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Application News

No. B88

Probe Electrospray Ionization Mass Spectrometer

Establishment of a Method for Direct Analysis of the Mouse Liver Metabolome Using the DPiMS™-8060

In the analysis of endogenous metabolites (metabolome analysis), it is difficult to perfectly remove biases caused by pretreatment and sampling. Therefore, in order to accurately grasp the changes in the metabolome of a biospecimen, the establishment of a method for direct analysis of the metabolome is indispensable. Probe electrospray ionization (PESI) is a new direct ionization method in which an ultrafine and minimally invasive probe is used for sampling. Acquired samples are ionized by applying a high voltage to the probe tip and therefore components can be analyzed without using a chromatograph.

By using the DPiMS-8060 probe electrospray ionization tandem mass spectrometer (Fig. 1), which combines PESI with tandem mass spectrometry, direct analysis of the metabolome of a biospecimen is possible.

This article introduces a method established to directly analyze the metabolome of a tissue sample (intact metabolome analysis) using a PESI tandem mass spectrometer together with the application of the method to the metabolome analysis of CCl_4-induced acute liver failure model mice.

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Fig. 1 **DPiMS™-8060**

Sample Preparation and Analytical Conditions

Standard metabolite samples including amino acids, organic acids, and sugars (26 metabolites) were prepared by diluting them with 50 % ethanol solution and dripping 10 µL of each sample into dedicated sample plates for liquid samples (Shimadzu Corporation). We then selected the MRM transitions for each compound and optimized the mass spectrometer conditions such as collision energy (CE). The information on the optimized MRM transitions of the 26 metabolites is listed in Table 1.

Next, liver samples were collected from mice by dissection. Square sections about 3 mm in size were taken from a healthy mouse and liver failure model mouse (liver failure induced by administering carbon tetrachloride) each. The sections were placed in dedicated sample plates for solid samples and then set on the instrument. Since solid samples can be analyzed by the DPiMS-8060 by simply placing them in a sample plate, there is no need for complex pretreatment.

Table 1 **MRM Transitions of 26 Metabolites**

Name	Polarity	Transition (*m/z*)	Collision Energy (V)
3-hydroxybutyrate	(–)	103.1>59.0	35
Citric acid/isocitric acid	(–)	191.0>111.1	20
D-glucose	(–)	179.1>59.2	20
Glucose-6-phosphate	(–)	259.1>96.9	20
Glutaric acid	(–)	131.0>87.3	20
Glycine	(–)	74.2>74.2	20
L-asparagine	(–)	131.0>113.3	20
L-asparatic acid	(–)	131.9>88.1	20
L-glutamic acid	(–)	146.0>102.1	20
L-lactic acid	(–)	89.0>43.2	20
L-malic acid	(–)	133.0>114.9	20
L-serine	(–)	103.9>74.2	20
Pyruvic acid	(–)	87.1>43.1	20
Succinic acid	(–)	117.1>73.0	20
Taurine	(–)	124.0>80.0	20
2-aminobutyric acid	(+)	104.1>58.1	20
L-glutamine	(+)	147.1>84.2	20
L-histidine	(+)	156.1>110.3	20
L-leucine/L-isoleucine	(+)	132.1>86.2	20
L-methionine	(+)	150.3>104.1	20
L-ornithine	(+)	132.9>70.0	20
L-phenylalanine	(+)	166.2>120.2	20
L-proline	(+)	116.2>70.0	20
L-threonine	(+)	120.1>74.0	20
L-tryptophan	(+)	205.2>146.1	20
L-tyrosine	(+)	182.1>136.1	20

Intact Metabolome Analysis of a Healthy Mouse and a Liver Failure Mouse

Carbon tetrachloride (CCl_4) is known to induce acute liver failure. Taking a CCl_4-induced acute liver failure model mouse group (hereinafter, model group) and a control group, we performed intact metabolome analysis by DPiMS/MS. Principal component analysis (PCA) of the obtained results revealed that the two groups are well separated along the first principal component axis as shown in the PCA score plot in Fig. 2 (a). Since the PCA loading plot in Fig. 2 (b) suggests that taurine is greatly influencing the separation, we created a box-whisker plot for taurine as shown in Fig. 2 (c) and performed a significance test. We found that there is a significant difference between the model group and the control group ($p<0.001$, Welch's t-test).

The amount of released enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are used as an index for the diagnosis of acute liver failure. Looking at these two enzymes, there is a significant increase in the model group and a significant negative correlation with the taurine level of the model group (Pearson correlation coefficient r=–0.975 (ALT) and –0.785 (AST)). The liver of a mouse that is administered CCl_4 produces trichloromethyl radicals from the CCl_4 by function of the metabolism enzyme CYP2E1 and it is regarded that these trichloromethyl radicals induce acute liver failure. On the other hand, it is understood that taurine acts as a scavenger of radicals in the liver. Therefore, the taurine concentration in the model group liver is likely to have decreased due to the trichloromethyl radicals that were produced from CCl_4.

Conclusion

The mouse liver metabolome was successfully analyzed without any complex pretreatment by using the DPiMS-8060.

Based on the fact that we successfully observed the changes in metabolites caused by liver failure in CCl_4-induced liver failure model mice, we were able to confirm the applicability of this method to practical use.

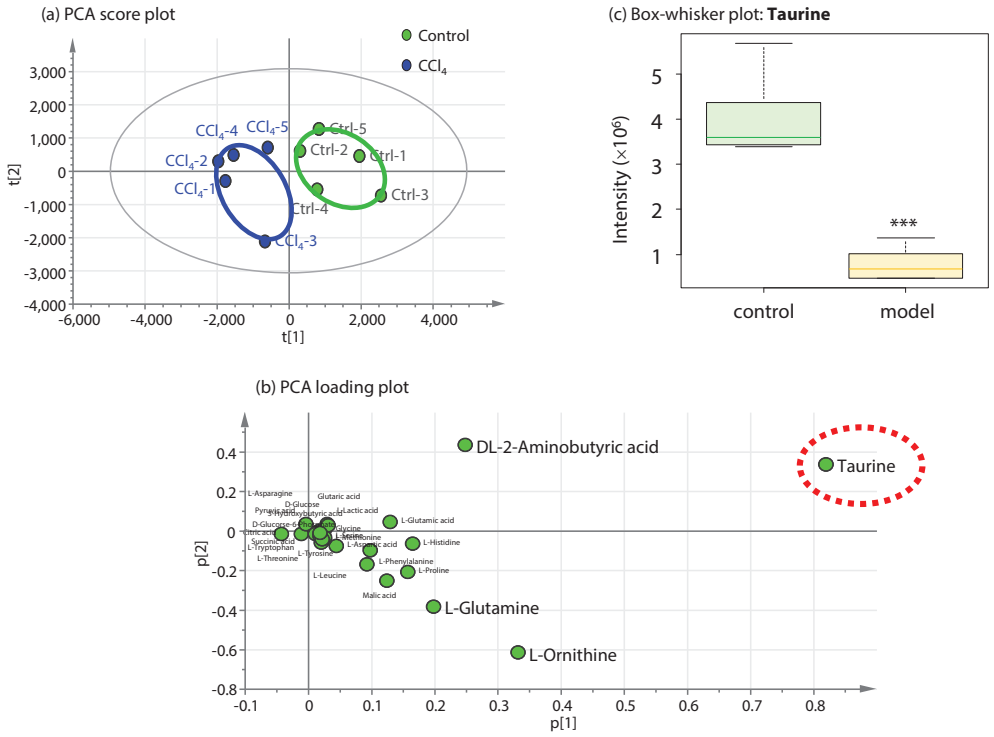

Fig. 2 **Results of Intact Metabolome Analysis of a Healthy Mouse and a Liver Failure Model Mouse**

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References

Zaitsu, K.; Hayashi, Y.; Murata, T., et al. *Anal. Chem.* **2016**, *88*, 3556-3561.

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