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Application News

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LC/MS

Phospholipid analysis using SimLipid software

Phospholipids (PLs) have a role of constituting a cellular membrane in a living cell and are also related to produce various fatty acids such as arachidonic acids, EPA and DHA which are precursors of bioactive lipids. Fluctuation of PLs concentration in a blood or a tissue is also known to be correlated with various disease. For example, Hyperlipidemia and arteriosclerosis are known to induce an elevation of lipid concentration in a blood and some nervous diseases are reported to change the ratio of fatty acid constitution of phospholipids. Thus although phospholipids are reported to be related with various disease, a number of phospholipid species is enormous. PLs are classified to glycerophospholipid and sphingophospholipid by the structural body. Furthermore, PLs are classified to PC, PE, PG, PI, PS, PA and SM by its characteristic head group. These PLs have diverse fatty acids different in a length of carbon chain, an number of double-bond.

Here we shows the analyzing results by LCMS-8060 of phospholipid changes in a liver tissue between a control and a mouse which a fluorescent probe has been administered by a tail vein injection. In this analysis, SimLipid software from PREMIER Biosoft, USA (www.premierbiosoft.com) was used to estimate the candidate of PLs fluctuated between a control and a probe administered mouse.

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Table 1 HPLC condition

Column	: Phenomenex Kinetex C8 (150 × 2.1 mm, 2.6 µm)
Mobile phase A	: 20 mmol/L Ammonium formate
Mobile phase B	Acetonitrile/2-propanol (1:1)
Flow rate of mobile phase	: 0.3 mL/min
Time program (B%)	: 20 % (0 min)→20 % (1 min)→40 % (2 min) →92.5 % (25 min)→100 % (26 – 30 min) Curved gradient from 2 min to 25 min.
Column temp.	: 45 °C
Injection volume	: 3 µL

Table 2 MS Condition (LCMS-8060)

Ionization	: ESI (+) / (−)
Nebulizer gas flow rate	: 3 L/min
Heating gas flow rate	: 10 L/min
Drying gas flow rate	: 10 L/min
Probe voltage	: 4 kV (+) / −3 kV (−)
Interface temperature	: 300 °C
DL temperature	: 250 °C
Block heater temperature.	: 400 °C

Sample preparation and analysis

Carbon nano tube (CNT) probe is known as a fluorescent probe for a long-wavelength to visualize an administered target molecule inside a living body. This probe was administered to a mouse at a concentration of 300 µg/mL by a tail vein injection (100 µL). After 5hr of administering, liver tissues were isolated from a control mouse and a administered mouse. The isolated tissues were rapidly frozen in liquid nitrogen and crushed to some blocks of an appropriate size. Then these tissue blocks were weighed. Furthermore, after crushing frozen tissue blocks by a bead type crusher, phospholipids were extracted by Bligh & Dyer method. Organic phase was recovered and then evaporated. The sample was dissolved with a solution of CHCl3/MeOH (1:1). Phospholipid profiling by precursor ion scan (PIS) and neutral loss scan (NLS) with LCMS-8060 were executed for the sample diluted with MeOH (Table 1). In this case, phospholipid analysis were carried out by PIS at *m/z* 184 focusing on the characteristic head groups of PC and SM or NLS of 141 for ethanolamine of PE (Figure 1). The candidate of phospholipids was estimated for each peak detected on PIS and NLS analysis as a result of database search by SimLipid software (Figure 2).

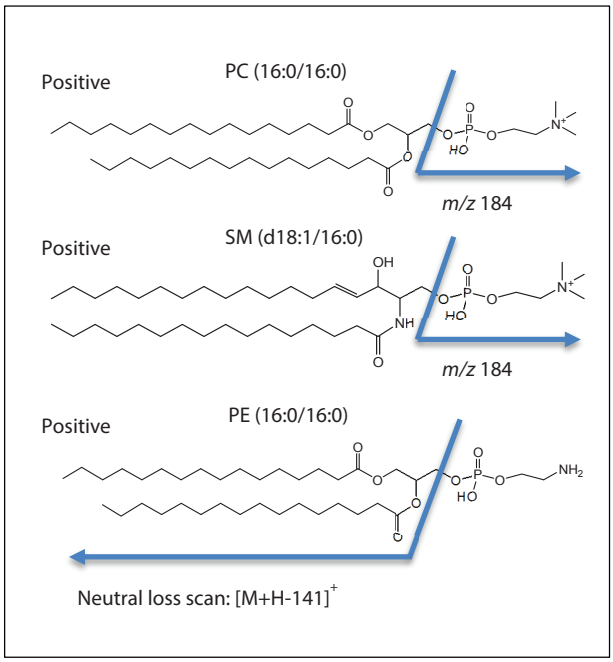

Fig. 1 Structural formula of PC, SM and PE

Database search of phospholipids by SimLipid

On the basis of analyzing results by precursor ion scan at *m/z* 184 for PC and SM, database search was executed to identify the detected peaks by SimLipid software. Fig. 2 shows the result of database search. Here the candidates of PLs are manually narrow down by considering the information of retention time of PL peaks and the length of carbon chain.

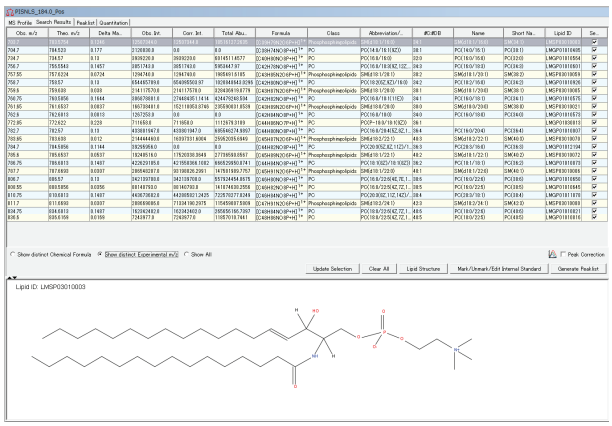

Fig. 2 Estimate of phospholipid candidates by SimLipid software

Phospholipid change in liver tissue by fluorescent probe dosing

Next, Figure 3 shows the graph plotted by the peak intensity (Total Abundant) of each phospholipid (PC, SM and PE) integrated among each sample group (a normal and a probe dosing, n=3). In this graph, only PL species for PC, SM and PE, which have been detected in all samples, are shown among all detected peaks of PLs. These peak intensity were normalized by the tissue weight

An increase of phospholipids which was considered to be the influence of probe administration, was confirmed as Figure 3 showed. In particular, it was confirmed notably in sphingophospholipids such as SM(38:1), SM(40:3) and SM(42:3). In addition, an increase of phospholipids such as PC(38:6), PC(40:6), PE(38:6) and PE(40:6) which were considered to contain polyunsaturated fatty acids, was observed as well. On the other hand, some phospholipids, PC(34:1) and PE(34:1) have reduced after probe dosing (5hr). These results suggest that increase and decrease in each class of PLs including the same fatty acid composition are correlated.

Thus the simultaneous analysis for phospholipids on PIS and NLS mode by LCMS-8060 enables to evaluate the fluctuation of PLs by narrowing down the candidate phospholipid species from the database search by SimLipid software.

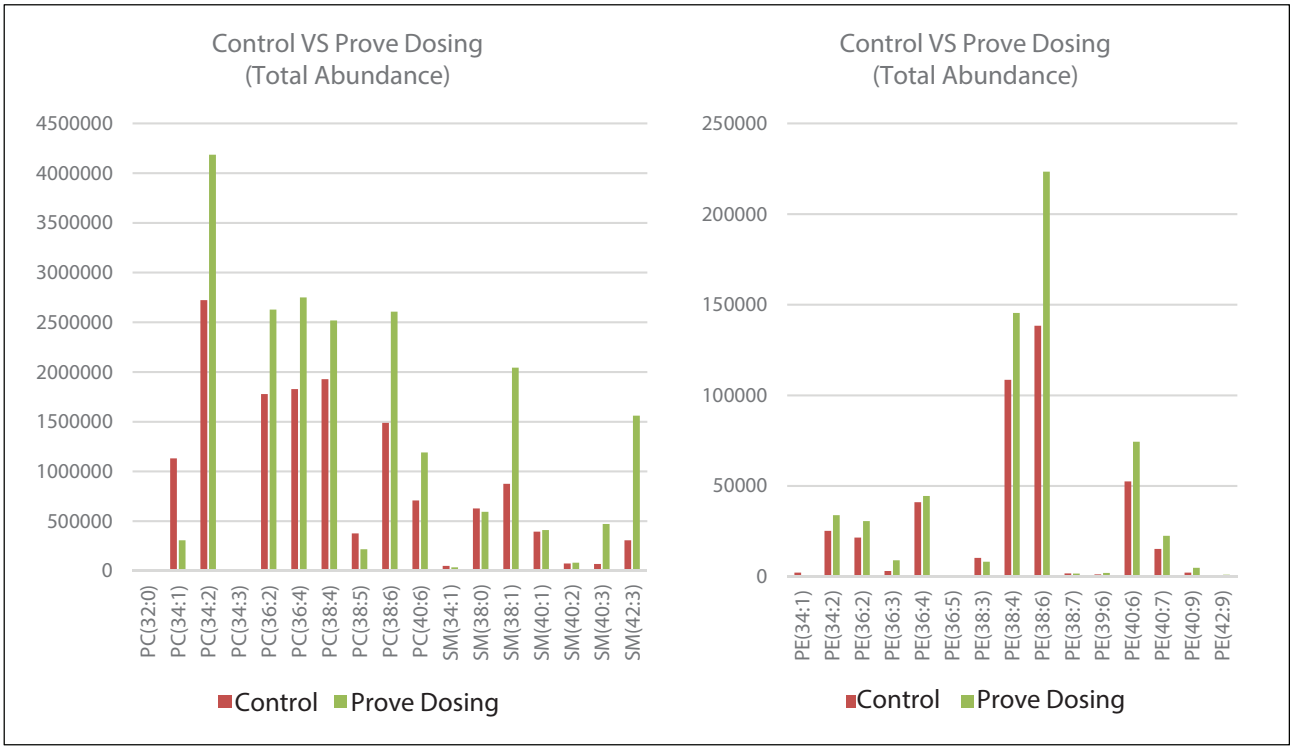

Fig. 3 Changes of phospholipids in liver tissues from a normal and a fluorescent probe dosing mouse.

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