Department of Laboratory Medicine

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General Summary

The members of our department performed studies about clinical laboratory medicine, with a focus on their individual specialties, as shown in the following *Research Activities*. Two research studies had started in clinical microbiology and clinical biochemistry with matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) from 2014. We also focused on insulin resistance, including nonalcoholic steatohepatitis (NASH). A simple method of using stable isotope breath tests to measure energy metabolism in the body will be developed in the near future.

Research Activities

Clinical microbiology

1. Identification of bacterial strains

Several clinically isolated, previously unidentified bacterial strains were identified though gene sequencing of polymerase chain reaction-amplified 16S ribosomal RNA. We investigated the relevance of the pathogen of *Staphylococcus aureus* and the phage open reading frame typing type. We have done research on the examination method of bacterial pathogens with MALDI-TOF MS.

2. Induction of the hepatitis B virus receptor Na^+ taurocholic acid cotransporting polypeptide in the FLC-4 human hepatocellular carcinoma cell line by retinoic acid

The cell culture system that can reproduce the infection, replication, growth, and release of the hepatitis B virus (HBV) was the human hepatocytes culture system. The Na^+ taurocholic acid cotransporting polypeptide (NTCP) on the cell membrane of hepatocytes was shown in 2012 to function as a HBV receptor. In the present study, we compared NTCP expression in several human hepatocellular carcinoma cell lines and examined the likelihood of establishing a new system for evaluating HBV infection. After comparing the NTCP messenger RNA expression in the FLC, Huh-7, and HepG2 cell lines, only FLC-4 cells significantly confirmed the onset of high NTCP, and the level of expression was higher than that by human hepatocytes but was 1/10 of that by differentiated HepaRG cells. Therefore, NTCP messenger RNA in FLC-4 cells was induced to a level similar to that of differentiated HepaRG cells with all trans-retinoic acid and the onset of the level of Na^+-dependent. Thetaurocholic acid transportation activity was increased by approximately 20%.

Clinical chemistry

1. Mass analysis of urinary β2 microglobulin in patients with proteinuria

We used using MALDI-TOF MS to analyze urinary β2 microglobulin in patients with high proteinuria. Before mass analysis we purified urine samples to obtain β2 microglobulin with the immunoprecipitation method. We detected the signal of 11.8-kDa intensity in the mass spectrograph, already reported as a reference. We also observed several different intensities in high molecular regions of the mass spectrograph. In patients with diabetes mellitus and proteinuria, we observed bimodal 11.8-kDa intensity. These results suggest that the part of the β2 microglobulin of this research might be the polymerized form and might be modified by endogenous substance(s).

2. Measurement of lipoprotein cholesterol with high-performance liquid chromatography

The vision of the high-performance liquid chromatography (HPLC) lipoprotein cholesterol measurement we developed was reported at a symposium of the 46_{th} annual scientific meeting of the Japan Atherosclerosis Society. The method to determine vitamin E concentrations of lipoprotein fractions developed with the modification of the HPLC method was published in *Scientific Reports* (2014; 4: 4086). In the studies of serum lipid-related reagents, the accuracy and precision of each high-density lipoprotein cholesterol direct method was published in *Atherosclerosis* (2014; 233: 253-9) and the reference interval of apolipoprotein B48 was published in *Journal of Atherosclerosis and Thrombosis* (2014; 21: 618-27). The study to determine the high-density lipoprotein function of cholesterol efflux with stable isotope and the risk assessment of atherosclerosis received a Department of Education, Culture, Sports, Science and Technology Research Grant, and the fundamental method is now being developed. The collaborative study results of statin-related adverse events for glucose metabolism and renal function were published in *Atherosclerosis* (2015; 241: 409-18) and *Public Library of Science One* (2014; 9: e9691), respectively. The clinical significance of the eicosapentaenoic acid/arachidonic acid ratio to explain the atherosclerosis development determined by the cardio-ankle vascular index in patients with high serum concentrations of arachidonic acid presumably expressive of inflammation was published in *International Journal of Cardiology* (2014; 177: 517-9).

3. Difference of hepatic energy metabolism of male and female rats

Sex is an important factor for the onset of diabetes, liver cancer, and NASH. In women, impaired glucose tolerance and diabetes easily develop after menopause. The fasting ^{13}C-glucose breath test has shown that the capacity of liver energy metabolism is higher in women than in men. Animal models are needed to clarify the causes of the sex gap in liver energy metabolism. The area under the curve until 6 hours of ^{13}C excretion of female Wistar rats was higher than that of male Wistar rats. However, the difference in Long-Evans rats was opposite. The comparison of both types of rat will be useful for clarifying the sex difference in the mechanisms of liver energy metabolism. (Funded by the Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities, 2011-2015)

Clinical pathology

Hepatic stellate cells that express lecithin:retinol acytransferase (LRAT) or cellular retinol binding protein 1 (CRBP-1) or both contribute to the development of fibrosis in patients

with NASH. The present study was performed to examine whether hepatic stellate cells (HSCs) positive for LRAT or CRBP-1 or both contribute to centrilobular fibrosis on NASH. Antibodies against LRAT and CRBP-1, a widely ascertained antibody to activated HSCs (α-smooth muscle actin [α-SMA]), and anti-R58 monoclonal antibody to latency-associated protein of transforming growth factor-β degradation products (LAP-D) in cells or the matrix were used for immunohistochemical studies to assess the distribution of cells that contribute to the development of fibrosis. The HSCs positive for LRAT or CRBP-1 or both were stained in centrilobular lesions in fibrotic livers with NASH. These cells were observed in fibrotic septa, which were stained with α-SMA or LAP-D or both. The present study provides evidence that functional HSCs expressing LRAT or CRBP-1 or both and continuing to maintain the ability to store vitamin A contribute to the development of centrilobular fibrosis and parenchymal fibrosis in patients with NASH. (Funded by Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities, 2011-2015) (In collaboration with the Departments of Internal Medicine and Pathology, The Jikei University School of Medicine)

Clinical psychiatry

We plan a clinical study of the management of pregnancy in woman with epilepsy. We discussed changes in serum concentrations of antiepileptic drugs during pregnancy. Furthermore, we reported a case of epilepsy that was induced by a specific situation and showed a peculiar clinical course. A study was performed to prevent the recurrence of depression in patients with epilepsy. We examined the safety and efficacy of psychotropic drugs in several forms of psychosis associated with epilepsy.

Addition

1. How to improve the certainty of laboratory communication

Because we work at the Department of Laboratory Medicine, transferring results to physicians correctly is important. However, we cannot confirm the receipt of the data. With oral transmission, the appropriate exchange and review of data are effective. Therefore, we investigate the frequency of review during daily work. To improve the accuracy of measurement and communication, we must pay more attention to the safety of transmission.

2. In the field of biochemical examination, we presented the effects of autoantibodies in tumor markers or hormone measurement, an examination of the SCC measurement method in an automated immunoassay analyzer (AIA-900, Tosoh Corp.), and the false-positive interference by human anti-mouse antibodies. We also announced in the field of microbiology a secular course on the sensitivity information for various antimicrobial agents in urine isolates and, in the physiological inspection area, we annouced a basic study of 10-m constant distance gait analysis with a walking spectrometer. With respect to laboratory management, we announced our approach for improving safety and shortening the inspection time.

Publications

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