction studies of the feet are useful for detecting early changes of diabetic neuropathy.

Neuroradiological studies with nuclear medicine

Cardiac sympathetic dysfunction was studied in mice with parkinsonism induced by 1-methyl-4-phenyl-1.2.3,6-tetrahydroxypyridine. The clinical utility of myocardial ¹²³I-MIBG scintigraphy and of ¹²³I-isopropyliodoamphetamine brain single-photon emission computed tomography studies was examined in parkinsonism and dementia. Early differential diagnosis in parkinsonian and dementing disorders was studied with positron emission tomography (PET) at the Positron Medical Center, Tokyo Metropolitan Institute of Gerontology. A significant Correlation was observed between presynaptic and postsynaptic nigrostriatal dopaminergic functions in MSA. The regional correlation between presynaptic and postsynaptic nigrostriatal dopaminergic functions in the MSA suggested pathophysiological mechanisms for responsiveness to levodopa. Intracerebral functional connectivity associated with occipital hypometabolism was evaluated in DLB. The results indicated that the visual hallucinations in DLB may be related to the hyperactivity of remote areas, such as limbic and brainstem regions.

Neuroimmunological disorders

The relationship between multiple sclerosis and Sjögren syndrome with neurological manifestations is controversial. To assess the prevalence of Sjögren syndrome in multiple sclerosis, the criteria of the American-European Consensus Group for Sjögren syndrome were used to study patients with multiple sclerosis. The authors questioned all patients about xerophthalmia and xerostomia. The following examinations were performed: measurements of anti-Ro/SS-A and anti-La/SS-B antibodies, antinuclear antibodies, and IgG in serum; the Schirmer test; salivary gland scintigraphy; and minor salivary gland biopsy.

Cerebrovascular disorders

In cerebrovascular disorders, a study of the utility of platelet-derived microparticles was performed in patients with cerebral infarction.

Neurosonological research concerning cerebral hemodynamics was performed with transcranial color flow imaging and carotid ultrasonography in patients with ischemic stroke. The hemodynamic state of intracranial arteries and the relationship between intracranial and extracranial arteries were evaluated with transcranial color flow imaging

and carotid ultrasonography. Stiffness parameter beta of the common carotid artery was measured with the echo-tracking method in patients with ischemic stroke and in healthy adults. One quantitative index of the elastic properties of large arteries was useful for evaluating early atherosclerotic changes before structural changes occur.

Neuropathological studies

Lewy body (LB)-related pathology (LBP) in the olfactory bulbs in human aging was evaluated. The olfactory bulbs are the target of neural progenitor cells and olfactory dysfunction in Parkinson's disease or AD, but few pathological studies have been performed. Pathological studies of the brain were performed in 270 consecutive autopsy cases by means of immunohistochemical staining for phosphorylated α synuclein. LBP was found in 83 (30.7%) of 270 cases; in addition, LBP was found in the olfactory bulbs in 66 cases (24.4%) and in the amygdala in 76 cases (28.1%). The cases with LBP in the olfactory bulbs also showed LBP in the amygdala. All cases that showed degeneration with LB of the substantia nigra or locus ceruleus also showed LBP in the olfactory bulbs. In only 5 of 270 cases were LBs found only in the olfactory Three of these cases also showed AD-type pathologic changes. These cases bulbs. showed only Lewy neurites or dots in the amygdala without LBs. One case showed LBP only in the amygdala without other senile changes. In the central nervous system, the olfactory bulbs are one of the regions that show LBs at the initial stage of LBD with or without tau pathology in human aging.

Basic research

Deficiency in energy supply, like that which occurs during hypoxia, anoxia, metabolic stress, and mitochondrial failure, strongly affects the excitability of central neurons. Such lowered energy supply evokes various changes in spontaneous synaptic input to hippocampal and cortical neurons. However, how this energy deprivation affects synaptic input to motor neurons, which are also vulnerable to energy deprivation, has not been addressed. We examined the effect of metabolic stress on synaptic input to motor neurons by recording postsynaptic currents in the hypoglossal nucleus. We found that, in hypoglossal motor neurons, chemical anoxia and anoxia increase the current mediated by N-methyl-D-aspartate (NMDA) receptors through activation of their glycine-binding sites by facilitated release of glycine in the absence of action-potential generation. To our knowledge, this study is the first to provide direct evidence linking metabolic disturbances and NMDA receptor potentiation through release facilitation of glycine and its spillover in motor neurons.

The caudal nucleus tractus solitarius (cNTS), in the dorsal medulla, specifically integrates respiratory, cardiovascular, and gastrointestinal afferents. Efferents from the cNTS are regulated by γ -aminobutyric acid (GABA) synapses within the cNTS. The proper function of the brain depends on a precise arrangement of excitatory and inhibitory synapses. The number of axosomatic GABA synapses decreases during postnatal development. Such a morphologic change could cause changes in electrophysiological activity and might contribute to reorganization of the local network within the cNTS from the neonatal to the adult type. These postnatal changes in the cNTS local network might be required for the cardiorespiratory reflexes of the adult type.

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Department of Internal Medicine Division of Kidney and Hypertension

Tatsuo Hosoya, Professor Iwao Ohno, Associate Professor Kimiyoshi Ichida, Lecturer Keitaro Yokoyama, Lecturer Makoto Ogura, Lecturer Yoichi Miyazaki, Lecturer Tetsuya Kawamura, Associate Professor Goro Tokudome, Associate Professor Yasunori Utsunomiya, Lecturer Yasuhiro Yamamoto, Lecturer Masato Ikeda, Lecturer Kazushige Hanaoka, Lecturer

General Summary

Major fields of research are 1) nephrology, 2) hypertension, and 3) uric acid metabolism. Published achievements and recent reports are summarized here.

Research Activities

Nephrology

1. Glomerulonephritis

The serum level of granulocyte-colony stimulating factor (G-CSF) might be a useful biomarker for predicting the course of diabetic nephropathy, and G-CSF might show antiapoptotic effects on renal vascular cells through the G-CSF receptor.

Glomerular density in renal biopsy specimens might be used as a long-term prognostic indicator in of IgA nephropathy. We reported the generation of a stem-cell-derived organoid that could produce erythropoietin and was sensitive to regulation by anemia, indicating a function in erythropoiesis. Podocyte-derived bone morphogenic protein 4 might act as a paracrine factor on the precursor cells of the glomerular capillary tuft, thereby regulating morphogenesis of the glomerulus.

2. Dialysis

We compared clinical features in the management of renal osteodystrophy between Japan and the United States. The clinical effectiveness of percutaneous injection therapy with ethanol and active vitamin D was verified for secondary hyperparathyroidism. We revealed that the function of L-type Ca^{2+} channels of cultured parathyroid cells depends on the extracellular Ca^{2+} concentration. We assessed the clinical value of combined therapy with peritoneal dialysis and hemodialysis; we found that combined therapy is a useful way to control body fluids and, moreover, that peritoneal function may be maintained long term. We studied the safety and efficacy of combination therapy with diltiazem and tacrorimus-based immunosuppressive treatment to maintain optimal serum tacrorimus concentrations in patients with poor bioavailability after renal transplantation. In transplant glomerulopathy, glomerular expression of plasmalemmal vesicle-associated protein-1 is positively correlated with the severity of transplant glomerulopathy and proteinuria. We are investigating the mechanism of cyst formation in polycystic kidney disease by using cells derived from the PKD knockout mouse.

Hypertension

The T-type Ca²⁺ channel blocker is a promising agent for the treatment of hypertension in chronic kidney disease. Candesartan is specifically indicated for patients with chronic kidney disease who require a more intensive treatment to prevent cardiovascular complications. Carotid-femoral pulse wave velocity, urinary albumin excretion, and brain natriuretic peptide were related to each other as predictors of and risk factors for cardiovascular diseases in patients with hypertension.

Uric acid metabolism

Renal function deteriorates more quickly in patients with gouty kidney than in patients with nongouty kidney. Allopurinol was mainly used to treat hyperuricemia in airline cockpit crew; however, the control of hyperuricemia was not sufficient, due, in part, to the complex procedure for changing prescriptions. The high incidence of renal hypouricemia in Japanese reflects the high incidence of the G774A mutation of URAT1 genes.

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Department of Internal Medicine Division of Rheumatology

Akio Yamada, Professor

Daitaro Kurosaka, Lecturer

General Summary

An internist must aim to practice patient-oriented medicine that is well grounded in medical science. Therefore, our department encourages its staff members to do basic and clinical research. Major fields of research are clinical and experimental immunology.

Research Activities

Clinical and experimental studies of autoimmune disease were performed

1. Analysis of the effects of neovascularization inhibitors in animal models of rheumatoid arthritis

Several studies have reported the arthritis-inhibiting effects of neovascularization inhibitors in animal models of rheumatoid arthritis. We evaluated the effects of theneovascularization inhibitor endostatin in an animal model of collagen-induced arthritis. Furthermore, we are analyzing the mechanism of the arthritis-inhibiting effects of endostatin.

2. Analysis of telomerase activity in peripheral blood mononuclear cells of patients with autoimmune disease

The activation of telomerase has been observed in healthy cells, including normal lymphocytes. An increase in telomerase activity is associated with the activation of lymphocytes. Much attention has been paid to the role of telomerase in immunocytes. We measured telomerase activity in peripheral blood mononuclear cells obtained from patients with autoimmune diseases, especially systemic lupus erythematosus.

3. Clinical studies aimed at standardizing immunosuppressant therapy for autoimmune disease

Many immunosuppressant drugs have been used to treat severe autoimmune diseases, such as amyopathic dermatomyositis with interstitial pneumonia, but the efficiency and treatment strategy of these drugs have not been clarified. We performed a clinical trial to establish a strategy for the treatment of severe autoimmune diseases. Clinical studies aimed at standardizing immunosuppressant therapy for autoimmune disease were performed.

Publications

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Department of Internal Medicine Division of Cardiology

Seibu Mochizuki, Professor Ikuo Taniguchi, Professor Masayuki Taniguchi, Associate Professor Teiichi Yamane, Associate Professor Satoru Yoshida, Lecturer Kenichi Hongo, Lecturer Makoto Kawai, Lecturer Mitsuyuki Shimizu, Professor Katsunori Ikewaki, Associate Professor Shingo Seki, Associate Professor Kazuhiko Ogawa, Lecturer Takahiro Shibata, Lecturer Hideki Sasaki, Lecturer Kimiaki Komukai, Lecturer

General Summary

The Japanese Investigation of Kinetic Evaluation in Hypertensive Event And Remodeling Treatment (JIKEI HEART) Study began registration in January 2002. By the end of November 2004, 3,081 patients had been enrolled, exceeding the original target enrollment. We could obtain meaningful results from the valsartan-treated group, and these results were accepted for publication in *The Lancet*. The Japanese Rhythm Management Trial for Atrial Fibrillation (J-RHYTHM), the trial in which our cardiac arrhythmia group is taking a lead role, announced that its study portion was completed on April 3, 2006. We are now waiting for the results to be summarized. Enrollment in the Assessment of β -Blocker Treatment in Japanese Patients with Chronic Heart Failure (J-CHF) trial has been stalled because only patients with untreated heart failure are enrolled. New registrations will be accepted until the end of 2006.

Individual research groups are continuously obtaining results from clinical and basic research. In the cardiac catheterization group, an increasing number of patients have received drug-eluting stents. Clinical follow/up data from 6 to 12 months is being accumulated. Utilizing a network system among the main university hospitals and 4 affiliated hospitals, data control and results analyses are under way. In the cardiac arrhythmia group, as the number of cases of radical therapy for atrial fibrillation (isolating pulmonary vein using a catheter) increases, new studies to address unexpected issues or points requiring improvement are being developed. Since 2005, the lipid metabolic group has been performing metabolic experiments with stable isotopes and has been performing research in collaboration with institutions overseas. Individual groups are also promoting their own basic research using an original experimental system and preparing for presentations next year at the scientific meetings of the Japanese Circulation Society, the International Society for Heart Research, the American Heart Association, and other groups. This year, graduate students in both basic and clinical courses powerfully demonstrated very vigorous research activities by studying domestically or abroad and announcing their research results.

Research Activities

Clinical research

- 1. Large-scale clinical trials
- 1) JIKEI HEART Study

The JIKEI HEART Study is a large-scale clinical trial that examined the effect of oral administration of valsartan on the prognosis of patients with hypertension and ischemic heart disease or heart failure. The study, which was performed by means of the prospective, randomized, open, blinded endpoint method, is Japan's first clinical trial in this field and has enrolled more than 3,081 patients at various Jikei University Hospitals. The study was completed in November 2005. We could obtain meaningful results from the valsartan-treated group, and these results were accepted for publication in *The Lancet*.

2) J-RHYTHM

J-RHYTHM is a multicenter randomized comparative trial that is examining drug therapies for atrial fibrillation. The trial is comparing the efficacies of therapy to sustain sinus rhythm and of therapy to control heart rates. J-RHYTHM was initiated by the Japanese Society of Electrocardiology with the support of the Japanese Circulation Society. The Ethics Committee of The Jikei University approved our division's participation. The arrhythmia team took the initiative, began study registration in April 2004, and observed the clinical time courses for the enrolled subjects. The study end was announced on April 3, 2006, and procedures for registering case data for study analysis have begun.

3) J-CHF

Underway since 2005, this large-scale clinical trial aims to establish a standard β -blocker therapy for patients with chronic heart failure. Specifically, the trial aims to determine the optimal dosage by comparing the efficacy and safety of 3 dosages of the β -blocker carvedilol: 2.5, 5, and 20 mg. Registration began on July 5, 2003, and will continue until December 2007. Four patients from our division have been enrolled in the study. 2. Arrhythmia

In 2005, catheter ablation therapy was first used to treat atrial fibrillation. This field still has many unknowns, and we have been exploring several topics, including novel therapeutic approaches, expansion of therapeutic indications, and assessments of treatment efficacy. We have passed on this newly acquired knowledge to various academic societies. This year, we examined the following topics.

1) Evaluation of therapeutic efficacy of isolating the pulmonary vein in patients with sustained or chronic atrial fibrillation

2) Identification of the intra-atrial matrix and the efficacy of local cauterization in patients with chronic atrial fibrillation

3) Examination of the frequency, significance, and effects of additional cauterization for transient conduction in ATP-induced isolation of the pulmonary vein

4) Development of a method to prevent phrenic nerve injury during operations that isolate the superior vena cava

5) Examination by means of vector electrocardiography of the effects of operations that

isolate the pulmonary vein on the process of excitability to electrical stimulation of the atrium

6) Preoperative and postoperative changes in serum brain natriuretic peptide values and their significance when isolating the pulmonary vein

- 3. Lipid metabolism
- 1) Triple tracer study using stable isotope

We continue in vivo kinetic studies to assess the effects of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), fibrates, and ezetimibe on lipid and glucose metabolism. Several international collaborations resulted in publication in prestigious journals, including the *New England Journal of Medicine* (University of Pennsylvania), the *Journal of Lipid Research* (Harvard School of Public Health), and *Kidney International* (University of Innsbruck). We are now focusing on several on-going projects.

4. Nuclear medicine

We are participating in a multicenter prospective cohort study to examine the prognosis for patients with heart failure using ¹²³I metaiodobenzylguanidine (MIBG) schintigraphy (Kanto MIBG Trial for Chronic Heart Failure; target number of cases to be enrolled: 300).

Basic research

1. Arrhythmia

Abnormalities in the expression of connexin, which controls intercellular transmission, are suggested to be an important underlining mechanism of atrial fibrillation. On the other hand, hypertension and heart failure are important factors in atrial fibrillation. This year, we used Dahl rats with hypertension-induced heart failure to observe changes in the expression of connexin due to decreased cardiac function and pressure loading. In the future, we will examine the localization of proteins, such as zona occludens 1, that are related to connexin.

2. Cellular cardiology

We have investigated the physiological and pathophysiological roles of intracellular Ca handling in cardiac excitation-contraction coupling. We are now using molecular biological methods combined with physiological methods.

This year, we published a paper regarding a new regulatory mechanism of α 1adrenoceptor stimulation on the L-type Ca channel in rat ventricular myocytes. We also estimated the function of the sarcoplasmic reticulum (SR) of genetically engineered mice in which the SR Ca pump function was selectively enhanced (SERCA-TG) or inhibited (SLN-TG). In addition, we have started to use a knock-in mouse model of dilated cardiomyopathy (DCM) to reveal its pathogenesis.

3. Myocardial metabolism

Impaired postischemic cardiac function and cytoplasmic ion movements in hearts from mice with type 2 diabetes: Myocardial function and cytoplasmic calcium movements were examined using fluorescence during ischemia-reperfusion in hearts from mice with type 2 diabetes. Exacerbation of postischemic cardiac dysfunction and cytoplasmic calcium overload during ischemia-reperfusion were shown. Systems for regulating ion movements associated with this phenomenon will be examined.

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Department of Internal Medicine Division of Diabetes, Metabolism and Endocrinology

Naoko Tajima, Professor and Chairperson Junichi Yokoyama, Professor Kazunori Utsunomiya, Associate Professor Katsuyoshi Tojo, Associate Professor Hideaki Kurata, Associate Professor Tamotsu Yokota, Lecturer Yoichi Sakamoto, Professor Kuninobu Yokota, Associate Professor Takashi Sasaki, Associate Professor Yutaka Mori, Associate Professor Masami Nemoto, Lecturer Rimei Nishimura, Lecturer

General Summary

Physicians should practice patient-oriented medicine based on the concept of evidencebased medicine, which consists of research evidence, clinical expertise, and patients' preferences. To accomplish this, we encourage the members of our staff to do basic and clinical research. Areas of research include diabetes, metabolism, and endocrinology.

Research Activities

Epidemiology and evidence-based medicine

A nationwide epidemiologic study of mortality in approximately 3,500 patients with type 1 diabetes was started in 1986 and has continued to provide much information about the prognosis of Japanese children with type 1 diabetes. A population-based interventional study of childhood obesity and glucose intolerance has also continued. Several clinical trials of the treatment of type 2 diabetes are being performed.

Genetic epidemiology of diabetes mellitus

To study the molecular mechanisms of the development of macrovascular complications, we have analyzed sets of human single nucleotide polymorphisms and have found that one antioxidant enzyme has a genetic association with increased coronary calcification. We will extend the analysis to other antioxidant enzymes.

Gene- and cell-based therapy for diabetes mellitus

For *in vivo* gene therapy to promote proliferation of pancreatic beta cells by activating the G1/S cell-cycle transition, we have developed an efficient gene-transfer method, direct injection of an adeno-associated virus vector expressing the CDK4R24C gene. We will present our results at the upcoming annual scientific session of the American Diabetes Association.

Diabetic vascular complications

Research has focused on the pathogenesis and treatment of diabetic vascular complications. Clinical studies have examined dietary therapy for type 2 diabetes. Experimental studies using vascular smooth muscle cells, mesangial cells, and retinal pericytes have investigated the role of the Rho/Rho-kinase-mediated signaling pathway in the pathogenesis of diabetic vascular complications and have provided evidence that these molecules are potential pharmacological targets in the treatment of diabetic vascular injury.

Insulin resistance and obesity

A series of basic research studies of insulin resistance were performed in Otsuka Long-Evans Tokushima Fatty rats. The effects of new oral hypoglycemic agents on insulin resistance were investigated.

Endocrinology

The possible relationship between tumor necrosis factor α and urocortin II was examined in HL1 cardiomyocytes, a cardiac cell line derived from mouse atrial cardiomyocytes. Clinical and pathological characteristics of preclinical Cushing's disease due to ACTH-precursor-producing pituitary macroadenoma were studied.

The effect of ghrelin, a stomach-derived hormone, on the generation of pancreatic beta cells was studied in streptozotocin-treated rats, an animal model of diabetes mellitus.

The cardioprotective role of heat shock transcription factor-1(HSF-1) were studied using HSF-1 transgenic and knockout mice.

Functional analysis of the novel S179R *POU1F1* mutation associated with combined pituitary hormone deficiency was performed.

A retrospective cohort analysis of patients with Grave's disease was performed to compare the efficacy and safety of antithyroid drugs.

Changes in levels of anti-thyrotropin receptor antibody after radioiodine therapy were evaluated in patients of childbearing age with Grave's disease.

Dietary therapy in patients with diabetes mellitus

The effects of pregerminated brown rice on postprandial blood glucose and insulin concentrations were compared with those of white rice in subjects with hyperglycemia. Postprandial blood glucose levels were lower with brown rice than with white rice. The incremental area under the curve of blood concentrations for 180 minutes after ingestion was significantly lower with brown rice than with white rice. The postprandial blood insulin concentration was slightly but not significantly lower with brown rice than with white rice.

Publications

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Department of Internal Medicine Division of Clinical Oncology/Hematology

Keisuke Aiba, Professor Fumi Mizorogi, Associate Professor Daisuke Inoue, Associate Professor Toshikazu Sakuyama, Lecturer Osamu Asai, Lecturer Shuichi Masuoka, Lecturer Tadashi Kobayashi, Professor Noriko Usui, Associate Professor Toshio Katayama, Lecturer Takaki Shimada, Lecturer Nobuaki Dobashi, Lecturer Yoshikazu Nishiwaki, Lecturer

General Summary

The Division of Hematology and Oncology and the Division of Clinical Oncology were merged into the new Division of Clinical Oncology/Hematology on September 1, 2006. The immediate goals of our clinical and basic research are to investigate malignant diseases and improve outcomes for patients with hematological malignancies and solid tumors, leading to the final goal of improving the natural history of malignant diseases. We performed several clinical trials and basic research studies in 2006.

Research Activities

Leukemias

We have conducted clinical trials as a member of the Japan Adult Leukemia Study Group (JALSG) for acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and chronic myeloid leukemia (CML). The JALSG AML206 protocol was developed to investigate gemtuzumab ozogamicin, a novel fusion agent comprising calicheamycin and a humanized monoclonal antibody to CD33, combined with cytarabine and idarubicin or daunorubicine for the treatment of relapsed or refractory adult AML. The JALSG AML201 VAL4 study was also performed to examine the correlation between expression of VAL4 molecules and the prognosis of patients with untreated adult AML. The JALSG performed a clinical trial, including our 3 patients, using a combination of standard induction chemotherapy and imatinib mesylate for Philadelphia-chromosome-positive (Ph+) ALL. The complete remission rate was 96%, and the event-free survival rate was 50%, which were both significantly higher than in previous studies. The results were reported in the Journal of Clinical Oncology (2006: 24: 460).

Phase I/II studies using nilotinib and dasatinib were also performed in patients with chronic-phase CML.

Lymphomas

We have performed clinical trials as a member of the Lymphoma Study Group of the Japan Clinical Oncology Group (JCOG-LSG). A phase I/II study of combinedmodality therapy consisting of the DeVIC regimen (dexamethasone, etoposide, ifosfamide, and carboplatin) and radiotherapy for localized nasal natural killer/T-cell lymphoma has recently been completed. We enrolled 1 patient, and the therapeutic result was satisfactory. The JCOG-LSG developed a new regimen named R-EPOCH, which is a combination of rituximab and the EPOCH regimen (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin), for a phase II study in patients with relapsed or refractory B-cell lymphoma. The study is now open for enrollment.

Myeloma

We examined the efficacy of the combination therapy of thalidomide and dexamethasone in a pilot study, and the target number of patients has been enrolled.

Hematopoietic stem cell transplantation

We have been investigating the efficacy of hematopoietic stem cell transplantation for hematological malignancies since the 1980's. The mechanisms of graft-versus-host disease encountered in hematopoietic stem cell transplantation were studied in 2006.

Solid tumors

Several of our protocols have been running throughout our university hospital, as we seek improved therapeutic outcomes in cooperation with related divisions or depart-The FEC100 regimen (5-fluorouracil, 500 mg/m², epirubicin, 100 mg/m^2 , and ments. cyclophosphamide, 500 mg/m^2) with or without docetaxel is an adjuvant therapy for patients with breast cancer after curative resection. FEC100 followed by docetaxel is a preoperative combination chemotherapy for patients with locally advanced breast cancer. AT followed by docetaxel and Herceptin is a first-line chemotherapy for patients with advanced metastatic breast cancer. A cooperative phase II study of lapatinib covering dual inhibition of epidermal growth factor receptor transmembranously was performed in our division, and 3 patients were enrolled. The standard care for operable, locally advanced esophageal cancer has been altered, resulting in the use of chemoradiation therapy instead of surgical resection. Therefore, since 2002 we have been investigating a combined-modality therapy of radiation and combination chemotherapy with low-dose cisplatin and 24 hours' continuous infusion of flurouracil (5-FU) for such patients. The results will be reported next year. For patients with advanced gastric cancer, the combination chemotherapy of S-1 and cisplatin has been administered. The regimens FOLFOX (leucovorin, 5-FU, and oxaliplatin) and FOL-FIRI (leucovorin, 5-FU, and irinotecan) have been our front-line chemotherapy regimens for patients with advanced colorectal cancer. Other important activities include palliative care using analgesic agents and consultation regarding psychiatric disorders accompanying cancer.

Basic research

One of our pivotal schemes is translational research covering hematological malignancies and solid cancers. The structural differences between the M protein produced by myeloma cells and that produced by malignant gastric ulcers have been examined, and the function of ATP-binding cassette transporter in cancer chemotherapy was studied in collaboration with the Kyoritsu University of Pharmacy.

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Department of Internal Medicine Division of Respiratory Diseases

Hisakazu Tai, Associate Professor Heiichi Yano, Lecturer Hiroshi Takeda, Lecturer Taichi Mochizuki, Lecturer Tetsuo Sato, Associate Professor Akira Kojima, Lecturer Futoshi Kotajima, Lecturer

General Summary

Several case reports were published concerning various lung diseases such as lung cancer, pulmonary fibrosis, chronic obstructive pulmonary disease, chronic respiratory failure, bronchial asthma, and respiratory infection. Some of these reports were presented at the Japanese Thoracic Society Congress.

Research Activities

Lung cancer; 2 case reports Pulmonary tuberculosis; 2 case reports Pulmonary fibrosis; 1 case report Chronic respiratory failure; 1 case report

Publications

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Department of Internal Medicine Division of General Medicine

Ken Hokkyo, Professor Nobuakira Takeda, Professor Akihiro Nishiyama, Associate Professor Hiroshi Yoshida, Associate Professor Futoshi Kotajima, Lecturer Jun Hiramoto, Lecturer Hidekatsu Yanai, Lecturer Kazuo Nagayama, Professor Norio Tada, Professor Masato Matsushima, Associate Professor Hideaki Suzuki, Lecturer Nobuyuki Furutani, Lecturer Chihiro Shikata, Lecturer

Research Activities

Division of General Medicine, Jikei University Hospital

Little is known about the relationship between anxiety and quality of life (QOL) in a primary care setting. The objective of this study was to determine whether the degree of anxiety affects physical complaints and the QOL physical status scale. The State-Trait Anxiety Inventory (STAI) and the 36-Item Short-Form Health Survey (SF-36) were employed to evaluate the degree of anxiety and general QOL scores, respectively. To date, 46 patients (26 men and 20 women) with a mean age of 40.3 ± 13.0 years have participated in the study. Of the 35 physical complaints, vertigo, chest pressure, cough, and sore throat were correlated with trait-anxiety. The physical component summary score of the SF-36 showed a statistically significant relation with trait-anxiety, but not state-anxiety, after we adjusted for sex and age.

Division of General Medicine, Jikei University Aoto Hospital

Experimental and clinical investigations were performed for cardiovascular abnormalities in hypertension and cardiomyopathy.

1. Effects of combination therapy with antihypertensive drugs were investigated in patients with hypertension associated with diabetes mellitus. Incidence of cardiovascular abnormalities increases if patients with hypertension also have diabetes. If patients show ischemic electrocardiographic changes antihypertensive agents should be selected carefully. In our study, the effects of angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, calcium antagonists, and beta blockers with or without intrinsic sympathomimetic action were examined in patients with diabetes-associated hypertension.

2. An experimental study was performed of alterations in myocardial subcellular organelles in cardiomyopathic J2N-k hamsters. Molecular biological examination was also done for laminin, which is a component of the extracellular matrix, in cardiomyopathy.

Division of General Medicine, Jikei University Daisan Hospital

1. Study of factors of infection in elderly hospitalized patients

To investigate the relation between infection and several factors in elderly hospitalized

patients, we studied the relation of infection with the nutritional state, administered drugs, and biochemical markers.

2. Study of the effects of hospitalization

We continued to study changes in the nutritional state, lean body mass, mental state in elderly hospitalized patients.

3. Study of fever of unknown origin

We attempted to clarify the cause of fever by measuring white blood cells, the erythrocyte sedimentation rate, and levels of C-reactive protein, adenosine deaminase, 2-5 oligoadenylate synthetase, soluble interleukin-2 receptor, and procalcitonin. We have treated 500 patients with fever since 2000. We found that viral infection can be distinguished from bacterial infection on the basis of the results of these measurements.

Division of General Medicine, Jikei University Kashiwa Hospital

Our research works consist of three parts: the first part is to investigate the role of the general medicine on the environmental health achievement in regional areas, the second part is to evaluate lipid abnormalities which cause premature atherosclerosis, and the third part is to develop educational tasks for teaching medical students and junior residents.

1. Investigation of the role of general medicine on environmental health achievement.

1) We became a central member of the DISCOVER, an epidemiological study, which is supported by Health and Labor Sciences Research Grants for Comprehensive Research on Aging and Health.

2) We established a regional nutritional education system for patients with diabetes mellitus or metabolic syndrome in the Kashiwa area.

2. Evaluation of lipid metabolism

1) We demonstrated that oxidized low-density lipoproteins activate platelets and induce serotonin release from platelets (Am J Hematol 2007; 82: 686-7).

2) We discovered that diacylglycerol ingestion elevates plasma serotonin levels (Obesity 2007, in press).

3) We showed that diacylglycerol is beneficial for patients with apolipoprotein C-II deficiency (QJM 2007; 100: 247-8).

3. Studies of mechanisms of atherosclerosis

1) The ameliorating effect of exercise on serum lipids was investigated in detail with high-performance liquid chromatography.

2) We measured levels of high-density lipoprotein cholesterol and low-density lipoprotein cholesterol with a homogenous high-performance liquid chromatography assay.

4. Medical Education

1) A new practical evidence-based medicine algorithm for the clinical setting was developed.

2) As an effective feedback system for undergraduate and postgradate medical education, a portfolio education system, was developed.

3) For easy and practical problem-oriented systems, a checklist audit report system was developed and the efficacy of this system was evaluated.

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Department of Psychiatry

Kazuhiko Nakayama, Professor Hiroshi Itoh, Associate Professor Hisatsugu Miyata, Associate Professor Kazutaka Nukariya, Lecturer Motohiro Ozone, Lecturer Tatsuroh Nakanishi, Lecturer Hiroo Kasahara, Professor Kei Nakamura, Associate Professor Hironari Sue, Lecturer Wataru Yamadera, Lecturer Kazuya Ono, Lecturer Toshihiko Hashizume, Lecturer

General Summary

Our research activities cover a wide range of topics: disorders at the psychologic and biologic levels, from childhood and adolescence, through adulthood, to the senile period. Sociologic, psychologic, physiologic, and biochemical methods were used.

Research Activities

Psychopathology, psychotherapy, and child psychiatry study group

A study of the personality structure of elderly patients with eating disorders suggests that the schizotypal personality disorder is involved in the severity of symptoms. A study of the borderline personality disorder indicated the effectiveness of short-term and moderately structured inpatient treatment. A text of dialectical behavior therapy was translated from English into Japanese. A collaborative study of neurosis in China clarified that obsessive-compulsive disorder is the most common disorder at outpatient clinics in urban areas in China. Furthermore, a study of a new treatment strategy for pervasive personality disorders and self-harm behavior was begun.

Morita therapy group

A study of the effectiveness of inpatient Morita therapy in 23 subjects with social anxiety disorder indicated that symptoms, social adaptation, and self-acceptance were improved. In addition, standardizing outpatient Morita therapy was started as an activity of the Japanese Society for Morita Therapy. We also performed a study of the personalities of patients with chronic depression, a study of the relationship between panic disorder and generalized anxiety disorder in terms of the personality and comorbidity, and a follow-up study of patients who showed improvement with inpatient Morita therapy.

Psychopharmacology group

In basic research, the mechanisms of the central actions of a new generation of antipsychotic agents and antidepressants were studied with a microdialysis technique, and the brain mechanisms underlying the processes of learning and the memory of drug dependence were studied in rats. In clinical research we studied clinical characteristics of craving in substance dependence, brain receptors in patients with obsessive-compulsive disorder, a new treatment strategy for mood disorders with concurrent administration of atypical antipsychotic drugs and selective serotonin reuptake inhibitors or mood stabilizers or both.

Psychophysiology group

In clinical research, we studied the effects of selective serotonin reuptake inhibitors on obstructive sleep apnea-hypopnea syndrome, the effects of the amino acid glycine on nocturnal sleep, the effects of cognitive behavioral therapy and Morita therapy on psychophysiological insomnia, and the effects of new nonbenzodiazepine hypnotics on human balance function. In chronobiological research, we studied the effects of vitamin B12 on nocturnal sleep, the effects of a 5-time-zone eastward flight on psychophysiological functions, and the chronobiological effect of psychiatric rehabilitation therapy on chronic schizophrenia.

Psychogeriatric group

The Itoigawa study, a longitudinal general-population study involving the 8-year followup of a representative sample of 8,000 persons 65 years or older in Itoigawa City (Niigata Prefecture) aimed to analyze the relationship between various risk factors, normal and pathological cerebral ageing, and risk factors for other conditions, such as mild cognitive impairment. A second area of research concerns the neuropsychological evaluation of neurodegenerative and psychiatric diseases. We believe the profiles of cognitive impairment derived from this research will improve the diagnostic accuracy and lead to new models of neurocognitive impairment in neurodegenerative and psychiatric diseases.

General hospital psychiatry

We have continued a study, in collaboration with the National Cancer Center Hospital East, of psychological distress in the families of cancer patients. The results suggest that early psychiatric intervention is needed, depending on the mental status of family members. We have also continued cognitive-behavioral therapy aimed at preventing recurrences of depression; our findings suggest that early intervention has strong educational and therapeutic effects.

Clinical electroencephalography group

The effects of atypical antipsychotics on electroencephalograms (EEGs) was investigated from the viewpoint of the glutamate hypothesis. A retrospective study was performed to clarify the clinical significance of the 6-Hz spike and wave on EEGs. Furthermore, studies examined epileptic seizures and chronological EEG changes in mentally handicapped patients and the characteristics of epilepsy in the elderly.

Clinical psychology group

We have continued to study the psychotherapy process, techniques of psychoanalytic psychotherapy, Morita therapy, and counseling. Furthermore, we have studied the characteristics of developmental disorders and many types of personality disorder through psychological assessments. In addition, we have devoted time to resident training.

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Department of Pediatrics

Yoshikatsu Eto, Professor Yasutaka Hoshi, Professor Nobuo Usui, Professor Toya Ohashi, Professor Yasuyuki Wada, Associate Professor Ichiro Miyata, Associate Professor Hiroaki Wakasugi, Lecturer Yoshihiro Saito, Lecturer Hiroshi Kobayashi, Lecturer Masaharu Kubo, Professor Fumiyuki Ito, Professor Hiroyuki Ida, Professor Toshio Katsunuma, Associate Professor Kazue Saito, Associate Professor Hisashi Tamaki, Lecturer Yoko Kato, Lecturer Yoshihiro Hayashi, Lecturer Masaharu Akiyama, Lecuturer

Research Activities

We have 8 subspecialty research groups: (1) the Medical Genetics, Endocrinology, and Congenital Metabolic Diseases group, (2) the Neurology group, (3) the Allergy and Immunology group, (4) the Cardiology group, (5) the Nephrology group, (6) the Infectious Diseases group, (7) the Hematology and Oncology group, and (8) the Neonatology group. The common goal of the groups is offering practical benefits to patients and families through research. To accomplish this, we encourage our staff members to engage in research.

Medical genetics, endocrinology and congenital metabolic diseases group

We focused on studies of the pathogenesis and treatment of genetic diseases, endocrinology, and digestive system disorders. We clarified the role of *shugosin* protein during mitosis. We successfully transduced genes into cultured neuronal cells using a baculoviral vector and corrected thalassemia with a retroviral vector in a mouse model. We identified a novel mutation of the POU1 gene and analyzed the function of mutated proteins in a patient with complex growth hormone deficiency. We established guidelines for children with Crohn's disease.

Neurology

We investigated the approach to rehabilitation in acquired brain injury, especially traumatic brain injury (TBI). Thirty-nine children with higher cortical dysfunction after TBI were evaluated. In children with diffuse brain damage the prevalence of memory disturbance was lower (p < 0.01) and that of attention deficit was higher (p < 0.05) than in children with focal brain damage. The prevalence of higher cortical dysfunction, especially the disturbance of visual perception, was higher in children with motor dysfunction. Intelligence quotient recovered mainly during the first 2 years after TBI, and profile patterns on the Wechsler Intelligence Scale for Children were almost the same 6 months after TBI. Support for re-entrance to schools should begin as soon as possible with cooperation among rehabilitation centers, schools, and homes. Because many problems were observed in study, behavior, communication, and other areas, considerable support from the rehabilitation center is necessary.

We studied human herpesvirus 6 (HHV-6) encephalopathy, to clarify diagnostic images

of HHV-6 encephalopathy. The findings of magnetic resonance imaging (MRI) and single photon emission computed tomography (SPECT) of HHV-6 encephalopathy were divided into 3 types: 1) frontal lobe predominantly damaged, 2) unilateral hemispheric damage, and 3) diffuse damage. Disturbances predominantly affecting the frontal lobes on MRI and SPECT images are considered to have similar pathophysiology as the diffuse type, which is more severe. The unilateral hemispheric type may be associated with pathophysiologic changes other than those found in the frontal lobe predominantly damaged type and the diffuse type.

We are studying various neurological disorders, including epilepsy; acute neurological disorders, such as acute encephalitis; and rehabilitation of patients with acquired brain damage. We have published several studies in the fields of rehabilitation, epilepsy, and acute encephalitis, as described below. We should strive to produce many reports, in rehabilitation, epilepsy, acute encephalitis, and other fields.

Allergy and immunology

We have been measuring several markers in exhaled breath condensate from young children with asthma. Levels of exhaled leukotriene (LT) E_4 were higher even in mild asthma than in control subjects without asthma. Exhaled LTE₄ levels were negatively correlated with the provocative concentration of methacholine causing a 15% fall in the forced expiratory volume in 1 second. These results suggest that airway cys-LTs play a role even in children with mild, asymptomatic asthma and reflect airway hyperreactivity based on chronic inflammation. These results are being published in *Chest*. We have recently started to measure other exhaled breath condensate markers with the Luminex system (Austin, TX, USA) and to measure exhaled NO levels. These measurements were made for the early diagnosis and treatment of allergic diseases, including asthma, especially in infants and younger children. The mechanism of asthma exacerbation due to upper respiratory infection with rhinovirus has also been investigated.

Cardiology

In the pediatric cardiology group, our studies are as follows.

- 1. Prenatal diagnosis of congenital heart disease
- 2. Diagnosis, treatment, and long-term postoperative follow-up of congenital heart disease
- 3. Imaging of congenital heart disease with multidetector row computed tomography

4. Evaluation of breath circulatory dynamics using expired gas analysis for children with heart failure

- 5. Therapy for the acute stage of Kawasaki disease
- 6. Evaluation of respiratory function in congenital heart disease
- 7. Treatment of arrhythmias detected with cardiac screening in school-aged children
- 8. Epidemiology of Kawasaki disease
- 9. Magnesium dynamics in pediatric cardiology
- 10. Magnesium therapy for arrhythmia in childhood
- 11. Molecular biology in congenital heart disease

12. Dynamics of nitric oxide in children with heart disease

13. Secretion kinetics of atrial and brain natriuretic peptides in children with heart failure

14. Catheter treatment for congenital heart disease, in particular, atrial septal defect We are engaged in our research after daily practice. In this year, we presented many findings at annual meetings. One of our staff has studied catheter treatment at Kobe Children's Hospital to extended our research in new field.

Nephrology

We performed a study of 11 patients with rhabdomyolysis with special emphasis on the relation to acute renal failure.

1. Causes of rhabdomyolysis were malignant hyperthermia, status epilepticus, hypernatremia, heat stroke, traffic accident, viral infection, and toxic shock syndrome.

2. In the development of acute renal failure associated with rhabdomyolysis, suspected risk factors were dehydration and a delayed treatment.

3. The severity of muscle damage seemed to be correlated with the severity of renal dysfunction, but the relationship was inconsistent and was affected by various factors.

4. The blood urea nitrogen/Cr ratio was 10 to 20 in patients with acute renal failure. The increase in Cr was not greater than that in adults. Hyperuricemia, hyperphosphatemia, and hypocalcemia, which are characteristic symptoms in adults, were not found.

5. The prognosis was poor in cases of rhabdomyolysis complicated by disseminated intravascular coagulation and other conditions.

Infectious disease

We researched into the new diagnosis and treatment based on our clinical experiences in the infections diseases, immunologic disorders and collagen diseases in children.

Our study of chronic granulomatous disease focused on gene therapy and made full use of our clinical experiences with bone marrow transplantation.

Expression of the CXCR4 gene in CD34-positive cells was increased to extremely high levels with a retrovirus vector, and the effect of CXCR4 is being examined in detail. Additionally, the method of diagnosing chronic granulomatous disease rapidly and with high precision is being studied continuously.

We have started studying DNA vaccines and performed clinical studies on the basis of a large number of cases, including those of bacterial meningitis and sepsis. Similar studies of collagen diseases have been performed. We are performing studies of various therapies that make use of our clinical experiences while assessing examinations showing disease activity in individual cases.

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Department of Dermatology

Hidemi Nakagawa, Professor Mariko Honda, Professor Arihito Ota, Lecturer Masaaki Kawase, Lecturer Ryoichi Kamide, Professor Takaoki Ishiji, Associate Professor Tsunemichi Takeuchi, Lecturer

General Summary

We have organized special clinics for selected skin diseases, including viral diseases, neurofibromatosis, photosensitive diseases, atopic dermatitis, psoriasis, acne, and skin cancers. Integrating concentrated clinical efforts and related basic research should provide a significant contribution to clinical practice.

Research Activities

Neurofibromatosis

We have had 99 new patients with neurofibromatosis (NF), including 87 patients with NF1, 1 patient with NF2, 9 patients with mosaic NF1, and 2 patients with schwannomatosis.

A constant itching sensation, known as pruritus, is associated with NF1. Why NF1 causes pruritus in some patients is unclear. According to one theory pruritus is caused by histamine released from proliferating mast cells in neurofibromas. Histamine. substance P, and tryptase induce pruritus, and nonsedating second-generation antihistamines, including epinastine, are widely used to treat pruritic skin disorders. To clarify the mechanisms and mediators involved in the pruritus of NF1, we have examined the inhibitory effects of epinastine using a visual analogue scale for the intensities of pruritus at 1, 2, and 3 months after the start of treatment with oral antihistamines in 12 patients with NF1. In addition, blood and urine samples were collected during antihistamine therapy to monitor levels of histamine, tryptase, substance P, and nerve growth factor. We reported on a 51-year-old woman presenting with malignant peripheral nerve sheath tumor (MPNST) associated with NF1. She showed multiple pulmonary metastases, which were successfully treated with weekly docetaxel therapy. A partial response was observed after 8 courses of chemotherapy without serious side effects. In addition, weekly docetaxel therapy in the outpatient clinic did not impair the patient's quality of life (QOL). This case suggests that docetaxel therapy could be an adjunct to a panel of chemotherapeutic regimens for MPNST.

Herpes virus infection

We have confirmed that the loop-mediated isothermal amplification method is an excellent alternative to conventional polymerase chain reaction assays for the rapid detection of herpes simplex viruses 1 and 2 and varicella zoster virus in clinical samples. Postherpetic neuralgia is a major sequela of varicella zoster virus infection and impairs the patients' QOL. We have been evaluating the efficacy and safety of various drugs,

including fluvoxamine, in the treatment of postherpetic neuralgia.

Human papillomavirus infection

In addition to standard cryotherapies, topical vitamin D3 and salicylic acid has been used to treat viral warts. Carbon dioxide laser evaporation has also been used to treat refractory viral warts, including condyloma accuminatum. Typing of human papillomaviruses with the polymerase chain reaction method has been performed regularly in cases of condyloma and rare viral warts.

Atopic dermatitis

Recently, psychosocial factors have been suggested to affect the exacerbation of atopic dermatitis. Therefore, we are trying to treat patients on the basis of both evidence-based medicine and narrative-based medicine. We obtain a detailed medical history and assess QOL in each patient. To support such an approach, we have been organizing monthly atopic dermatitis forums, which include lectures and group meetings. Each time, about 10 patients and their family members attend the forum.

Photosensitivity diseases

Since 1983, more than 1,000 patients have been examined and treated at our special clinic for photosensitivity diseases. Narrow-band ultraviolet (UV) B irradiation at a newly established skin care clinic could induce tolerance in a patient with intractable solar urticaria and could help improve QOL. Clinical findings from 2 cases of Rothmund-Thomson syndrome were reported in detail. Recently, an increasing number of patients complain of photosensitivity, but many patients have no objective evidence of photosensitivity. For these patients, a psychosomatic approach will be required. The adverse effects of long-term sun exposure (photoaging) has been publicized through the Internet, newspapers, magazines, and public seminars.

Psoriasis

Various systemic therapies, including oral cyclosorin, methotrexate, and etretinate, in addition to topical vitamin D3 and steroids, have been used. Also phototherapy, including psoralen UV A and narrow-band UV B irradiation, is known to be effective and has been performed in a newly organized skin-care clinic. We have evaluated patients' QOL reflecting social backgrounds and developed a Japanese version of the Psoriasis Disability Index. In this special psoriasis clinic, we select patient-based treatments according to the patients' preferences. Clinical trials of new biologic agents have been performed.

Malignant skin tumors

We have been studying the clinical courses and postoperative outcomes of patients with malignant melanoma, extramammary Paget's disease, squamous cell carcinoma, basal cell carcinoma, and malignant schwannoma.

Sentinel lymph node biopsy is performed for patients with stage II or III melanoma.

Contact dermatitis/drug eruption

We have performed patch testing to identify causes of contact dermatitis and drug eruption. Recently, patients with anaphylaxis including oral allergy syndrome have been increasing. Patients with contact allergy to over-the-counter medications containing bufexisamac are often referred to our clinic. These patients have widespread severe contact dermatitis. Topical nonsteroidal anti-inflammatory agents are apparently not effective for controlling dermatitis, and these side effects should be publicized because these drugs are, unfortunately, commonly used.

Laser

The Q-switched ruby laser is useful for treating nevus Ota because of its selective photothermolysis. Superficial pigmented lesions, such as senile pigment freckles are usually successfully treated with one treatment. Nevus spilus is difficult to treat with the Q-switched ruby laser because it often recurs 1 to 2 months after treatment. The efficacy of a pulsed dye laser for treating hemangiomas and teleangiectasia depends on the clinical type, location, patient age, and other factors. This type of laser was effective for hemangioma simplex developing on the face or neck of young adults. The size and intensity of the strawberry mark can be reduced if treatment is started before the age of 6 months. The recently introduced V-beam laser is expected to be effective for refractory vascular lesions. Because the ultrapulse CO_2 laser has higher energy and a shorter pulse width, it can vaporize at a fixed depth and can be used to quickly remove actinic keratosis lesions that have been increasing in elderly persons.

Self-assessment

Neurofibromatosis: Many patients with NF1 are still being referred to our special clinic. We are now doing inheritance consultation for pediatric patients. Diffuse plexiform neurofibroma is believed to transform into MPNST. Although the incidences of loss of heterozygosity and allelic imbalance were elevated in MPNST, chromosomal hot spots have not been detected.

Herpes virus infection: We have developed the loop-mediated isothermal amplification method. Selective serotonin reuptake inhibitors have also proven effective for the treatment of postherpetic neuralgia.

Human papillomavirus infections: We have employed new therapeutic interventions, including topical vitamin D3, to treat viral warts in addition to ordinary surgical treatments. Typing of human papillomavirus is also regularly performed.

Photosensitivity: We have been studying the etiology of the photoexacerbation in various diseases. It is important to publicize the risk of UV irradiation so that photocarcinogenesis and photoaging can be prevented.

Contact dermatitis: Severe cases of anaphylaxis caused by food allergy have been studied. We also studied severe contact dermatitis cases caused by bufexamac. Atopic dermatitis: We have been investigating the psychosocial background of patients. To help patients understanding their illness, we have been organizing monthly atopic dermatitis forums, which include lectures and group meeting.

Psoriasis: To improve patients' QOL, we have developed a Japanese version of the

Psoriasis Disability Index. Phototherapy using narrow-band UV B irradiation is being introduced.

Malignant skin tumors: We have been treating many patients with skin cancers including melanomas and extramammary Paget's disease by surgical operation and chemotherapy.

Laser: We have been treating many patients using several different types of laser. On the basis of extensive clinical results, the most appropriate treatment can be selected. Further clinical investigation using the V-beam laser will be necessary to improve therapeutic results for refractory vascular lesions.

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Department of Radiology

Kunihiko Fukuda, Professor Junta Harada, Professor Shunichi Sadaoka, Associate Professor Mayuki Uchiyama, Lecturer Norio Nakata, Lecturer Chihiro Kanehira, Professor Toru Sekiya, Associate Professor Yukio Miyamoto, Associate Professor Manabu Aoki, Lecturer Hiroya Ojiri, Lecturer

General Summary

The division of diagnostic imaging

1. Diffusion-weighted magnetic resonance imaging in neoplastic lesions

Diffusion-weighted imaging (DWI) is a new magnetic resonance (MR) technique that evaluates the diffusion capacity of water molecules. With recent advances, the usefulness of DWI for detecting neoplastic conditions has been explored. We evaluated the tumors described below to clarify the usefulness and practicality of DWI.

• Detectability of hepatocellular carcinoma (HCC) with DWI in comparison with findings with dynamic computed tomography (CT) and CT portography/CT hepatic arteriography.

• Usefulness in evaluation of primary lesions, nodal metastases, and metastatic liver tumors of colorectal cancers in comparison with surgical specimens.

• Usefulness in evaluation of disease extent of breast cancers in comparison with surgical specimens.

• Usefulness in evaluation of prostate cancer: Setting of optimal b factor for detection of prostate cancer in high-risk groups and the adequate prostate-specific antigen level for performing DWI.

2. Multidetector-row CT as preoperative evaluation for partial hepatectomy

Liver parenchyma/volumetry, vascular structures, and the biliary system should be assessed before partial hepatectomy. To establish imaging strategies for evaluation before partial hepatectomy we obtain imaging data on the arterial and portal phases of the liver with dynamic CT after drip-infusion cholangiography-CT. Both 2-dimensional (D) and 3D displays of the biliary system and vascular structures are provided to surgeons.

3. Evaluation of the lymphatic system of the trunk with heavily T2-weighted images A study to evaluate the usefulness of nonenhanced 3D heavily T2-weighted images obtained with 2D prospective acquisition and correction in visualization of the lymphatic system of the trunk.

4. Evaluation of the enhancement of the deep venous system in the lower extremities with different total doses and concentrations of iodine in contrast media.

The study to assess the effects on enhancement in the deep veins of the lower extremities of different total iodine doses and concentrations with spiral CT venography.

5. Sonazoid (NC100100; Nycomed Amersham, Oslo, Norway) is a second-generation sonographic contrast agent. We investigated the mechanical index of the ultrasound beam and several postprocessing image-reconstructive methods and evaluated the appli-

cation of software to obtain the most useful images with various types of sonographic equipment, using contrast materials.

6. In collaboration with the Division of Gastroenterology and Hepatology, Department Internal Medicine, we investigated the most useful timing for liver tumor imaging with contrast agents.

7. To obtain images of sentinel lymph nodes using contrast material, we performed harmonic sonographic imaging in pigs after subcutaneous injection of contrast material.

8. To clarify the method for obtaining precise 3D images of superficial soft-tissue tumors with vascular structures, we examined tumors of the breast and thyroid gland using 3D/4D transducers with B-flow imaging and developed the most useful 3D imaging method.

9. In collaboration with The Department of Clinical Examination, Tokyo Rika University, and the Aloka Co. (Tokyo), we developed a microbubble contrast agent containing antibodies to enable molecular imaging and establish a new drug-delivery system.

10. We evaluated the usefulness of several elastic imaging methods to correctly diagnose superficial soft-tissue tumors.

11. Investigation of the physical properties of microcatheters smaller than 2.2-Fr

Various types of medical equipment are used for interventional radiology, and microcatheters are required to reach narrow, distal vessels for such techniques as transcatheter arterial embolization. We reported the physical properties of an advanced microcatheter previously. The physical properties of microcatheters with tip diameters of 1.8-Fr to 2.2-Fr were reviewed. We measured tip hardness, the smoothness of the interior and exterior surfaces, the flow rate, flexibility of the guide wire, the ability to maintain shape, resistance to kinking, visibility, intensity of pulling, and pressure resistance. The apical flexibility of the catheters was good, but flow rate, visibility, and pressure resistance were problematic.

12. The analysis of the lung deposition of inhaled technetium-99m diethylenetriamine pentaacetic acid aerosol

Technetium-99m diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) radioaerosol scintigraphy can be used to evaluate the transport system of ciliated epithelium in the proximal airways and the permeability of the alveolar capillary membrane. We investigated whether mucociliary clearance affects the evaluation of alveolar capillary membrane clearance. The half-time (T_{1/2}) of DTPA clearance was categorized into 6 groups (group 1: clearance from all lung field including the hilar proximal airways; group 2: clearance excluding 5×5 pixels of the hilar proximal airways; group 3: clearance excluding 8×8 pixels; group 4: clearance excluding 11×11 pixels; group 5: clearance excluding 14×14 pixels; and group 6: clearance excluding 17×17 pixels). There was no significant difference in the T_{1/2} of DTPA clearance between the groups (p=0.481). The ^{99m}Tc-DTPA clearance from the peripheral alveolar capillary membrane does not affect clearance from the ciliated epithelium transport system of the proximal airways.

13. To evaluate the therapeutic response of cortical bone and bone marrow metastases from neuroblastoma: Comparison with ¹²³I-metaiodobenzlguanidine and ^{99m}Tc-

methylene diphosphonate scintigraphy

In studies of stage IV neuroblastoma, ¹²³I-metaiodobenzlguanidine (¹²³I-MIBG) scanning or MR imaging have shown diffuse or multifocal nodular bone marrow abnormalities. We have speculated that ¹²³I-MIBG-positive lesions without abnormalities on ^{99m}Tcmethylene diphosphonate (^{99m}Tc-MDP) bone scintigraphy represent bone-marrow metastasis and that ¹²³I-MIBG-positive lesions with abnormalities on ^{99m}Tc-MDP bone scintigraphy represent cortical bone metastasis with marrow metastasis. The aim of this study was to evaluate the therapeutic response of cortical bone and bone marrow metastases. There were no lesions in the skeletal system with positive findings on bone scanning that ¹²³I-MIBG imaging failed to detect. Cortical bone metastases showed a good response to induction chemotherapy at least once previously. Diffuse and multifocal bone marrow metastases showed a response behind cortical bone metastases or was refractory to therapy. This outcome suggests that bone metastases from neuroblastoma progress to diffuse and multifocal nodular bone marrow lesions and then advance to dissemination to the cortical bone.

The division of radiation therapy

1. Prognostic factors of T2N0 laryngeal cancer

The purpose of this study was to review treatment results and determine prognostic factors related to local outcomes in 48 patients with T2N0 squamous cell carcinoma treated with definitive radiotherapy. The cumulative probabilities of local-relapse-free survival and larynx-preserving survival were 61.4% and 76.4%, respectively. Multivariate analyses showed that the number of subsites involved and overall treatment time significantly affected the probability of local-relapse-free survival and that age, impaired mobility, and overall treatment time affected the probability of larynx-preserving survival.

2. Standardization of postoperative dosimetry for prostate brachytherapy with I-125

We compared the postoperative variables of 2 patients who had been treated with prostate brachytherapy to review differences between 30 institutions. The difference between institutions in prostate volume was as high as a factor of 2.61. The effect of prostate volume was D90>V100>U-D30. Postoperative dosimetry in prostate brachytherapy varied greatly between institutions, suggesting that standardization of postoperative dosimetry is needed.

3. Hematopoietic stem cell transplantation following preparation with total body irradiation, busulfan, and cyclophosphamide for nonlymphocytic leukemia

Seventy-three patients with nonlymphocytic leukemia (30 with acute myeloid leukemia, 29 chronic myeloid leukemia, and 14 with myelodysplastic syndrome) were treated with transplantation during the past 15 years. They underwent hematopoietic stem cell transplantation after a pretransplant conditioning regimen including 10 Gy of total body irradiation, 8 mg/kg of busulfan, and 120 mg/kg of cyclophosphamide. Treatment failed in 16 patients. Ten patients died of leukemia. The 5-year overall survival rate was 56.6% (standard, 71.0%; high, 41.9%). This strategy was especially effective for high-risk leukemia.

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Department of Surgery Division of Digestive Surgery

Katsuhiko Yanaga, Professor and Chairperson Susumu Kobayashi, Professor Nobuyoshi Hanyu, Associate Professor Kazuo Matai, Lecturer Toru Kuroda, Lecturer Norio Mitsumori, Lecturer Shigeki Unemura, Lecturer Kouji Nakada, Lecturer Yuichi Ishida, Lecturer Yuichi Ishida, Lecturer Yutaka Suzuki, Lecturer Hidejirou Kawahara, Lecturer Sadao Anazawa, Professor Hideyuki Kashiwagi, Associate Professor Tetsuji Fujita, Associate Professor Yoshihiko Takao, Lecturer Akira Yanagisawa, Lecturer Yoshiyuki Furukawa, Lecturer Yuji Ishii, Lecturer Shuzo Kono, Lecturer Tomoyoshi Okamoto, Lecturer Yoshio Ishibashi, Lecturer Noburo Omura, Lecturer

General Summary

Publications in peer-reviewed journals are the main measure of research activity. According to a recent article, Japan is the second most productive country behind only the United States in terms of the absolute number of articles (9.1% of the total) published in 15 major surgical journals (as determined by impact factor). However, when corrected for population size, Japan ranks low in clinical medicine and surgery. According to a recent article in *The Lancet*, there is a considerable difference in activity between basic research and clinical research in Japan, which contributes to 8% of papers in *Nature* but is responsible for less than 4% of contributions to *The Lancet*.

We must take the initiative to fill the gap between basic research and clinical trials. Using a newly developed integrated system for searching the data of patients admitted to each Jikei University hospital, we are now performing or planning prospective clinical trials regarding outcomes of surgery, perioperative management, and adjuvant therapy for gastrointestinal and hepatobiliary malignant tumors.

Research Activities

Laparoscopic surgery for achalasia and reflux esophagitis has been intensely studied to clarify the significance of surgical treatment for such diseases. As the effectiveness of laparoscopic surgery becomes better known, the number of such operations are increasing nationwide. Also, hand-assisted laparoscopic-thoracoscopic surgery has been performed to treat esophageal cancer, and the advantages of less-invasive surgery and shorter hospitalization have been achieved in selected patients. Basic research in esophageal cancer has led us to identify molecular markers that are indicators of prognosis.

For early gastric cancer, we have established a new technique of sentinel node navigation surgery using indocyanine green without radioisotopes. A multicenter trial to evaluate our technique with radioisotopes has been completed and has obtained encouraging results. We have also started to examine sentinel node navigation surgery in patients with early Barrett's esophageal cancer.

To improve the quality of laparoscopic operations we are evaluating the usefulness and reliability of the Virtual Reality Surgical Simulator for laparoscopic colectomy. We are also examining the relationship between reactions of various immunoglobulins in the serum of patients with cancer by means of enzyme-linked immunosorbent assay and several factors pertinent to clinicopathological data. Furthermore, we are evaluating bowel function by $[^{13}C]$ -breath testing after colorectal surgery to determine the appropriate duration of postoperative bowel rest. Preoperative diagnosis of lymph node metastasis for colorectal cancer by diffusion magnetic resonance is ongoing. Interim results are as follows: sensitivity, specificity, the positive predictive value, and the negative predictive value were 71%, 63%, 55%, and 77%, respectively. Various types of lymph node (>10 mm) could be diagnosed with increasing frequency.

The ongoing research topics in the field of hepatopancreatic-biliary surgery are as follows: 1) living donor liver transplantation (LDLT), regenerative medicine, and artificial liver (especially, implantable artificial liver); 2) chemotherapy for pancreatic cancer; 3) expansion of surgical indications for multiple hepatic tumors by hepatic resection; 4) laparoscopic hepatic resection; 5) development of navigation systems for intraoperative evaluation of biliary surgery; and 6) the significance and clinical application of lipid mediators and high mobility group box-1 for hepatocellular carcinoma and liver diseases. Regarding topic 1, the first LDLT was successfully performed for a patient with postnecrotic cirrhosis with hepatocellular carcinoma on February 9, 2007. Our ongoing research on regenerative medicine and artificial organs is expected to have a synergistic effect with liver transplantation medicine. Regarding topic 2, we are carrying out a unified clinical trial for pancreatic cancer at the 4 Jikei University hospitals. Furthermore, at The Jikei University Hospital, we have started translational research by combination chemotherapy with generitabine and a naive protease inhibitor, FUT-175, which has dual function of NF κ -B inhibition and apoptosis induction in pancreatic cancer cell lines. Research into topics 3, 4, 5, and 6 are in progress after approval was obtained from the Ethics Committee of our university.

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Department of Surgery Division of Chest Surgery, Breast and Endocrinology Surgery

Toshiaki Morikawa, Professor Kazuhiko Yoshida, Associate Professor Tadashi Akiba, Lecturer Satoki Kinoshita, Lecturer Shuji Sato, Lecturer Ken Uchida, Professor Toru Kuroda, Lecturer Hiroshi Takeyama, Lecturer Yasuo Toriumi, Lecturer

General Summary

The Divisions of Chest Surgery and of Breast and Endocrinology Surgery were established in June 2005. Since then, all staff members have been active in surgical practice, research, and education. Many studies are ongoing.

Research Activities

Chest surgery

Thoracoscopic surgery is the focus of our clinical activity. This minimally invasive surgery produces fewer postoperative complications and sequelae and is especially beneficial for elderly, high-risk patients. Thoracoscopic surgery requires advanced skills, and we have independently developed total thoracoscopic surgery using only a thoracoscopic surgery can be used to treat many chest conditions, such as juvenile pneumothorax, peripheral lung nodules, mediastinal tumors, and lung cancer.

Operative procedures, including wedge resection, segmentectomy, lobectomy, and pneumonectomy of the lung, are all safely performed, in addition to resection of mediastinal tumors or the thymus. Surgery for lung cancer requires much more advanced skills and oncological considerations, which have also been independently developed. We select high-risk patients with such complications as advanced pulmonary emphysema, impaired pulmonary function, and extremely high age, as candidates for thoracoscopic surgery. Of the mediastinal procedures, thymectomy is usually performed via thoracoscopy rather than via a conventional median sternotomy. As our thoracoscopic skills have advanced, the indications for thoracoscopic surgery have been safely expanded to include higher-risk patients. In our department more than 90% of the chest operations are performed via thoracoscopy, which we assume to be the highest rate in the world. The minimal invasiveness of thoracoscopic surgery is being investigated with prospective clinical studies. These studies include a comparative study of video-assisted lung cancer surgery with open surgery, an evaluation of video-assisted surgery for bullous lung diseases in elderly persons with impaired lung function, an evaluation of video-assisted surgery for thymic tumors, and an evaluation of videoassisted thymectomy for myasthenia gravis. New devices are also being evaluated in our

clinical studies, such as narrow band imaging for the thoracoscopic diagnosis of benign and malignant lung diseases, and LaparoSonic Coagulating Shears for small thoracotomy.

Many basic research studies are also underway. In the morphological expressionrelated advancement of molecular genetic analysis of lung cancer, we are investigating whether carcinogenesis of the lung as reflected by CA19-9 activity is an important marker of de novo carcinogenesis. Biological and genetic characteristics of peripheral adenocarcinoma of lung are investigated to establish the most appropriate surgical procedures.

Breast and endocrinology surgery

Current treatment modalities for cancer include surgery, radiation therapy, and anticancer chemotherapy. However, responses to anticancer chemotherapy vary with individual tumors, and adverse reactions to therapy may outweigh clinical benefits in some cases. To allow the most effective anticancer agent to be chosen for a given neoplasm, a joint study is in progress at this and other institutions to seek effective drugs for individual patients or cells by analyzing microarrays by means of DNA chips comprising anticancer genes conferring drug susceptibility extracted from tumors.

Sentinel lymph-node navigation has become a standard procedure in breast cancer surgery in Western countries as well as in Japan. However, the use of sentinel lymph node biopsy after preoperative chemotherapy remains controversial. We are investigating its feasibility for standard use, especially after preoperative chemotherapy.

The usefulness of postoperative endocrine therapy for patients with hormone-receptorpositive breast cancer is recognized, but adverse effects, such as osteoporosis caused by aromatase inhibitors, must be considered. A trial to redeem the best therapeutic effect is being performed.

Various antihormonal therapeutic agents have been used for hormone-receptor-positive breast cancer. We are now evaluating some of these new antihormonal agents for patients with metastatic breast cancer refractory to previous antihormonal therapies.

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Department of Surgery Division of Pediatric Surgery and Vascular Surgery

Takao Ohki, Professor Yuji Kanaoka, Lecturer Joji Yoshizawa, Lecturer

General Summary

Vascular surgery

1. Development of angiogenic therapy in ischemic disease

Angiogenesis, the process of postnatal neovascularization, is a critical component of several human diseases, including ischemic heart disease, cancer, diabetic microvascular disease, and rheumatoid arthritis. Moreover, angiogenesis is believed to be mediated by the proliferation, migration, and remodeling of endothelial progenitor cells (EPCs). Recent studies have shown that EPCs increase by direct injection of EPCs and stimulation with various cytokines and that administration of drugs have lead to improvement in ischemic diseases. Here, we investigated the effects of new drugs or stem cells on angiogenesis in ischemic disease.

2. Development of new vascular grafts and stents

The patency rate of small-caliber artificial blood vessels remains low, and the use of autologous venous grafts is inevitable, yet large venous autografts, such as the great saphenous vein, are often not available. Therefore, we are performing a study to develop new vascular grafts with intraluminally grown autologous vascular endothelial cells and with biodegradable materials. Moreover, we will develop biodegradable stents and drug-eluting stents.

3. Research on hemostatic fleece and closure devices in endovascular aortic aneurysm repair

We have found that collagen patches coated with components of fibrin glue significantly reduce blood loss and the time required for hemostasis at the operation site in endovascular aortic aneurysm repair. Moreover, percutaneous aortic aneurysm repair has been shown to be technically feasible and to be associated with a low morbidity rate. Complications from percutaneous arterial closure are not insignificant, however, and can be life-threatening. We have evaluated our experiences with this technique, compared them with previously published results, and identified factors associated with complications and conversion to open repair.

4. Clinical study of specific antibody for heparin-platelet factor 4 complexes

Heparin is commonly used for anticoagulation in vascular surgery. Heparin-induced thrombocytopenia is a rare but life-treating complication with thrombosis of veins and arteries. Even if heparin use is limited, it occasionally induces the production of specific antibodies against heparin-platelet factor 4 (PF4) complexes. Patients with such antibodies are at increased risk for heparin-induced thrombocytopenia. The prevalence of these antibodies in patients receiving heparin is presumably underestimated. Accordingly, we prospectively measured antibodies against heparin-PF4 complexes

and the activity of PF4 and investigated whether they are related, particularly in patients undergoing major vascular surgery. The results of this study will be published in late 2007.

Pediatric surgery

The Jikei University Hospital is authorized (has been certified?) by the Japanese Society of Pediatric Surgeons. Operations for children at The Jikei University Hospital are performed by a highly trained, expert team of surgical professionals who specialize in the diseases and conditions affecting young people. Four specialists in pediatric surgery work at our hospital. In 2006, 346 patients were admitted, and 272 patients underwent surgery. The numbers of patients are increasing yearly.

Our pediatric surgery team for minimally invasive surgery is a leader in developing and performing minimally invasive procedures on children in Japan. To perform minimally invasive surgery, our team uses specially designed instruments that are inserted through small, bandage-sized incision, especially for gastroesophageal reflux, inguinal hernias, funnel chest, and vesicourethral reflux. Minimally invasive procedures are as effective as open operations, take no more time, and cost no more. They typically require shorter hospitalization after surgery, reduce postoperative pain, shorten recovery time, and leave smaller, less noticeable scars.

Research Activities

Vascular surgery

1. Therapeutic angiogenesis has emerged as a promising therapy to treat patients with ischemic diseases. Transplantation of bone marrow cells is reported to augment development of collateral circulation in ischemic organs either by differentiating into vascular cells or by secreting angiogenic cytokines. Recent evidence suggests that adipose tissues secrete several humoral factors and contain pluripotent stem cells. We evaluated the therapeutic potential of cells derived from adipose tissue to promote angiogenesis in cases of ischemia.

2. Plasma levels of high-density lipoprotein (HDL) are negatively correlated with the incidence of ischemic diseases. However, the molecular mechanism by which HDL prevents atherosclerosis is not fully understood. We investigated the effect of HDL on differentiation of EPCs and angiogenesis in ischemia.

3. There is an increasing need for functional small-diameter grafts for surgical revascularization. However, smaller vascular grafts made from synthetic biomaterials, particularly smaller than 6 mm in diameter, are associated with a high incidence of thrombosis. Fibroin is a biodegradable protein derived from silk which provides an antithrombotic surface and serves as a scaffold for various cell types in tissue engineering. We evaluated the potential of fibroin to generate artificial vascular prostheses for small arteries.

Pediatric surgery

To improve treatment results, new approaches are essential. One new approach is the use of gene therapy, and another new method is the inhibition of tumor angiogenesis. This year, we focused on gene therapy with tumor immununology using dendritic cells that induced mouse interferon (IFN)-alpha/beta with an adenovirus vector (Adm IFN-Alfa) in mice bearing GL26 sarcoma. Tumor-specific cytotoxic T lymphocytes were induced after injection of Adm IFN-alpha into GL26 sarcoma. Also when we used mice bearing MCA205 sarcoma, tumor size was decreased after injection of Adm IFN-alpha.

We presented these results at the annual meetings of the Japan Surgical Society, the Japanese Cancer Association, and the Japanese Society of Pediatric Surgeons. Our article entitled "Inhibitory effect of drug-free hybrid liposomes on metastasis of human neuroblastoma" won the annual best-article prize of the Japanese Society of Pediatric Surgeons.

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Department of Orthopaedic Surgery

Keishi Marumo, Professor Kazuo Asanuma, Lecturer Hiroki Funasaki, Lecturer Makoto Kubota, Lecturer Fumiaki Masui, Lecturer Mitsuru Saito, Lecturer Shigaku Sai, Associate Professor Takuya Otani, Lecturer Shigeru Soshi, Lecturer Yasuhiko Koyano, Lecturer Mamoru Yoshida, Lecturer

General Summary

We have focused on topics that require originality of the highest level, necessitate a long-term vision, and call for constant reconsideration of research validity in clinical practice, i.e., topics within the field of translational research.

We developed the world's first system for comprehensive analysis of collagen cross-links. Using the technology we developed, we discovered a biochemical compound that forms upon excessive aging of collagen fibers. In a clinical study we confirmed that this substance can be used as a marker to predict fractures. This marker is now routinely analyzed at medical institutions throughout Japan. Furthermore, in a research project on beta-tricalcium phosphate, a bone-filling material previously designed at our laboratory, we developed a novel carrier material with unique structural characteristics. Its successful application in animal studies allowed for clinical trials in joint surgery, which are now under way.

As interest in minimally invasive surgery has increased in recent years, so has the need for improved surgical skills to obtain better postoperative outcomes. With these developments in mind, we installed a computer-assisted surgery system in our department and are now examining its efficacy for simulating knee and hip reconstruction operations with artificial joints.

Research Activities

Morphologic and immunohistologic study of the acromion in patients with rotator cuff tears

It was thought that spur formation is induced by various mechanical stimuli acting at the coracoacromial arch. Prolonged stimulation results in proliferation of fibrous cartilage, its transformation into hyaline cartilage and simultaneous enchondral ossification, especially in patients with a small angle of the acromial slope.

Distal radius fractures

A morphometric evaluation was performed in a case of transformed distal radius fracture reconstruction. We obtained good results with a conventional plate instead of a locking plate. Further analysis based on fracture examination is under way.

Postoperative degeneration at adjacent segments following L5/S1 fusion for isthmic spondylolisthesis at L5

The L5/S1 fused angle and preoperative L5/S1 range-of-motion percentage were considered to be risk factors, according to stepwise logistic regression analysis. It became clear that surgical methods (posterolateral fusion/posterior lumbar interbody fusion) did not affect the clinical results or degeneration at adjacent segments.

Clinical results of cementless total hip arthroplasty with the Trifix-J Cup and the Primaloc Stem

We found that large spikes were beneficial for initial fixation of the acetabular cup but that strict indications of this cup must be established because complications occurred in cases of severe hip dysplasia. On the femoral side, excellent biological fixation was obtained in the proximal region of the Primaloc stem (Ortho Development Corp., Draper, UT, USA).

Quantitative assessment of postoperative range-of-motion during computer-assisted total knee arthroplasty

Through the use of a tension balancer designed exclusively for computer-assisted surgery, the soft-tissue balance and various joint angles produced by joint repositioning procedures could be quantitatively measured during total knee arthroplasty. Thus, in comparison with previous nonrepositioning reconstruction techniques, computer-assisted surgery allowed analysis of range of motion, an additional postoperative outcome factor that could easily be examined intraoperatively. Research on the usefulness and accuracy of the measurement is under way.

Achilles tendon ruptures in patients older than 50 years

In all cases we recognized edema of the leg and claudication. The Thompson test was positive. The patients could walk but could not stand tiptoe on the affected side. We concluded that Achilles tendon rupture might occur due to application of a relatively slight force in patients older than 50 years.

Reduction surgery with intraoperative irradiation for high-grade malignant soft-tissue tumors

Intraoperative irradiation of the neurovascular bundle was considered useful for reduction surgery of high-grade malignant soft-tissue tumors.

Involvement of neutrophils and natural killer cells in reactive arthritis after bacille Calmette-Guerin vaccination

Leukocytes, most of which were neutrophils, increased in number at the onset of reactive arthritis. The number of lymphocytes was unchanged, but the numbers of natural killer (NK) cells and NK T cells increased. The levels of neutrophils, NK cells, and NK T cells decreased as arthritis resolved.

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Department of Neurosurgery

Toshiaki Abe, Professor Shizuo Oi, Professor Tsutomu Koyama, Associate Professor Tetsuro Kikuchi, Associate Professor Shigeyuki Murakami, Associate Professor Satoshi Ikeuchi, Lecturer Yoshiaki Miyazaki, Lecturer Satoshi Sawauchi, Lecturer Haruo Sakai, Professor Yuichi Murayama, Professor Satoshi Tani, Associate Professor Hisashi Onoue, Associate Professor Masami Kamio, Lecturer Masato Nakajima, Lecturer Tatsuhiro Joki, Lecturer Robert Tomohiko Numoto, Lecturer

General Summary

The research studies in our department, examining such topics as syringomyelia, endovascular surgery, mechanism of head injury, and pediatric neurosurgery, made good progress in the past year. Research in these areas is performed to international standards. Clinical research on brain tumors, hypothalamic disorders, and spine and spinal cord diseases has also continued.

Research Activities

Cerebrovascular disorders

Although cerebral vasospasm is an important cause of morbidity and mortality in patients with subarachnoid hemorrhage (SAH), the precise mechanisms responsible for pathogenesis of cerebral vasospasm remain unclear. Recent electrophysiologic and pharmacological studies have shown the importance of potassium channels in the hyperpolarization and relaxation of vascular smooth muscle. Therefore, we have attempted to determine the role of potassium channels in the relaxation of cerebral arteries and arterioles. Recent studies suggest that functions of potassium channels are potentiated in arteries exposed to SAH and that the role of potassium channels may be more important in small arterioles than in large cerebral arteries.

In thrombolytic therapy for acute ischemic stroke, thrombolysis must be performed rapidly before ischemic neuronal injury occurs. To develop a new technique of thrombolysis for acute stroke, the effect of transcranially applied ultrasound on thrombolysis has been examined. We have reported that low-frequency and low-intensity transcranially applied ultrasound could enhance thrombolysis by tissue plasminogen activator in a rabbit model of femoral artery occlusion. Furthermore, our recent results have disclosed that ischemic neurological deficits could be reduced by transcranially applied ultrasound in a rabbit model of middle cerebral artery occlusion without an increase in the rate of hemorrhagic complications. We have reported these results in an international journal (*Stroke*). We are now attempting to confirm the safety of ultrasonification for vascular and neuronal tissue and to develop a clinically applied ultrasonication probe.

Development of a new endovascular opening system

We conducted several clinical and basic research studies regarding endovascular therapy.

Development of a new endovascular operating system

We developed a state-of-the-art endovascular neurosurgery suite that offers integrated neurosurgical and radiological capabilities. A specially designed biplane digital subtraction angiography system was installed in the neurosurgery operating room. The new suite, which facilitates three-dimensional digital subtraction angiography imaging and microsurgery, allows neurosurgeons to perform a wide array of neurosurgical and endovascular procedures.

Development of bioactive coils (Matrix coil)

In collaboration with the University of California Los Angeles (UCLA) School of Medicine, we developed a biodegradable, bioabsorbable polymer coil for treating brain aneurysms. This device has been approved by the United States Food and Drug Administration and been used to treat more than 30,000 patients in the United States and Europe. We are collaborating with UCLA, and the next generation of biocoilsis being investigated at the Laboratoy of Animal Facility.

Development of Mebiol gel

We have developed a thermoreversible polymer as a tissue-engineering therapeutic device. This polymer can be used as a drug delivery embolic material for treatment of malignant or as a hemostatic device.

We obtained a grant for this project from the New Energy and Industrial Technology Development Organization. We have used this device to treat cerebral aneurysms, and preliminary data suggest promise for clinical application.

Brain tumor

In the treatment of malignant glioma, local recurrence determines the prognosis. The principal of therapy becomes the control of local recurrence. However, treating malignant glioma with chemotherapy has been difficult because the blood-brain barrier prevents chemotherapeutic agents from reaching brain tumors. To avoid these problems, a method has been developed for the local sustained release of chemotherapeutic agents by their incorporation into biodegradable polymers. Gliadel Wafer (MGI Pharma, Bloomington, MN, USA), which contains carmustine, has been authorized for the treatment of patients with malignant glioma in Europe and the United States. On the other hand, recent advances in liposome technology have shown promise because of the introduction of chemotherapeutic agents with reduced toxicity, extended longevity, and the potential for cell-specific targeting. In some previous studies, liposomal doxorubicine was used systemically to treat malignant glioma. In our study, we performed intracranial implantation of a thermoreversible polymer containing doxorubicine, a strategy that has been shown to be safe and effective for the treatment of malignant glioma. We will investigate the release kinetics, toxicity, distribution, and efficacy of this treatment in vitro and in vivo.

We have investigated the safety and effectiveness of immunotherapy with fusions of dendritic cells and glioma cells with interleukin (IL)-12 in patients with malignant glioma. The subjects were 15 patients with malignant glioma, ranging in age from 40 to 62 years. Dendritic cells were generated from peripheral blood. Cultured autologous glioma cells were obtained from surgical specimens in each case. Fusions of dendritic cells and glioma cells were prepared with polyethylene glycol. All patients received 3 to 7 immunizations with fusion cells with IL-12 at intervals of 3 weeks. Fusion cells were injected subcutaneously close to a cervical lymph node, and IL-12 was injected transvenously. There were no severe adverse effects, and two partial responses have been observed so far.

Neurotrauma

Traumatic acute subdural hematomas in the Japan Neurotrauma Data Bank were categorized into a focal brain injury group and a diffuse brain injury group and were analyzed to clarify the pathophysiological and therapeutic aspects of these injuries. The analysis suggested that the pathophysiological and therapeutic aspects of acute subdural hematoma associated with diffuse brain injury differ from those of acute subdural hematoma associated with focal brain injury alone.

During the past decade neurobiochemical markers of brain damage have attracted increasing interest in neurotraumatology. The aim of this study was to investigate S-100B protein and neuron-specific enolase (NSE) as serum markers of brain cell damage after traumatic brain injury. Venous blood samples for assessing levels of S-100B protein and NSE were obtained on the day of admission and on the following day. Serum levels of S-100 protein and NSE were compared with Glasgow Coma Scale scores, computed tomographic findings, and outcomes after 3 months. Serum concentrations and kinetics of S-100B protein and NSE allow the clinical assessment of primary brain damage and the prediction of outcome after traumatic brain injury.

Syringomyelia

About 50 cases of syringomyelia are surgically treated in our department each year. We have been investigating the following subjects.

1. Evaluation of the cerebrospinal fluid (CSF) obstruction at the craniovertebral junction of patients with Chiari malformation

We have been measuring the pressure volume index and outflow resistance to reveal CSF blockage, before and after surgery. The aim of this study was to determine the proper surgical procedure before the operation.

2. Electrophysiological study in patients with syringomyelia

The purpose of the surgical treatment of syringomyelia is to collapse the syrinx. However, even after the syrinx has been collapsed, some patients still have intractable pain. This pain is thought to be caused by damage to the dorsal horn of the spinal cord and is difficult to relieve. We examined sensory evoked potentials with median nerve stimulation to investigate the relation of pain relief and changes in sensory evoked potentials before and after surgery.

3. Fluid in the syrinx

The mechanism of syrinx enlargement remains unclear. The composition of syrinx fluid is believed to be the same as that of CSF, but the origin of the fluid is unknown. We are investigating the fluid by measuring cytokine and antibiotic concentrations.

4. Analysis of predictive factors in patients with syringomyelia

Because of improvements in magnetic resonance imaging and neurosurgical techniques, the prognosis of patients with syringomyelia has significantly improved. However, outcomes are determined by variable preoperative conditions as well as by the surgical result. We are performing multivariate statistical analysis of predictive factors in patients with syringomyelia.

Pediatric neurosurgery

The Division of Pediatric Neurosurgery, The Jikei University Hospital Women's and Children's Medical Center, was established in October 2002. In the last 6 years, we have collected more than 1,000 new cases of various entities, including hydrocephalus (27%), spina bifida (25%), brain tumors (13%), and craniofacial anomaly (8%). Since April 2003, the clinical research fellows, 12 from other domestic universities and 9 from other countries (including Germany, Italy, Austria, Jordan, and Bulgaria) have taken part in our research activities.

In hydrocephalus research, we have performed pathophysiological analyses of CSF dynamics, both in fetal and postnatal periods, along with extensive clinical investigations in a large series of cases. On the basis of the results we have proposed a new theory for the specificity of CSF dynamics in the immature brain, i.e., "Evolution Theory in CSF Dynamics."

We have also completed the development of a new neuroendoscope and proposed a new surgical technique (J Neurosurg : 102, 2005) and a specific technique for treating intracranial cyst (J Neurosurg : 103, 2005), with a specific navigational endoscope trajectory called the "Oi clear Navi Sheath" (J Neurosurg : 07, 2007). We have been assembling the largest series of patients. A member of our department has been nominated as chairman for the National Study Group on Spina Bifida and Conducting nationwide and international cooperative studies on controversial issues in this field.

In craniofacial anomaly research, we have extensively applied the distraction method for Japan's largest series of cases; the clinical efficiency has been summarized, and our extensive study received the honorable prize of the International Society for Pediatric Neurosurgery, Raimondi's Award in 2004, and the Kawabuchi Award in 2005.

Our clinical and research activities have been also well maintained both in Tokyo (JWCMC: Jikei University Women's & Children's Medical Center) and Hannover, Germany (the International Neuroscience Institute) on the basis of firm international collaboration exchanging leading pediatric neurosurgeons and related research workers. Our department has continued as the headquarters of the International Study Group on Neuroendoscopy, the Japanese Society for Pediatric Neurosurgery, the Japan Academy of Hydrocephalus Research, as well as the Presidency of the Executive Board Committee of the International Society for Pediatric Neurosurgery, and the Japan Association of Medical English Education) as the President in each Executive Board.

Spine and spinal cord

Numerous conditions, including syringomyelia, degenerative spine diseases, spinal cord tumors, and spinal vascular lesions, have been the major concerns of our department. The departments of orthopedic surgery and neurosurgery often collaborate in the interests of patient-orientated treatment in our hospital.

As a clinical research activity, the analysis of pain in patients with neuropathic pain has started. The Dyna CT scanning system in operating rooms 4 and 5 seems to be one of the most sophisticated and unique image-guided surgery systems, especially when used with a navigation system.

Basic research, including research on spinal cord injuries and regeneration technology, has just begun in our group.

Hypothalamopituitary disease

In 1995, we developed the endoscopic endonasal trasethmosphenoidal technique, a new operative approach for parasellar diseases, in collaboration with otolaryngologists. Using this method, developed on the basis of the theory and practice of functional endoscopic sinus surgery, we use only an endoscope without a nasal speculum to go through ethmoid sinuses via the nostril to reach the sphenoid sinus. Thereafter, we have continued to improve the safety and effectiveness of our operative method by introducing a navigation system for nasal sinus surgery and developing surgical instruments. Now we are working to establish technical standards in collaboration with several other institutions.

Numerous therapeutic drugs for hormone-producing pituitary adenomas are being developed. The standard treatment for prolactinomas is pharmacotherapy with bromocriptine, terguride, and cabergoline, but definitive criteria for treatment selection have not been established. We studied the relation between the results of drug-loading tests and therapeutic effects, performed a long-term analysis of many cases, and studied the problems of pharmacotherapy. These studies should prove useful for establishing criteria for treatment selection in pharmacotherapy for prolactinoma.

We investigated the mechanism of action of a somatostatin analogue for growthhormone-producing pituitary adenoma and clarified one part of the mechanism of action.

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Department of Plastic and Reconstructive Surgery

Kunihiro Kurihara, Professor Meisei Takeishi, Associate Professor Takeshi Miyawaki, Associate Professor Mitsuru Uchida, Professor Kunitoshi Ninomiya, Associate Professor Kimihiro Nojima, Lecturer

General Summary

The main research topics of our department are congenital hand and foot anomalies, craniomaxillafacial surgery, hand surgery, reconstructive surgery after excision of malignant tumor and severe trauma, malignant skin tumors, laser treatment, and burns. These topics were investigated both experimentally and clinically. These treatments require knowledge of nervous, vascular, and integumentary systems, the physiological mechanisms of metabolic change, and skills for the atraumatic and microsurgical techniques. Basic research and clinical technique is require taking authorization of the plastic surgeons medical specialist during first 6 years.

Research Activities

Clinical research

1. Cleft lip and palate

A long-term follow-up study revealed satisfactory results after rotation-advancement lip repair for unilateral cleft lip. A long-term follow-up study of the push-back method for cleft palate also revealed satisfactory results for speech. A long-term follow-up study of early-stage bone grafting for alveolar cleft revealed good results for maxillary growth. These results were analyzed with dental models and cephalograms and by speech therapists.

2. Congenital hand and foot malformations

Because hands are often said to serve as a second pair of eyes, repair of congenital hand and foot malformations requires good functional results in terms of both sensation and motion. The purpose of our clinical research was to analyze the morphological and functional conditions of abnormal hands and feet and to analyze the long-term results after treatment. Long-term follow-up after repair of malformations with triangular flaps, a method we developed to separate the digits in syndactyly, revealed excellent functional and cosmetic results.

3. Distraction osteogenesis

Distraction osteogenesis has been used to treat several hand anomalies and syndromic craniosynostosis and has shown great potential for the reconstruction of deformities of the hand and cranial vault.

Open reduction and fixation using a bioabsorbable system were used to treat 41 cases of facial bone fracture. The mean follow-up period was 8.1 months (range, 3 to 21 months). Malar bone fractures were present in 31 patients with a mean age of 32.7 years (range, 10 to 72 years). We have encountered no severe complications with this system, and the fixation force was sufficient. After this analysis, we have continued to use this

method and have obtained good results.

4. Breast reconstruction

Autologous tissue transfer was used for breast reconstruction in more than 100 patients and yielded satisfactory results. A long-term follow-up study of breast reconstruction with free flaps in 210 patients revealed excellent results. Transplanted soft tissues were harvested from the abdominal wall and back wall with vascular pedicles and were anastomosed to the thoracodorsal or internal thoracic vessels.

5. Head and neck reconstruction

Microsurgical free flap reconstruction was performed in more than 40 patients after resection of head and neck tumors and achieved satisfactory results. Reconstructive surgery increases a patient's quality of life. The flap survival rate was greater than 90%. 6. Laser treatment

Cooling jelly is used with dye lasers to decrease skin damage when port wine stain is treated with high-density irradiation. This technique achieves good results and shortens the period of treatment.

Basic research

1. Tissue engineering

Cultured autogenous mucous cell transplantation on the fascia lata is used to restore the bladder, the full thickness of the cheek wall, and the pharyngeal wall. The goal of this research was restoration of these areas after the excision of malignant tumors.

2. Mechanism of extremity formation using gene induction in mouse embryos

The whole-embryo culture system, which can completely reproduce development, is an established method of inducing genes of the mouse embryo bud. Twelve-day-old embryos of the Std-ddt mouse were dissected out of the yolk sac, amnion, and chorioallantoic placenta. A plasmid (cytomegalovirus enhancer+ β -actin promoter) with 0.1 μ l of green fluorescent protein (GFP) was injected via a microcapillary tube into the yolk sac. Immediately after injection of the material, the embryo was pinched with the pincette-type element. In Tyrode's solution, an electric shock (30, 40, or 50 V, 50 milliseconds, 3 pulses) was applied to the embryo. The rotating bottles were incubated at 37°C, with fresh 95% O₂ and 5% N₂ supplied to the bottle twice a day. The bottles were rotated at 30 rpm. After 48 hours the frozen sample was resected. With electric shocks of 40 V or 50 V, GFP was detected throughout the body, but with shocks of 30 V, GFP was observed only in the bud portion.

3. Repair of calvarial defects with artificial bone

The repair of calvarial defects with artificial bone (α - or β -tricalcium phosphate) was studied. Healing of bone defects was observed.

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Department of Cardiovascular Surgery

Kazuhiro Hashimoto, Professor Keno Mashiko, Associate Professor Yuzuru Nakamura, Associate Professor Yoshimasa Sakamoto, Lecturer Hiromitsu Takakura, Lecturer Kouji Nomura, Lecturer Kiyozo Morita, Professor Kouji Kawahito, Associate Professor Tatsuumi Sasaki, Associate Professor Hiroshi Okuyama, Lecturer Kei Tanaka, Lecturer

General Summary

The main clinical investigations in our department involved evaluation of alterations in cardiac performance after corrective surgeries. Clinical investigations, including followup studies, of valvular and ischemic heart diseases are our main research activities, as are studies of complex congenital anomalies. New treatment approaches utilizing new surgical techniques, new devices, and research outcomes have been attempted and evaluated. We are also conducting several experimental studies with *in vivo* and *in vitro* models. The experimental projects include gene therapy for angiogenesis in ischemic myocardium, a new application of autologous skeletal muscle as an assist device in heart failure, and improvement of cardioplegic solutions used during cardiac arrest. The major activities are described below.

Research Activities

Basic research

1. Experimental studies of right ventricular dynamic cardiomyoplasty with autologous skeletal muscle

We have performed an experimental study to test the feasibility of right ventricular (RV) dynamic cardiomyoplasty with a paced skeletal muscle graft wrapped around the RV to augment depressed ventricular function. In 5 beagles, RV dysfunction was produced by ligating the right coronary artery. A pedicled latissimus dorsi muscle graft was transferred to the thorax and wrapped onto the RV free wall as an on-lay patch and electrically stimulated in synchrony with the cardiac beats using a newly developed pacemaker system. Regional function of the RV (percent segmental shortening), assessed with a sonomicrometry real-time functional analysis system, was augmented from 2.6 to 12.1 by pacing the grafts. These results suggest the possible application of the autologous skeletal muscle graft for reconstructive surgery of the RV.

2. A preliminary experimental study of a tissue-engineered dynamic patch utilizing biodegradable polymers

We have performed a preliminary experimental study to establish a tissue-engineering technique for producing a biocompatible dynamic patch utilizing biodegradable polymers seeded with autologous swine cells.

The double-layered scaffold sheets composed of biodegradable polymers (polyglycolic acid mesh) were seeded with different types of cell (myoblasts, skeletal myoblasts

(satellite cells), and bone marrow cells) and compared with non-cell-seeded sheets as a control.

These biodegradable polymers were implanted in the lattissimus dorsi muscles of adult pigs for 4 weeks, after which they were explanted to assess histological and biochemical characteristics. The polymers seeded with bone marrow cells and satellite cells became lined with cells immunoreactive to antibodies against alpha-smooth muscle cell antigens, suggesting the angiogenesis of the polymers.

Adult cardiac surgery

1. Comparison of inflammatory and coronary-graft responses, and quality of anastomoses after off-pump and on-pump coronary bypass surgery

Off-pump coronary artery bypass grafting (OPCAB) has become popular because of potential benefits obtained by elimination of the pump. In this study, we investigated differences in the severity of systemic inflammatory responses by evaluating activation of blood components and the production of proinflammatory substances between OPCAB and conventional coronary artery bypass grafting (cCABG). Changes in eicosanoids and vasoactive substances, the quality of anastomosis, and endothelial smooth-muscle functions of coronary grafts were also assessed and compared. The subjects were 120 patients undergoing OPCAB and 42 patients undergoing cCABG. The mean flow and the pulsatile index were determined for all grafts through measurements of transit time flow. Blood samples were obtained at several points for comparison. The pulsatile index was used to indicate the quality of the anastomosis. The two treatment groups were similar in terms of characteristics, except for evidence of aortic calcification. The average number of grafts did not differ between the groups. However, levels of the creatine phosphokinase MB isozyme were significantly lower in patients undergoing OPCAB. Neither graft flow nor the pulsatile index differed between the groups or among the bypass territories. cCABG was disadvantageous with regard to neutrophil activation (on-pump); however, trends of proinflammatory substances and vasoactive substances (eicosanoids, endothelin) were the same with OPCAB and cCABG. The quality of anastomosis with OPCAB was equivalent to that with cCABG in all territories. OPCAB showed an advantage over cCABG in myocardial protection. Systemic inflammatory responses were somewhat apparent on-pump with cCABG, but the difference disappeared off-pump. Neither coronary-graft responses nor changes in vasoactive substances differed between OPCAB and cCABG.

2. Mitral valve disease: Surgical management of perivalvular leakage after mitral valve replacement

Perivalvular leakage (PVL) is a serious complication after mitral valve replacement. From 1991 through 2006, 9 patients with mitral PVL underwent reoperation. All patients showed severe hemolytic anemia before the operation. The serum level of lactate dehydrogenase decreased from $2,366\pm780$ to 599 ± 426 IU/L after operation. The locations of PVL could be accurately determined with echocardiography in 7 patients. PVL occurred around the posterior annulus in 3 patients, the anterior annulus in 2 patients, the anterior annulus in 1 patient. The most frequent cause of PVL was annular calcification, which

occurred in 5 patients. Postoperative infection developed in only 1 case. The prosthesis was replaced in 4 patients, and the leak was repaired in 5 patients. There was 1 operative death due to multiple-organ failure and 4 late deaths, due to cerebral infarction, subarachnoid hemorrhage, sudden death, and congestive heart failure due to persistent PVL in 1 patient each. Reoperation for PVL due to extensive annular calcification is associated with a high mortality rate and a high recurrence rate, making such procedures a frustrating challenge for surgeons.

3. Aortic valve disease: Patient-prosthesis mismatch may be irrelevant after aortic valve replacement with the 19-mm Perimount pericardial bioprosthesis in patients 65 years or older

We evaluated valve function and the effects of patient-prosthesis mismatch (PPM) on the medium-term results after implantation of a 19-mm Carpentier-Edwards Perimount (CEP) pericardial aortic valve in patients 65 years or older. From August 1996 through May 2005, 51 patients underwent aortic valve replacement with a 19-mm CEP valve. The mean follow-up period was 2.4 ± 1.8 years, with a total follow-up of 134.4 patientvears. The mean age and body surface area at the time operation were 74.0 ± 5.0 years and 1.41 ± 0.14 m², respectively. There were 2 (3.9%) operative deaths. Three patients (5.9%) underwent enlargement of a small aortic annulus. The actuarial survival rate after 8 years (including operative mortality) was $90.2 \pm 4.7\%$. The mean rates of freedom from thromboembolism, from reoperation, and from valve-related mortality at 8 years were 75.0 \pm 21.7%, 97.8 \pm 2.2%, and 95.3 \pm 3.2%, respectively. The peak and mean transvalvular pressure gradients were significantly improved after surgery (peak: 93 ± 35 vs. 28 ± 12 mmHg; mean: 58 ± 19 vs. 17 ± 7 mmHg; p<0.01). The mean left ventricular mass index had decreased from 192 ± 44 to 142 ± 46 g/m² at late follow-up (p<0.01). The prevalence of PPM was low (17.6%) when an indexed effective orifice area of less than $0.85 \text{ cm}^2/\text{m}^2$ was used to indicate significant PPM. The clinical outcome, postoperative pressure gradient, and reduction of left ventricular mass index did not differ between patients with and without PPM. Use of the 19-mm CEP valve achieved satisfactory medium-term clinical outcomes (regardless of PPM) in patients 65 years or older, who are presumed to be less active than are younger patients. Moderate PPM was rare and did not have an adverse effect on the medium-term results. Annulus enlargement was needed only in a few patients when a 19-mm CEP valve could not be inserted.

4. A 10-year study of the durability of triangular resection for prolapsing mitral anterior leaflets

The reconstruction technique of using artificial chordae for prolapsing anterior leaflets has been widely adopted. Since 1991, we performed triangular resection with or without wrapping and shortening of elongated chordae whenever possible instead of using artificial chordae. The aim of this study was to assess long-term results of mitral valve repair, with a focus on the resection of anterior leaflets. From October 1991 through December 2006, we performed mitral valve repair for 57 patients (22 patients have been followed up for more than 10 years) with prolapsing anterior leaflets. Thirty-nine patients underwent triangular resection (14 patients have been followed up for more than 10 years),16 patients underwent wrapping and shortening of elongated

chordae (13 patients have been followed up for more than 10 years), 9 patients underwent artificial chordal replacement (2 patients have been followed up for more than 10 years), and 4 patients were treated with the flip-over method. Both the anterior and posterior leaflets were resected in 18 patients (8 patients have been followed up for more than 10 years), including 9 patients who underwent wrapping and shortening of elongated chordae. Ring annuloplasty was performed in all patients. A Carpentier-Edwards rigid ring was used in 49 patients. A Physio and Cosgrove ring or band was used in 6 and 2 patients, respectively (average ring size, 30 mm). The mean follow-up duration was 6.2 years (range, 0.5-14 years). The mean age was 51.7 ± 15.9 years. The mean postoperative mitral valve area did not differ significantly among the 3 groups (anterior leaflet resection: 2.86 cm²; posterior leaflet resection: 2.86 cm²; anterior and posterior leaflet resection: 3.09 cm²). Reoperation was required in 4 cases. Two cases were observed within 2 years after we began to perform mitral reconstruction (when we did not perform intraoperative transesophageal echocardiography). One of the reoperated cases, reconstructed with artificial chordae, showed mitral tethering with progression The other case showed Barlow's disease and was treated with of cardiomyopathy. artificial chordae replacement. Fifty-three patients (92%) were in New York Heart Association class I. The linearized rate of thoromboembolism did not differ significantly between patients who underwent triangular resection of anterior leaflets (0.57%) patient-year) patients who underwent quadrangular resection of the posterior leaflets (0.27% patient-year). There were 2 late deaths: 1 was due to pneumonia and 1 was due to sudden death. The rates of survival and of freedom from reoperation at 10 years were 91.7% and 92.3%, respectively, in patients who underwent resection of the anterior leaflet and both the anterior and posterior leaflets and were 96.4% and 90.4%, respectively, in patients who underwent quadrangular resection of the posterior leaflets; the rates did not differ significant difference between the treatment groups. Moreover, the rates of survival and freedom from reoperation in patients who underwent triangular resection of anterior prolapsing leaflets and were followed up for more than 10 years were 95.2% and 90.0%, respectively. There was no significant difference in the rate of survival or the risk of reoperation between patients who underwent resection of only the anterior leaflet and patients who underwent resection of both the anterior and posterior leaflets. In conclusion, with follow-up extending beyond 10 years, there was no significant difference in the rates of reoperation and survival after resection of the anterior leaflet, the posterior leaflet, and both leaflets. The mitral valve area was in the lower-normal range after anterior leaflet resection, and the remaining mitral valve area was large enough to allow valvular function. Long-term follow-up shows that triangular resection of the prolapsing anterior leaflet is a reliable procedure in terms of obtaining an ideal, smooth coaptation zone.

Surgery for congenital cardiac defects

1. Studies of myocardial protection during open-heart surgery

1) Experimental studies of a new strategy for myocardial protection against ischemia/ reperfusion injury:

On the basis of the results of a series of experimental studies of cardiac ischemia/

reperfusion injury in an in-vivo pig model, we established the efficiency and reliability of intraoperative myocardial protection with integrated myocardial protection using a modification of the combined use of modified St. Thomas solution. Furthermore a recent experimental study of a hybrid cardioplegia solution (blood and crystalloid) demonstrated the critical importance of lowering Ca^{2+} content during blood cardioplegic reperfusion (terminal hot shot). More recently, we have performed experimental studies to examine the effects on reperfusion injury of a phosphodiesterase (PDE) III inhibitor added to the cardioplegic solution.

2) A high-dose PDE inhibitor in terminal warm-blood cardioplegia

To test the hypothesis that myocardial ischemia/reperfusion injury can be limited by adding a PDE inhibitor to the terminal warm-blood cardioplegia (TWBCP) solution, 25 piglets were placed on cardiopulmonary bypass (CPB) and subjected to 90 minutes of ischemia with a single dose of cold crystalloid cardioplegia solution and reperfusion with or without TWBCP before aortic declamping. Left ventricular functional recovery, assessed with left-ventricular-pulmonary-vein loops with sonomicrometry, and biochemical myocardial injury, evaluated with troponin-T, creatine kinase, and lipid peroxide, were compared among control piglets that did not receive a TWBCP solution, piglets receiving a low-Ca TWBCP solution, and piglets receiving a low-Ca TWBCP solution.

A significant improvement in cardiac function and a reduction in reperfusion-induced biochemical injury, associated with an increase in myocardial cAMP levels and suppression of lipid peroxide levels, were observed in piglets receiving a low-Ca TWBCP solution with amrinone or orprinon. On the basis of these results, we conclude that a high dose of a PDE III inhibitor in the TWBCP solution replenishes the myocardial cAMP that is depleted during ischemia/reperfusion and promotes rapid and sustained myocardial functional recovery due to the inhibition of oxidative damage.

We are now conducting a dose-response study with the PDE inhibitor orprinon for a future clinical trial of this new therapeutic modality.

3) Efficiency of ischemic postconditioning

We have performed experimental studies of ischemic postconditioning in an in vivo pig model of ischemia/reperfusion on CPB. Postconditioning of the ischemic myocardium was applied in 6 pigs, by 10 cycles of transient (10 seconds) ischemia/reperfusion before aortic declamping following 90 minutes of ischemia (aortic clamping) on CPB. Reversal of reperfusion injury, assessed on the basis of left ventricular functional recovery and biochemical injury (l- p- o- , creatine phosphokinase, and troponin), was noted only marginally in 6 piglets. Further studies using an alternate postconditioning strategy with TWBCP are in progress.

2. Clinical studies of management of CPB in infants

1) Deleterious effects of hyperoxemia and the role of normoxemic management of CPB Because CPB simultaneously alters many factors, including cytokine, vasoactive mediators, and free radical generation, oxidative injury may occur owing to conventional hyperoxic CPB in the heart and lungs of infants. To test the hypothesis that the extremely high pO_2 level during CPB provokes oxidative cardiopulmonary changes that can be prevented by normoxemic CPB management, we performed a clinical study to

compare functional and biochemical effects after CPB in infants who underwent normoxemic management (pO_2 , 100 to 150 mmHg) or hyperoxemic management (pO_2 , 200 to 300 mmHg). Respiratory and myocardial function and results of routine enzymatic evaluation did not differ significantly between the treatment groups. However, normoxemic management was more beneficial for cytokine generation and pulmonary vasoconstriction after CPB in infants with pulmonary hypertension. The present study suggests cardiopulmonary bypass causes a substantial inflammation stress, including oxidative lipid peroxidation, and that these deleterious effects are prevented by normoxemic CPB management

2) Effects of modified ultrafiltration after operation for congenital heart disease with pulmonary hypertension

The systemic inflammatory response after CPB is a key factor in the incidence of postoperative pulmonary hypertensive crises in children. To evaluate the effect of modified ultrafiltration (MUF) on the ability to remove chemical mediators, hemodynamics, and pulmonary function, we studied infants with a ventricular septal defect and pulmonary hypertension who underwent a venovenous MUF after CPB. Chemical mediators (levels of thromboxane B_2 , interleukin 6, and endothelin 1), pulmonary function (PaO₂/FiO₂, A-aDO₂, and the respiratory index), and the reduction in the ratio of pulmonary arterial pressure to aortic pressure were evaluated before and after MUF.

The use of MUF produced significant improvements in respiratory function and pulmonary artery pressure, associated with marked reductions in the levels of thromboxane B_2 , interleukin 6, and endothelin 1. In addition, the use of MUF significantly decreased the incidence of postoperative pulmonary hypertensive crises compared with that in a historical control group without MUF. On the basis of these findings, we conclude that MUF is useful for avoiding pulmonary disorders due to CPB, especially in pediatric patients with preoperative pulmonary hypertension.

3. Clinical studies on pediatric heart surgery

1. Retrospective study of the indications and outcomes of the Fontan procedure

The clinical studies were undertaken by reviewing clinical records and data of patients who underwent staged univentricular repair, including the bidirectional Glenn procedure and the Fontan procedure, and examined: 1) the efficacy of the staged approach for high-risk Fontan candidates and selection criteria; 2) risk analysis with a newly proposed index: the Fontan index; 3) indications for final Fontan conversion after the staged approach assessed on the basis of superior vena cava pressure at Glenn circulation; 4) the clinical importance and limitations of the early volume reduction strategy as a infantile surgical policy for Fontan candidates; and 5) persistent hypoxia after the bidirectional Glenn procedure, and therapeutic management.

2. New approach for intraoperative evaluation of hemodynamic candidacy for the Fontan procedure after the bidirectional Glenn procedure

In 6 patients in whom the staged Fontan procedure was indicated after the bidirectional Glenn procedure, we measured superior vena cava flow, which is equivalent to pulmonary artery flow in bidirectional Glenn procedure physiology, by means of a transit-flow meter intraoperatively. Measurement of pulmonary flow and pumonary vascular resistance, incorporated with serial volume loading, allows the pulmonary vascular reserve capacity to be assessed in response to an increase in pulmonary flow to simulate Fontan circulation. In 4 patients in whom pulmonary artery flow increased to 2.0 L/min/body surface area under the acceptable range of central venous pressures (i.e., less than 15 mmHg), Fontan completion was successfully performed with excellent hemodynamic status.

3. Ross procedure

The surgical outcome and long-term results of the Ross procedure were reviewed, with a focus on autograft durability, in 33 patients who underwent the Ross procedure from 1995 through 2007 with total aortic root replacement and use of a pulmonary autograft. Autograft function was assessed with periodic echocardiographic evaluation for up to 12 years after the operation. There were no operative or acute deaths, but late reoperation for autograft regurgitation was necessary in 3 patients: (rate of freedom from reoperation for autograft failure, 87% at 12 years). The durability of implanted pulmonary autograft valves was excellent, especially in pediatric patients and patients with preoperative aortic stenosis.

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Department of Obstetrics and Gynecology

Tadao Tanaka, Professor Makoto Yasuda, Professor Takekazu Onda, Associate Professor Naoki Kamiya, Associate Professor Shigeki Niimi, Lecturer Aikou Okamoto, Lecturer Akihiko Watanabe, Lecturer Hiroshi Nishii, Lecturer Hirokuni Takano, Lecturer Kazunori Ochiai, Professor Kazuhiko Ochiai, Professor Hiroshi Sasaki, Associate Professor Seiji Isonishi, Associate Professor Shigemitsu Kobayashi, Lecturer Takekazu Saitou, Lecurer Kyousuke Yamada, Lecturer Kuniaki Ohura, Lecturer Satoshi Takakura, Lecturer

General Summary

The main research topics of our department are the development of molecularly targeted agents for ovarian cancer, clarification of the mechanisms of successful pregnancy, and the development of assisted reproductive techniques. These topics were investigated both experimentally and clinically.

Research Activities

Gynecologic oncology

1. Increased synthesis of indoleamine-2, 3-dioxygenase protein is associated with impaired survival in patients with serous, but not with other types, of ovarian cancer We have previously reported that indoleamine-2,3-dioxygenase (IDO) is associated with paclitaxel resistance and that IDO serves as a marker of poor prognosis in ovarian serous adenocarcinomas. We further examined IDO expression of serous adenocarcinomas and of other types of ovarian cancer. After informed consent was obtained, expression of IDO protein was analyzed with immunohistochemical techniques for a total of 136 ovarian cancers, including 40 serous adenocarcinomas, 67 clear cell adenocarcinomas, 12 mucinous adenocarcinomas, and 17 endometrioid adenocarcinomas. Staining for IDO was positive in 57% of serous adenocarcinomas, 49% of clear cell adenocarcinomas, 25% of mucinous adenocarcinomas, and 76% of endometrioid adenocarcinomas. The Kaplan-Meier survival curve showed a clear relationship between staining score and overall survival for patients with advanced (stage III and IV) serous adenocarcinoma (n=33) who underwent optimal surgery and paclitaxel-carboplatin chemotherapy as a first-line regimen. There was no association between IDO staining score and overall survival in cases of clear cell adenocarcinoma. Eight of 11 cases (72.7%) of clear cell adenocarcinomas with endometriosis showed identical IDO staining patterns between clear cell adenocarcinomas and endometriosis. In 43 of 60 cases (71.6%) with lymphnode metastasis, the IDO staining patterns of the primary lesion and the metastatic site were identical. These results suggest that increased synthesis of IDO protein is associated with impaired survival only in serous ovarian cancer.

2. Study of CD147 expression as a novel biomarker in malignant gynecological tumors Immunohistochemical studies have shown expression of CD147 (extracellular matrix metalloprotease inducer, basigin) and matrix metalloproteinase (MMP) in several types of cancers and have suggested that they play important roles in tumor invasiveness and metastasis. We showed that CD147 is expressed in 97.3% of endometrial carcinomas and is correlated with various clinicopathological factors and with recurrence-free survival. The aim of our study was to further analyze CD147 expression in other malignant gynecological tumors with links to clinicopathological factors, including patient outcomes. In addition, we would like to clarify the functional significance of CD147 in cancer progression and examine the possibility of using CD147 as a novel biomarker or as a therapeutic target.

3. Pattern recognition in serum to diagnose ovarian cancer in a Japanese population: Preliminary results

This small proof-of-principle study demonstrates that there are profiles in the serum of Japanese patients with ovarian cancer that can be used to classify the presence of cancer. The information is similar to that in the sera of patients with ovarian cancer in the United States because N-dimensional clusters built on United States sera spectra were used to create cluster maps predictive of the Japanese samples. The model performed better than any existing single biomarker assay, although truly useful models await a much larger sample size and the use of independent validation sample sets to demonstrate the robustness of the models. These results encourage us to start a large scale, multisite collection of serum from Japanese patients with ovarian cancer to develop a serum profile assay for ovarian cancer.

4. MicroRNA expression profiles for cancers, including lung, breast, stomach, prostate, and colon, were examined to investigate the involvement of microRNA in carcinogenesis.

We found that microRNAs were differentially expressed in normal tissues and cancers and could contribute to cancer development and progression. The microRNA molecular profiles were also correlated with patient survival. These results indicate that microRNA expression profiles are diagnostic and prognostic markers of cancers.

5. Mesenchymal-to-epithelial transition during inclusion-cyst formation from human ovarian surface epithelium

Most surface epithelial-stromal tumors of the ovary are thought to arise from epithelial inclusion cysts. Thus, these cysts are the precursor lesion of ovarian carcinoma. On the basis of this hypothesis, we aimed to characterize human ovarian surface epithelium in which the mesenchymal-to-epithelial transition occurs in the process of inclusion cyst formation. We used specimens from 9 patients with endometrial cancer who underwent hysterectomy and bilateral salpingo-oophorectomy. Immunohistochemical studies were performed with 10 normal ovaries containing 92 inclusion cysts and 4 normal tubes to examine the expression of antigen markers, including calretinin, podoplanin, D2-40, thrombomodulin, human bone marrow endothelial (HBME)-1, vimentin, epithelial membrane antigen (EMA) WT1, CA125, MOC31, TAG-72, Ber-EP4, and E-cadherin. We found that the positive staining rates for mesothelial markers in normal ovarian surface epithelium were 100% (10 of 10) with calretinin, 80% (8 of 10) with podoplanin, 80% (8 of 10) with D2-40, 70% (7 of 10) with thrombomodulin, 100% (10 of 10) with HBME-1, and 100% (10/10) with vimentin. In tubal epithelium the positive staining

rates for epithelial markers were 100% (4 of 4) with HBME-1, 100% (4 of 4) with vimentin, 100% (4 of 4) with EMA, 75% (3 of 4) with TAG-72, and 100% (4 of 4) with Ber-EP4. Inclusion cysts showed positive staining for both markers with an incidence of 51.1% (47 of 92) with HBME-1, 44.6% (41 of 92) with vimentin, 65.2% (60 of 92) with TAG-72, and 88.0% (81 of 92) with Ber-EP4. Ovarian surface epithelium has both mesencyhmal and epithelial characteristics. In contrast, inclusion cysts gain epithelial characteristics and lose mesencyhmal characteristics. These findings support the observation of a mesenchymal-to-epithelial transition during inclusion cyst formation from the ovarian surface epithelium.

6. Characterization of mitochondria in cisplatin-resistant human ovarian carcinoma cells

Examination of mitochondrial transmembrane potentials revealed greater depolarization of platinum-resistant cells than of platinum-sensitive cells. Treatment of these cells with cisplatin or hydrogen peroxide (H_2O_2) induces complete destruction of mitochondrial DNA damage in sensitive cells, whereas only partial destruction of DNA was observed in resistant cells, suggesting mitochondria *per se* are resistant to cisplatin. Continuous oxygen consumption was significantly greater in sensitive cells than in resistant cells, and the amount of oxygen consumption was decreased 30% in resistant cells, suggesting mitochondrial DNA to cisplatin might be a main characteristic of the lower-level apoptosis induced by cisplatin.

Fetomaternal medicine

1. Which types of antiphospholipid antibody affect miscarriage or pregnancy-induced hypertension?

The aim of this study was to determine which types of antiphospholipid antibody affect miscarriage or pregnancy-induced hypertension. The results suggest that different types of of antiphospholipid antibody affect the pregnancy-induced hypertension group and miscarriage group, respectively.

2. Introduction of experimental intrauterine growth restriction in naive mice with purified IgG B_2 -glycoprotein I-dependent anticardiolipin antibody

Antiphospholipid syndrome (APS) is a clinical entity manifested by arterial and venous thromboses and recurrent miscarriages and is caused by antiphospholipid antibodies. Recently, APS has also reported to be observed in some complications with pregnancy i.e., pregnancy-induced hypertension, intrauterine growth restriction (IUGR), and late fetal death. However, little is known about how APS is involved in these complications. The Fc receptor for IgG (Fc γ receptor) is implicated in some autoimmune diseases. To investigate the pathological significance of the Fc γ receptor in APS and complications of pregnancy, we established an experimental model for APS using Fc γ receptor knock-out mice.

3. Interferon- γ -mediated IDO expression in peripheral monocytes is up-regulated by prolactin

IDO plays essential roles in successful pregnancy and is induced by type 1 cytokines. The receptors of type 1 cytokines share a structure and a signal transduction pathway with prolactin. Because physiological levels of prolactin increased markedly during pregnancy, we examined the possible cooperation between prolactin and IDO expression. Therefore, to investigate IDO expression in CD14 cells, we obtained mononuclear cells from 12 healthy volunteers and cultured the cells for 24 to 48 hours in the presence or absence of stimuli (interferon [IFN]- γ : 5,100 IU/ml; prolactin: 10, 100, or 500 ng/ml). IDO expression was up-regulated only when prolactin were applied at physiological concentrations observed during pregnancy in combination with 5 IU/ml of IFN- γ ; in contrast, prolactin or 5 IU/ml of IFN- γ alone had no effect. These findings suggest prolactin plays important roles in pregnancy maintenance, thereby up-regulating IDO induction. These findings also suggest that the physiological concentration of IFN- γ is important for pregnancy.

4. Does maternal exercise encourage the progression of uterine contractions and lead to premature delivery?

A rat model of excessive exercise during pregnancy was used to examine uterine smooth-muscle contractility and the effects on the uterine muscle of excessive exercise during pregnancy. Contraction of the uterine muscle was not induced by exercise on the 18th day of pregnancy. Even if excessive exercise caused a decrease in fetal-placenta blood flow, because fetal weight was smaller in exercised rats, contraction of the uterine muscle was not induced. Exercise did not cause premature delivery.

5. *IGFBP1* and *follistatin-like* 3 genes are significantly up-regulated in expression profiles of the IUGR placenta.

The clinicopathological features of IUGR remain unclear, and no effective therapy has been established for IUGR. To our knowledge, this is the first study to use microarray analysis to identify differentially expressed genes in the IUGR placenta. The expression profiles of 9121 genes were examined with cDNA microarray analysis using mRNA from an appropriate for gestational age (AGA) placenta and an IUGR placenta from discordant dichorionic twins. Up-regulation of the IGFBP1 and follistatin-like 3 genes was detected in the IUGR placenta, with a balanced differential degree of 20.7 ± 1.3 and 13.1 ± 2.1 , respectively, whereas the balanced differential degrees of other genes were 2.6 or less. The expression of the *IGFBP1* and *follistatin*-like 3 genes in 4 single IUGR and 4 AGA placentas was also examined with the reverse transcriptase polymerase chain reaction. Consistent with our data in discordant chorionic twin placentas, 3 of 4 IUGR placentas showed up-regulation of the IGFBP1 gene, and all 4 IUGR placentas showed up-regulation of the *follistatin*-like 3 gene when compared with AGA placentas. Our results suggest that *IGFBP1* and *follistatin*-like 3 are highly up-regulated in the placenta in IUGR. IGFBP1 and *follistatin*-like 3 are known critical regulators of fetal growth and differentiation. Pathways associated with these genes might be promising targets in molecularly-targeted therapy for IUGR

Reproductive endocrinology

We reported outcomes of treatment with laparoscopic ovarian drilling in cases of polycystic ovary syndrome, which causes follicular growth disorder, ovulation disorder. The effects of mental pressure during treatments were also examined.

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Department of Urology

Shin Egawa, Professor Haruki Yamazaki, Associate Professor Koichi Kishimoto, Associate Professor Tetsuro Wada, Associate Professor Yasuyuki Suzuki, Lecturer Takashi Hatano, Lecturer Shoichi Onodera, Professor Isaho Ikemoto, Associate Professor Hiroshi Kiyota, Associate Professor Nozomu Furuta, Lecturer Koji Asano, Lecturer Kenta Miki, Lecturer

General Summary

We performed research in the following areas: urologic oncology, urinary tract infection and sexual transmitted diseases, urodynamics and erectile dysfunction, kidney and adrenal gland, endourology, and extracorporeal shockwave lithotripsy.

Research Activities

Urologic oncology

1. Basic research: We performed several projects to clarify the biology of urological malignancies and to develop new therapeutic tools. Most projects were reported at the annual meetings of the Japanese Urological Association and the American Urological Association. The projects are as follows.

- 1) Proteomics analysis for a new biomarker for prostate cancer and urothelial cancer
- 2) Gene therapy against urological malignancies
- 3) Research on prostate cancer stem cells

2. Clinical research: Several clinical studies are in progress at our institution. Some works have already been reported at the annual meeting of the Japanese Urological Association.

1) Study of seeds and hormones for intermediate-risk prostate cancer.

2) Clinical study of high dose rate brachytherapy with external beam radiation therapy against high-risk prostate cancer

Genitourinary tract infection

1. Basic research on the antimicrobial resistance of *Neissseria gonorrhoeae*, especially resistance mechanisms in cephem-resistant *N. gonorrhoeae*.

2. Clinical surveillance of gonococcal urethritis in the Tokyo metropolitan area.

3. Clinical study of combination therapy with clarithromycin and cefteram for male gonococcal urethritis

4. Psycological education for chronic prostatitis/chronic pelvic pain syndrome

Neurourology and urodynamics

1. Study for relation between dysuria and a phosphodiesterase type 5 inhibitor We showed the clinical benefits of a phosphodiesterase type 5 inhibitor for the patients with dysuria. 2. Assessment of overactive bladder by means of the Overactive Bladder Symptom Score

We assessed overactive bladder by means of the Overactive Bladder Symptom Score, which is the latest and most reliable tool for assessing overactive bladder.

3. Study of nocturia

The relation between cardiac insufficiency and nocturia was shown by means of brain natriuretic peptide and the frequency-volume chart.

Kidney and adrenal gland

1. The usefulness of ^{99m}Tc-mercaptoacetyltriglycine scintigraphy for preoperative prediction of renal function remaining after nephron-sparing surgery

2. Laparoscopic surgery for adrenal tumor

Endourology and extracorporeal shochwave therapy

- 1. Extracorporeal shockwave therapy for Peyronie's disease
- 2. Holmium-YAG laser ablation of the prostate for benign prostatic hypertrophy

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Department of Ophthalmology

Kenji Kitahara, Professor Hiroshi Tsuneoka, Associate Professor Hisato Gunji, Associate Professor Masaki Yoshida, Lecturer Akira Watanabe, Lecturer Tsutomu Sakai, Lecturer Katsuya Mitooka, Lecturer Osamu Taniuchi, Professor Keigo Shikishima, Associate Professor Genichiro Takahashi, Associate Professor Tadashi Nakano, Lecturer Kenichi Kohzaki, Lecturer Takaaki Hayashi, Lecturer

General Summary

The main research interest of our department is the pathophysiology of the visual processing system. The following topics are the subjects of basic and clinical studies: color vision, ocular oncology, histopathology, biochemistry, eye movement, neuroophthalmology, corneal and refractive surgery, cataract, glaucoma, electrophysiology, diabetes, and vitreoretinal diseases.

Research Activities

Color vision defects and inherited retinal diseases

We evaluated the usefulness of the Waggoner Hardy-Rand-Rittler (HRR) pseudoisochromatic plate as a screening test for anomalous trichromacy in patients with congenital red/green color vision deficiencies. The Richmond HRR and the Waggoner HRR tests were performed in 16 protanomals and 38 deuteranomals. Results of the Waggoner HRR test were statistically compared with those of the Richmond HRR test for each severity scaling. In addition, colorimetric measurements of the figure dots and the background dots of the HRR tests were made with a spectrophotometer and plotted on a CIE 1976 (L*, u*, v*) chromaticity diagram. Severity scale scores were identical on the Waggoner HRR and Richmond HRR tests in 87.5% of the protanomals and in 73.7% of the deuteranomals. No significant differences in severity scaling were seen between the tests. Subjects whose scale scores differed between the tests showed milder severity on the Waggoner HRR test than on the Richmond HRR test. Chromaticity differences between the figure and background dots were greater on the Waggoner HRR test. Our results suggest that the utility of the Waggoner HRR test may be equivalent to that of the Richmond HRR test for evaluating the severity of anomalous trichromacy. The differences in severity outcomes in some cases may be due to chromaticity differences of the figure and background dots between the tests.

Ocular oncology and histopathology

1. We demonstrated the pathological features of an extremely rare case of transitional cell carcinoma (TCC) of the bladder metastatic to the orbit and reviewed the literature on such cases. A man presenting with proptosis underwent transseptal anterior orbitotomy. Histopathological examination of the orbital lesion revealed nests of carcinomatous cells. Atypical pleomorphic cells with vacuolated cytoplasm were evident.

The cellular morphology of the orbital lesion was identical to that of the primary TCC. There have been 12 previously reported cases of TCC of the bladder metastastic to the orbit, with the time from onset of primary TCC to observation of ocular symptoms ranging from 3 weeks to 11 years. Mean survival after orbital metastasis is 3.0 months. This study presented a detailed description of the pathological features of TCC metastatic to the orbit. In such cases the prognosis is extremely poor.

2. We reported rare cases of optic neuropathy caused by an intraorbital tumor in a patient with von Hippel-Lindau disease and a schwannoma presenting with clinical features of lacrimal gland tumor.

Biochemistry

Staphylococcal enterotoxin B (SEB) is a type of superantigen, which is delivered by bacteria, viruses, and mycoplasmas. Superantigens are powerful T-cell activators which are regarded as a leading cause of infectious etiology in autoimmune disease. We investigated the effects of SEB in experimental autoimmune uveoretinitis (EAU). SEB increased clinical and histological scores of EAU. Clinical were significantly higher scores in the SEB-treated group than in the control group on day 28. The invasion of T cells and the proliferation of Muller cells were increased in the SEB-treated group. Furthermore, SEB increased T-cell proliferation specific for interphotoreceptor retinoid-binding protein and increased interferon γ production. An antibody against V β 8 showed significant anti-inflammatory and immunosuppressive effects in EAU. SEB may play a role in the development of EAU via T-cell receptor V β 8.

Eye movement

Binocular summation on the visual cortex was investigated by comparing cortical responses to binocular and monocular visual stimulation by means of functional magnetic resonance imaging (fMRI). Signal intensities were markedly higher with the binocular condition than with the monocular condition. We believe this result reflects the binocular summation process in the visual cortex.

Neuro-ophthalmology

1. Hemianopia respecting the vertical meridian generally results from a disturbance in the optic chiasm or the postchiasmal visual pathway. We described 5 unusual patients with visual field defects respecting the vertical meridian that were not related to distinct chiasmal or postchiasmal lesions, as determined with MRI. Optic neuritis and the effects of the testing algorithm for perimetry were possible causes in 2 cases. The symptoms of 1 patient with homonymous hemianopia were a functional deficit. In the 2 other cases, the visual field defects respecting the vertical meridian, and without any evidence of distinct disease, are uncommon, neurologists and neuro-ophthalmologists should consider the differential diagnosis if MRI does not show distinct lesions in the optic chiasm or the postchiasmal visual pathway.

- 2. Several advantages of kinetic perimetry in neuro-ophthalmology were reviewed.
- 3. We examined the association between a polymorphism of the endothelin-1 gene and

the occurrence of nonarteritic anterior ischemic optic neuropathy and found an increased prevalence of G/T polymorphism of the gene.

4. We evaluated the efficiency of long-term, low-dosage steroid therapy for ocular myasthenia gravis (MG). This therapy would be effective for reducing or eliminating symptoms in patients with ocular MG and may also prevent progression to generalized MG.

5. We reported rare cases of Gradenigo syndrome presenting with typical findings on MRI, midbrain ptosis caused mainly by a lesion in the periaqueductal gray matter, and reversible posterior leukoencephalopathy syndrome with bilateral visual impairment.

6. Conventional MRI cannot directly visualize wallerian degeneration of the optic radiation resulting from proximal axonal injury, such as that caused by temporal lobe lesions. A new technique, diffusion tensor imaging (DTI) can show the extent of conservation of axonal fibers in the white matter. fMRI and DTI were simultaneously performed to explore the trajectory of the optic radiation and cortical activation in a patient with a right-sided hemianopia and a temporal lobe lesion. Although the left occipital cortex was anatomically preserved, fMRI showed weaker cortical activation on the left side than on the right side. DTI tractography showed that the left optic radiation did not reach the occipital pole.

Our technique suggests that the hemianopia in this patient was caused by wallerian degeneration of the optic radiation. fMRI and DTI were useful for clinical evaluation of cerebral visual disability.

Cornea and refractive surgery

Automated lamellar therapeutic keratectomy, in which a microkeratome is used to make a lamellar flap, was performed in several cases of corneal opacity. We found that automated lamellar therapeutic keratectomy enables earlier suture removal and induces less astigmatism than does conventional lamellar keratoplasty.

We studied the clinical outcomes of secondary implantation of the iris-clip intraocular lens for aphakic eyes 5 years postoperatively. Clinically significant complications were not found with specular microscopy or laser flaremetry.

Glaucoma

1. We evaluated the Glaucoma Progression Analysis, which is a new event-type method of judging the progression of early glaucoma, in 152 eyes of 76 outpatients. The results suggest that detection with Glaucoma Progression Analysis was more sensitive than that with the MD slope, which is an analytical method of the trend type.

2. Studies in Europe and the United States have shown a high prevalence of glaucoma in patients with sleep apnea syndrome (SAS). However, a detailed examination of this relation has not been performed in Japan. Therefore, we examined the relationship between SAS and frequency-doubling technology (FDT) visual field abnormality in 182 outpatients attending the sleep clinic of The Jikei University School of Medicine. We found that the prevalence of FDT visual field abnormalities was high in patients with SAS, suggesting a relation between SAS and glaucoma.

3. We evaluated a stimuli of FDT perimetry by using fMRI. The results suggested

that the FDT stimuli detected the response of the subtype (My cell) of the M cell.

4. We examined the detection precision of an FDT screening program and compared it with Humphrey Matrix perimetry, which is considered a second-generation FDT, in terms of sensitivity, specificity, and inspection time.

5. We determined the diagnostic value of FDT for glaucoma by comparison of funds photograph and intraocular pressure (IOP) measurement by non-contact tonometer. In conclusion, the FDT and funds photograph had high sensitivity to detect glaucoma. In contrast, the IOP measurement had low sensitivity.

Electrophysiology

We recorded electroretinograms in patients with hereditary retinal degenerative diseases. We follow international standards and record cones and rods separately. In addition, we use a colored light-emitting diode built in to the contact electrode, which can separate long- and middle-wavelength cones from short-wavelength cones. Such separation can be effective for recording rare cases, such as enhanced s-cone syndrome. In addition, we placed rats in a box made from materials similar to a colored intraocular lens (yellow lens) and created a light-damage model of the rat retina. We plan to record electror-etinograms in these rats.

Diabetes and vitreoretinal diseases

We have used 23-gauge and 25-gauge transconjunctival vitrectomy systems to treat macular hole, epiretinal membrane, macular edema, and rhegmatogenous retinal detachment. The 25- and 23-gauge sutureless vitrectomy techniques decrease surgical trauma and improve patients' postoperative comfort. The 25- and 23-gauge instrumentation is effective for a variety of vitreoretinal surgical indications. Although the infusion and aspiration rates of the 25- and 23-gauge instruments are lower than those for the 20-gauge high-speed vitrectomy system, the use of 25- and 23-gauge transconjunctival vitrectomy systems may effectively reduce operative times of selected patients who do not require the full capability of conventional vitrectomy techniques.

Visual neuropsychology

1. Neuropsychology

Data from 3 patients with focal lesions suggest a relationship between the recognition of Mooney faces and the lateral surface of the right occipital lobe. We reported on patients with polyopia or metamorphopsia caused by cortical damage. We reported on 3 patients without vection perception. We commented on the neuroscientific theory proposed by Zeki.

2. fMRI study

We developed a software program for perimetry analysis, mrFA, and we reported on visual stimuli and analysis methods to estimate the hemodynamic delay. The mrFA can be used as an objective method of perimetry and a decoder of brain activity, and we reported its practicality. We observed the selective activation of the magnocellular pathway associated with simple flickering visual stimuli. We observed activation during the pseudoisochromatic test and the color arrangement test; activation during the

latter test was correlated with activation in the more anterior region of the ventral occipital lobe. We observed nonhomogeneous responses in area V1 even with homogeneous stimuli with Ganzfelt. We assumed that these responses were due to the distribution of retinal ganglion cells. We could not detect activation in the foveal projection cortical region of patients with macular degeneration. We summarized studies of binocular visual function with fMRI. We commented on the loci of visual area maps on ordinary MR images based on visual area mapping with the recent fMRI technique. 3. We evaluated cyanopsia by means of achromatic-point settings before and after intraocular lens implantation for cataracts; we then found a large shift of the achromatic point observed within 1 hour after eyepatch removal.

Low vision (poor visual acuity)

We performed stabilometry for 40 patients with low vision and found that tunnel vision might contribute to decreased control of posture in patients. We found that patients with oscillopsia have poor visual short-term memory. We measured the reading profiles of patients with oscillopsia and found that severe oscillopsia does not contribute to visual acuity loss but does cause severe deterioration of maximal reading speed; therefore, we reported that such patients read more easily when the text size is larger. We introduced the activities of the Kanagawa Low Vision Network, which was established to improve the quality of low-vision care in Kanagawa Prefecture. We tried short-term admissions for the rehabilitation of patients with low vision and evaluated outcomes with the 25-Item Visual Function Ouestionnaire. We summarized dos and don'ts of perimetry for patients with unilateral spatial inattention and introduced the quantitative measurement of visual extinction. We showed in patients with brain damage that testing with Teller Acuity Cards showed significantly higher visual acuity than did ordinary visual acuity testing with Landolt rings. We reported on trends in the study of artificial eves.

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Department of Otorhinolaryngology

Hiroshi Moriyama, Professor Takakuni Kato, Professor Hiromi Kojima, Associate Professor Nobuyoshi Otori, Lecturer Kazuhiro Soeda, Lecturer Mamoru Yoshikawa, Lecturer Yuji Umezawa, Professor Atsushi Hatano, Associate Professor Yoshinori Tomiya, Lecturer Makoto lida, Lecturer Yasuhiro Tanaka, Lecturer Yoshinori Matsuwaki, Lecturer

General Summary

Our basic and clinical studies have examined: the pathogenesis of cholesteatoma and adhesive otitis media, surgery of the middle ear, navigation medicine, space motion sickness, nasal allergy, endoscopic endonasal sinus surgery, sleep apnea syndrome, and deglutition reconstructive surgery for head and neck tumors.

Research Activities

Otology

We have conducted a basic experiment on regeneration of the middle ear mucosa, which plays an important role in the improvement after middle ear surgery, and a study aimed at clinical applications. We have been developing our own navigation system that uses virtual reality with the aim of safely performing surgery of the middle ear and the petrous pyramid. We have used the records of cholesteatoma operations, approximately 200 of which are performed annually, to create a database, have analyzed the pathology and surgical procedures, conducted an epidemiological survey, and evaluated the postoperative results. In otology, we have conducted a study of internal-ear lesions in metabolic diseases in experimental animals, and we have carried out a genetic analysis of patients with otosclerosis in collaboration with Shinshu University.

Cochlear implant surgery continues to be performed. Surgery for lesions of the base of the cranium, such as cholesteatoma of the pyramidal area and internal auditory meatus, is performed in cooperation with the Department of Neurosurgery to preserve hearing and facial nerve function. Approaches across the posterior cranial fossa, middle cranial fossa, or labyrinth are employed, depending on the case.

We have been measuring changes in total middle ear cavity pressure associated with transmucosal gas exchange in relation to the duration of intubation for secretory otitis media and have been deciding on the timing of extubation.

In studies of equilibrium, we have been cooperating with the Department of Neuropsychiatry in an analysis of dizziness after ingestion of ultra-short-acting and short-acting sleep-inducing agents. Dizziness was quantitatively measured with a GANGAS mobile analyzer (T & T medilogic Medizintechnik GmbH, Schoenefeld, Germany) and the film-type pressure sensors Huge-Mat and Mat-Scan (Nitta Corp., Osaka), and we have been continuing to demonstrate associations with drug concentrations in the blood and with drowsiness caused by sleep-inducing agents. In addition, we are analyzing the
results of experiments demonstrating relationships between fingertip contact pressure and posture control by means of a mobile analyzer that utilizes a force plate and analysis software.

We are assessing methods of treating patulous Eustachian tube which achieve long-term therapeutic results, such as treating the pharyngeal orifice with salicylic acid, insertion of Gelfoam or Merocel into the pharyngeal orifice, and physiological saline nose drops.

Rhinology

With the aim of expanding the indications for endoscopic sinus surgery and improving its safety, a high-tech system that superimposes stereoendoscopic images and stereonavigation is under development. We have also been assessing the involvement of fungi in the development of eosinophilic paranasal sinusitis, which is often refractory. The fungus *Alternaria* induces the production of interleukin 5, interleukin 13, and interferon γ by peripheral blood mononuclear cells isolated from patients with paranasal sinusitis, but no such responses have been observed in cells isolated from healthy subjects. We have also demonstrated that *Alternaria* directly induces activation and degranulation of isolated human eosinophils and that the aspartate protease secreted by *Alternaria* induces various immune reactions mediated by the protease-activated receptor 2 expressed on the surfaces of eosinophils and airway epithelial cells.

A comparative assessment of the association between cell expression type and clinical disease type by means of a comprehensive gene analysis of fibroblasts cultured from the inferior nasal concha of patients with perennial allergic rhinitis and healthy subjects performed to clarify pathogenetic factors in nasal allergy has shown that many of the gene expression patterns differed with the presence or absence of allergy.

We have been studying the relationship between the quality of life of patients with pollinosis and methods of oral administration of antiallergy drugs.

Head and neck cancers

The main constituents of the treatment of cancers of the head and neck are: 1. surgery, 2. radiotherapy, 3. chemoradiotherapy, and 4. radiotherapy combined with super-selective intra-arterial chemotherapy.

1. To perform curative resection designed to maximize preservation of function in cases of advance cancer, reconstruction is performed with free flaps (rectus abdominis flaps, free jejunal grafts, anterior axillary flaps, and anterolateral thigh flaps). In addition to performing reconstruction (50 to 60 cases a year), we perform partial laryngectomy for postradiotherapy recurrences (T2 to T3 cases) and strive to preserve as much of the larynx as possible.

2. Radiotherapy is primarily used to treat early cancers on an outpatient basis. In some cases chemoradiotherapy is performed by adding the oral anticancer agent S-1. In advanced cases, radiotherapy is performed as postoperative adjuvant therapy after surgical treatment.

3. Chemoradiotherapy consists of radiotherapy and simultaneous treatment with cisplatin or 5-fluorouracil as chemotherapy and is followed by adjuvant chemotherapy. Patients selected for chemoradiotherapy have cancer of the middle and lower pharynx or

the cervical portion of the esophagus and cannot undergo larynx-preserving surgery or curative resection.

4. Radiotherapy combined with superselective intra-arterial chemotherapy may be effective in cases in which curative resection is impossible, especially in highly advanced cases, and possible use in our hospital is being considered.

In addition, early diagnosis is an important factor in improving the survival rate of patients with cancers of the head and neck. We are making an effort to detect superficial cancer of the middle and lower pharynx at an early stage by means of narrow band imaging endoscopy (i.e., endoscopy performed with a narrow band filter).

Research on phonation and swallowing function

We have been performing endoscopic outpatient day surgery with flexible endoscopes, as in the past, to treat vocal cord polyps, vocal cord nodules, and vocal cord cysts.

We have also been performing atelocollagen injections as outpatient day therapy for unilateral recurrent laryngeal nerve palsy and have obtained favorable results.

Botulinum therapy, the treatment of first choice for spastic dysphonia, has been approved by the ethics committee of the university. In addition to continuing clinical assessments of diagnosis and treatment, we are assessing surgical therapy for cases in which botulinum therapy fails.

Cooperation with other departments, such as the Department of Neurology and the Department of Rehabilitation Medicine, and teamwork with co-medical staff, including nurses, are necessary to evaluate and treat dysphagia. We have been evaluating cases by means of video endoscopy and videofluoroscopy, assessing treatment policy, and implementing swallowing exercises.

Research on sleep apnea syndrome

We used acoustic rhinometry and rhinomanometry to investigate the effects of nasal breathing disorders on breathing disorders during sleep. Multivariate analysis with the apnea-hypopnea index as an objective variable and with background factors, such as body mass index and age, nasal cavity cross-sectional area, and volume variables as explanatory variables has shown that the apnea-hypopnea index increases as nasal cavity volume decreases, in other words, that breathing disorders during sleep tend to become more severe. Follow-up of children with breathing disorders during sleep who had undergone surgical treatment showed that when 2 or more years had passed since treatment, the maxillofacial morphology of many patients had improved to the standard values of healthy children, suggesting that the breathing disorders during sleep may have affected their maxillofacial morphology. Preoperative and postoperative evaluations in patients who had undergone uvulopalatopharyngoplasty suggested that the cyclic alternating pattern, a new method of analysis that differs from the conventional method of sleep electroencephalographic analysis (Rechtschaffen and Kales rules), is a very sensitive method that identifies arousal responses at the subcortical level.

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Department of Anesthesiology

Shoichi Uezono, Professor Sachiko Omi, Associate Professor Masaki Kitahara, Lecturer Ichiro Kondo, Lecturer Yasushi Mio, Lecturer Takehiko Nezu, Professor Masanori Takinami, Associate Professor Chieko Fujiwara, Lecturer Kazuhiro Shoji, Lecturer

General Summary

The year 2006 was the second academic year that Shoichi Uezono, M.D., served as the Chairperson of the Department of Anesthesiology. Our department provides a wide spectrum of medical care, from conducting preoperative anesthesia assessment to performing the most advanced life-supporting techniques in all perioperative settings, such as cardiac surgery, thoracic surgery, interventional neuroradiology, comprehensive pain management, postanesthesia care unit, and the intensive care unit (ICU). The Department continues to grow in all these areas.

A new recruitment strategy started by Dr. Uezono aims to attract academically oriented physicians with excellent clinical backgrounds. In the past year, we successfully recruited two new faculty members to the Department: Dr. Kitahara was recruited from Teikyo University to direct our new pain management program, and Dr. Uchino, a board certified intensive-care specialist and internist, joined the staff of the ICU to establish a clinical research program in intensive care medicine.

Research Activities

Research continues as a growing and important component of the Department's activities. Research opportunities are available in a variety of areas, including cardiac anesthesia, vascular anesthesia, interventional neuroradiology, pediatric, acute and chronic pain management, critical care medicine, and medical informatics. Department faculty members continue to be invited as visiting professors and guest lecturers at national and international institutions.

Listed below are the ongoing research projects in which the principal investigators are faculty members in the Department of Anesthesiology.

Clinical research

1. Dr. Uezono has been interested in outcome research in pediatric anesthesia. His main focus is to develop a new monitoring device to measure the depth of anesthesia in children to avoid the overdosing or underdosing of anesthetic agents. This year he was invited to write a review article on the usefulness and the limitations of bispectral index monitoring in pediatric patients.

2. Dr. Shoji and his colleagues continue to search for better postoperative pain management. They have found that a new alpha-2 agonist, dexmedetomidine, is a promising drug for postoperative pain relief, and his group completed a clinical trial to

evaluate the efficacy and safety profiles of this new drug.

3. Dr. Kase has developed a new method for detecting small amounts of endotoxin which are not detectable by conventional methods. He is now studying whether extremely minute amounts of endotoxin have any clinical significance in ICU patients. 4. Disseminated intravascular coagulation is a common pathological condition in the ICU. Dr. Uchino, a newly recruited intensive care specialist, has addressed this vexing problem. He is performing a prospective study to validate new guidelines issued by the Japanese Society of Intensive Care Medicine for the treatment of disseminated intravascular coagulation.

Dr. Kitahara and his colleagues from the pain clinic have been interested in neuropathic pain after mastectomy for breast cancer. They have found that antidepressant drugs are effective for treating postmastectomy pain.

Basic science research

1. Dr. Uezono has established a pulmonary hypertension laboratory to investigate gene therapy (antisense nucleoside therapy) in experimental pulmonary hypertension. This year he was invited to the Medical College of Wisconsin in October 2006 to lecture on new treatments for experimental pulmonary hypertension. He received a new Grant-in-Aid for Scientific Research (*kakenhi*) for this research.

2. Dr. Kondo and his group studied the effects of dexmedetomidine on the release of substance P in the dorsal horn of the spinal cord after tissue injury. Their results suggest that the spinal cord is an important site for the analgesic effects of alpha-2 agonists.

The appended bibliography of the department shows that a wide range of investigative and scholarly activities were performed during the past year.

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Department of Rehabilitation Medicine

Satoshi Miyano, Professor Masahiro Abo, Lecturer Tetsuo Ikai, Associate Professor Itaru Takehara, Lecturer

General Summary

The main research projects in our department have been focused on neurorehabilitation, stroke, brain injury, cognitive science, and dysphagia.

Research Activities

Facilitated beam-walking recovery during the acute phase by kynurenic acid treatment in a rat model of photochemically induced thrombosis causing focal cerebral ischemia We previously demonstrated the presence of activated areas in the uninjured contralateral sensorimotor cortex in addition to the ipsilateral sensorimotor cortex of the area surrounding a brain infarction, using a rat model of focal photochemically induced thrombosis (PIT) and functional magnetic resonance imaging. Using this model, we next applied gene expression profiling to screen key molecules upregulated in the activated area. RNA was extracted from the sensorimotor cortices ipsilateral and contralateral to the focal brain infarction and from the sham-controlled cortex and hybridized to gene-expression profiling arrays containing 1322 neurology-related genes. We found that glycine receptors were upregulated in the cortices both ipsilateral and contralateral to the focal ischemic lesion. To prove the preclinical significance of upregulated glycine receptors, kynurenic acid, an endogenous antagonist of glycine receptors on neuronal cells, was administered intrathecally. The kynurenic acid significantly improved behavioral recovery within 10 days from paralysis induced by the focal PIT (p < 0.0001), as evaluated with beam walking. These results suggest that intrathecal administration of a glycine receptor antagonist may facilitate behavioral recovery during the acute phase after brain infarction.

Laterality of swallowing in healthy subjects by AP projection using videofluoroscopy Although anteroposterior projections in videofluoroscopic examination of swallowing provide clinically important information, the laterality of swallowing in healthy subjects has not been fully examined fully. A total of 167 healthy volunteers were prospectively studied. The subjects were asked to swallow 5 ml of a barium solution 3 times while X-ray images were obtained showing the pathway of the solution from the pharynx to the esophagus to assess the laterality of swallowing. We classified patterns of swallowing into 3 types according to the passage of the barium solution in the pharyngoesophageal segment as indicated by width: right-side-dominant flow, left-side-dominant flow, and no laterality in flow. The swallowing pattern was classified as no laterality in 58%, left-side dominant in 35%, and right-side dominant in 7%. The ratio of the 3 swallowing patterns in women was 7:2:1. There were no significant differences in swallowing patterns according to age. Although swallowing patterns in young men (aged 20-30 years) tended to be the same as in women regardless of age, the prevalence of the left-side-dominant pattern tended to increase with age: 71% of older adults (aged 51-75 years) showed this pattern. These results demonstrate laterality in normal swallowing and will be helpful in determining treatment strategies for the patients with dysphagia.

Is the ipsilateral cortex surrounding the lesion or the uninjured contralateral cortex important for motor recovery in rats with photochemically induced cortical lesions? Primary Objective: To determine whether the ipsilateral cortex surrounding the lesion or

the uninjured contralateral cortex is more important for motor recovery after brain damage in the PIT model.

Research Design: We induced PIT in the sensorimotor cortex of rats and examined the recovery of motor function using the beam-walking test.

Methods and Procedures: In 24 rats, the right sensorimotor cortex was lesioned after 2 days of training for the beam-walking test (group 1). After 10 days, PIT was induced in the left sensorimotor cortex. Eight additional rats (group 2) received 2 days of training in beam walking and then underwent the beam-walking test to evaluate function. After 10 days of testing, the left sensorimotor cortex was lesioned, and recovery was monitored with the beam-walking test for 8 days.

Main outcomes and results: In group 1, left hindlimb function impaired by a right sensorimotor cortex lesion recovered within 10 days after the operation. Right hindlimb function impaired by the left-sided lesion recovered within 6 days. In group 2, right hindlimb function impaired by the left-sided lesion after a total of 12 days of beam-walking training and testing recovered within 6 days, as with the double-PIT model. The training effect may be relevant to reorganization and neuromodulation. Motor recovery patterns did not indicate whether motor recovery was dependent on the ipsilateral cortex surrounding the lesion or on the contralateral cortex.

Conclusion: The results emphasize the need to select appropriate programs tailored to the area of cortical damage to enhance motor functional recovery in this model.

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Department of Emergency Medicine

Takeki Ogawa, Professor Joji Ohtsuki, Associate Professor Satoshi Takeda, Lecturer Tsutomu Koyama, Associate Professor Masahiko Uzura, Lecturer

General Summary

Education system for junior residents in emergency medicine Establishing a database of severe traumatic brain injury in Japan The etiology of syncope

Research on laboratory assessment of heart attack in the emergency room

Research Activities

1. Director of Japan Neurotrauma Data Bank Committee

2. Prognostic value of heart fatty acid binding protein for patients with chest symptoms in the emergency room

3. Research committee on higher cerebral function after traumatic brain injury

4. Research committee on impact biomechanics in automobile accidents

5. Published a revised edition of *Guidelines for the Treatment and Management of Severe Head Injury* (The Japan Society of Neurotraumatology).

6. Research and development of ultrasound devices in diagnosis and treatment of cerebrovascular disorders.

7. Secretary of research group on surgical maneuver for trauma

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Department of Endoscopy

Hisao Tajiri, Professsor Hiroshi Kakutani, Lecturer Takeshi Suzuki, Lecturer Muneo Kawamura, Lecturer Mitsuru Kaise, Associate Professor Tomohiro Kato, Lecturer Matsuda Koji, Lecturer

General Summary

Our main fields of research are clinical studies of endoscopic diagnosis and treatment of gastrointestinal (GI), hepatobiliary, and pancreatic diseases. In addition, we performed basic research for the development of novel instrumentation, image processing and analysis, and optical devices, such as autofluorescence imaging (AFI), narrow band imaging (NBI), supermagnified imaging, confocal endoscopy, and high degree of freedom therapeutic endoscopes. Published achievements and recent reports are summarized below.

Research Activities

Pharyngeal, esophageal and gastric malignancies

1. Endoscopic diagnosis for esophagogastric neoplastic lesions

Early detection and accurate diagnosis of esophagogastric premalignant and malignant lesions are essential for selecting the most appropriate therapeutic strategy for each patient. At our institution, the following novel optical technologies are used in addition to conventional white-light endoscopy.

1) Magnifying endoscopic observation using an NBI system: This new diagnostic system consists of a magnifying (\times 80) endoscope and an NBI light source and provides detailed morphological information about capillaries on the mucosal surface. Our present goal is to develop algorithms for NBI technology, which may allow accurate analysis of the histological type of gastric carcinoma and the tumor extent without biopsy and allow the early detection of precancerous changes in the specialized columnar epithelium of Barrret's esophagus. The preliminary achievements have already been published and reported at several conferences.

2) Endoscopic ultrasound-guided fine needle aspiration biopsy: Endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) biopsy (EUS-FNA) allows histopathological analysis of endoscopically undetectable lesions within and outside the walls of the GI tract such as esophageogastric submucosal tumors and tumors in mediastinal and abdominal lymph nodes. In EUS-FNA, the biopsy needle can be precisely guided into the lesions with real-time ultrasonographic imaging. The tissues obtained with EUS-FNA are immediately examined by a cytologist or a pathologist to evaluate the presence of malignant cells. Evaluation of the technical safety and usefulness of this technique is underway.

3) AFI

AFI is a technology to visualize autofluorescence emitted from the GI mucosa. AFI is

expected to enable early detection of GI tumors. We are comparing regular endoscopy, NBI, and AFI in prospective clinical studies to determine the features of each modality.2. Endoscopic treatment of esophageal and gastric malignancies

With recent advances in endoscopic diagnostic techniques and instruments, the indications for endoscopic therapy for early gastric and esophageal carcinomas have been increasing. Research on the following endoscopic therapeutic modalities are now under way to standardize these endoscopic techniques as treatments for upper GI tumors.

1) New indications for endoscopic mucosal resection: Current indications for mucosal resection (EMR) are limited by the size, depth, and histological type of the lesions. Our recent efforts have been focused on expanding the indications of EMR for early gastric cancer on the basis of the histopathological analysis. Being evaluated as new EMR indications for gastric cancer are small, poorly differentiated adenocarcinomas without ulceration, well-differentiated adenocarcinomas 30 mm or larger confined to the mucosa, and carcinomas with microinvasion to the submucosal layer. In esophageal cancer the current indications for EMR are epithelial cancer (m1) and cancer partially invading the lamina propria mucosae (m2) with negligible risk of lymph node metastasis. Being evaluated as new indications are mucosal cancer invading the lamina muscularis mucosae (m3) and lesions with slight submucosal invasion within the first third of the submucosal layer (sm1).

2) Therapeutic interventions with innovative endoscopy systems

The multibending endoscope (M-scope) is a new type of endoscope with a higher degree of freedom. We have previously reported that the M-scope is useful for treating tumors in the lesser curvature, greater curvature, posterior wall of the gastric body, and the cardiac region, which are poorly accessible with a conventional endoscope. Studies using an M-scope with magnifying capability are now under way to develop safer and more accurate procedures. In addition, clinical studies using a newly developed therapeutic endoscope (R-scope), which has a special mechanism allowing the forceps to move laterally and vertically in addition to the multibending function, are now under way to advance endoscopic therapy.

3. The role of *Helicobacter pylori* (*H. pylori*) infection in the development of gastric cancer

Many studies have demonstrated an association between H. pylori infection and the development of gastric cancer. However many aspects of this association remain Therefore, it is imperative to clarify this association in our department, in unclear. which endoscopic treatment of gastric cancer is routinely performed. The experiments on this topic, especially on DNA methylation due to H. pylori infection, have been performed in cooperation with Department of Gastroenterology, Toshiba General Hospital. We have also been exploring the roles of inducible nitric oxide synthase in the pathogenesis of *H. pylori*-associated diseases and have found that the treatment to eradicate *H. pylori* plays an important role in the repair of methylated DNA. The interim results have already been reported at several conferences and have been published in Japan and internationally. In addition, we have reported that the diverse topographical patterns of *H. pylori*-induced inducible nitric oxide synthase expression may contribute to the development of gastric cancer caused by H. pylori infection.

4. Diagnosis of oropharyngeal and hypopharyngeal malignancies

Endoscopic screening with iodine staining has allowed esophageal cancer to be detected at an early stage and has improved prognoses. However, metachronous or synchronous cancer of the oropharynx or hypopharynx has become the main factor affecting the prognosis and quality of life of patients with esophageal cancer. Although detecting such cancers at early stage is, of course, important, performing chromoendoscopy is difficult because of their locations, unlike in cases of esophageal cancer. Magnifying endoscopy performed in combination with the NBI system has enabled us to detect hard-to-find cancers at an early stage without performing chromoendoscopy. A multicenter randomized, controlled study on the clinical value of this new combination endoscopy is now under way.

Functional disorders of the upper GI tract

The etiology of gastroesophageal reflux diseases, including nonerosive reflux disease and GI motility disorders, is difficult to determine. Methods must be established to evaluate hypersensitivity and dysmotility disorders of the GI tract to understand the pathophysiology of these disorders and treat them.

We developed a new method for evaluating esophageal functions using a small-caliber endoscope. We started basic experiments for analyzing the motility and sensitivity of the esophagus with the goal of applying this technique to clinical practice.

Diagnosis and treatment of esophagogastric varices

Recently, we have been involved in color-Doppler endoscopic ultrasonography (CD-EUS) studies of the hemodynamics of the portal venous system in patients with esophagogastric varices. These studies have clarified some of the factors associated with an increased likelihood of recurrence of esophagogastric varices after endoscopic treatment. When all the factors are identified, we can expect to be able to predict and prevent early recurrence of varices after treatment. We have also started a study to confirm the factors that aggravate hemorrhagic gastritis and cardiac varices. Studies of CD-EUS are multidirectional. CD-EUS is a highly accurate technique for detecting gastrorenal shunts and can clearly delineate shunt status after the treatment of esophagogastric varices. Therefore, this diagnostic system could be useful for selecting patients with esophagogastric varices who are candidates for interventional radiology and for predicting its therapeutic effects.

Enteroscopy and colonoscopy

1. Diagnostic techniques

Capsule endoscopy is a breakthrough modality that allows the detection of diseases in the small intestine that are inaccessible with ordinary endoscope systems. In western countries, capsule endoscopy has been performed in 300,000 cases and is recommended as a first-line examination for detecting diseases of the small intestine. Our department is 1 of 12 major endoscopy centers participating in a multicenter study to evaluate the usefulness of capsule endoscopy. Accurate preoperative evaluation of tumor invasion is essential for selecting the most appropriate and effective therapeutic strategy. To improve diagnostic accuracy, we use a magnifying endoscope with NBI/AFI technology. 2. Treatment using colonoscopic techniques

Surgical resection has been the first choice of treatment for large sessile tumors in the colon. Endoscopic *en bloc* resection, which has become a standard treatment for gastric lesions, might also be a treatment option for colonic lesions. However, endoscopic resection of large lesions in the narrow colonic lumen is technically challenging and is accompanied by a higher risk of severe complications, such as perforation and bleeding. Our present efforts are focused on establishing safe and reliable methods to remove large colonic lesions endoscopically. Accordingly, we have started to apply endoscopic submucosal dissection to colonic lesions. Additionally, we have used an infrared endoscopy system to evaluate potentially troublesome vessels on the ulcer base to prevent postoperative bleeding after endoscopic submucosal dissection.

Pancreatobiliary endoscopy

1. Diagnosis of biliary and pancreatic diseases

Due to the recent introduction of duodenopancreatectomy the establishment of standardized systematic diagnostic algorithms for biliary and paricreatic diseases has become more important than ever. We are clinically comparing diagnostic accuracy for hepatopancreatic diseases among EUS-FNA, multidetector computed tomography, magnetic resonance cholangiopancreatography, and endoscopic retrograde cholangiopancreatography. We are also evaluating the usefulness of immunohistological examination of EUS-FNA samples to determine prognosis in cases of hepatopancreatic malignancy. Aiming to improve diagnostic accuracy of EUS-FNA and reducing sampling errors, we have started to compare 22-G and 25-G needles for tissue sampling. In the diagnosis of ampullary tumors of the duodenum, we perform detailed characterization of the mucosal surface structures by means of NBI with magnifying capabilities to determine if the lesion is benign or malignant. In addition, a convex-array EUS study is being performed to evaluate the depth of tumor invasion. On the basis of these findings, indications for endoscopic papillectomy are determined. Favorable clinical outcomes have been obtained so far.

2. Treatment using endoscopic techniques of pancreatobiliary diseases

A randomized, controlled study was conducted to compare the usefulness of endoscopic sphincterotomy and endoscopic papillary balloon dilation for the removal of stones from the common bile duct. Data obtained from the comparative study are used for selecting the most appropriate treatment for patients with common bile duct stones. The appropriateness of the procedure selection has also been reviewed and examined, for further improvement of the therapeutic results. Now studies of the long-term results, such as the recurrence rate and long-term complications, are under way. External biliary drainage, or endoscopic nasobiliary drainage, and internal biliary drainage using a plastic stent (endoscopic biliary drainage) have been widely adopted for the treatment of obstructive jaundice. No criteria, however, have been established to facilitate the selection of the most appropriate treatment for individual patients. We are now conducting a randomized, controlled study to compare the two different endoscopic treatment methods. For cases of inoperable cancers of the bile duct and pancreatic

head, we employ a metallic stent made of shape-memory alloy and performed historical comparison with standard stents. Although EUS-guided celiac plexus block has been performed to control benign but persistent pain due to chronic pancreatitis, the pain relief was short-lived. Therefore, we have applied EUS-guided celiac plexus neurolysis with injections of small amounts of ethanol and are evaluating its feasibility.

Palliative care

More and more interest is being shown for palliative care. Various techniques have been developed to provide the best quality of life for critically or terminally ill patients. Endoscopic procedures may play an important role, especially in supporting food intake. In our department, percutaneous endoscopic gastrostomy is performed for patients who cannot maintain sufficient oral intake. Although percutaneous endoscopic enterostomy is usually not indicated for patients who have undergone gastric surgery, we have extended the use of this procedure to include such patients since 1994 and have investigated its clinical usefulness. Kits developed by us for placing percutaneous endoscopic gastrostomies have reduced the frequency of complications associated with percutaneous endoscopic enterostomy placement. To alleviate stenosis attributable to tumors of the digestive tract and bile ducts, we perform endoscopic ballooning/ bougienage and subsequent metallic stenting. The therapeutic results have been good. To reduce the pain associated with chronic pancreatitis and inoperable pancreatic cancer, we perform transgastric celiac plexus block with EUS. These endoscopic procedures may greatly improve the quality of life of patients who are not candidates for radical surgery. The cost-effectiveness of these interventions is an additional benefit.

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Department of Infection Control

Shoichi Onodera, Professor

Masaki Yoshida, Lecturer

Research Activities

Epidemiological research on sexually transmitted diseases

From 2003 through 2005, the "Study Group on Prevention of the Spread of Sexually Transmitted Diseases," with Dr. Onodera as the chief investigator, was supported by a Grant-in-Aid for Scientific Research of the Ministry of Health, Labor and Welfare (Research Project on Emerging and Re-emerging Infectious Diseases). Since 2006, however, part of this research has continued as a new research project of the Ministry of Health, Labor and Welfare under the title "Research on Promotion of Policies for Prevention of Specified Infectious Diseases Related to Sexually Transmitted Diseases," with Dr. Onodera as the chief investigator. Based on the contents of the "Policies for Prevention of Specified Infectious Diseases related to Sexually Transmitted Diseases" revised in 2006, the objective of this study group was research and development to prevent the onset and spread of sexually transmitted diseases (STDs) and to promote measures against STDs. The main research topics included: 1) epidemiological survey of onset trends for STDs, 2) exploratory research on early discovery of STDs in young people and links to treatment, 3) development of rapid and high precision test methods for genital herpes and condyloma acuminata (genital warts), and 4) surveillance of drug-resistant gonococci and development of diagnostic and therapeutic methods for gonococcal infections of the pharynx. Topics 2, 3, and 4 are research continued from the previous study group, but the epidemiological survey of onset trends for STDs is a newly developed research topic from this year. At present, trends for 6 STDs in Japan are being surveyed: syphilis, human immunodeficiency virus/acquired immunodeficiency syndrome, genital Chlamydia infections, gonococcal infections, genital herpes virus infections, and condyloma acuminata. For the first 2 diseases, notification of all cases is required, and for the other 4 diseases, onset trends are surveyed with a fixed-point method. Selection of the designated reporting institutions for the fixed-point surveys is the responsibility of each local government. No problems with the method of selection have been found, and surveillance to validate the survey method has not been performed recently. The Study Group surveyed all cases of STD in a limited area to validate the fixed-point survey, and the suitability of the fixed-point survey was examined. This year, 4 model prefectures were selected: Chiba, Ishikawa, Gifu, and Hyogo. With the cooperation of the Japan Medical Association, prefectural medical associations, and associations of clinicians in each region, all-case surveys of STDs were performed for each region, and the suitability of the fixed-point surveys was confirmed.

Hospital-acquired infections by drug-resistant bacteria and measures against them In the 3-year period from April 1, 2003, to March 31, 2006, detection conditions of *Pseudomonas aeruginosa*, drug sensitivity, and doses of injectable antibacterial agents used were surveyed with *P. aeruginosa* isolated from inpatients at affiliated hospitals and patients in whom *P. aeruginosa* was detected as subjects. The doses of injectable antibacterial agents and resistance to *P. aeruginosa* were surveyed in each hospital department, and correlations were examined. The detection conditions of multidrugresistant P. aeruginosa (MDRP) and doses of injectable antibacterial agents were surveved and compared in patients in whom MDRP was detected and in patients showing resistance to 1 or 2 agents among imipenem, amikacin, and levofloxacin. As the doses of the antibacterial agents increased, the resistant bacteria also increased. The doses of the agents were higher in patients in whom imipenem-resistant bacteria were detected than in patients in whom MDRP was detected. If MDRP-positive patients are carriers, the bacteria can be eliminated by discontinuing the antibacterial agent. The methods of using antibacterial agents differ markedly among hospital departments; therefore, the bacteria detected and the doses of agents used must be understood, and guidelines are necessary. In our hospital, there are no restrictions on the use of antibacterial agents, but we recommend that guidelines be established.

Measures against norovirus outbreaks

Outbreaks of norovirus in medical institutions have become a problem in recent years. When cases of such infections were analyzed in our hospital, the possibility that the virus is spread by inappropriate treatment of vomited matter cannot be ruled out; therefore, adding preventive measures against airborne infections appears necessary. When outbreaks do occur, the cost of using personal protection equipment (PPE) is clearly less than the loss to hospitals by admission restrictions. In molecular biological surveys of patients, outbreaks in the winter of 2005 and 2006 were caused mainly by the G (Kan??) 4 type virus. Long-term virus excretion was observed more often in children than in adults, and virus reservoirs have been found in hospitals. The transcription-reverse transcription concerted assay has been introduced as a diagnostic method for norovirus and has been suggested to be as sensitive as the reverse transcription-reverse transcription concerted assay is a useful measure against hospital-acquired infections because of its simplicity.

Study of antibacterial drug treatment of abdominal typhus

The current first-line therapy for abdominal typhus in Japan is new quinolones, but patients not responding to new quinolones have increased in recent years. The usefulness of various antibacterial agents and drug-sensitivity tests were examined in patients with typhus at our hospital. Many of the strains of *Salmonella typhi* and *Salmonella paratyphi* A detected from these patients showed resistance to nalidixic acid and were classified as having low sensitivity to new quinolones. In patients with bacterial infections showing low sensitivity to new quinolones, the response rate to new quinolones was low, and coadministration of third-generation cephems or switching to a sulfmethoxazole-trimethoprim combination drug was necessary. Therapeutic effects on abdominal typhus were consistent with the results of in vitro drug sensitivity tests. An analysis with the checkerboard method revealed either synergistic or additive effects for

coadministration of a new quinolone with a third-generation cephem. In treatment of abdominal typhus, drug-sensitivity tests should be performed promptly, and drugs should be selected on the basis of the results.

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Department of Dentistry

Haruyasu Tanabe, Professor Akihiro Ikai, Associate Professor Shigeru Suzuki, Lecturer Masashi Sugisaki, Professor Kazuo Ioroi, Associate Professor

General Summary

1. Clinical studies of temporomandibular disorders

We have continued studies of the evaluation of quality of life and screening questionnaires for temporomandibular disorders (TMDs) and the development of new therapies. We also examined the background of patients with TMDs.

2. Basic studies of oral mucosal keratinocytes in wound healing

We examined the effects of various growth factors, including trefoil factor 3 (TFF3) and nerve growth factor, on human oral mucosal keratinocytes in vitro, focusing on cell proliferation and migration.

Research Activities

Clinical studies of TMDs

1. Sex-related differences in daily functions of patients with TMDs

We developed a questionnaire of the pain-related limitations of daily function (LDF-TMDQ) for Japanese patients with TMDs. Furthermore, we reported on the validity of the LDF-TMDQ, using results from 2 groups of subjects (from 2000 and 2004).

Objective: To examine the homogeneity of the 2 groups, and sex-related differences in the LDF-TMDQ scores in Japanese patients with TMDs under conditions of homogeneity.

Methods: A total of 1,073 outpatients were recruited, of whom 866 patients (81%), including 421 patients from 2000 (88 men and 333 women) and 445 patients from 2004 (139 men and 306 women), completed the questionnaire and were eligible for the analysis. In this study, we addressed the issues of homogeneity and sex-related differences by means of multigroup analysis and mean structure modeling (SPSS version 12, AMOS version 5).

Results: On multigroup analysis, the 2 groups showed homogeneity. On mean structure modeling, male patients showed significantly lower scores for all latent variables. Conclusions: In female patients, limitations of daily function (executing a certain task, mouth opening, and sleeping) are more severe than in male patients.

2. Comparative study of limitations of daily functions between patients with TMDs and those with other dental diseases

Purpose: To compare limitations of daily functions between patients with TMDs and patients with other dental diseases.

Methods: A questionnaire survey was administered to 1,575 patients who had consulted our hospital in the year after January 16, 2006. A total of 1,535 valid responses (TMD prevalence, 13%) were obtained. The items of the questions could be classified into 3 groups: limitations in executing a certain task (5 items), limitations of mouth opening (3 items), and limitations of sleeping (2 items).

Results: Patients with TMDs showed higher values for limitations in executing a certain task, limitations of mouth opening, and limitations of sleeping than did other patients. However, limitations of sleeping were rare in patients with TMDs and patients with other dental disorders.

Conclusion: Limitations in executing a certain task and limitations of mouth opening are important disorders of daily functions in patients with TMDs.

3. Validity of the grading of the severity of chronic pain in Japanese patients with TMDs

Objectives: Recently, many authors have evaluated the performance of the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD). The RDC/TMD have been translated into 18 languages and is being used by a consortium of RDC/TMD-based international researchers. We set out to validate the Grading of Severity of Chronic Pain (GSCP) in RDC/TMD for Japanese patients with TMDs. Methods: We used the GSCP in the Japanese version of the RDC/TMD. A total of 448 of 542 patients with TMD were analyzed. The GSCP included 7 questions for 3 items: Characteristic Pain Intensity (item 1, 3 questions), and Disability score (item 2, 3 questions), and Disability days (item 3, 1 question). To assess the cross-validity of the GSCP, patients were randomly divided into 2 halves (group 1 and 2). We used 3 statistical methods to check the validation: Mokken analysis for a cross-validity, structural equation modeling for factor validity, and the Spearman correlation coefficient for criterion-based validity.

Results: Using Mokken scale analysis, item coefficient H ranged from 0.64 to 0.72 in group 1 and from 0.43 to 0.62 in group 2, and scale coefficient H was 0.70 and 0.57, respectively, in these groups. The results indicated monotone homogeneity. Furthermore, the reliability of the scales in groups 1 and 2 was 0.83 and 0.77, respectively, indicating double monotonicity. Structural equation modeling yielded a substantially excellent model fit. The GSCP showed criterion-based validity with a visual analog scale and a questionnaire to assess pain-related limitations of daily functions.

Conclusion: In this series of Japanese patients with TMDs, cross-validity, factor validity, and criterion-based validity of the GSCP were shown, and the GSCP subscales were used to compare Japanese and English-speaking patients with TMDs.

4. Teeth-contacting habit as a factor contributing to chronic pain in patients with TMDs

Many different factors are known to cause and perpetuate the symptoms of TMDs. However, the roles of parafunctional factors have not been clarified. We found one of these habits in the clinical setting. This parafunctional habit involves daily light touching of the upper and lower teeth when the mouth is closed. We named this habit the teeth-contacting habit (TCH).

Objectives: To investigate the following hypotheses: 1) TCH is associated with perpetuation of chronic pain in patients with TMDs; and 2) TCH is associated with other behavioral factors.

Methods: A total of 229 outpatients with TMDs and chronic pain were analyzed with

multivariate logistic regression models.

Results: The TCH was present in 52.4% of patients. Patients with TCH and pain lasting for more than 4 months were less likely to show a decrease in pain at the first visit (odds ratio [OR] = 1.944, p=0.043). Other factors associated with TCH were unilateral chewing (OR=2.802) and involvement in a precision job (OR=2.195).

Conclusion: The TCH can prolong TMD pain and is associated with other behavioral factors.

5. Selection of question items for screening of patients with TMDs

No questionnaire has been available for epidemiologic studies estimating the crossvalidation of question items for screening for TMDs.

Purposes: To estimate selection methods for screening for TMDs disorders.

Methods: We created 5 physical estimation items, 4 psychologic estimations (5-point numeric rating scale), and 11 exclusion items (binary scale) of TMDs. We analyzed 222 patients (TMD prevalence rate: 10.2%) who visited either of 4 dental treatment facilities. We used factor analysis, receiver operating characteristic (ROC) curves, and a non-parametric item scalability test (Mokken analysis) for item selection.

Results: Four psychologic items and 5 physical items (5-point numeric rating scale) were selected from 20 items predetermined with factor analysis as the first and second factors, respectively. With Mokken analysis, 4 physical items were selected (Scale H=0.53, Rho=0.79), and their ROC curve value was 0.908. Three psychologic items were selected with Mokken analysis (Scale H=0.72, Rho=0.86), and their ROC curve value was 0.595.

Conclusions: These results show that factor analysis, Mokken analysis, and ROC curves are useful for selecting screening items for TMDs.

Basic study of oral mucosal keratinocyte in wound hearing

1. Salivary TFF3 enhances migration of normal oral keratinocytes

Purpose: TFF3 is a small peptide (7 kDa) secreted by the submandibular gland and is 1 of 3 members of the trefoil factor family. Studies of the gastrointestinal tract have shown that members of this family play important roles in maintaining and protecting mucosal surfaces. TFF3 promotes restitution, i.e., the rapid movement of neighboring epithelial cells into a wound area. We investigated the potential positive role of TFF3 in oral wound healing.

Methods: We performed a wound-healing assay of keratinocytes isolated from oral mucosa (n=3). Cells in serum-free medium at passages 2 to 4 were grown to confluence. The monolayer was wounded with the tip of a 200- μ l pipette and allowed to migrate for 12 hours after stimulation with 0.25 or 0.5 μ M of TFF3. The wounded areas were photographed before and after migration, and the area recovered by migrating cells was measured. To demonstrate that the observed cell movement was not related to an increased number of cells, we added mitomycin C (an inhibitor of mitosis) to the cultures before wounding and stimulation. Conversely, to demonstrate that the cells had actively migrated, we added cytochalasin B (an inhibitor of migration) to the cultures before wounding and stimulation.

Results: Cells stimulated with TFF3 showed a higher migration index than did un-

stimulated controls (P=0.005, paired t-test). Treatment with mitomycin C did not impair the observed cell migration and indicates that the movement is independent of mitosis. Treatment with cytochalasin B completely inhibited cell movement.

Conclusion: Our results show that stimulation of normal keratinocytes with TFF3 promotes migration. On the basis of these findings, we hypothesize that TFF3 is one of many factors involved in the rapid wound healing of the oral mucosa.

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Department of Pathology, Clinical Service

Makio Kawakami, Professor Masaharu Fukunaga, Associate Professor Masafumi Suzuki, Associate Professor Koichi Nomura, Lecturer Yutaka Yamaguchi, Professor Akihiko Sakata, Associate Professor Hiroyuki Takahashi, Lecturer Yukiko Kanetsuna, Lecturer

General Summary

Although improvements in the personnel loan system, which decreases motivation, are urgently needed, such improvements were not made this year. Therefore, the research of mainly non-loan employees is described here. Readers who are interested in the research of loan employees can find their achievements in the records of the Department of Pathology.

Research Activities

Main Hospital

Kawakami's research focused on cardiovascular issues and aging problems of the gastric mucosa and ovaries.

1. Natural history of the myocardium

Twelve separate myofibrils in a subcytomembranous monocoque arrangement in the sponge framework of the cytoplasm during the prenatal period become independent myofibril units as the cardiac muscle develops. The paleomyocardium, as pace-making cells, takes an axial myofibrillar position, and neomyocardium, as the ventricles, takes a peripheral position in the so-called monocoque alignment. After the age of 65 years, the power of longitudinal splitting stops and the volume of the myofibrils increases.

2. The participation of thrombus in myocardial infarction

Coronary artery thrombosis occurs in 50% of cases of myocardial infarction, but many cases of even advanced infarction are without thrombus. Mixed thrombi tend to appear in chronic sclerosis, and red thrombus tends to appear in acute sclerosis. Therefore, thrombosis in myocardial infarction is more strongly influenced by rheological factors, such as blood flow delay, than by factors of vasculomural injury.

3. The natural history of the ovary

The cross-sectional area of the ovary increases exponentially until the 30's and then decreases. The ovarian medulla shows similar changes. The volume of the corpora albicans increases most in the 20's and in the 50's, although the corpora albicans is larger in the 20's than in the 50's. In the cortex, the distinction between the inner active and the inner inactive strata is greatest in the 20's and after 40 years.

4. Peritoneal dialysis and encapsulating peritoneal sclerosis

The peritoneum, which is a revival of the dialysis membrane caused by the reral impairment is exposed to a state of hyperfiltration as "flat" glomerulus, analogous to the renal glomerulus. The additional development of skeletal collagenosis an essential trend, decreases with the progression of vasculopathy after 6 years, and the basis of the

encapsulating peritoneal sclerosis is established.

The achievements of loan personnel are described below.

Suzuki performed a comparative immunohistochemical study of renal cell carcinoma and renal adenoma. The characteristics of cystic renal cell carcinomas were also reported. Suzuki also assessed the validity of the immunohistochemical Hercep test and the fluorescence in situ hybridization method for selecting the optimal method of molecular target therapy of breast cancer.

Nakayama started a study of morphological changes of lymphatic channels in normal, hepatitic, and cirrhotic livers by means of D2-40 and CD34. The degree of lymphatic dilation was weakly correlated with the grade of fibrosis.

Nakano studied genes in prostatic carcinoma with special emphasis on metastasis.

Koike also started his debut study of the natural history of the vertebra. He found that growth continues until 30 years of age and then gradually decreases.

Aoto Hospital

Sakata has been studying the process of liver restructuring. To find antigens related to proliferation and oxidative stress, he introduced 8-hydroxydeoxyguanosine and proliferating-cell nuclear antigen, resulting in their frequent expression unrelated to causes or location and magnitude of inflammation. The expression of proliferating-cell nuclear antigen was predominant, and 8-hydroxydeoxyguanosine activity was present in the nucleus and in the cytoplasm. Damage and repair of DNA were found to have progressed further than expected in the cirrhotic stage.

Kanetsuna, who returned from the United States, continued an experimental study of the diabetic kidney. She clarified the relationship between endothelial nitric oxide synthase and vascular endothelium in diabetic and nondiabetic kidneys.

Daisan Hospital

Fukunaga performed research in obstetric and gynecologic pathology. The discrepancy of histological diagnoses among pathologists is substantial in atypical hyperplasia and grade 1 endometrioid adenocarcinoma after treatment with medroxyprogesterone acetate. New criteria that can be applied to hormonally effected lesions are needed. He also examined hepatoid carcinoma of fallopian tube, hydatidiform mole, and perivascular epithelioid cell tumors of the uterus and soft tissue.

Takahashi compared separate and global scorings for Gleason grades in prostatic cancer and showed the effectiveness of separate scoring. He also performed molecular loss-ofheterozygosity analysis of minute lesions of prostatic cancer and found a lower value in minute cancer.

Kashiwa Hospital

Yamaguchi analyzed hypertensive nephropathy semiquantitatively by means of image analysis software. He investigated the correlation of glomerulosclerosis, tubulointerstitial lesions, and vascular lesions was investigated and found that arteriolar lesions influence of the progression of glomerulosclerosis. Lesions of interlobular arteries correlated with acute tubuloepithelial lesions and interstitial fibrosis and influenced renal prognosis.

With repeated biopsies for nephritis related to myeloperoxidase antineutrophil cytoplasmic antibodies Yamaguchi found that the active lesions of the glomerulus and tubules disappear after treatment.

Yamaguchi's histological analysis of renal grafts in chronic rejection showed that basement membrane thickening of the peritubular capillary was related to moderate and severe peritubular capillaritis.

Ohmura analyzed the development of cancer in the alimentary canal from the viewpoints of the environmental conditions of vessel invasion and local circulatory disturbance.

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Institute of DNA Medicine Department of Gene Therapy

Yoshikatsu Eto, Professor and Director

Toya Ohashi, Professor

General Summary

Our research focuses on efforts to develop methods of molecular therapy and cell therapy for genetic diseases, diabetes, kidney diseases, and cancer. Members of the laboratory staff were recruited from various departments of The Jikei University, including the departments of internal medicine, surgery, and gynecology. All members are working to develop novel therapies for intractable diseases.

Research Activities

Molecular therapy for genetic diseases

Fabry disease is a lysosomal storage disease characterized by deficient alphagalactosidase A activity. This deficiency results in progressive accumulation of globotriaosylceremide (GL-3), mainly in vascular endothelial cells, leading to renal and cardiac failure. Enzyme replacement therapy is now available. However, antibodies form against infused enzyme in some male patients. Our studies this year revealed that these antibodies neutralize the enzyme and inhibit its cellular uptake. Although normalization of urinary GL-3 levels was achieved more efficiently in seronegative patients than in seropositive patients, the clinical outcome was not affected by antibody formation. Finally, our experiment showed that neonatal intravenous infusion of the enzyme induced immune tolerance in a murine model of Fabry disease.

Phosphorylation of shugoshin by aurora B

The conserved protein shugoshin plays a role in the maintenance of centromeric cohesion in mitosis and meiosis. In a previous study, we demonstrated that shugoshin accelerates kinetochore-driven formation of kinetochore microtubules for spindle assembly.

This year, we investigated the mechanism of bipolar attachment via shugoshin. Aurora B is a mitotic kinase that regulates bipolar attachment. Thus, we examined the phosphorylation of shugoshin by aurora B. We found that two highly conserved serine residues of shugoshin, located at the mid-portion and the C-terminal, were phosphorylated by aurora B.

Gene transfer to pancreas for the regeneration of islet cells

In our study of regeneration therapy for the endocrine pancreas in diabetes mellitus, we have developed a novel gene-delivery system: *in vivo* direct injection to the murine pancreas using an adeno-associated virus (AAV) vector. When the gene for cyclin-dependent kinase 4, a gene crucial for cell-cycle regulation and beta-cell proliferation,

was transduced to the remaining beta cells by the direct injection method, we observed improvement of metabolism in mice with diabetes. We are now investigating the precise mechanism for the therapeutic effects so that clinical application will be possible in the future.

Generation of an erythropoietin-producing organoid derived from human mesenchymal stem cells

Differentiation of autologous stem cells into functional tissue for organ regeneration is a promising regenerative therapeutic approach for many human diseases, including renal failure. Yet to be accomplished, however, is differentiation into tissue capable of producing erythropoietin. We report the generation of a stem-cell-derived organoid capable of producing erythropoietin and sensitive to regulation by anemia, indicating a function in erythropoiesis.

Mesenchymal-to-epithelial transition during the inclusion cyst formation from human ovarian surface epithelium

Most surface epithelial-stromal tumors of the ovary are thought to arise from epithelial inclusion cysts. Thus, these cysts are the precursor lesion of ovarian carcinoma. On the basis of this hypothesis, we aimed to characterize human ovarian surface epithelium in which the mesenchymal-to-epithelial transition occurs during inclusion cyst formation. We used specimens from 9 patients with endometrial cancer who underwent hysterectomy and bilateral salpingo-oophorectomy. Immuohistochemical study was performed with 10 normal ovaries containing 92 inclusion cysts and 4 normal tubes to examine the expression of antigen markers, including calretinin, podoplanin, D2-40, thrombomodulin, human bone marrow endothelial (HBME)-1, vimentin, epithelial membrane antigen (EMA) WT1, CA125, MOC31, TAG-72, Ber-EP4, and E-cadherin. We found that the staining rates for mesothelial markers in normal ovarian surface epithelium were 100% (10 of 10) for calretinin, 80% (8 of 10) for podoplanin, 80% (8 of 10) for D2-40, 70% (7 of 10) with thrombomodulin, 100% (10 of 10) for HBME-1, and 100% (10 of 10) for vimentin. In tubal epithelium the staining rates for epithelial markers were 100% (4 of 4) for HBME-1, 100% (4 of 4) for vimentin, 100% (4 of 4) for EMA, 75% (3 of 4) for TAG-72, and 100% (4/4) for Ber-EP4. Inclusion cysts showed staining for both markers with an incidence of 51.1% (47 of 92) for HBME-1, 44.6% (41 of 92) for vimentin, 65.2% (60 of 92) for TAG-72, and 88.0% (81 of 92) for Ber-EP4. Ovarian surface epithelium has both mesencyhmal and epithelial characteristics. In contrast, inclusion cysts gain epithelial characteristics and lose mesencyhmal characteris-These findings support the observation of a mesenchymal-to-epithelial transition tics. during inclusion cyst formation from the ovarian surface epithelium.

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Institute of DNA Medicine Department of Oncology

Yoshikatsu Eto, Professor and Director Sadamu Homma, Associate Professor Tetsuro Kikuchi, Associate Professor Mikio Zeniya, Professor Junko Horiguchi-Yamada, Associate Professor

General Summary

We continue to study immunological therapies for cancer and a model of leukemia cell differentiation. To further develop immunotherapy, we investigated the mechanisms of immunological reactions against cancer and strategies to modify them to enhance clinical responses.

Research Activities

Mass spectrometric identification of a tumor-associated antigen expressed on mouse hepatocellular carcinoma cells that regulates host antitumor immunity

Tumor-associated antigens might stimulate or suppress host antitumor immunity depending on host tumor burden or immune environment. To identify a tumor antigen expressed on cancer cells, MHC class II binding peptide was isolated from I-A^k/peptide complexes obtained from bone marrow-derived dendritic cells (DCs) loaded with MIH-2 mouse hepatocellular carcinoma (HCC) cells, and peptide ion peaks were analyzed by means of mass spectrometry. One representative peptide, EMTK, was considered to be derived from amino acids 284-287 of the cytochrome P450 2j subfamily (CYP2js) expressed in MIH-2 cells. Amino acids adjacent to EMTK showed an I-Ak-binding structural motif. Accordingly, the peptide consisting of EMTK and the I-A^k-binding motif (DFIDAFLKEMTKYPE) was considered to be an antigenic peptide presented to CD4⁺T cells in the context of MHC class II. Administration of several doses of the synthesized CYP2js peptide to naïve mice induced interferon (IFN)-gamma production by splenocytes. However, repeated immunization of mice with CYP2 is peptide suppressed IFN-gamma production and enhanced MIH-2 tumor growth in vivo. Production of interleukin (IL)-4, IL-10, and transforming growth factor beta was not involved in this immune-suppression. Increased frequency of CD4+FoxP3+regulatory T cells and CD11b+Gr-1+immature myeloid cells was observed in splenocytes from mice repeatedly immunized with CYP2js peptide. These results indicate that CYP2js expressed on HCC cells could promote antitumor immunity at an early stage of tumor growth with low or moderate antigenic stimulation but would suppress antitumor immunity in an advanced stage of tumor growth with excess antigenic stimulation due to a large tumor burden.

Clinical immunotherapy for brain tumors

Several clinical trials of immunotherapy with DCs have been published. We investigat-

ed the safety and clinical response of immunotherapy with fusions of DCs and glioma cells and IL-12 for patients with malignant glioma. Fifteen patients with malignant glioma participated in this study. No severe adverse effects were observed. In 4 patients, magnetic resonance showed a reduction in tumor size of more than 50%. The results of the Phase I clinical trial of IL-12 and fusion cells indicated that this treatment safely induced immune responses, resulting in a clinically significant antitumor effect in some patients. We are now analyzing the antitumor effects of DC therapy in patients with both primary and recurrent malignant gliomas. Stat-3 is a potential negative regulator of inflammatory responses. Blocking Stat-3 in tumor cells increases expression of proinflammatory cytokines that activate innate immunity and DCs, leading to tumor-specific T-cell responses. We are investigating the antitumor effects of fusion cells transfected with Stat-3 small interfering RNA to block Stat-3 expression.

Differentiation of leukemia cells by adhesion

JASR is a novel leukemia cell line with megakaryoerythroid features. JASR cells adhere to extracellular matrices and acquire megakaryocytic features, including morphological changes. Among genes expressed in parallel to this alteration, FLI1, a transcription factor of the ets family, is a target of particular interest. FLI1 has been shown to be a key regulator for megakaryocytic progenitors. Furthermore, the adherent cells show greater resistance to chemotherapeutic agents. The precise mechanism of the cell-adhesion-mediated drug resistance is being investigated.

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Institute of DNA Medicine Department of Molecular Genetics

Hisashi Yamada, Professor and Director

General Summary

Over the last few decades, many genes involved in the etiology of cancers have been found. These findings must contribute to the improvement of cancer therapy, but cancer is still the leading cause of death in Japan. Our goal is to advance cancer medicine on the basis of molecular genetics.

Research Activities

Exploring human leukemic stem cells and the development of anticancer therapy Leukemic stem cells represent a small percentage of human leukemia cells in vivo but play a critical role in prognosis. In general, leukemic stem cells are resistant to chemotherapeutic drugs and support the production of leukemic cells in vivo. The ability of leukemic stem cells to be transferred sequentially from mouse to mouse has been demonstrated, but the genetic and biochemical natures of these cells remain largely unknown. To address these issues we first studied how the growth environment affects leukemic cells. Cells of the megakaryocytic-erythroid leukemic cell line JAS-R show a lineage shift depending on culture conditions. The interaction between fibronectin and integrin elicits the megakaryocytic phenotype and leads to the loss of erythroid characteristics in JAS-R cells. Moreover, adherent cells express CD34 and CD9, which are markers of hematopoietic stem cells. These findings demonstrate that the growth microenvironment controls the leukemic phenotype. We are now studying, by means of a microarray method, the regulatory mechanism that determines the lineage of the two groups. The results will provide useful information about the nature of leukemic stem cells and the mechanisms of chemoresistance.

Pharmacology of anticancer drugs

The telomere is a protein-DNA complex that protects the ends of double-stranded DNA. Its shortening accelerates aging and cell senescence. Generally, somatic cells do not have telomerase activity, while most cancers do. Therefore, telomerase is a promising molecular target for cancer therapy. 5,10,15,20-Tetrakis(*N*-methyl-4-pyridyl)porphyrin (TMPyP4) is a compound that binds to telomere DNA stably and tightly and blocks telomerase. Thus, we studied the anticancer activity of TMPyP4 in cells over-expressing the telomerase catalytic gene human telomerase reverse transcriptase (hTERT). A short-term growth inhibition assay showed that the anticancer activity of TMPyP4 was not related to telomerase activity, but transcriptional dysregulation of growth-related genes, including the c-Myc oncogene, might play a critical role in cell death. These finding may lead to the appropriate application of TMPyP4 as an anticancer agent.

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Institute of DNA Medicine Department of Molecular Immunology

Saburo Saito, Associate Professor and Director Yuji Ohno, Associate Professor Daitaro Kurosaka, Associate Professor

General Summary

Our research interests have focused on the analysis of the basic immune system protecting against disease and of immune disorders, such as hypersensitivity diseases and autoimmune diseases.

Research Activities

Enhanced IgE levels in interleukin 31 transgenic mice

Interleukin (IL)-31 is a newly discovered T-cell-derived cytokine whose overexpression in transgenic mice produces a phenotype closely resembling Alzheimer disease. In Nc/ Nga mice high expression levels of IL-31 are associated with increased levels of serum IgE. Enhanced expression levels of IL-31 correlate with IL-4 and IL-13 but not with serum IgE levels in atopic dermatitis or allergic contact dermatitis.

To investigate the function of IL-31 and to confirm the possible association of IL-31 with serum IgE levels, transgenic mice that overexpress IL-31 were established under the control of cytomegalovirus enhancer/chicken beta-actin promoter.

Comparison of transgenic mice with littermate nontransgenic mice at 7 weeks of age showed a tendency for serum IgE levels to be increased in transgenic mice. Serum IgE levels in IL-31 transgenic mice with severe dermatitis were significantly higher than those in nontransgenic or other transgenic mice with mild or moderate dermatitis. Total IgE levels in 13-week-old transgenic mice were significantly higher than those in 7-week-old transgenic mice, but no age-related changes in serum IgE levels were observed in nontransgenic mice. However, no significant difference in the production of IL-4 or interferon- γ were found between nontransgenic and transgenic mice. Although our data do not reveal the mechanism of IgE expression in IL-31 transgenic mice, they do suggest that IgE levels in the mice correlate with the severity of the dermatitis induced by IL-31.

Abnormal telomerase activity and telomere length in T and B cells from patients with systemic lupus erythematosus

To evaluate the clinical significance of telomerase activity and telomere length in T and B lymphocytes from patients with systemic lupus erythematosus (SLE), CD3+ (T cell) and CD19+ (B cell) lymphocytes were isolated from the peripheral blood of patients with SLE and healthy control subjects by means of magnetic-bead-coupled antibodies. The telomerase activity of lymphocytes was measured with the telomeric-repeat amplification protocol. Telomere length was measured with flow cytometry fluorescence *in*

situ hybridization.

The telomerase activity of T cells was significantly higher in patients with either active or inactive SLE than in control subjects but was lower than the telomerase activity of B cells in patients with active SLE and was not correlated with the SLE disease activity index. The telomerase activity of B cells was significantly higher than in controls only in patients with active SLE and was strongly correlated with the SLE disease activity index. Four laboratory results, the anti-double-strand DNA antibody titer, the IgG level, the C3 level, and the CH50 level, were correlated with B-cell telomerase activity. Telomere length in T cells was significantly shorter than in control subjects. In contrast, the telomere length in B cells did not differ significantly from that in control subjects.

These results suggest that: 1) T cells are continuously activated in patients with SLE, 2) this activation alone does not cause the disease to develop, and 3) the disease manifests when B cell activation also occurs. Although it is not clear whether B-cell activation is mediated by T cells, the inhibition of B-cell activation may at least lead to the suppression of disease activity.

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Institute of DNA Medicine Department of Molecular Cell Biology

Yoshinobu Manome, Associate Professor and Director Hiroyuki Sasaki, Associate Professor Toru Obata, Associate Professor

Michiko Watanabe, Lecturer

General Summary

Our research activities include analysis and visualization of cell biological events under physiological and pathological conditions. For this purpose molecular biological, fine morphological, and biochemical techniques were applied.

Research Activities

Development of sonodynamic therapy for malignant glioma

Ultrasound is widely used in clinical medicine, and, thus, its safety is well established. In addition to diagnostic applications, therapeutic applications are also possible. When combined with a contrast agent containing microbubbles, such as Levovist (Schering AG, Berlin, Germany), ultrasound can induce cell death in nearby tissues by the cavitation effect. Using this effect, we established a new therapeutic application of ultrasound for central nervous system malignancies, such as malignant glioma. We found 210 kHz and 2.61 W/cm² of insonation could efficiently induce cell death when combined with 30 mg/ml of Levovist. Moreover, the effect was also confirmed with *in vivo* animal experiments. We are now proposing therapeutic/diagnostic, or theragnostic, applications of ultrasound and are attempting to refine the system.

Soft ionization

Soft ionization processes, such as electrospray ionization, in mass spectrometry, have used proton transition from water molecules. This condition may cause proton substitution in the target molecule. The isotope dilution method of quantification is commonly used in mass spectrometry. This method is based on the stability of the labeled compound during the preparation and assay processes. The study of stability check with labeled compounds during soft ionization shows the possibility of substitution of deuterium label to proton in water molecules.

Functional analysis of tight junctions

Tight junctions (TJs) are the most apical components of junctional complexes and play a vital role in cell-cell adhesion in epithelial and endothelial cells. Evidence is accumulating that TJs in the granular layer of the epidermis contribute to the epidermal barrier. To further clarify the role of TJs in the barrier function of human epidermal keratinocytes, we investigated the behavior of TJ components by means of a keratinocyte culture system in which differentiation was induced by transfer to a high-calcium medium. TJ-related molecules became localized at the cell membrane, and transepithelial resistance was elevated with the progression of keratinocyte differentiation. The epidermal barrier function was clearly suppressed when the expression of claudin-1 or occludin was blocked by RNA interference. Furthermore, suppression of claudin-1 inhibited occludin expression in the cell membrane, whereas suppression of occludin did not affect the localization of claudin-1.

Morphological analysis with a 3-dimensional cell-culture system

To clarify the *in vivo* behavior of malignant glioma cells, we investigated morphological changes in a novel 3-dimensional (3D) cell-culture system by means of scanning electron microscopy. Four glioma cell lines, T98G, U118MG, A172 and KNS42, were used. When cells were cultivated in 3D, their morphologies changed markedly. Both A172 and KNS42 cells showed self-adherence, aggregated, and formed balloon-like structure, whereas T98G cells were partly conglomerated. The tight attachment of U118MG cells to the scaffold was observed. Although overall characteristics of these cells were similar to those of cells in ordinary two-dimensional cell culture and each line was used as a standard model for malignant brain tumor the morphology and adherence or fiber formation differed in the 3D cell culture system. This observation might be related to proliferation signals, such as phosphorylation of Akt, which were enhanced in A172 cells.

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Institute of DNA Medicine Department of Molecular Neurobiology Division of Morphology and Organogenesis

Masataka Okabe, Lecturer

General Summary

We are interested in the developmental and evolutionary aspects of vertebrate organs. By comparing organ development in humans and other animals, we are attempting to reconstitute the evolutionary path that each of our organs has taken, at both the molecular and morphological levels, thus identifying fundamental molecular mechanisms that shape each organ.

Research Activities

Evolutionary origin of the vertebrate lung

Terrestrial vertebrates and fish both possess an organ that is filled with air: the lungs and the swimbladder, respectively. These organs have been postulated to be homologous. Both the lungs and the swimbladder are air-filled sacs that are derived from the digestive tract. On the other hand, the lungs are a paired structure while the swimbladder is a The lungs extend from the ventral side of the digestive trace, whereas the single sac. swimbladder extends from the dorsal side. Due to a lack of fossil evidence, it has been difficult to determine whether the lungs and the swimbladder are actually homologous organs and if so, what they were like when they were first acquired in the ancestral vertebrate. We are comparing gene expression patterns during development of the lungs and the swimbladder in Xenopus, Australian lungfish, Polypterus, and zebrafish to test this hypothesis. So far, we have found that TBX4, FGF10, and NKX2.1, which have been identified as key regulators of aminiote lung development, are also specifically expressed in the swimbladder of the zebrafish. Knockdown of FGF10 results in loss of NKX2.1 expression and in swimbladder hypoplasia. These results suggest that both the lungs and the swimbladder evolved from a lung-like organ that was present in the common ancestor of teleosts and tetrapods.

Identification of ureteric bud progenitors in chicken embryo

Our goal is to generate kidneys exclusively derived from autologous human mesenchymal stem cells (hMSCs). However, we have not induced differentiation of hMSCs into the ureters and collecting ducts, both of which are derivatives of the ureteric bud. Last year, we identified the ureteric bud progenitor region, where hMSCs should be transplanted for their differentiation into the ureters and collecting ducts, in chicken embryos by lineage tracing with 3,3'-dioctadecylindocarbocyanine iodide. This year, we demonstrated that *Pax2*-transfected hMSCs differentiated into the Wolffian ducts, which are the parent tissue of the uteric bud when transplanted into the chicken ureteric bud progenitor region. This result suggests that with further research we may be able to induce differentiation of hMSCs into ureters and the collecting ducts.

Apoptosis and compensatory proliferation

Coordination of cell death and cell proliferation during development is critical for consistently producing organs of the correct size and shape. Accidental cell death in *Drosophila* larval imaginal discs leads to "compensatory proliferation." We found that the *Drosophila* initiator caspase DRONC simultaneously triggers cell death and induces compensatory proliferation. When cells are stimulated to undergo apoptosis but are simultaneously kept alive, the "undead cells" induce excessive compensatory proliferation. This abnormal tissue overgrowth is completely suppressed in *dronc* mutants. We also found that jun kinase (JNK) signaling is also required for compensatory proliferation. Genetic epistasis analysis suggests that JNK signaling is activated downstream of DRONC activation. These results suggest that the apoptosis signaling bifurcates at DRONC, with one branch leading to apoptosis and the other branch leading to compensatory proliferation through activation of JNK signaling.

How to make figures and presentations that are friendly to color-blind people

In scientific presentations and publications, color has become a significant vehicle for information and presentation effect. However, color perception varies greatly among individuals; in particular, red-green color blindness is present in 4% to 9% of males in various populations, a frequency comparable to that of the AB blood type. Thus, inappropriate color choices can cause unexpected difficulty in understanding color figures. We are examining how color and color combinations are perceived by various color-vision types to develop a method for presenting color information that can convey maximal information to most color-vision types, including color blindness. We introduced this method on our web site: http://www.nig.ac.jp/color

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Department of Neuroscience Division of Neuropathology

Satoshi Kurihara, Professor and Director Junko Fujigasaki, Lecturer Takahiro Fukuda, Lecturer

General Summary

Our research projects have concerned neurodegenerative disorders caused by intracellular accumulation of abnormal proteins. We are also studying mouse models of neurodegenerative disorders and autopsy cases by means of standard morphologic analysis and molecular biological analysis.

Research Activities

Localization and dynamic changes of the MLC1 protein in the central nervous system Mutations of the MLC1 gene are responsible for a form megalencephalic leukoencephalopathy with subcortical cysts (MLC). The function of the MLC1 protein is unknown. To characterize MLC1, we generated polyclonal antibodies against the MLC1 protein. The MLC1 protein and aquaporin 4 (AQP4) were expressed in the astrocyte end-feet membranes adjacent to blood vessels, the pia matter, and ependymal cells. In cerebral infarctions of spontaneously hypertensive/stroke-prone rats, we observed temporary loss of MLC1 and AQP4 during the edema phase and overexpression of them during the reactive astrocyte phase. Although a case of MLC examined at autopsy showed no mutation of MLC1 mRNA, a low expression level of MLC1 protein was shown by immunohistochemical study. MLC1 protein is suggested to have functions similar to those of AQP4, and further elucidation of the functions of MLC1 will be necessary to understand the precise molecular mechanisms whereby mutations of the MLC1 gene lead to myelin vacuolation.

Amyloid precursor proteins in spinocerebellar ataxia 7

Spinocerebellar ataxia 7 (SCA7) is a polyglutamine disease caused by polyglutamine expansion within a causative protein, ataxin-7. Recent evidence suggests that ataxin-7 regulates transcription and that aberrant regulation of transcription is related to the pathogenesis of SCA7; however, additional investigation is needed to clarify the pathogenesis of SCA7. Amyloid precursor-like protein (APLP) 2, a member of the amyloid precursor protein (APP) family, was identified as a partner protein of ataxin-7. We examined the subcellular distribution of APP-related proteins in SCA7 brains by immunohistochemical analysis.

Results: APP, APLP1, and APLP2 were localized mainly in the cytoplasm of normal control neurons. In SCA7 brains, nuclear APLP2 immunoreactivity was observed in neurons, although APP and APLP1 did not show clear nuclear localization. No neuronal intranuclear inclusions were immunostained with antibodies against APP,

APLP1, or APLP2. However, a few inclusions were immunostained with an antibody against caspase-cleaved APP/APLP proteins.

Discussion: The relation of the cleavage processes of APP or APLPs or both in the pathogenesis of Alzheimer disease has recently been demonstrated. Our data showing nuclear relocalization of APLP2 in SCA7 brains indicates that subcellular localization of APLP2 could be modified by the expression of mutated ataxin-7. In addition, recruitment of APP/APLPs caspase-3 cleavage products suggests that cleaved APP/APLPs might be enhanced in SCA7.

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Department of Neuroscience Laboratory of Neurophysiology

Fusao Kato, Professor and Director

General Summary

We are attempting to clarify the mechanisms underlying dynamic cell-to-cell signaling in the central nervous system. We use approaches at the molecular, cellular, and network levels, including the patch-clamp recording of synaptic currents and the real-time imaging of the intracellular Ca^{2+} concentration in living brain tissues from normal animals, animal models for various types of disease, and animals with experimental manipulation of gene expression.

Research Activities

Regulation of synaptic transmission in the brain network

1. Molecular mechanism of neurotransmitter release

To clarify the roles played by specific molecules in transmitter release in brain synapses, we developed a novel method for *in-vivo* gene silencing with RNA interference against genes coding presynaptic proteins, which is followed by functional analysis of synaptic transmission with patch-clamp recording in brain slices.

2. Glia-neuron interaction at synapses

To identify the functional role of gliotransmitters in the regulation of synaptic transmission, we developed a novel method of applying ATP, a "gliotransmitter" released from astrocytes, onto synapses in a time- and space-limited manner using laser-based photolysis of caged ATP compounds in brain slice preparations. Time-limited application of ATP onto synapses in the nucleus of the solitary tract immediately facilitated glutamate release through direct activation of presynaptic P2X receptors.

3. Central mechanism of frequency-dependent decoding of afferent information

To understand how the brain analyzes sensory signals from visceral organs, we analyzed the postsynaptic responses of second-order neurons in the nucleus of the solitary tract and the dorsal motor nucleus of the vagus nerve to repeated stimulation of afferent fibers in brainstem slices. These synapses showed distinct types of short-term plasticity with distinct Ca^{2+} -dependency, which might underlie the frequency-dependent "tuning-in" of visceral information.

Central mechanisms of pain-related negative emotion

Using rat models of chronic neuropathic pain, we demonstrated that excitatory synaptic transmission in the central nucleus of the amygdala, a structure playing principal role in the expression of emotional behaviour, is markedly potentiated.

Synaptic responses to metabolic failures in motor neurons

To elucidate the mechanism underlying selective vulnerability of motor neurons in various types of diseases involving mitochondrial dysfunction, we analyzed the responses to metabolic stress of hypoglossal motor neurons in brainstem slices. Hypoxia and NaCN markedly facilitated synaptic glycine release, which in turn potentiated N-methyl-D-aspartate-receptor-mediated currents by glycine-binding site activation.

Molecular target of volatile anesthetics

We found by analyzing membrane potentials and currents in pontine slice preparations that volatile anesthetics, but not intravenous anesthetics, directly excite neurons in the locus coeruleus through a mechanism involving the opening of gap-junction channels.

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Institute for High-Dimensional Medical Imaging

Naoki Suzuki, Professor and Director

Asaki Hattori, Lecturer

General Summary

The goal of our research is to develop new imaging systems that can be applied to clinical medicine now and in the future. High-dimensional, i.e., 3-dimensional (3D) and 4-dimensional (4D), imaging techniques have enabled noninvasive, realistic, uninhibited, and accurate observations of human spatial structures and their dynamics. The availability of real-time imaging using high-performance computers and medical virtual reality systems has expanded the possibilities for diagnosis, treatment, surgery, and medical education. The Institute for High-Dimensional Medical Imaging has therefore has established a system that facilitates cooperative research and development with international researchers and organizations.

Research Activities

Clinical application of high-dimensional medical imaging with real-time imaging techniques

The purpose of this project was to develop and clinically apply high-dimensional medical imaging with noninvasively obtained functional and morphological data of a living human. In this project, research was focused on developing an analysis system for real-time 4D ultrasonic images and a 4D dynamic visualization system for cardiovascular medicine. Clinically applicable methods of high-dimensional imaging were developed in collaboration with various departments of The Jikei University School of Medicine and with the Mayo Clinic (Rochester, MN, USA).

Development of a data-fusion system for image-guided surgery

A data-fusion system that enables surgeons to observe the inner structures of the surgical field during operations and data-fusion systems for laparoscopic surgery and robotic surgery have been developed with this project. A "video see-through" navigation system with a C-arm-mounted computed tomography scanner has been developed for a high-tech navigation operating room at Daisan Hospital. This year, the video see-through navigation system was applied to 2 liver operations and 1 vascular surgery operation in collaboration with the Department of Surgery.

Development of a temporal-spatial human motion-analysis system based on measurements of unrestrained whole-body movement

The research focused on temporal-spatial measurements of the whole human body in motion and the temporal-spatial quantitative analysis of motion. The whole-body movements of a subject were measured and volumetrically rendered with 3D models that were reconstructed from magnetic resonance data by means of a dynamic spatial video camera developed by the High-Tech Research Center Project. A new technique was

developed to visualize the dynamics of a patient-specific 4D skeletal system using a standardized human skeletal model and a 4D patient-specific body-surface model. We are aiming to improve the speed and accuracy of analysis for efficient application of the system for orthopedics, rehabilitation, and sports medicine.

Development of an endoscopic robot system

An endoscopic robot has been developed to overcome difficulties that occur during endoscopic mucosal resection: limited degrees of freedom, wound-closure area, and forceps-permitted torque. The robot was also built to extend the operative field for at-once safe resection of a lesion and closure of the mucosal wound within a broad area. In addition to endoscopic mucosal resection, natural orifice transluminal endoscopic surgery (NOTES), a new surgical method that penetrates the stomach wall for access to abdominal organs, was attempted. The robot consists of a stereoscopic endoscope and two manipulators. We also attempted to use the robot for vascular surgery. Several new mechanisms were developed to ensure a clear view within blood vessels, and intraoperative navigation with 4D ultrasonography was achieved this year.

Development of a surgery-simulation system and its use for laparoscopic surgery

A surgery-simulation system for laparoscopically assisted colorectal surgery has been developed in collaboration with the Department of Surgery. The system allows the spatial distribution of blood vessels to be visualized and their positions relative to other organs to be recognized. We intend to use this system for surgical training and for the preoperative planning of surgical procedures. With this system, the surgeon can manipulate patient-specific organs as elastic models and can perform simulations interactively in real-time. This year, the system was applied to the preoperative simulation of laparoscopically assisted colorectal surgery with the consent of our university's ethics committee. With this system, the range of lymph node removal and the vessels that should be processed could be decided preoperatively. We believe this system will improve the safety of surgical procedures.

Development of real-time visualization and motion-analysis system for implanted artificial joints

A real-time imaging system that can visualize the 3D location of hip joints and the skeletal system of patients and analyze their motion has been developed. This year, we developed an intraoperative pressure-measurement system for estimating postoperative hip dislocation after total hip arthroplasty. The system can be used to measure the pressure distribution of the hip joint surface during surgery. With this system, the surgeon can estimate the risk of dislocation and select the most suitable implant for each patient. This research is a collaborative study with the Graduate School of Medicine, Osaka University.

Development of a 4D human model, "Virtual Anatomia"

A 4D human model that contains the dynamics of the inner structure of the heart or the skeletal structure as well as the detailed anatomical structure of the "living human" has

been developed as a collaborative work with a software company. The human model was constructed with the whole-body magnetic resonance data set of a healthy volunteer and contains 421 parts, including the skeletal system, internal organs, and the vascular system. The model enables the structure of the entire body to be interactively visualized and analyzed in the virtual environment from any point of view. Furthermore, this 4D models allows the real-time observation of the inner structure of the beating heart and the analysis of the dynamics of the whole-body skeletal system.

Publications

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Institute of Clinical Medicine and Research

Kiyotaka Fujise, Professor and Director Akihito Tsubota, Lecturer Sadayori Hoshina, Associate Professor Koich Nariai, Lecturer

General Summary

The Institute of Clinical Medicine and Research conducts research with a focus on clinical applications as its basic approach. In 2006, the Institute thus conducted medical research associated with clarifying the etiology, diagnosis, and treatment of intractable diseases. As such, we carried out research on the relationship between oxidative stress and disease, including an analysis of the effects of oxidative stress on carcinogenesis, the role of reactive oxygen species (ROS) in physiological and pathological conditions (which was detected *in vivo* using a sensing system developed in collaboration with the Tokyo University of Science), and the usefulness of antioxidant agents. We have also performed basic research into cancer therapies, including the enhanced antitumor effect of 5-fluorouracil (5-FU) with photoactivated dihydropyrimidine dehydrogenase (DPD)-small interfering (si)RNA transfection, prevention of liver metastasis by NK-4 gene transduction, magnetically enhanced drug delivery systems, and the development of immunotherapy using fusions of dendritic cells and cancer cells. Additionally, we have carried out studies to define the pathological conditions underlying hyperlipidemia and heart disease, to clarify the actions of anesthetics against substances associated with pain, to prepare nanoparticles containing poorly insoluble drugs using an innovative procedure, and to define the pathological conditions underlying viral diseases.

Research Activities

Liver disease and oxidative stress

1. Comprehensive RNA gene expression analysis of the contribution of oxidative stress in chronic liver disease: Our group investigated how ROS produced in oxidationreduction (redox) reactions would affect the pathogenesis of fulminant hepatitis and oncogenesis in chronic liver disease, using an animal model with persistent exposure to ROS and comprehensive RNA gene expression analyses. Based on our experiments, we are now analyzing whether the findings can be applied clinically.

2. Development of a novel antioxidant agent: A candidate agent, which was developed by a venture capital company, was investigated as a novel antioxidant in an animal model. A patent application has been submitted.

Role of reactive oxygen in ovulation

Immunohistochemical detection of markers for oxidative stresses in the ovulating ovary: ROS containing superoxide are believed to play a role in ovulation. We have recently confirmed production of superoxide by using a specific sensor for it in the ovulating ovary. This year, we observed localization of oxidative stresses by using several markers for it (8-hydroxydeoxyguanosine, 4-hydroxynonenal, and hexanoyl-lysine). We observed heavy oxidative stresses in the theca interna and the theca externa and concluded that these stresses are involved in formation of stigmata as the ovum exits during ovulation.

Basic research for development of cancer therapy

1. The enhanced antitumor effect of 5-FU with the photo-activated DPD-siRNA transfection: We devised photosensitive nanoparticles by which the delivery of DPD-siRNA to gastric cancer lesions can be enhanced. 5-FU is the most widely used chemotherapeutic agent for gastrointestinal cancers; however, DPD strongly decomposes and inactivates 5-FU. To overcome these difficulties, we developed an efficient DPD-siRNA transporter through photochemical internalization.

2. The prevention of liver metastasis by NK-4 gene transduction using novel lipid nanoparticles: We developed a novel lipid-nanoparticle vector that selectively delivers the NK-4 gene to liver. NK-4 binds to the c-Met receptor without activation and competitively antagonizes hepatocyte growth factor c-Met-mediated biological activities. Moreover, NK-4 inhibits angiogenesis by blockade of basic fibroblast growth factor and vascular endothelial growth factor.

3. Magnetically enhanced drug-delivery system: We devised magnetic nanoparticles as an active targeted drug-delivery system. Through the use of a magnetic field these nanoparticles outperformed present drug-delivery systems. We are now investigating the therapeutic potential of these nanoparticles in tumor-bearing mice.

Trial for preparation of nanoparticle

An innovative procedure for preparing lipid nanoparticles for poorly water-insoluble drugs: We developed a safe and simple method of preparing lipid nanoparticles using a novel grinding mill. The greatest advantage of this method is that it does not require a harmful organic solvent. Moreover, with this method, we could obtain smaller, more-uniform nanoparticles than with current methods.

Analysis of hepatitis virus in liver disease

Analysis of the pathogenesis of chronic viral hepatitis and the development of treatment: We are aiming to improve the efficacy of antiviral treatment for chronic viral infection by analyzing viral kinetics and applying a new clinical protocol in collaboration with the Division of Gastroenterology and Hepatology, Kashiwa Hospital.

Publications

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Medical Engineering Laboratory

Hiroshi Furuhata, Professor and Director

General Summary

The Medical Engineering Laboratory has developed new ultrasonic therapeutic technologies. This year, we focused on the development of new thrombolytic treatments for patients with acute ischemic stroke; research work was supported by a research grant from the Ministry of Health, Labor and Welfare. Basic research studies in molecular medical engineering have also been continued to develop an ultrasonic transdermal drug delivery system. We have contributed to the establishment of a national nanomedicine database that includes nanothechnology information from around the world and gathers the needs of clinicians. We have also improved previously developed diagnostic techniques for measuring cerebral circulation and hemodynamic variables by means of noninvasive transcranial ultrasonography. In addition, safety problems related to electromagnetic compatibility between medical equipment and mobile telecommunication equipment have been investigated in collaboration with telecommunications and medical-equipment associations and government-related organizations.

Research Activities

Integrated system of diagnosis, analysis, and treatment for acute stroke

This system can enhance thrombolytic effects using low-frequency therapeutic ultrasound. For clinical application of this system, we have developed an instrument for holding the US probe near the patient's head and have studied the detection rate of intracranial arteries with transcranial color flow imaging. The patients with an insufficient temporal bone window can be treated with this system and a virtual sonography method.

Ultrasonic thrombolysis

We have developed a much safer and more efficient method of dual-mode ultrasonic thrombolysis combining the diagnostic power of M-mode Doppler ultrasound with low-frequency ultrasound-mediated thrombolysis with tissue plasminogen activator. We have shown that lipid bubbles produced by mixing liposomes and perfluoropropane can enhance the thrombolytic effect of recombinant tissue plasminogen activator by low frequency, 500-kHz, continuous-wave ultrasound.

Study of an ultrasound drug delivery system

We have been developing novel strategies of drug delivery using physiological energy, which includes focused ultrasound and microbubbles of acoustic cavitation, to approach the target as quickly as possible.

Ultrasonic generation of nitric oxide in tissue

We measured the nitric oxide (NO) concentration of the thigh adductor muscle of rabbits in real time using an NO electrode. The NO concentration increased with the intensity of ultrasound exposure. Ultrasonic NO generation was inhibited by inhibitors of NO synthase.

Development of a new antithrombotic stent using ion beam surface modification

Twelve commercially available bare-metal stents were implanted into the coronary arteries (left anterior descending, circumflex, and right coronary arteries) of 4 swine to compare the thickness of the neointima 1 month after implantation with administration of antiplatelet drugs. There was no significant difference in the thickness of the neointima between bare-metal stents and He⁺-ion-coated bare-metal stents.

Establishment of a nanomedicine database

A database for nanomedicine was established to gather various nanotechnologies and various clinical requirements. This database can be accessed via the Internet and includes a special nanomedicine forum.

Electromagnetic compatibility between various types of radio-wave devices and medical electrical devices

Electromagnetic interference with cardiac pacemakers by radio frequency identification devices and electric assurance systems was investigated in collaboration with associations related to such equipment. The rate of electromagnetic interference was low at short distances with several electric assurance systems and was diminished in radio frequency identification systems.

Publications

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Division of Clinical Pharmacology and Therapeutics

Shigeru Kageyama, Professor and Director

General Summary

The Division of Clinical Pharmacology and Therapeutics was established in July 1995. The aim of the division is to investigate drug treatment in internal medicine, whereas other departments of clinical pharmacology in Japan focus on registration trials, particularly phase I trials. Although evidence-based medicine has been advocated for the past 10 years, most of the evidence has come from the United States and Europe. We have performed a pilot study for a large-scale clinical trial of the treatment of hypertension to obtain evidence in the Japanese population. With the evolution of pharmacogenomics, our recent research activities in humans have focused on the relationship between drug effects and gene polymorphisms.

Research Activities

An important issue of medicine in the 21st century is to distinguish patients who are responsive and who are unresponsive and to distinguish patients who show and who do not show adverse reactions to drugs. We started a collaborative study with other institutions to examine the relationship between polymorphisms of genes of drug-metabolizing enzyme and the effects of drugs on inhabitants of an isolated island.

We have analyzed drug-metabolizing enzymes CYP2C9 and CYP2C19. Some of our results have already been applied to drug therapy.

In the treatment of hypertension, calcium channel blockers have been used in more than 70% patients in Japan. That drugs of this class reduce cardiovascular events by lowering blood pressure has only recently been confirmed in Europe and the United States. However, because cardiovascular events differ by ethnicity, confirmation in a Japanese population is mandatory. Therefore, we planned a large-scale clinical trial (Optimal Combination of Effective Antihypertensives Study) to find the best agent to use with the calcium channel blocker amlodopine. A pilot study has been completed. To obtain a sufficient sample size, we performed this study in collaboration with a Chinese group. We introduced site visits to a drug company and an institution for phase I trials for 3rd-year students assigned to our division. Although information on these sites is not included in the current medical education system, the visits were well received by students.

Since the introduction of the new good clinical practice (GCP) guidelines, clinical trials of new drugs in Japan have encountered many problems. An administrative office for registration trials was established in the hospital in February 1999, and the system for registration trials in the hospital has been reformed to meet the demands of the new GCP. Seven clinical research coordinators (CRCs), six nurses, and a pharmacist now facilitate clinical trials. The CRC have started to assist investigator-initiated trials as

well as registration trials. CRCs were introduced for all registration trials in 2004; the quality and speed of these trials were much improved. Efforts have been made both inside and outside the university to resolve problems of registration trials for developing new drugs.

I have been working as a principal investigator of the GCP study group organized by the Ministry of Health, Labour and Welfare to improve clinical trial systems.

Publications

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DDS Institute

Megumu Higaki, Professor and Director Akinori Ueno, Professor Yutaka Mizushima, Professor Tsutomu Ishihara, Lecturer

General Summary

We are now investigating a new drug delivery system (DDS) using nanotechnology. We have developed fabrication methods for 1) (poly)ethyleneglycol (PEG)-poly (D, L-lactic acid) (PLA)/PLA nanoparticles for targeting and sustained release of steroids, 2) CaCO₃ nanoparticles with insulin, and 3) intelligent antigen-responsive nanoparticles. These studies were supported in part by a grant from the Ministry of Education, Culture, Sports, Science and Technology.

Research Activities

Nanoparticle preparations of a steroid for targeting and sustained release

We have examined the therapeutic activity of betamethasone phosphate (BP) encapsulated in biocompatible and biodegradable nanopaticles consisting of PLA homopolymers and PEG-block-PLA copolymers (stealth nanosteroid), which are targeted to inflamed joints and have shown slow release and prolonged blood circulation after intravenous administration, in experimental arthritis models, including rats with adjuvant arthritis (AA rats) and mice with arthritis induced by anti-type II collagen antibodies (AbIA mice).

First, we determined the characteristics of nanoparticles, such as diameter, PEG density, BP encapsulation efficiency, BP release rate, and cellular uptake, because various types of nanoparticle can be prepared depending on different compositions and molecular weights of polymers and by various blend ratios using an oil-in-solvent diffusion method. The pharmacokinetic and biodistribution profiles were examined in normal rats, AA rats, normal mice, and AbIA mice. Furthermore the biodistribution of nanoparticles with Cy7 was determined with an *in vivo* imaging system (Optix) in CIA mice. Uptake in the RAW and LYM1 cell lines was lower and slower with stealth nanosteroid (24 hours) than with non-stealth nanosteroid without PEG (3 hours). With stealth nanosteroid nanoparticles blood circulation time was markedly higher (24 hours) and liver uptake was lower (20%) than with non-stealth nanosteroid nanoparticles (5 minutes and 70%, respectively). Furthermore, stealth nanoparticles specifically accumulated in inflamed joints in AbIA mice and remained for at least 1 week. In AA rats, the highest anti-inflammatory activity was exhibited by stealth nanosteroid nanoparticles composed of 80% PLA (molecular weight, 6,200) and 20% PEG-PLA (68: 32; molecular weight, 10,000) with a diameter of 115 nm, encapsulation efficiency of 7.1%, modest PEG density, slower release rate, and lower cellular uptake; a 35% decrease in paw inflammation was obtained in 1 day and maintained for 2 weeks with a single injection of 30 μ g of this stealth nanosteroid. In AbIA mice, a single injection of 3 μ g of this stealth nanosteroid resulted in complete resolution of the inflammatory response after 1 week. In contrast, non-stealth nanosteroid and free BP did not reduce the severity of inflammation with the same dose in both models. The observed strong therapeutic benefit obtained with the stealth nanosteroid may be due to the prolonged blood circulation, to the targeting of the inflamed joint, and to its slow release *in situ*.

$CaCO_3$ nanoparticles with insulin

This study evaluated the pharmacokinetic and pharmacodynamic effects of a transdermally delivered insulin using novel CaCO₃-nanoparticles in normal and diabetic mice. The CaCO₃-nanoparticle encapsulating insulin (nanoinsulin) was transfermally applied to the back skin of normal ddY mice and diabetic dB/dB and kkAy mice after 1 hour Serum insulin levels in ddY mice were analyzed with enzyme immunoassay, of fasting. and blood glucose levels in normal and diabetic mice were monitored with a transdermal sensor (Diasensor, Diasense, Inc., Midland, TX, USA). Maximum serum insulin was $67.1 \pm 25.9 \,\mu \text{IU/ml}$ at 4 hours with 200 μg of transdermal nanoinsulin in ddY mice, whereas that after subcutaneous injection of $3 \mu g$ of monomer insulin was 462 ± 20.9 μ IU/m at 20 minutes. Transdermal nanoinsulin decreased glucose levels in a dosedependent manner. Maximum decreases in blood glucose with 200 μ g of transdermal nanoinsulin observed after 6 hours were $48.3 \pm 3.9\%$ (ddY), $32.5 \pm 9.8\%$ (dB/dB), and $26.2\pm7.6\%$ (kkAy), whereas maximum decreases observed after 1 hour with 3 μ g of subcutaneous monomer insulin were $64.1\pm1.0\%$ (ddY), $57.9\pm3.4\%$ (dB/dB), and $24.1\pm$ 6.7% (kkAy). Insulin bioavailability until 6 hours with transdermal nanoinsulin in ddY mice was 0.9% based on serum insulin levels and 2.0% based on pharmacodynamic blood-glucose-lowering effects. This CaCO₃-nanoparticle system successfully delivered insulin transdermally, as evidenced by a significant sustained decrease in blood glucose in normal and diabetic rats. These results support the feasibility of developing transdermal nanoinsulin for human applications.

In collaboration with other institutions, we developed an immunosensor using a quartz crystal microbalance to detect dioxin, after preparing monoclonal antibody and singlechain variable fragments against tetrachlorodibenzodioxin.

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Division of Clinical Research and Development

Satoshi Kurihara, Professor and Director Masato Matsushima, Associate Professor Mitsuyoshi Urashima, Associate Professor

General Summary

Clinical study is a strategy and a science for implementing 1) efficient diagnosis/treatment/prevention, 2) discovery of etiology, and 3) understanding pathophysiology. Baron Kanehiro Takaki prevented beriberi by a change in diet based on evidence obtained from clinical study. Thus, we at The Jikei University have a responsibility to carry on and expand his passion using epidemiology and biostatistics to save lives and maintain peace in the world. However, most clinicians are busy treating patients. In addition, modern epidemiology and biostatistics have been changing along with computer science. Therefore, we have launched the Division of Clinical Research and Development as project base to support clinical studies at The Jikei University School of Medicine. Our mission is to find *veritas* in a complicated clinical world and to contribute to society with our findings. To accomplish our mission, education and promotion are major strategies.

Research Activities

The Jikei clinical research course

From September 2006 through March 2007, we held 13 seminars about strategies for clinical studies for health-care practitioners at The Jikei University School of Medicine. In 2006, small-group study courses targeting postgraduate students will be started from the principles of epidemiology and biostatistics by reading textbooks and analyzing real clinical data using STATA software and designing clinical studies. Our goal is for postgraduate students to develop the skills to construct hypotheses, design protocols, monitor trials, and analyze data.

Consulting for clinical studies

1. Inside The Jikei University School of Medicine: Most clinical departments

2. Outside The Jikei University School of Medicine: Tokyo Women's Medical University

As shown in the publications section, we published several original articles in English by collaborating with clinical departments. For fiscal year 2004, we accelerated the process from consultation to paper submission. Next year, we are planning to launch a clinical data-pooling system in our division to support high-quality prospective clinical studies.

Umbilical cord blood study

We hypothesized that exposure of fetuses to low concentrations of hazardous heavy

metals during pregnancy can cause changes in the behavior and intelligence of children. We have started to collect umbilical cord blood to measure heavy metals and have sent a questionnaire to ask about daily habits and the conditions of children after birth. We have finished enrollment, since we could collect more than 1,000 samples of umbilical cord blood. We have finished measuring levels of heavy metals in umbilical cord blood and questionnaires when the children were 2 years of age. In addition, we are performing a study of twins to examine the genetic and environmental effects on children's behavior.

Diagnosis of complication in diabetes mellitus

We could draw receiver operating characteristics curves with high sensitivity and high specificity in diagnosing complications of diabetes mellitus.

Global environmental change and human health

We have been dedicated to research on global environmental change and human health with the Japanese government.

Homeland security

Prevention is one of our missions. An extreme example of prevention may be homeland security. We attended a workshop on "G8 Forensic Epidemiology" in London. We have held a series of lectures on National Security Affairs and Crisis Management for the Cabinet and have advised the Deputy Chief Cabinet Secretary for Crisis Management.

Publications

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Laboratory Animal Facilities

Kiyoshi Ohkawa, Professor and Director Koichi Nariai, Lecturer Takamasa Iwaki, Associate Professor

General Summary

Our laboratory animal facilities have 3,000 m² of floor space and 67 rooms (including 29 animal breeding rooms, 8 laboratories, 3 operating rooms, 2 cage-washing rooms, an X-ray room, and a sterilization room) and have the capacity to breed about 10,000 laboratory animals per day. Our tasks include establishing suitable environments for new technological studies using laboratory animals and offering the knowledge we have obtained to medical and biological research. Our activities range over wide fields of basic sciences, from laboratory animal anatomy and physiology to technical development applicable to clinical medicine.

Research Activities

Publishing anatomic color atlases of the laboratory rat and mouse

We have published 3 series of the macroscopic and sectional anatomy atlases of laboratory animals (*Color Atlas of Sectional Anatomy of Rabbit*, Chikusan Publishing Co., Ltd., Tokyo, Japan, 1993; *Color Atlas of Sectional Anatomy of Rat*, Chikusan Publishing Co., Ltd., Tokyo, Japan, 1997; and *Color Atlas of Sectional Anatomy of Mouse*; Maruzen Co., Ltd., Tokyo, Japan, 2001). Plans to republish English versions of the rat and mouse atlases are advancing in collaboration with the Department of Anatomy (I).

Bactericidal activities and safety of chlorine dioxide

In June 2004, the International Agency for Research on Cancer classified formaldehyde as carcinogenic to humans. For this reason, we have explored other gas compounds that could be usedfor disinfection instead of formaldehyde gas.

We have reported several methods for generating chlorine dioxide gas and have explored whether this gas could be used for disinfection instead of formaldehyde gas. Chlorine dioxide gas is strongly oxidizing and is corrosive to steel (at concentrations greater than 100 ppm); however, we have used concentrations of approximately 30 ppm, so the adverse effects were tolerable. We believe that chlorine dioxide gas could be used for disinfection instead of formaldehyde.

Spermatozoa and spermatids retrieved from frozen whole bodies of male mice can produce normal offspring

Cryopreservation of male germ cells is a strategy for conserving animal species and strains of animals valuable for biomedical research. We tested whether male mouse germ cells could be cryopreserved without cryoprotection techniques by simply freezing

epididymides, testes, or whole bodies. Testicular spermatozoa retrieved from the bodies of male mice (BALB c nude and C3H He strains) that had been kept frozen (-20° C) for 15 years could produce normal offspring through microinsemination. We reported that spermatozoa or spermatids, retrieved from frozen reproductive organs or frozen bodies of mice, can produce offspring through microinsemination.

Immunohistochemical detection of markers for oxidative stresses in the ovulating ovary Reactive oxygen species containing superoxide were believed to play a role in ovulation. By using a specific sensor for superoxide we have recently confirmed production of superoxide in the ovulating ovary. This year, we attempted to observe the localization of oxidative stresses by using several stress markers (8-hydroxydeoxyguanosine, 4hydroxynonenal, and hexanoyl lysine). We observed heavy oxidative stresses in the theca interna and the theca externa and concluded that these stresses were involved in stigmata formation during ovulation.

Establishment and characterization of strains originated from the Japanese wild mouse and the Phodopus hamster

Originally established inbred strains derived from Japanese wild mice (*Mus musculus molossinus*) and Phodopus hamsters (*Phodopus sp.*) were maintained in this laboratory. *Phodopus* hamsters are small rodents that differ taxonomically from Syrian hamsters, which are the most commonly used laboratory hamster. We recently determined that the *Phodopus* hamster is a good candidate for a new laboratory animal and have established an inbred strain. Furthermore, we continue to establish new inbred strains or congenic strains, to develop models of human disease, and to research these strains' biomedical characteristics.

In the collaboration with the Department of Biochemistry 2, we developed 2 new mouse strains using our original wild-derived inbred strain MSKR. One strain is a congenic strain having a knockout allele of *Oaz1* derived from the B6.129-*Oaz1*tm to the MSKR background, and the other strain is a consomic strain that has chromosome 10 derived from the above-mentioned strain to the MSKR background. We have confirmed that these newly established strains are useful for researching genetic modification of *Oaz1* knockout mice.

Publications

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Radioisotope Research Facility

Kunihiko Fukuda, Professor and Director

Yukio Yoshizawa, Lecturer

General Summary

The Radioisotope Research Facility was established to support medical and biological research with isotopes. We have supported researchers by suggesting methods and practical techniques for experiments. Lectures and training sessions were held for researchers and for medical students and graduate students who are starting to work with radioisotopes. In 2006, 62 researchers from 13 departments consulted this facility for 29 studies.

We have received authorization to use 21 nuclides, including a new nuclide, ¹⁰⁹Cd.

Research Activities

The second active site of teicoplanin

Glycopeptide antibiotics inhibit peptidoglycan synthesis by binding to the terminal D-Ala-D-Ala stem of peptidoglycan precursors. Despite the many similarities in chemical and microbiological properties, teicoplanin is more efficient than vancomycin when combined with beta-lactams. The strong synergistic activity of teicoplanin with beta-lactams against methicillin-resistant *Staphylococcus aureus* suggests an additional mechanism of action. We examined the possible second action point of teicoplanin that may appear when combined with beta-lactams. The minimum inhibitory concentrations (MICs) of teicoplanin and vancomycin against *S. aureus* TS670 were determined with Mueller-Hinton agar and a hypertonic medium CYLS agar. The MIC of teicoplanin was $0.125 \,\mu$ g/ml both on Mueller-Hinton agar and on CYLS agar. In contrast, the MIC of vancomycin increased from $0.25 \,\mu$ g/ml to $1 \,\mu$ g/ml. Although CYLS medium protects bacteria from osmotic pressure, the excellent properties of teicoplanin on CYLS agar may indicate that teicoplanin can act on the bacterial membrane.

Isolation of fibronectin binding protein-deficient mutants of S. aureus

Fibronectin binding proteins (FnBPs) are expressed on the cell surface of *S. aureus* and play important roles in colonization. We isolated FnBP-A-deficient mutants by inserting a tetracycline-resistance gene into the *fnb* A gene. The deficiency was confirmed with Western blotting as a lack of the FnBP A band. These mutant strains are useful for studying the roles of FnBPs in pathogenicity.

A study of radioactivity in consumer products

Some daily products are said to contain radioactive ores that have various effects on health. We have examined 8 wallpapers that are claimed to have so-called negative-ion effects to estimate external and internal radiation doses after exposure to them.

Gamma-ray spectrometry revealed that the wallpapers contain 0.03 to 0.35 $Bq \cdot g^{-1}$ of Th-series nuclides (²⁰⁸Tl, ²¹²Pb, ²¹²Bi, and ²²⁸Ac) and of a U-series nuclide (²¹⁴Pb). Distribution of radioactive nuclides in the samples was measured with an imaging plate and a phosphoimage analyzer (FLA-2000, Fuji Photo Film Co., Ltd., Tokyo). The radiation doses from the printed side of the wallpapers were 5 to 15 times higher than those of the back side. The ²²²Rn concentrations emanating from the wallpapers in a 50-L sealed container were measured with Pico-Rad radon detectors (Accustar Labs, Medway, MA, USA). One wallpaper showed ²²²Rn concentrations 2 to 5 times higher than the background value.

Research on marine bacteria

The habitat distribution of several marine bacteria was surveyed during the *Tansei Maru* cruise KT-06-31 off Izu Peninsula. To study the metallic transport system of microbes, we focused on marine bacteria that produce siderophores in a low-iron environment. We are attempting to cultivate such bacteria from seawater and the sediment of the sea floor at a depth of 3,600 m.

Publications

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Department of Rehabilitation Medicine Division of Physical Fitness

Satoshi Miyano, Professor and Director

Hideki Yamauchi, Lecturer

General Summary

The research of our division has been focused on skeletal muscle plasticity, neuroscience, and exercise physiology.

Research Activities

Neuroscience

We have previously demonstrated the presence of activated areas in the uninjured contralateral sensorimotor cortex in addition to the ipsilateral sensorimotor cortex of the area surrounding a brain infarction using a rat model of focal photochemically induced thrombosis (PIT) and functional magnetic resonance imaging. In this model, we next used gene expression profiling to screen key molecules upregulated in the activated area. RNA was extracted from the sensorimotor cortices ipsilateral and contralateral to the focal brain infarction and from the sham-controlled cortex; the extracted RNA was hybridized to gene-expression profiling arrays containing 1322 neurology-related genes. We found that glycine receptors were upregulated in the cortices ipsilateral and contralateral to the focal ischemic lesion. To determine the preclinical significance of upregulated glycine receptors, kynurenic acid, an endogenous antagonist of glycine receptors on neuronal cells, was administered intrathecally. The kynurenic acid significantly improved behavioral recovery, as evaluated with beam walking, within 10 days after paralysis was induced by focal PIT. These results suggest that intrathecal administration of a glycine receptor antagonist may facilitate behavioral recovery during the acute phase after brain infarction.

We determined whether the ipsilateral cortex surrounding the lesion or the uninjured contralateral cortex is more important for motor recovery after brain damage after PIT in the sensorimotor cortex in rats. Recovery of motor function was examined using the beam-walking test. Motor recovery patterns did not indicate whether motor recovery was dependent on the ipsilateral cortex surrounding the lesion or the cortex of the contralateral side. The results emphasize the need for selection of appropriate programs tailored to the area of cortical damage to enhance functional motor recovery in this model.

Exercise physiology

We investigated the effects of long-term wheel running exercise and food restriction on blood adiponectin levels in male Otsuka Long-Evans Tokushima Fatty rats (26 weeks old). Both running exercise and food restriction induced similar reductions in body weight, abdominal fat volume, and plasma leptin concentrations compared with ad libitum control. Although plasma adiponectin levels were increased with food restriction, adiponectin levels did not change with running exercise. Plasma testosterone levels were higher in the running-exercise group than in either of the other two groups. A significant inverse relationship existed between plasma levels of adiponectin and testosterone for all groups. Our results suggest that 12 weeks of voluntary wheel running exercise induces different effects on plasma adiponectin level than does food restriction, despite similar reductions in body weight, fat tissue mass, and plasma leptin concentrations. We speculate that the elevated plasma testosterone concentration offsets any hyperadiponectinemic effects of body weight and fat volume reduction in exercising rats.

Publications

Abo M, Yamauchi H, Suzuki M, Sakuma M, Urashima M. Facilitated beam-walking recovery during acute phase by kynurenic acid treatment in a rat model of photochemically induced thrombosis causing focal cerebral ischemia. *Neurosignals* 2006-2007; **15**: 102-10. **Takata K, Yamauchi H, Tatsuno H, Hashimoto K, Abo M.** Is the ipsilateral cortex surrounding the lesion or the non-injured contralateral cortex important for motor recovery in rats with photochemically induced cortical lesions? *Eur Neur-* o/ 2006: 56: 106-12.

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Department of Physiology (II) Division of Aerospace Medicine

Satoshi Kurihara, Professor and Director Hiroko Toshima, Lecturer Masamichi Sudoh, Associate Professor

General Summary

Our main interests are research on gravitational physiology, aerospace medicine, and stress reactions.

Research Activities

Body fluid distribution during parabolic flight

Changes in body fluid distribution in response to the levels or the direction or both of gravity created by the parabolic flight of a jet airplane (Gulfstream II; Diamond Air Service, Aichi, Japan), were investigated in 5 healthy male subjects (22-52 years old). Distribution of body fluid was estimated with impedance plethysmography. Electrodes were placed around the chest, abdominal, thigh, and calf regions. A total of 5 experiments were performed. The period of each experiment in each subject was 1 hour per day. The gravity levels were altered from 1 g to 1.8 g during the ascending phase and then microgravity was created for 23 seconds. The levels were recovered to 1.5 g and then to 1 g during the descending phase. Such parabolic flights were repeated 12 to 15 times during a 1-hour period. The recordings were made with each subject in the sitting, upright standing with both feet, and horizontal positions. For the sitting position, the subject's knees were either bent at 120° on a seat or fully extended on a bed. Recordings were also made with the subjects in the supine and prone positions on a bed with their head pointed toward either the nose or the tail of the airplane. The greatest shift in fluid toward the lower limbs was noted when the standing subjects were exposed to 1.8 g, and the shift was reversed toward the upper body at microgravity. Similar shifts were observed in the sitting position on a seat, even though the magnitude was minor. A profound shift was not induced when the subjects were sitting on a bed. Furthermore, a slight but significant shift from the upper body toward the lower extremities was seen in a microgravity environment when the subjects were in a horizontal position with their head toward the nose of the airplane, or vice versa. These results suggest that body fluid clearly shifts toward the upper body when the Gz gravity becomes zero. The results also indicate that a slight but significant Gx-gravitydependent shift occurs in a microgravity environment, even though such a shift is not detectable in a hypergravity environment because of the greater Gz gravity.

Stress

1. The processing of stress in the human brain and stress-related disorders We performed near-infrared optical topography and recorded P300 event-related potentials, when subjects were stressed. We then investigated information processing in the human brain.

2. Brain imaging in *karuta* players by means of near-infrared optical topography and recording of event-related potentials

We recorded event-related P300 potentials and performed near-infrared optical topography in players of *karuta* (a traditional Japanese card game) during an auditory oddball task that is similar to *karuta*, and we investigated the information processing in the brains of *karuta* players.

3. Stress facilitates spontaneous platelet aggregation in healthy young persons

After arithmetic stress loading, the concentration of norepinephrine was significantly increased. Spontaneous platelet aggregation was also accelerated by arithmetic stress, which was correlated with the increase in norepinephrine concentration. Stress releases norepinephrine from sympathetic nerve terminals, and the binding of norepinephrine to α 2A-adrenoreceptors of platelets triggers platelet aggregation.

Publications

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Department of Rehabilitation Medicine Division of Sports Medicine

Minoru Shiraishi, Lecturer

General Summary

Since the Division of Sports Medicine was established as the Sports Clinic in October 1985, we have made research on sports medicine in the following subjects: 1. Elite amateur and professional athletes; 2. Middle-aged and elderly people who do sports routinely; 3. Children and adolescents who participating in sporting activities at school or sports clubs; 4. the other general sports medicine.

Research Activities

- 1. Series of researches on the long-distance runners were done. We reported about oxidation stress changes and psychological changes after long-distance running.
- 2. We reported about medical support for the road racing team in a stage race.
- 3. Studies of football contents go in many divergences. The content of the researches at this year reached many topics like, medical activity on the Japan national football team, medical care during overseas expedition, and so on.
- 4. And the other reports were established in the fields of various sports, such as cycle sports, dance sports, and ultra marathon.
- 5. Series of researches on hyperthermia were also done. We reported about changes in muscle temperature during hyperthermia. And the other studies were published about effect of hyperthermia on blood creatine kinase activity and muscle damage after long-distance running.

Publications

Ichinoseki-Sekine N¹, Naito H¹, Saga N¹, Ogura Y¹, Shiraishi M, Giombini A (Italian Natl Olympic Committee), Giovannini V (Restek Medical Device), Katamoto S¹ (¹Juntendo Univ). Changes in muscle temperature induced by 434 MHz microwave hyperthermia. Br J Sports Med 2007; **41**: 425–9.

Endoh T¹, Saga N¹, Ichinoseki-Sekine N¹, Shiraishi M, Naito H¹, Yoneda T¹ (¹Juntendo Univ). Effect of hyperthermia on muscle damage after long-distance running. Adv Exer Sport Physiol 2006; **12:** 99.

Saga N¹, Ichinoseki-Sekine N¹, Endoh T¹, Shiraishi M, Naito H¹, Katamoto S¹ (¹Juntendo Univ). Effect of hyperthermia on blood creatine kinase activity. Adv Exer Sport Physiol 2006; **12**: 106. Ichinoseki-Sekine N¹, Saga N¹, Endoh T¹, Shiraishi M, Naito H¹, Katamono S¹ (¹Juntendo Univ). Changes in muscle temperture during microwave hyperthermia. Adv Exer Sport Physiol 2006; **12**: 106.

Ushijima F, Shiraishi M, Sato M, Nakajima Y, Nakamura Y. Medical support for the road racing team in a stage race (in Japanese). Tokyo Jikeikai Ikadaigaku Zasshi (Tokyo Jikeikai Med J) 2006; 121: 278. Nakajima Y, Shiraishi M, Ushijima F, Sato M, Nakamaura Y. Physical performance of deaf adult (in Japanese). Tairyoku Kagaku 2006; **55:** 724. Kohno T¹, Fujiya H¹, Kato H¹, Seki H¹, Yatabe K¹, Terawaki F¹ (¹St Mariana Univ), Nakajima Y, Kawasaki Y (Chu-o Gakuin Univ), Ohta M (Daito Bunka Univ). Oxidative stress changes after long-distance running (in Japanese). Tairyoku Kagaku 2006; 55: 769. Ushijima F, Shiraishi M, Sato M, Nakajima Y. Nakamura Y. Medical support for the road racing team in a stage race (in Japanese). Tairyoku Kagaku 2006; 55: 860. Yatabe K¹, Kohno T¹, Fujiya H¹, Kato H¹, Seki H¹, Terawaki F1 (1St Mariana Univ), Nakajima Y,

Reviews and Books

Shimizu K¹, Aoki H¹, Morikawa T (Kawatetsu

Chiba Hosp), Shiraishi M, Kohno T, Kato H¹, Beppu M¹ (¹St Mariana Univ). Medical activities on the Japan National football team. Seikeigeka Sports Igakushi 2006; **25:** 350-4.

Shiraishi M. Medical care during overseas expedition of the Japan National football team. *Rinsho Sports Igaku* 2006; **23:** 513-20.

Health-Care Center

Mikio Zeniya, Professor and Director Takashi Wada, Associate Professor Yoichi Sakamoto, Professor Takekazu Onda, Associate Professor

General Summary

Shimbashi Medical Checkup Office: The research efforts of our center are focused on lifestyle-related diseases.

Research Activities

Shimbashi Medical Checkup Office

1. Preventing lifestyle-related disease through healthy habits

Our mnemonic or motto for promoting healthy habits is "none of one, less of two, more of three." The "one" behavior is smoking. The "two" things are intake of food and alcohol. The "three" things are exercise, rest, and enjoyable activities. We studied the correlation of these six healthy habits with serum triglyceride levels. A questionnaire about compliance with our motto was filled out by 5,584 Japanese men undergoing medical health check-ups. Serum triglyceride levels were measured. Persons receiving medication were excluded. The decrease in triglyceride levels was correlated with the number of healthy habits practiced (161-mg/dl for 0, 156 mg/dl for 1, 140 mg/dl for 2, 127 mg/dl for 3, 115 mg/dl for 4, 104 mg/dl for 5, and 94 mg/dl for 5). We also investigated the correlation of the number of these healthy habits practiced with serum high-density lipoprotein (HDL)-cholesterol levels. The increase in HDL-cholesterol levels was correlated with the number of healthy habits practiced (53 mg/dl for 0, 56 mg/ dl for 1, 58 mg/dl for 2, 58 mg/dl for 3, 60 mg/dl for 4, 62 mg/dl for 5, and 63 mg/dl for 6). Practicing these 6 healthy habits helps prevent low-HDL cholesterolemia and dyslipidemia.

Publications

Wada T, Fujishiro K (Toho Univ). The first strategy for metabolic syndrome: healthy habits "Give up one, Reduce Two, and Increase Three" (in Japanese). *J Jpn Coll Angiol* 2006; **46**: 341–4.

Reviews

Wada T, Fukumoto T. Glucose tolerance examination in medical checkup and ningen dock (in Japanese). *Prog Med* 2006; **26:** 2097–100.
Premedical Course

Japanese

Ikuko Noro, Associate Professor

General Summary

Suitability for patients of printed materials written in Japanese

Two kinds of informed-consent documents used in a regional hospital in Nishinomiya City were evaluated by 400 patients. At the same time, 100 physicians and nurses working at the hospital attempted to predict the results of patients' evaluation. There were 3 main findings: patients understood only 60% of the contents, patients regard the feeling of ease to be as important as readability when reading informed consent documents, and physicians and nurses underestimated the degree of patients' understanding.

Methodology for analyzing medical interviews in Japanese

I held a workshop about how to use the Roter Interactional Analysis System, a well-known method for analyzing medical interviews, with other researchers.

Research Activities

Presentations

"Modifying SAM's readability component for the Japanese language." Bull Hijiyama Univ No. 13, 2006.

Social Science (Law)

Ryuichi Ozawa, Professor

General Summary

Problems of constitutional law in present-day Japan.

Research Activities

I addressed the problems of constitutional law in present-day Japan, especially pacifism, parliamentary democracy, the welfare state, and the judicial system. I presented a paper at a seminar titled "Democracy Representative and Government of Opinion" at the 7th seminar on French-Japanese public law sponsored by the University of Montpellier I. I also reported at a seminar titled "Unequal Society and Constitutional Law" at the

"Unequal Society and the Safety-Net" working party of the Legal Committee of the Science Council of Japan.

Publications

Ozawa R. Judicial reform and Japanese constitutional law. *Ho no Kagaku* 2006: **36:** 14-20. *Ozawa R.* Constitutional reform vision of Japanese business circles. *Jichi to Bunken* 2006; **25:** 78-86.

Ozawa R, Legal profession in critical era. *Kenpou Riron Sosho* 2006: **15:** 151-63. *Ozawa R.* A case of posting in Katushika. Extra edition of Houritu-jihou. 2006. p. 279-83.

Human Science

Takao Fukuyama, Professor

General Summary

The study of modern German philosophy and ethics.

Research Activities

I have been working with other Japanese scholars of philosophy on a Japanese translation of Jürgen Habermas's anthology, *Between Naturalism and Religion*, which is to be published in 2008. In particular, I worked on the two chapters, "Public Space and Political Publicity" and "Pre-political Foundation of the Democratic Constitutional State?" Habermas is a leader of the Frankfurt School, which was founded 1924 and studies the relation of economy, culture, and the individual in a comprehensive program called "critical theory." Recently Habermas published *Facticity and Validity*, in which he searches for the ethics of discussion in the constitutional state and emphasizes the importance of solidarity in democracy.

English

Osamu Ohara, Professor

Tetsuro Fujii, Associate Professor

General Summary

English audiovisual education and the history of the English language (Ohara)

English Language Communication and Education: Material Analysis and Development (Fujii)

Ohara continued his study of graphology concerning the letters of Margaret Paston and the letters of the Celys in the fifteenth century. Ohara also continued an investigation

of how to make useful digitized images and XML files of fifteenth century manuscripts, especially of the *Cely Letters*, together with Dr. Karina H. van Dalen-Oskam of the Huygens Institute (The Hague, the Netherlands) and Professor Katsuhide Sonoda of the University of Hokkaido. The results of this investigation were presented at a session of the 78th Conference of the English Literary Society of Japan at Chukyo University. Fujii studied the communicative and learning strategies for second-language learners. He also analyzed materials for learners, with a focus on dictionaries, vocabulary, testing, and extensive reading. He took an action research approach to find ways of making students autonomous language-learners and to develop materials that facilitate language acquisition.

Research Activities

Ohara read a paper at a session titled "Making Electronic Files of Medieval Documents and their Use" at the 78th General Meeting of the English Literary Society of Japan held at Chukyo University in Nagoya. In this paper, Ohara compared the graphemic and graphetic characteristics of Richard I and Richard II, showed that there are three different hands under the name of Richard I, and demonstrated that we could revise the current edition and make the useful XML text of the letters.

Fujii compiled two vocabulary textbooks for second-language learners which are based on teaching theories and research findings on vocabulary usage on the Test of English for International Communication. Fujii also presented about lexicography in "The Characteristics of Sample Sentences that Best Illustrate Target Lexemes" at the 45th national conference of the Japan Association of College English Teachers in Osaka in September 2006 and about "Comprehensible Example Sentences from Learner Perspectives" at the 3rd Vocabulary Research Conference of the Japan Association of College English Teachers in Tokyo in December 2006.

Publications

Ohara O. "Re-examination of the amanuenses of Margaret Paston." in: English Corpus Studies, No. 13. 2006. p. 131–5.

Books

Fujii T, Miyano T. Ultimate vocabulary for TOEIC TEST basic 2200 (in Japanese). Tokyo: Goken Publishing Co; 2006.

Fujii T, Miyano T. Ultimate vocabulary for TOEIC TEST advanced 2700 (in Japanese). Tokyo: Goken Publishing Co; 2006.

German

Yoshiaki Shirasaki. Associate Professor

Research Activities

Literature in the era of poetical realism

On the basis of earlier historical and regional research on German poems of the 19th century, I have concentrated on clarifying realism as a Pan-European concept of literature with reference to the traditional thoughts of the ancient Greeks and the scholarly ideas of realism.

Schiller

Friedrich Schiller has been regarded merely as a representative poet of German classicism or as a typical dramatist of German idealism. I intend to show his versatility by using a comparative method that would clarify his various influences upon European literature and political history.

Noh and German literature

Noh, one of Japan's traditional theater forms with a consitent style and high artistry, includes a general character that is understood in the West. Noh is traditionally discussed in relationship with modern European theatres, and Paul Claudel and Yeats have also produced some great works that show Eastern influences. However, in addition to direct connections, both theatres' forms can be identified in German literature, such as the works Brecht and Hofmansthal. Here, I intend to clarify the relationship between Noh and Baroque theater through structural comparison.

Reviews

Shirasaki Y. Schiller und Europa, Eine Forschungsbericht aufgrund eines Aufsatzes von P.M. Luetzerer Schiller u. Europa, Tokyo 2006.

Mathematics

Kanji Suzuki, Professor

General Summary

1. Mathematical Logic: We have considered how the if-then statement $P(x) \rightarrow Q(x)$ should be defined and concluded that, at the first stage of higher mathematical education, the definition in mathematical logic should not be introduced. Our assertions are

described below.

2. Calculus: In the double integral, there is a pair of changes of variables: one change is to polar coordinates, and the other change is x=uv, y=u(1-v). It is reasonable to teach these two concepts correspondingly.

Research Activities

1. The statement $P(x) \rightarrow Q(x)$ means $[\forall x, (P(x) \rightarrow Q(x))]$ in mathematical logic, while, in our assertions it means [When we put $A = \{x \mid P(x)\}, \forall x \in A, P(x)]$.

2. The measure of angles and the ratio of line segments are two fundamental concepts in elementary geometry. Using the angle θ , the change to polar coordinates is described geometrially. Similarly, the change x=uv, y=u(1-v) can be expressed geometrically using the ratio of line segments and should not be omitted.

Publications

Suzuki K, Fukuda K (Tokyo Metrop Univ), Eto K (Nippon Technol Univ). On 'any' and 'some' : a proposal of teaching methods different from that in mathematical logic (in Japanese). Tokyo: Mathematics Education Society of Japan (a special edition); 2006, 9. Suzuki K, Fukuda K, Eto K. Two typical ways concerning change of variables in multiple integral: polar coordinates and 'simplicial' coordinates (In Japanese). Tokyo: Mathematics Education Society of Japan (a special edition); 2007, 3.

Physics

Koichi Satoh, Associate Professor

Katsumi Kasono, Lecturer

General Summary

Phospholipid membranes have been studied from several points of view, including form formation, phase transitions, interaction with ions, and optical characteristics.

Research Activities

Investigations of the biaxial liquid crystal phase of rectangular molecules A simple simulation model is assumed to be presented by tightly connected rigid spheres. We have performed Monte Carlo simulations with constant pressures on a system of 512 molecules consisting of 5×2 spheres. We have found a crystal phase and smectic phases at low densities (<50%).

Review and Books

Satoh K, Fujishiro T. Physics for college students in medical and its related fields (in Japanese). Tokyo: Tokyo Kyogakusha; 2007.

Chemistry

Tomoyoshi Takahashi, Professor

Chikao Hashimoto, Associate Professor

General Summary

The research of our laboratory is focused on the synthesis of biologically active compounds and the modification of synthetic reactions.

Research Activities

Reaction of α -halo ketone with 2-aminothiol

We previously reported a new type of condensation between α -bromosteroidal ketones and 2-aminothiol which yields α -ketospirothiazolidine derivatives. In the resulting product, the oxo group has migrated to the original position occupied by the bromine atom. Moreover, to clarify the generality of the migration of the oxo group, the reaction of cyclic and acyclic α -halo alkanones with aminothiol yielded the corresponding thiazolidine derivatives. In addition, the use of microwaves was found to improve yields and shorten the reaction time in comparison with the usual conditions.

Synthesis of N-protected dipeptide acids using amino acid-metal salts

The application of amino acids (H_2 N-AA-COOM; M: metal ion) with a carboxyl group protected by a metal ion as an amino component has been investigated for the synthesis of *N*-protected dipeptide acids. The use of metal ions to protect the carboxyl group saves the time needed to introduce and remove the protecting group and prevents side reactions caused by the use of esters. Therefore, we examined the couplings between various amino-acid-alkali-metal salts and active esters of Boc amino acids. We found that the coupling of Boc-Ala-ONp and H_2 N-AA-COONa in dimethylformamide water as a reaction solvent yields a desired dipeptide, Boc-Ala-AA-OH, in large quantities.

Publications

Hashimoto C, Takeguchi K¹, Kodomari M¹ (¹Shibaura Inst Technol). An efficient synthesis of *N*-protected peptide acids using the Cacarboxylate salts of amino acids and peptides. *Peptide Sci* 2006; 40–1.

Horiuchi CA, Saitoh Y, Utsukihara T, Takahashi S, Matushita M, He L, Takahashi T, Hashimoto C,

Sugiyama T¹ (¹Kyoto Univ), Nozaki S² (²Josai Univ). Reaction of α , β -unsaturated ketones with cerium (IV) salts in alcohol. Appl Organometal Chem 2006; **20:** 663–8.

Biology

Osamu Terasaka, Professor

Rie Hiratsuka, Lecturer

General Summary

The main research subject of our laboratory is the reproductive system of seed plants. Our research is now focused on the relation between pollen tube growth and the programmed cell death of pollen tube conducting tissue.

Research Activities

Involvement of nucellar cell death in pollen tube elongation in gymnosperms: A comparison between Pinus densiflora and Cryptomeria japonica

To clarify the relation between pollen tube elongation and nucellar cell death in gymnosperms, we compared P. densiflora and C. japonica, which require 14 months and 3 months, respectively, from pollination to fertilization. Pollen tube branches formed in both species, but the tube protrusion that invades the nucellar cell was observed only in *P. densiflora*. The release of vesicles from dying nucellar cells and endocytosis of the vesicles by tube occurred in both species. Tube walls in both species were labeled with the monoclonal antibody JIM13 (against arabinogalactan protein) but not with JIM7 (against pectin). In *P. densiflora*, nucellar cells in the layer around the tube died and were TUNEL-positive, but in C. japonica only cells in direct contact with the tube died and were TUNEL-negative. During nucellar cell death, chromatin condensation, autophagy, and secondary wall formation were observed only in *P. densiflora*. Starch grains accumulated in the nucellar cells around the tube tip of *P. densiflora* but not in C. japonica. These findings suggest that nucellar cell death in P. densiflora is apoptosis-like and participates in the formation of the aisle and the supply of nutritional materials for tube elongation. In C. japonica, nucellar cell death is not apoptosis-like, and the dead cells supply nutrient materials to the pollen tube less actively than do those in *P. densiflora*. We assume that the differences between *P. densiflora* and *C. japonica* result from differences in the required interval from pollination to fertilization.

School of Nursing

Basic Nursing I

Sawako Haga, Professor Mayumi Kikuchi, Lecturer Machiko Hirao, Associate Professor

Research Activities

The research activities of the basic nursing group can be divided into the following 3 areas.

Haga has been investigating the effects of physical assessment by nurses, the evidence of nursing skill, and the history of nursing.

Hirao has been investigating the history of nursing and Florence Nightingale's thoughts about nursing.

Kikuchi has been investigating the effects of nursing education, methods of teaching, and nursing diagnosis.

Reviews and Books

Haga S. The Jikei nurses which were produced the nursing practice of Jikei Hospital (in Japanese). J Nurs Educ 2006; 47: 1024-33.

Ebina M, Hirao M, Haga S. A study of Dr. Kanehiro Takaki's thought on health education (1): from his opinions related to the improvement of elementary school education (in Japanese). *J Jpn Soc Med Hist* 2006; **52:** 62–3.

Nakamura S, Hirao M. Hirano J. Author of "Youjyou Ketu"(1835), and his thought on health regimens (in Japanese). *J Jpn Soc Med Hist* 2006; **52:** 120–1.

Oishi S, Kita K, Hirao M, Haga S. Censorship in occupied Japan(2)—Publication of "Magokoro" and "Hokenfu" in Kyousyu (in Japanese). *J Jpn Soc Med Hist* 2006; **52:** 54-5.

Kikuchi M, Hanyu C, Kita K, Hirao M, Haga S. Recognition of student nurses before clinical practicum for medical accident-a comparison before and after the study class (in Japanese). Proceedings of the 16th Academic Conference Japan Academy of Nursing Education. 2006. p. 154.

Oishi S, Haga S, Hirao M. The realities of the Japanese journal "Hokenhu Jigyou" from the prange collection of the University of Maryland(2) (in Japanese). Proceedings of the 26th Academic Conference Japan Academy of Nursing Science. 2006. p. 128.

Oishi S, Kita K, Hirao M, Haga S. Publication Circumstances of the JNA Bulletin "Kango" Revealed on Analysis of Materrials in the Gordon W. Prange collection (in Japanese). J Jpn Soc Nurs Hist 2006; **19:** 18-23.

Hirao M, Haga S. The significance and contents of the journal "Kangohu" of which published by Hora Seki before world war II (in Japanese). Proceedings of the 26th Academic Conference Japan Academy of Nursing Science. 2006. p. 126.

Fujino S, Sakurai M, Hanyu C, Hukaya C. An evaluation of all classes by students for the teaching improvement (in Japanese). Proceedings of the 16th Academic Conference Japan Academy of Nursing Education. 2006. p. 88.

Kita K, Kikuchi M, Hanyu C, Hirao M, Haga S. Temperature measurements of disposable Glycerin Enema-I-using radiation thermometer (in Japanese). Proceedings of the 5th Academic Conference Japan Society of Nursing Art and Science. 2006. p. 101.

Kita K, Kikuchi M, Hanyu C, Hirao M, Haga S. Temperature measurements of disposable Glycerin Enema-II-difference of surface temperature and central temperature (in Japanese). Proceedings of the 5th Academic Conference Japan Society of Nursing Art and Science. 2006. p. 102. Saito A, Kuroda Y, Takahashi Y, Yanagiya H, Kikuchi M. Qualitative inquiry for appropriateness of nurse's clinical judgment that was diagnosed for "acute pain" to patients conditions-case analysis from nursing records (in Japanese). J Jpn Soc Nursing Diagn 2007; 12: 14-26.

Basic Nursing 2

Sugino Oishi, Associate Professor

General Summary

To clarify the characteristics of the Japanese nursing system after World War II, I have been studying the history of nursing and analyzing the factors affecting the Japanese nursing system.

Research Activities

Analysis of factors affecting the Japanese nursing system

I created a database about the nursing system, nursing education, the medical system, public health, and factors affecting the nursing and medical systems in Japan from 1974 through 2004. I intend to revise the database annually.

To predict changes in the nursing education system and nursing, I analyzed the data and identified factors that had significant effects on Japanese nursing and the nursing education system. I presented the results in my lecture on informatics science.

Study of the policy of the Nursing Affairs Division GHQ by analyzing censorship by the GHQ

The results of censorship of medical and nursing publications from 1945 through 1949 in Japan are thought to be housed in the Gordon W. Prange Collection of the University of Maryland. The purpose of this study was to investigate the circumstances of publishing and the criteria for censorship by General Headquarters Supreme Commander for the Allied Powers (GHQ) in occupied Japan. The Nursing Affairs Division of GHQ censored the Japanese Nurses Association's journal before it was censored by the Civil Censorship Detachment of GHQ.

Historical research on Japanese nursing reform after World War II

The Nursing Affairs Division of GHQ revised the nursing law and the nursing system during period of occupation. The present Japanese nursing system is based on the nursing reforms of the GHQ but has been modified because of economic and educational problems. I obtained information from the GHQ records and related persons to investigate nursing reform. I introduced the methods of historical investigation and presented the results of my study to students in my lecture on nursing management.

Publications

Oishi S, Kita K, Hirao M, Haga S. Publication Circumstances of the JNS Bulletin "Kango"

Revealed on analysis of the Materials in the Gordon W. Prange Collection (in Japanese). J

Jpn Assoc Historical Studies Nursing 2006; **19:** 18–23.

occupied Japan(2): publication of "Magokoro" and "Hokenfu" in Kyushu. *J Jpn Soc Med Hist* 2006; **52:** 54-5. *Oishi S.* The biography of C. Sumiyoshi (in Japanese). *Jpn J Nurs Educ* 2006; **47:** 1012-9.

Reviews

Oishi S, Kita K, Hirao M, Haga S. Censorship in

Adult Nursing

Chieko Fukaya, Professor Yukie Kitayama, Lecturer Shoko Fujino, Associate Professor

Research Activities

We study the acquisition of nursing skills in clinical adult-nursing practice. We examined the achievements of nursing skills after clinical adult nursing practice. We have reported that our students have successfully acquired nursing skills and that

their skills compared favorably with those of students from other schools. We reported our findings at the 17th Japan Academy of Nursing education meeting in 2006.

Fujino has investigated and discussed the use of purposeful touch by nurses in cancer care.

Kitayama is investigating the nursing of patients with various skin conditions, such as pressure ulcers, stoma, wounds, and foot ulcers.

Reviews

Fujino S. Touch on terminal patients (in Japanese). Hoken no Kagaku 2006; 48: 509-14.
Fujino S. Caring and touch on nursing 1 (in Japanese). Rinsho Kango 2006; 32: 1350-5.

Fujino S. Caring and touch on nursing 2 (in Japanese). Rinsho Kango 2006; 32: 1505-10.

Fujino S. Skills of caring touch (in Japanese). Rinsho Kango 2006; 32: 1641-7. Fujino S. The effective of touch on patients (in Japanese). Rincho Kango 2006; 32: 2011-7. Fujino S. Characteristics and coping patterns using purposeful touch by male nurses (in Japanese). Rinsho Kango 2006; 32: 2147-52.

Gerontological Nursing

Miyoko Sakurai, Professor

Kumiko Date, Associate Professor

General Summary

Since we added new staff members to our teams, we have had a marked increase in educational and research activities. The main research interest of our teams is nursing interventions for elderly persons with chronic health problems and for their family

members. The main subject in our division is preventing pressure ulcers in elderly persons.

Research Activities

Research on fatigue of family caregivers for elderly persons with dementia Sakurai et al. have been investigating the psychological conflicts of family caregivers for the elderly persons with dementia.

Relationship between health and lifestyle in elderly adults

Date continued a study of acquisition of health behavior in elderly adults with lifestylerelated diseases and nursing intervention. In recent years, Date has been investigating the health status of middle-aged and elderly adults and various factors influencing it for primary and secondary prevention of lifestyle-related diseases from a comprehensive perspective, including nutrition, exercises, and rest.

Publications

Natori S¹, Miyazawa K¹, Tuji K¹, Nagasaki H¹, Motizuki E¹, Fushimi M¹, Date K (¹Yamanashi Univ Hosp). A comparison of difficulties of self-, management of patients of total laryngectomy with their families' care (in Japanese). Yamanashi Nursing J 2006; **5**: 49–55.

Shida K¹, Yoda Y¹, Takahashi H¹, Saito M¹, Ishikawa M¹, Date K (¹Yamanashi Univ Hosp). Practice and perception of the chest physical therapy of nurses on preoperative nursing (in Japanese). Yamanashi Nursing J 2007; **5**: 25– 9

Miyamoto T¹, Date K, Iijima S² (¹Health Center, Kofo City² Yamanashi Univ). The way how municipal public health nurses grasp the child: care problem at the regular health checkups (in Japanese). *J Child Health* 2006; **63:** 322–30.

Reviews and Books

Date K. The meaning of communication in nursing care (in Japanese). In: Communication in nursing. Tokyo: Kyoiku-Shuppan; 2006. p. 97–9.

Date K. The main factors of nursing communication (in Japanese). In: Communication in nursing. Tokyo: Kyoiku-Shuppan; 2006. p. 146-7. **Date K.** The communication process in nursing. (in Japanese). In: Communication in nursing. Tokyo: Kyoiku-Shuppan; 2006. p. 148-51.

Mental Health and Psychiatric Nursing

Toshiko Ikebe, Professor

Setsuko Hayashi, Lecturer

Research Activities

We studied the support given to persons with mental disorders in the community. This time, we investigated feelings of difficulty about the single life of group-home residents with mental disorders and the support given to outpatients with mental disorders in the nursing-support room.

Hayashi investigated the teachers' conflicts related to nursing students with mental health problems.

Publications

Hayashi S, Shibata M (Kitasato Univ). Teacher conflict related to students with mental health problem: interviews with the nursing school

teacher (in Japanese). *Nippon Kangokenkyu Gakkaizasshi* (J Jpn Soc Nurs Res) 2006; **29:** 49–57.

Child Nursing

Kiyo Hamanaka, Professor

Kayo Cho, Lecturer

Research Activities

The present conditions and problems of basic education in child nursing Last year a questionnaire survey on education about the death of a child was performed, and this year an interview survey study was performed with the cooperation of nursing schools for 3 years.

The present conditions and problems of postgraduate education in child nursing Semistructured interview to head nurses showed that effects of a decrease in training time and the specificity of child care were factors of difficulty of new face education. The findings were presented at a congress.

A study of the organization of practice in nursing with outpatients and outpatient nursing to promote health promotion of children in basic education

This year, Hamanaka started a joint study with many institutions with the support of a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology. A nursing basic education organization (nursing program in university) and a medical institution (medical offices of the entire country, a polyclinic, a pediatric hospital) were investigated to clarify the present conditions and problems of basic education for outpatient nursing. In addition, through a visit and exchange of opinions with British universities and four pediatric hospitals, cooperation in basic education and the practice of health promotion support of children was examined.

The present conditions of the social growth process of children with end-stage renal and drafting a support plan by offering information

Cho conducted interviews of children with end-stage renal disease. The purpose of this study was to clarify methods to deal with problems that patients faced in the process of social growth and to make a pamphlet for patients and families about a school life entering a school of a higher grade, and finding employment. This information may become fundamental knowledge for support organizations for patients and families.

Maternity Nursing

Kimiko Kayashima, Professor

Marie Shimada, Associate Professor

Research Activities

This study examined various health issues experienced by women at each stage of their lives and explores how nursing assistance may be provided to address them.

1. Use of postpartum lactation consultation and its effect on the rate of breastfeeding 1 month after childbirth

A survey was conducted of 138 women up to 1 month after childbirth concerning their use of postpartum lactation consultation and rate of breastfeeding. We found that 52.2% of mothers used postpartum lactation consultation, with primiparous women using the service at a higher rate than did multiparous women. The results also indicted that primiparous women who did not use the consultation service had a lower breast-feeding rate. Also, mothers with light-for-dates babies switched from breastfeeding to combined feeding at a higher rate.

2. Trend of research concerning breastfeeding: Analysis of Japanese documents over the past 5 years

The promotion of breastfeeding is cited in the national campaign "Sukoyaka Family 21" by the Ministry of Health, Labor and Welfare, but the rate of breastfeeding has yet to increase. A total of 53 research papers concerning breastfeeding over the past 5 years have been examined to identify the direction and tasks for future research and initiatives. This examination identified factual studies based on clinical practice, including, in particular, a study of continuous follow-up from childbirth to the 1-month checkup. The studies categorized study findings into "care factors for facilitating the continuation of breastfeeding" and "factors inhibiting the establishment of breastfeeding."

Community Nursing

Noriko Okuyama, Professor

Miki Shimada, Associate Professor

Research Activities

Community health nursing education

In a society with an aging population and a falling birthrate, nurses engaged in community health nursing must possess rapid responsiveness, cooperation, ethics, morals, and, most importantly, the ability to respond to drastically changing social demands. The aim of our research is to examine whether early exposure is an effective learning method for student nurses in community health nursing education.

Community health activity based on the health promotion Okuyama performed a study of preventive care for bedridden patients. Shimada performed a study of a community-based end-of-life care system. Shimizu performed a study of the lifestyle-related-disease prevention and evaluated an activity community health group.

Publications

Hatono Y, Hirano K, Shimada M. A survey of community health care programs for the prevention of housebound elderly. Science and technology research project in Ministry Health and Welfare, 2007.

Reviews and Books

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Research Activities 2006

The Jikei University School of Medicine

〔非売品〕

平成20年3月1日 発行

発 行	人	栗 原 敏
編集	者	「教育・研究年報」 編集委員長 兼 平千裕
		英文研究年報担当(JMJ 編集委員長) 松藤千弥
発	行	東京慈恵会医科大学
		〒105-8461 東京都港区西新橋 3-25-8 雪手 03-2423 1111
印 刷	所	笹氣出版印刷株式会社
		〒 984-0011 仙台市若林区六丁の目西町 8-45 電話 022-288-5555

Published by THE JIKEI UNIVERSITY SCHOOL OF MEDICINE Tokyo, Japan