Department of Laboratory Medicine

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General Summary

Research projects in our department in 2010 were concerned with clinical physiology, clinical microbiology, clinical chemistry, hematology, cardiology, clinical cell biology, and clinical psychiatry. Research achievements in each division are described below.

Research Activities

Clinical physiology
This study examined the effects of long-tem caffeine intake and regular exercise, as well as their combination, on muscle triglyceride and glycogen content in the liver and skeletal muscle, an indicator of insulin resistance. Twenty-four Otsuka Long-Evans Tokushima fatty rats, an obese-diabetic animal model, were assigned to a sedentary group, an exercise group, a caffeine group, and a caffeine and exercise group for 5 weeks of treatment and then compared with a group of control rats. The caffeine group and the caffeine and exercise group were fed rat chow containing 0.25% caffeine, and the exercise group and the caffeine and exercise group were encouraged to exercise every day. The pretreatment and posttreatment levels of serum biochemical components were measured after overnight fasting, and the posttreatment triglyceride and glycogen contents in the liver and quadriceps femoris were measured. Combined long-term caffeine intake and exercise expenditure improves insulin resistance in both the liver and skeletal muscles and metabolic syndrome risk factors in Otsuka Long-Evans Tokushima fatty rats more effectively than does caffeine intake or exercise alone.

Clinical microbiology
Blood stream infection in children was studied with the Multiplex PCR System (GenScript USA, Inc., Piscataway, NJ, USA) (in combination with Seeplex sepsis tests [Seegene, Seoul, Korea] and the MultiNA DNA/RNA analysis system [Shimadzu Corp., Kyoto]). This system has the potential to rapidly and easily identify bloodstream infections.
Molecular epidemiologic analysis of *Clostridium difficile* was performed. Most strains were characteristic of toxin A-negative, toxin B-positive variant strains.
Some clinically isolated, previously unidentified bacteria strains were identified though gene sequencing of polymerase chain reaction-amplified 16S ribosomal RNA.
Methods have been improved for the molecular detection and identification of bacteria species from paraffin-embedded tissues.

Clinical chemistry

1. Principal research interests are to clarify the pathophysiology of atherosclerosis in relation to impaired lipoprotein metabolism and oxidized low-density lipoprotein and to develop methods to assess cardiovascular disease risk, including the application of our high-performance liquid chromatography (HPLC) method to determine cholesterol levels of lipoproteins.

We reported the following study findings.

(1) The significance of very low density lipoprotein (VLDL) cholesterol measurements with our established HPLC lipoprotein analysis method for monitoring the exercise-induced amelioration of lipid metabolism and taking longer time for the improvement of adiponectin and insulin resistance than for the amelioration of VLDL metabolism were verified and published in the *Journal of Atherosclerosis Thrombosis*.

(2) The HPLC method was revised to measure lipoprotein a in a collaborative project and reported in the *Journal of Lipid Research*.

(3) Because of the advantages of this HPLC method, Hiroshi Yoshida was awarded the Life Science Prize at the 56th National Congress of Japanese Society of Laboratory Medicine, and his paper was published in *Rinsho Byori*.

(4) The clinical features of malondialdehyde-modified LDL, a novel method of oxidized LDL measurement, and the clinical significance of oxidized lipoproteins were presented at a symposium of the 56th National Congress of Japanese Society of Laboratory Medicine and was published in *Rinsho Byori* in an article entitled "Frontlines of oxidized lipoprotein research."

(5) Atherosclerosis-related serum lipid markers (remnant lipoprotein cholesterol and small, dense LDL) presented at a symposium of the 41st Annual Meeting of the Japan Society of Clinical Laboratory Automation was published in the *Japanese Journal of Clinical Laboratory Automation*.

(6) The ameliorating effects of astaxanthin on triglycerides, high-density lipoprotein cholesterol, and adiponectin were presented in a workshop at the 15th Congress of the International Atherosclerosis Society (Boston, MA, USA) and were described in a paper published in *Atherosclerosis.*

(7) Subanalysis by sex from findings of the Jikei Heart Study was published in the *Journal of Hypertension*; (8) A review paper entitled "Mechanisms of LDL oxidation" was published in *Clinica Chimica Acta* in response to an invitation from the journal's editor.

2. Successful eradication of *Helicobacter pylori* is extremely important for preventing the progression of gastroduodenal diseases. A triple regimen combining a proton-pump inhibitor (PPI) and 2 antibiotics (clarithromycin and amoxicillin or metronidazole) is now considered the gold standard therapy for eradicating *H. pylori*. However, the eradication rate of first-line treatment is only 70% to 85% and has tended to decrease because of increasing resistance to clarithromycin and metronidazole. In the present study we examined the susceptibility of *H. pylori* to antibiotics and a PPI using *in vitro* determination of the minimum inhibitory concentration (MIC) and the patient's genetic polymorphism of cytochrome P450 2C19 (CYP2C19), an enzyme that metabolizes PPIs, in 40 patients with *H. pylori* infection and dyspeptic diseases in whom first-line eradication therapy had failed. Then we have eradicated such infected *H. pylori* by more suscept-

able triple selected regimen. The rate of successful eradication with second-line or third-line therapy was lower in homo/hetero extensive metabolizers than in poor metabolizers of CYP2C19. The selection of 2 antibiotics determined with the MIC *in vitro* appeared to result high eradication rate.

Hematology

More attention should be paid to hematological neoplasms with the translocation derivative (1;7) (q10;p10).

The translocation derivative (1;7) (q10;p10) is sometimes observed in myeloid neoplasms, but its precise characteristics are unknown. We evaluated 13 patients who had hematological neoplasms with derivative (1;7). Six of the 13 patients had a history of chemotherapy, indicating the importance of secondary malignancy. Most patients were men, and all of the patients with *de novo* disease were men and had a median age of 74 years. Laboratory data revealed that mean corpuscular volume was high, thrombocytopenia was not apparent, dysplasia was common, and complex chromosome abnormalities were not present. The prognosis was not as poor as previously reported: the estimated 3-year-survival rate was 35%. Although the patients were too few for clear conclusions to be made, more attention should be paid to this chromosome aberration.

Cardiology

We are studying catheter intervention for atrial fibrillation. To eliminate atrial fibrillation, we use the technique of segmental ostial catheter ablation (SOCA). We have developed 2 new methods for SOCA. One method is SOCA with a large lasso catheter, and the other is intravenous administration of ATP to induce transient pulmonary vein (PV) reconduction (dormant PV conduction) following PV isolation. This year we revealed the detailed characteristics of dormant PV conduction in patients with atrial fibrillation who underwent catheter ablation.

Clinical cell biology

1. ^{13}C-glucose breath test for diagnosing hepatic insulin resistance

To evaluate the hepatic insulin resistance with a simple and highly sensitive method, we developed the fasting ^{13}C-glucose breath test (FGBT). The area under the curve until 6 hours (AUC_{360}) of the kinetic curve for ^{13}C excretion was significantly lower in patients with impaired glucose tolerance than in healthy subjects. Also, the FGBT could be used to diagnose insulin resistance, with the homeostasis model assessment of insulin resistance as the gold standard, and to sensitively diagnose diabetes with fasting plasma glucose and HbA1c. We were able to set each cut-off value in the AUC_{360}. FGBT is a simple and highly sensitive and specific clinical test for evaluating hepatic carbohydrate metabolism.

(In collaboration with Meiji University, National Defense Medical College, and the Department of Surgery, The Jikei University)

2. New diagnostic marker transforming growth factor β latency-associated protein degradation products for diagnosis of fibrotic activity in the liver

We have developed a novel, quantitative, and specific assay of plasma latency-associated

protein (LAP) of transforming growth factor (TGF)-β degradation products (LAP-D), which are produced during proteolytic activation of TGF-β. We have previously validated this assay as a marker of hepatic stellate cell activation in *in vivo* liver fibrosis. Also, immunostaining was performed for LAP-D in liver tissue sections, which were obtained at biopsy at the same time as blood sampling. Staining for LAP-D was positive in fibrotic bundles in cases of chronic active hepatitis C and cases of autoimmune hepatitis. For both these liver diseases LAP-D might be used as marker of the activity of liver fibrosis. (Supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation and performed in collaboration with the Institute of Physical and Chemical Research.)

3. Development of the human plasma protein high generating system using bioartificial liver

Plasma protein high-productive cells (functional cell line [FLC] 4) and FLC‐4M#1 cells, which incorporates a strong promoter and a hybrid of the albumin gene, were cultured at high density in a 3-dimensional radial flow bioreactor to produce medical human albumin. In ASF-104 serum-free culture medium (Ajinomoto Pharmaceuticals Co. Ltd., Kawasaki), the quantity of albumin produced by FLC‐4M#1 cells was approximately 3 times that produced by FLC-4 cells. However, in enhanced RDF serum-free culture medium (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo), the quantity of albumin produced by FLC-4 cells increased by a factor of 3. In contrast, the quantity of albumin produced by FLC-4M#1 cells decreased by one-third. Because 40% to 50% of the protein released by FLC-4 cells is albumin, the quantity of albumin produced can be further increased through gene manipulation. The efficiency of the albumin secretion must be improved through posttranscriptional regulation.

(Supported by the Human Science Foundation and performed in collaboration with the National Institute for Infectious Diseases, Waseda University, and the Department of Biochemistry, The Jikei University)

4. Development of methods for the diagnosis and treatment of fatal encephalopathy with acute hepatic failure

A mouse model of fatal encephalopathy with acute hepatic failure was produced in the toxin receptor-mediated cell knockout (TRECK) hepatitis mouse. With magnetic resonance imaging we were able to confirm edema of the entire brain. Also, the plasma TGF-β LAP-D concentration was positively correlated with alanine aminotransferase activity and total bilirubin concentration and was negatively correlated with the total protein level. Immunohistochemical staining for LAP-D was correlated with that for α-smooth muscle cell actin in the TRECK hepatic failure model. This result suggests that the liver tissue of TRECK hepatitis mouse shows augmented fibrosis activity.

(Supported by Grant in Aid, Ministry of Education, Culture, Sports, Science and Technology and in collaboration with Advanced Institute of Science and Technology, Nara, and Medical Engineering Research Group, The Jikei University).

5. Development of an ultrasonic molecular imaging system

To develop an ultrasonic molecular imaging method, we performed a study of the application of high-sensitivity detection technology using micro-nano bubbles that were highly stable and provided a high degree of contrast with high-level harmonics. The stability

of microbubbles in the serum was increased by a mixture of phospholipids and a newly developed detergent.
(Performed in collaboration with the Tokyo University of Science and the Departments of Radiology and Biochemistry, The Jikei University).

Clinical psychiatry
Psychotropic drugs give rise to some concerns in clinical practice because of their ability to reduce seizure threshold; therefore, we examined the safety and efficacy of psychotropic drugs in several forms of psychosis associated with epilepsy. We reported a case of epilepsy in a patient with ring chromosome 20 syndrome (R 20 synd), and from a review of the literature, we discussed the characteristics of patients with R 20 synd. Furthermore, we reported a case of adult epilepsy and ictal apnea during sleep. A study was performed to prevent the recurrence of depression in patients with epilepsy.

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