Department of Laboratory Medicine

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General Summary

Research projects in our department in 2011 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

In the OLETF rat, a model of obesity-related diabetes, we found that rats that exercised regularly at the age of 5 to 19 weeks had a significantly lower body weight than sedentary control rats at the later age of 20 to 45 weeks.   In contrast, rats that received food-restriction treatment showed a rapid gain in body weight when the treatment was discontinued.   Immediately after the completion of the experiment, at the age of 46 weeks, exercised rats showed significantly lower body weight and body fat mass and enhanced activities of energy metabolism-related enzymes and uncoupling protein 3 mRNA expression in skeletal muscle compared with those of the sedentary and food-restricted groups.   These results strongly indicate that many individuals who receive diagnoses of obesity or metabolic syndrome during adolescence to middle age may have had insufficient physical activity during childhood.

Clinical microbiology

Phage open reading frame typing (POT), a new typing method for methicillin-resistant *Staphylococcus aureus*, was compared with pulsed-field gel electorophoresis (PFGE), which is the standard method.   The results suggest that the POT method has a discrimination power equal to that of PFGE.

A polymerase chain reaction (PCR) method targeted to the internal spacer region (ITS) was developed to detect *Cunninghamella* spp.

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

The isolation rate of *S. aureus* carrying the Panton-Valentine leukocidine (PVL) gene from patients with skin infections at Daisan Hospital investigated with PCR *luk-S-PV* and *luk-F-PV* amplification was 7%.

Clinical chemistry

1.   To evaluate the clinical usefulness of the serum level of procalcitonin, a precursor of

calcitonin, for determining whether bacterial infection is present in patients with systemic inflammatory response syndrome (SIRS) caused by bacterial sepsis, the level of procalcitonin was measured concommitantly with the white blood cell count and levels of tumor necrosis factor-α, interleukin 6, E-selectine, and C-reactive protein.   The subjects were 5 patients with SIRS not complicated by Gram-negative bacterial infection.   We analyzed the time to reach peak level (C_{max}) from the clinically diagnosed time-point of SIRS (T_{max}) for the above 6 inflammatory markers.   The mean T_{max} was shortest for tumor necrosis factor-α (18.0 hours) and was followed by those for procalcitonin (32.9 hours), interleukin 6 (36.0 hours), the white blood cell count (36.7 hours), and C-reactive protein (43.0 hours), and was longest for E-selectin (46.5 hours).   These findings suggest that measuring levels of procalcitonin in patients with SIRS is useful for diagnosing bacterial infection and the severity of illness.

2.   Our 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 for assessing cardiovascular disease risk, including the application of our method of high-performance liquid chromatography, to determine levels of lipoprotein cholesterol.

Our studies obtained the following results.   1) The method of high-performance liquid chromatography we developed has excellent quantitative performance for lipoprotein cholesterol in samples with increased remnant lipoprotein (*Atherosclerosis*, in press); 2) The pleiotropic effects of hydroxymethyl glutaryl coenzyme A reductase (statin) on oxidized lipoproteins vary, and pitavastatin can markedly decrease malondialdehyde-modified low-density lipoprotein/apolipoprotein B, whereas atorvastatin can decrease oxidized high-density lipoprotein/apolipoprotein A1 (under submission).

Hematology

Because intravascular large B-cell lymphoma (IVLBCL) is difficult to diagnose, we evaluated the clinical characteristics of IVLBCL.   In many cases hemophagocytic histiocytosis showing hemophagocytic syndrome is the most prominent finding that leads to the diagnosis, whereas in some cases small collections of lymphoma cells in the bone marrow and skin are an important sign of IVLBCL.   Because many types of IVLBCL exist, systemic evaluation, including bone marrow aspiration and skin biopsy, is extremely important.

Cardiology

We studied 2 topics in 2011.   One was T-wave abnormalities in electrocardiograms, and another was the recurrence of atrial fibrillation after pulmonary vein isolation.   We published several papers regarding new methods to prevent the recurrence of atrial fibrillation after pulmonary vein isolation.

Clinical cell biology

1.   We investigated the convenience and sensitivity of the fasting ^{13}C-glucose breath test (FGBT) for evaluating hepatic insulin resistance.   Healthy, nonobese subjects and a disease group of patients with mild glucose intolerance were given 100 mg of ^{13}C-glucose

after an overnight fast.   A series of breath samples was collected until 360 minutes after ingestion, and the $^{13}CO_2/^{12}CO_2$ ratio was measured with an infrared spectrometer and plotted as a kinetic curve of ^{13}C excretion.   The area under the curve until 360 minutes (AUC_{360}) of the ^{13}C excretion kinetic curve of the FGBT reflects the efficiency of energy production of the liver.   The AUC_{360} of the healthy subjects was consistently higher than that of patients with glucose intolerance.   Insulin resistance in males and females could be diagnosed with a cut-off value.   In a similar manner, diabetes mellitus could be diagnosed with cut-off values.   The FGBT is a novel glucose metabolic test that can be used to conveniently and safely evaluate the balance of glucose metabolism in the liver.   This test has excellent sensitivity for diagnosing alterations in hepatic glucose metabolism.   In particular, the FGBT was useful for evaluating liver insulin resistance in cases of fatty liver. (Supported by the 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, National Defence Medical College, and the Departments of Internal Medicine and Surgery, The Jikei University)

2.   We have developed a quantitative and specific assay for plasma latency-associated protein of transforming growth factor (TGF) β (LAP) degradates (LAP-Ds), which are produced during proteolytic TGF-β activation.   LAP-Ds would be novel markers in blood and tissues reflecting fibrogenetic activity but not the amount of accumulated fibrosis, particularly in patients with chronic hepatitis C virus infection and autoimmune hepatitis, and can be used to estimate fibrogenesis, in which TGF-β activation and activation of hepatic stellate cells are more frequent, and to assess the effect of treatment.   The plasma LAP-D concentrations decreased significantly when patients with chronic hepatitis C virus infection were treated with interferon and ribavirin.   A sustained virological response was obtained when levels of LAP-D continued to be undetectable low.   (Supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation) (In collaboration with the Institute of Physical and Chemical Research)

3.   Because a large quantity of energy, particularly ATP, is necessary for protein secretion in hepatocellular carcinoma (HCC), we examined the production of energy from glucose in the FLC-4 and FLC-7 HCC cell lines.   Glucose transporter (GLUT) 2 and glucokinase (GK), were expressed by FLC-4.   This result indicates that FLC-4 cells are hepatocyte-type energy-producing cells that use the tricarboxylic acid cycle.   Therefore, we speculated that FLC-4 uses energy for metabolism, not cell proliferation, under stable culture conditions in a radial-flow bioreactor (RFB).   In contrast, FLC-7 showed carbohydrate metabolism depending on GLUT1 and hexokinase II.   The findings suggest that energy-producing efficiency was poor and that glucose is used for cancer cell growth rather than energy production (Warburg effect).   Because brain-type GLUT3 has high glucose-uptake efficiency expressed in the RFB culture, much glucose is used for cell proliferation.   Therefore, it is necessary to consider culture methods other than RFB for protein production by FLC-7.   (Supported by the Human Science Foundation) (In collaboration with the National Institute for Infectious Diseases, Waseda University, and the Department of Biochemistry, The Jikei University)

Clinical psychiatry

The ability of psychotropic drugs to reduce the seizure threshold is a cause of concern in clinical practice; therefore, we examined the safety and efficacy of psychotropic drugs in several forms of psychosis associated with epilepsy.   We reported on 2 patients with epilepsy and reflex seizures caused by their psychological state.   Furthermore, we examined the characteristics of epileptic falls and dental and orofacial injuries in mentally handicapped patients.   A study was performed to prevent the recurrence of depression in patients with epilepsy.

Publications

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