SHIMADZU

Application News

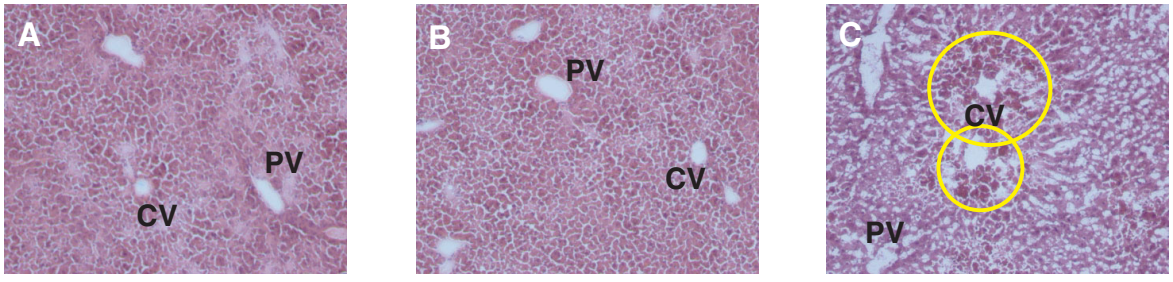
MALDI-TOF Mass Spectrometry No.B04

On-Tissue Direct Analysis: Analysis of Changes in Amount of Lipids in Murine Hepatopathy Model due to Administration of Carbon Tetrachloride (CCl_4)

Recently, the use of the MALDI technique to analyze target biomolecules (low-molecular-weight metabolites, lipids, peptides, proteins, etc.) directly from biological tissue sections is attracting increasing attention for its potentially strong utility in disease-related biomarker discovery. Generally, tissue sections are frozen during preparation, and then the matrix solution used in the MALDI technique is applied uniformly on the tissue section using a spotter (CHIP-1000) or other such applicator. After deposition of the matrix, analysis is conducted on the tissue section by MALDI-TOF MS to detect the *m/z* values of target biomolecules. Then, variations in MS peaks between the site of pathology and the normal site are compared in the search for disease biomarkers.

Here we introduce an example of on-tissue direct detection of phospholipid changes using MALDI-TOF MS. The sample consisted of a mouse model hepatopathological tissue section in which injury was induced by administration of carbon tetrachloride, and on-tissue direct MS analysis was conducted at time intervals following administration of the carbon tetrachloride.

Fig. 1 shows the H&E-stained hepatic tissue sections of 5-week-old ICR mice (♂) in which hepatopathy was induced by interperitoneal administration of carbon tetrachloride (1.0 mL/kg). The sections were taken at dissection conducted at 15 minutes and 48 hours following administration of CCl_4, respectively. Necrosis and cellular infiltration are recognizable at the periphery of a central vein in the liver after 48 hours, as shown in Fig. 1. Next, 500 pL of matrix solution consisting of 5 mg/mL a-cyano-4-hydroxycinnamic acid (60 % acetonitrile, 0.1 % trifluoroacetic acid) was deposited 25 times at 300 μm intervals on each of the hepatic tissue sections using a chemical printer (CHIP-1000). Fig. 2 shows each of the hepatic tissue section images and the deposited matrix.



A: reference murine hepatic tissue, B: 15 min post-administration, C: 48 hr post-administration, CV: central vein, PV: portal vein
A～C: X100, Yellow-encircled areas: cellular infiltration

Fig. 1 H&E Staining of Murine Liver with Hepatopathy Induced by Administration of Carbon Tetrachloride (CCl_4)

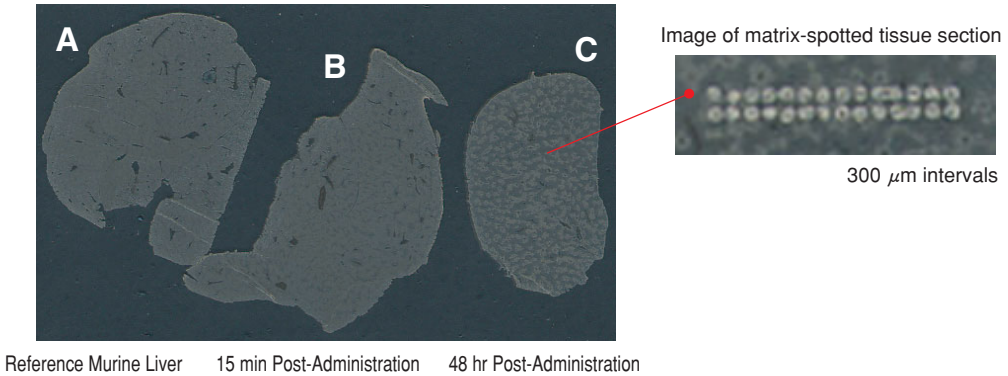


Fig. 2 Matrix Deposition onto Tissue Sections of Murine Liver

Fig. 3 shows typical MS spectra obtained from on-tissue direct MALDI-MS analysis of the respective tissue sections following administration of carbon tetrachloride, in addition to those obtained from the reference liver tissue section. In the MS spectra of the 48-hr-elapsed tissue section results of Fig. 3, six distinctive MS peaks were confirmed to show increase and decrease. From the *m/z* values of these six MS peaks and the results of MS/MS analysis, they were determined to be the protonated molecular ions and potassium adducts of PC 32:0, PC 34:2 and PC 34:1 of phosphatidyl choline (PC).

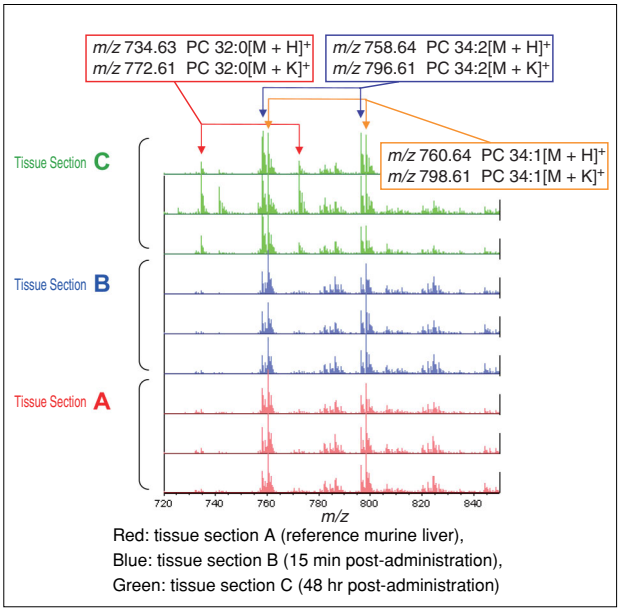


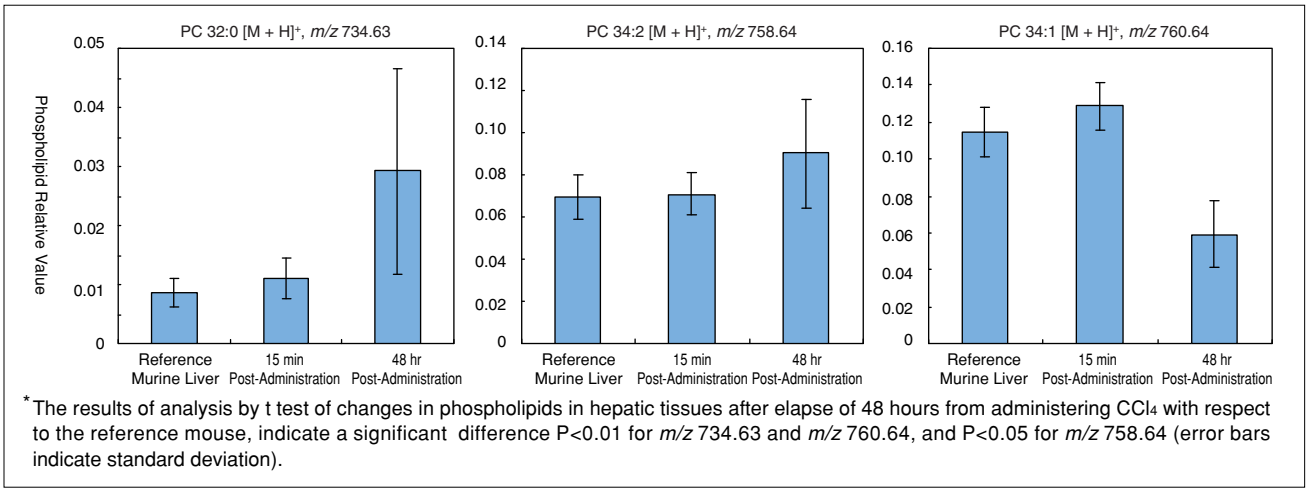
Fig. 3 MS Spectra of Respective Tissue Sections

Next, in order to conduct more in-depth analysis of the three types of PC related to the confirmed increases and decreases, ten points each of the respective tissue sections were measured, and relative comparison of the respective phospholipids $[M+H]^+$ was performed based on the obtained MS spectra. Assuming that the Relative Value of Phospholipids = Area of MS Peak of Interest / Sum of all MS Peak Areas, the relative value of phospholipids in the respective tissue sections can be plotted as shown in Fig. 4.

As indicated in Fig. 4, PC 32:0 $[M+H]^+$ = 734.63 is the highest value at 48 hours following the administration of carbon tetrachloride ($P = 0.0051$ according to t test). In addition, PC 34:2 $[M+H]^+$ decreased greatly after the elapse of 48 hours, however PC 34:1 $[M+H]^+$ with one less double bond decreased greatly after the elapse of 48 hours ($P = 0.036$, $P<0.0001$ according to respective t tests).
Furthermore, the same trend was confirmed for the respective phospholipid potassium ion adducts.

It is generally known that after the acute phase of liver cell necrosis in the carbon tetrachloride-induced liver-injured mouse model, the course eventually shifts to hepatic cell division and liver regeneration. Such on-tissue direct mass analysis allowed direct observation of the distinctive changes in phospholipids in the liver regeneration phase.
These results demonstrate that the on-tissue MALDI-MS analytical technique incorporating the chemical printer and MALDI-TOF MS is an effective approach to in-vivo metabolite change analysis and in-depth disease biomarker search.

This article is prepared based on collaborative study with Dr.Masaya Ikegawa , Kyoto Prefectural University of Medicine.



*The results of analysis by t test of changes in phospholipids in hepatic tissues after elapse of 48 hours from administering CCl_4 with respect to the reference mouse, indicate a significant difference $P<0.01$ for *m/z* 734.63 and *m/z* 760.64, and $P<0.05$ for *m/z* 758.64 (error bars indicate standard deviation).

Fig. 4 Changes in Amount of Phospholipids due to Administration of Carbon Tetrachloride (CCl_4)

NOTES:
✻This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.

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