Department of Laboratory Medicine

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General Summary

Research projects in our department in 2012 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-term caffeine intake and regular exercise, alone and in combination, on triglyceride and glycogen contents in the liver and skeletal muscle, an indicators of insulin resistance. Otsuka Long-Evans Tokushima fatty rats were divided into sedentary, exercise, caffeine-intake, and caffeine-intake and exercise groups for 5 weeks of treatment from 25 to 29 weeks of age. The caffeine-intake group and the caffeine-intake and exercise group were fed rat chow containing 0.25% caffeine, and the exercise group and the caffeine-intake and exercise group were encouraged to exercise every day. Treatment with caffeine only, exercise only, or both caffeine and exercise reduced body weight, visceral fat mass, and metabolic syndrome-related variables. Combination of long-term caffeine intake and exercise expenditure more effectively decreases insulin resistance in both the liver and skeletal muscles and risk factors for metabolic syndrome in Otsuka Long-Evans Tokushima fatty rats than does caffeine intake or exercise alone.

Clinical microbiology

Several clinically isolated, previously unidentified bacterial strains were identified though gene sequencing of polymerase chain reaction-amplified 16S ribosomal RNA.

Panton-Valentine leukocidin (PVL) secreted by *Staphylococcus aureus* causes severe infections of the skin, soft tissue, and lung. To assess the prevalence and genetic characteristics of PVL-positive *S. aureus*, we investigated 86 *S. aureus* isolates obtained from skin and soft-tissue pus from September 2011 through May 2012 at Daisan Hospital. A total of 6 PVL-positive strains (6.9%) were detected: 3 methicillin-sensitive *S. aureus* isolates and 3 methicillin-resistant *S. aureus* isolates. The PVL prevalence was 11.1% in methicillin-resistant *S. aureus* and 5.1% in methicillin-sensitive *S. aureus*.

Clinical chemistry

1. 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 (amoxicillin and clarithromycin or metronidazole: the first-line treatment) is now considered the gold standard of therapy for eradicating *H. pylori* in Japan. However, the eradication rate of the first-line treatment is only 70% to 85% and has tended to decrease because of increasing rates of 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 genetic polymorphism of cytochrome P450 2C19 (CYP2C19), an enzyme that metabolizes PPIs, in 28 patients with *H. pylori* infection and dyspeptic diseases in whom the first-line eradication therapy had failed. The rate of resistance, on the basis of MIC, was 64% (18 of 28 *H. pylori* isolates) to clarithromycin and 52% (14 of 27 isolates) to metronidazole. On the basis of these results we have chosen a new regimen of antibiotics and a PPI for eradicating *H. pylori*. With this new regimen second-line or third-line therapy successfully eradicated *H. pylori* in 20 of 28 patients (72%). According to the CYP2C19 genotype, the eradication rate was 100% (6 of 6 patients) with the CYP2C19 homozygous poor metabolizer genotype, 69.2% (9 of 13 patients) with the heterogenous extensive metabolizer genotype, and 55.6% (5 of 9 patients) with the homozygous extensive metabolizer genotype. Use of MIC susceptibility to select the drug regimen increased the eradication rate, even in patients who had failed first-line eradication therapy. Such an increase in the eradication rate appears to depend on CYP2C19 genetic polymorphism, although the mechanism remains unclear.

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 of assessing cardiovascular disease risk, including the application of our high-performance liquid chromatography (HPLC) method to determine cholesterol levels of lipoproteins.

We investigated and reported the following. 1) The HPLC method we developed can provide excellent quantitative performance for lipoprotein cholesterol in samples with increased remnant lipoprotein (*Atherosclerosis*, 2012 Jun; 222(2): 541-4). 2) Intermediate-density lipoprotein cholesterol may serve as a useful marker for the risk of coronary heart disease risk in Japanese men with increased levels of non-high-density lipoprotein (HDL)-cholesterol (*Int J Cardiol*, in revision). 3) Pleiotropic effects of hydroxymethyl glutaryl coenzyme A reductase inhibitors (statins) on oxidized lipoproteins are divergent, and pitavastatin can markedly decrease malondialdehyde-modified LDL/apolipoprotein B, whereas atorvastatin can decrease oxidized HDL/apolipoprotein A1 (*Atherosclerosis*, in press). (4) An LDL cholesterol homogenous assay agrees with the LDL-C beta-quantification assay in healthy subjects but exhibits positive bias for subjects with hypertriglyceridemia among unhealthy subjects for some reagents (*Atherosclerosis*, 2012 Jun; 225(1): 208-15).

Hematology

We evaluated the efficacy of the body-fluid (BF) mode of an automated hematology analyzer in measuring leukocyte counts in samples of whole blood that contain only a

few leukocytes. Although in some cases leukocyte counts falsely increased in the BF-mode with a Flag message, there was a good correlation between BF mode and eye count. These results indicate that the BF mode of an automated hematology analyzer is useful for counting leukocytes in samples of whole blood with very few leukocytes if we pay attention to the Flag message.

Cardiology

We researched 2 topics in 2012. One topic was the meaning of T-wave abnormalities on electrocardiograms, and the other was the recurrence of atrial fibrillation after pulmonary vein isolation. We published several papers about new methods for preventing the recurrence of atrial fibrillation after pulmonary vein isolation.

Clinical cell biology

1. We developed the fasting ^{13}C-glucose breath test to evaluate the insulin resistance of the liver. In the present study, we attempted metabolic simulation with a computer to estimate *in vivo* glucose metabolism from the dynamics curve of the ^{13}C excretion rate on the fasting ^{13}C-glucose breath test. First, we established an *in vivo* metabolic model of glucose and adopted 5 compartment models. The dynamics curve of the exhalation ^{13}C excretion by 5 compartment models fitted well that with the actual value. In healthy subjects, the area under the curve until 360 minutes of the ^{13}C excretion dynamics curve of female subjects was greater than that of male subjects. The *in vivo* simulation suggested that the glucose metabolism in the liver of female subjects is more efficient than that in male subjects. This simulation is also useful for judging the effects of drugs before and after treatment. (Supported by a Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities, 2011-2015) (In collaboration with Meiji University, the National Defense Medical College, and the Departments of Internal Medicine and Surgery, The Jikei University)

2. We have developed a novel, quantitative, and specific assay of plasma latency-associated protein of transforming growth factor β degradates (LAP-D), which are produced during proteolytic activation of transforming growth factor β. The level of LAP-D in blood and tissues would be a novel marker reflecting fibrogenesis activity but not the amount of accumulated fibrosis in patients. We found that even if nucleotide analogs inhibited alanine aminotransferase release from livers infected with hepatitis B virus, the plasma LAP-D level increased in some cases. Most likely, the effect of nucleotide analogs is not sufficient to inhibit fibrogenesis in the liver. (Supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation and the Research on the Innovative Development and the Practical Application of New Drugs for Hepatitis B provided by the Ministry of Health, Labour and Welfare of Japan) (In collaboration with the Institute of Physical and Chemical Research)

Clinical psychiatry

We reported a case of epilepsy in a patient with ring chromosome 20 syndrome, and

from a review of the literature, we discussed the characteristics of patients with ring chromosome 20 syndrome. Furthermore, we reported a case of elderly-onset partial epilepsy with manic state due to postictal psychosis. A study was performed to prevent the recurrence of depression in patients with epilepsy. We are planning a study of the management of epilepsy in pregnant women.

Publications

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Reviews and Books

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