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General Summary

Research projects in our department in 2009 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

The daily intake of coffee enhances fat metabolism by altering lipolytic hormones and inhibits the development of metabolic syndrome. Therefore, a combination of coffee ingestion and moderate exercise might greatly enhance fat metabolism and decrease risk factors for metabolic syndrome. Eight healthy male volunteers ingested 250 ml of coffee containing 4 mg/kg body weight of caffeine or 250 ml of hot water as a control fluid. The present study suggests that moderate-intensity exercise after temporary ingestion of coffee inhibits glucose metabolism and preferentially enhances fat metabolism and energy metabolism during the recovery period.

Clinical microbiology

Blood stream infections and respiratory tract infections in children and adults with febrile neutropenia were studied with a magnetic filtration system for DNA/RNA extraction (Magtration, Precision System Science, Co., Ltd., Chiba, Japan), dual priming oligonucleotide polymerase chain reaction, and DNA microchip electrophoresis (MultiNA, Shimadzu Corp., Kyoto, Japan). Multiple infectious agents have been identified in both cases. Bioremediation of dioxins with thermophilic bacteria has been established for contaminated soil and has achieved reductions of 90% (W/W). Methods have been developed for treating infectious waste and chemical hazardous materials in hospitals and other institutions. Epidemiological studies were performed with the DNA diagnosis of pathogens.

Clinical chemistry

1. Genetic polymorphism of enzymes related to the therapeutic effectiveness of fluorouracil in patients with hepatocellular carcinoma
We investigated the genetic polymorphisms of 3 enzymes related to the therapeutic effectiveness of fluorouracil (5-FU) in 58 patients with hepatocellular carcinoma as follows: 1) cytochrome P450 2A6 (CYP2A6), which activates the prodrug of 5-FU

(tegafur): CYP2A6*4 (enzyme deletion type); 2) dihydropyrimidine dehydrogenase (DPD), which metabolizes and inactivates 5-FU: DPD*9AB (no activity); and 3) thymidylate synthase (TS; target enzyme): divide between TS high and low expression types by TS tandem repeat (variable number tandem repeat) and G/C polymorphism within the 28-bp repeat component of TS (single nucleotide polymorphism) (reported by Kawakami K, et al., *Cancer Res* 6004, 2003). Only 13 of 58 patients with hepatocellular carcinoma had normal activities of CYP2A6 and DPD and the low-expression type of TS.

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

We reported the following findings:

1. We verified the significance of measuring levels of very low density lipoprotein (VLDL) cholesterol with our established HPLC lipoprotein analysis method for monitoring the exercise-induced amelioration of lipid metabolism and the longer time required for the improvement of adiponectin and insulin resistance than for the amelioration of VLDL metabolism; a manuscript was submitted to the *Journal Atherosclerosis and Thrombosis*. The results were presented, in part, at a symposium of the 17th annual meeting of the Japan Society of Exercise and Sports Physiology.
2. A revised HPLC method for measuring lipoprotein A was developed with a collaborative project and submitted for publication in the *Journal of Lipid Research*.
3. Because of the advantages of our revised HPLC method, Hiroshi Yoshida was awarded the Life Science Prize at the 56th National Congress of the Japanese Society of Laboratory Medicine.
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 the Japanese Society of Laboratory Medicine
5. Atherosclerosis-related serum lipid markers (remnant lipoprotein cholesterol and small dense LDL) was presented at a symposium of the 41st annual meeting of the Japan Society of Clinical Laboratory Automation, and our findings concerning cholesterol and atherosclerosis were presented at a seminar of the 49th annual meeting of the Japan Society of Clinical Chemistry.
6. The ameliorating effects of astaxanthin on triglyceride, high-density lipoprotein cholesterol, and adiponectin were verified and presented at a workshop of the 15th congress of the International Atherosclerosis Society (Boston, MA, USA) and at a symposium of the 7th annual meeting of the Japanese Society for Medical Use of Functional Foods and was submitted for publication in the journal *Atherosclerosis*.
7. Subanalysis by sex from the findings of the Japanese Investigation of Kinetic Evaluation in Hypertensive Event And Remodeling Treatment (JIKEI HEART) study was presented at the annual congress of the European Society of Cardiology (Barcelona, Spain) and was submitted for publication in the *Journal of Hypertension*.

Hematology

Evaluation of the QuantiFERON TB-2G test for detecting tuberculosis infection
We evaluated the efficacy of the QuantiFERON TB-2G (QFT) test (Cellestis Limited, Melbourne, Australia) in detecting tuberculosis infection by comparing the results obtained with QFT, liquid culture, the polymerase chain reaction, and direct staining. The QFT test was found to be an easy-to-use method, but some differences were found. Positive results on the QFT test were obtained in 20% of patients. The detection rate of tuberculosis with the QFT test was sufficiently high in patients younger than 50 years, but the detection rate was lower in older patients. These results indicate that the results of the QFT test depend on the history of tuberculosis infection and that the QFT test should be accompanied by bacterial tests.

Cardiology

This year we researched the following 3 topics.

- 1) As participants in a multicenter study, we analyzed the effect on health of longtime exercise with respect to oxidant stress.
- 2) From the standpoint of perioperative cardiovascular evaluation for noncardiac surgery, we studied the significance of electrocardiography as a preoperative examination.
- 3) We evaluated the effectiveness of catheter ablation for atrial fibrillation and our improvement of this method.

Clinical cell biology

1. ^{13}C -glucose breath test

The ^{13}C -glucose breath test was established as a diagnostic test for insulin resistance and as a liver function test.

2. Enzyme-linked immunosorbent assay for measurement of latency-associated protein of transforming growth factor β degradates in plasma

We have developed a novel, quantitative, and specific assay for plasma latency-associated protein of transforming growth factor (TGF) β degradates (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. Plasma levels of LAP-D significantly decreased in 24 patients 3 months after the start of successful combination therapy for hepatitis C virus infection.

3. Plasma protein production system using a bioartificial liver

We cultured cells producing higher levels of human plasma protein in the radial-flow bioreactor for development of plasma protein production for medical care and reviewed culture conditions.

4. Retinoid metabolism and liver fibrosis

The cellular distribution of lecithin: retinol acyltransferase and cellular retinol-binding protein I was examined in biopsy specimens from patients with chronic viral hepatitis. Ten percent to 20% of double-positive cells were distributed in fibrous lesions in pathological livers. The results suggest that these cells are still able to store vitamin A even if they transform into myofibroblasts.

5. Diagnosis and therapy for lethal encephalopathy in acute hepatic failure
Toxin receptor-mediated cell knockout mice were used as an experimental model of lethal hepatic encephalopathy in acute hepatic failure.

6. Ultrasound molecular imaging system

We performed research to develop stable nanobubbles for use in contrast media. Our research into the application of nanobubbles to a high-sensitivity ultrasound molecular imaging system was advanced through the use of harmonic imaging.

Clinical psychiatry

Psychotropic drugs have caused some concern in clinical practice because of their ability to reduce seizure thresholds and provoke seizures. Therefore, we examined the therapeutic attitudes regarding several forms of psychoses associated with epilepsy.

Because little attention has been paid in the past, we reported the characteristics of patients with epilepsy who demonstrated social withdrawal. Moreover, we reported a case of symptomatic generalized epilepsy in which long-term control was achieved by the addition of zonisamide therapy.

Publications

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