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Phospholipid characterization by a TQ-MS data based identification scheme

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Overview

Phospholipids play a wide variety of roles such as the structural units of the cellular membrane, the storage and transport of lipids, the precursor of the lipid mediators and the energy source *in vivo*. To deeply understand the biological function of phospholipids, quantitative analysis for the phospholipid composition ratio in the blood and tissues is required. Phospholipids species have many types of combination of its characteristic head groups and fatty acid composition. Although identification of phospholipid species typically is performed for the data acquired by a high resolution mass spectrometer, in this study, we report phospholipids database search and quantitative analysis by coupling LC-TQ MS with SimLipid software.

Introduction

Phospholipids constitute a lipid bilayer in a cellular membrane in a living cell and the number of phospholipid species reaches up to several thousands due to the combination of its characteristic head groups and its fatty acid composition. It has been also reported that change of phospholipid levels in blood and tissues correlates with various diseases. Therefore, quantitative analysis for phospholipids has been often performed to evaluate the change of phospholipid species at a concentration. In this study, the carbon nano tube (CNT) probe, known as a long wavelength fluorescent probe, was administered to mice to evaluate the changes in phospholipid in a liver tissue. To analyze the fluctuation of phospholipids, a precursor ion scan/neutral loss scan was performed by a LC-TQ MS. The change of identified phospholipid levels was compared between control and a probe dosing mouse.

Methods

Carbon nano tube (CNT) probe is known as a fluorescent probe for a long-wavelength. This probe was administered to a mouse at a concentration of 300 mg/mL by a tail vein injection (100 mL). After 5hr of administering, liver tissues were isolated from a control mouse and a ministered mouse under anesthesia. 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 $CHCl_3$/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 . In this measurement, phospholipid was analyzed by PIS at *m/z* 184 focusing on the characteristic head groups of phosphatidylcholine (PC) and sphingomyeline (SM) or NLS at *m/z* 141 for ethanolamine of phosphatidylethanolamine (PE). The candidate of phospholipids was estimated for each peak detected by PIS and NLS analysis as a result of database search by SimLipid software from PREMIER Biosoft, CA, USA (www.premierbiosoft.com).

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Table 1 Analytical conditions

HPLC condition	
Column	: Phenomenex Kinetex C8 (150 X 2.1 mm, 2.6 mm)
Mobile phase A	: 20 mM Ammonium formate - water
Mobile phase B	: Acetonitrile/2-Propanol (1:1)
Time program (B%), Curved gradient	: 20% (0 min) - 20% (1.0 min) - 40% (2.0 min) - 92.5% (25 min) - 100% (26.0-35.0 min)
Flow rate	: 0.3 mL/min
Injection volume	: 3 mL
Column oven	: 45˚C
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
Interface voltage	: 4 kV(+)/-3 kV(-)
Interface temp.	: 300˚C
DL temp.	: 250˚C
Block heater temp.	: 400˚C

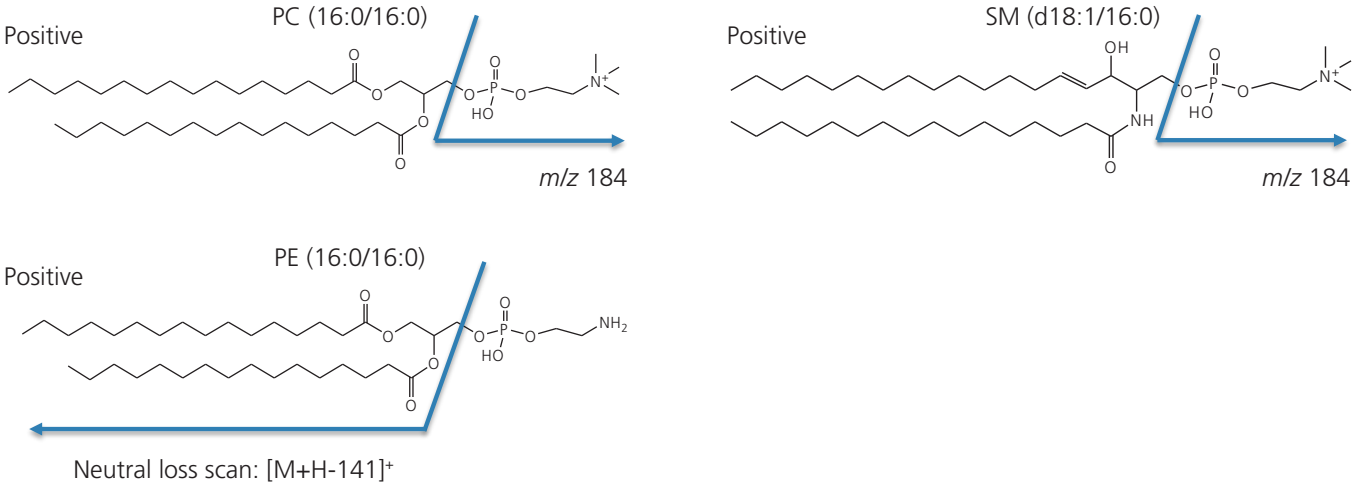

Figure 1 PIS for PC and SM at *m/z* 184 and NLS for PE of $[M+H-141]^+$

Results

PIS and NLS measurements to detect phospholipid class of PC, SM and PE

To detect phospholipid species in tissue extracts, we performed PIS at *m/z* 184 as a product ion for PC / SM and NLS of $[M+H-141]^+$ for PE. Both measurements were performed in positive mode. A typical structural formula for PC, SM and PE are shown in Figure 1. As shown in Figure 1, PIS and NLS were measured by utilizing a loss of its characteristic head group on MS/MS. Figure 2 shows typical mass chromatograms of PIS and NLS for liver tissue extracts. We can see some detected peaks corresponding to PC, SM and PE.

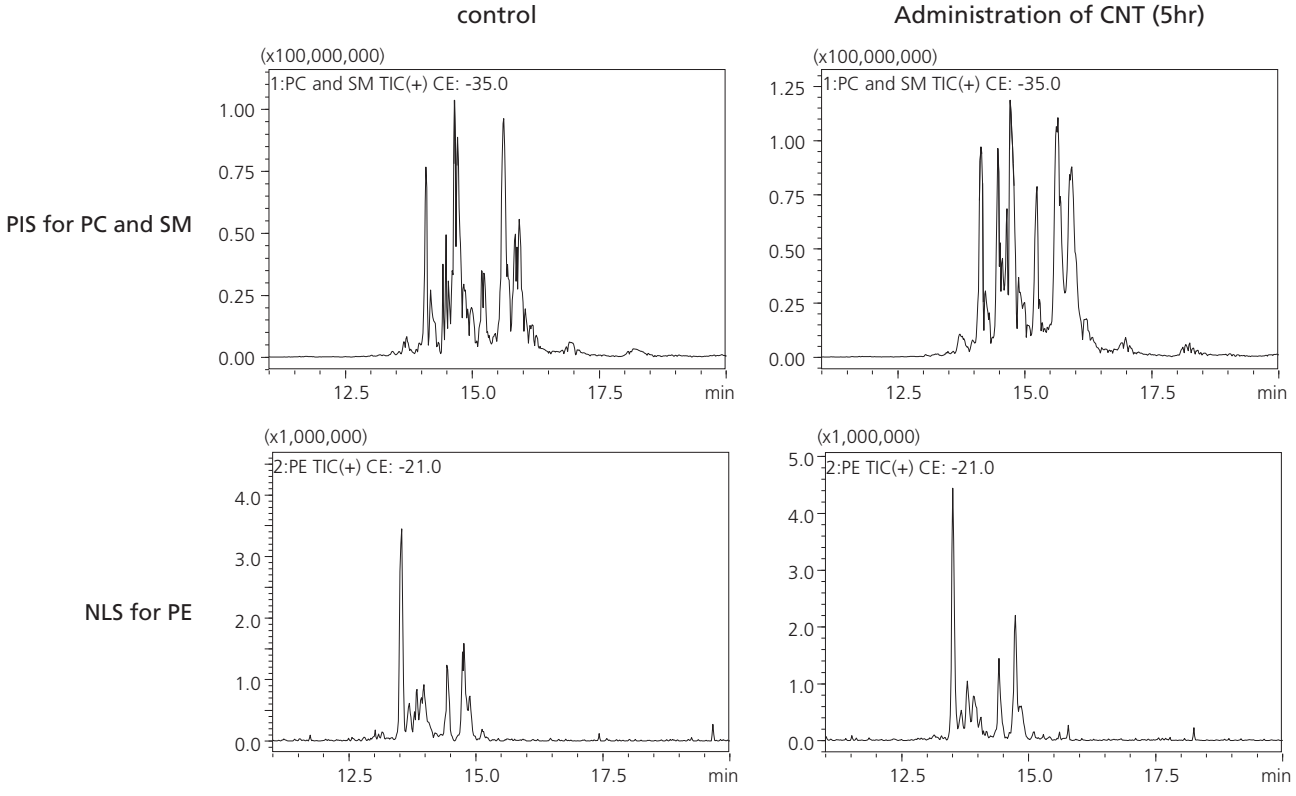

Figure 2 MS chromatograms of PIS for PC and SM at *m/z* 184 and NLS for PE of $[M+H-141]^+$

In Figure 2, the change of some phospholipids, especially PC and SM can be also confirmed between control and administration of CNT sample. To evaluate the change of phospholipids at a concentration, we first performed a database search to identify the detected phospholipid species. A database search and quantitative analysis were performed by SimLipid software. Scheme 1 shows a workflow of phospholipid profiling by SimLipid software. TQ-MS data (.lcd file) is imported into the program and a database search is carried out on the basis of a observed mass value of precursor ion/product ion on PIS/NLS measurement. SimLipid software has a theoretical mass value of total product ion/neutral loss masses corresponding to >5,000 species in the TQ database. In this study, a database search was executed at a range of 0.2 Da as a tolerance. As a result, the candidate phospholipid species were estimated for the detected peaks and the intensity for the detected peaks in a group of control or administration of CNT was integrated for quantitative analysis.

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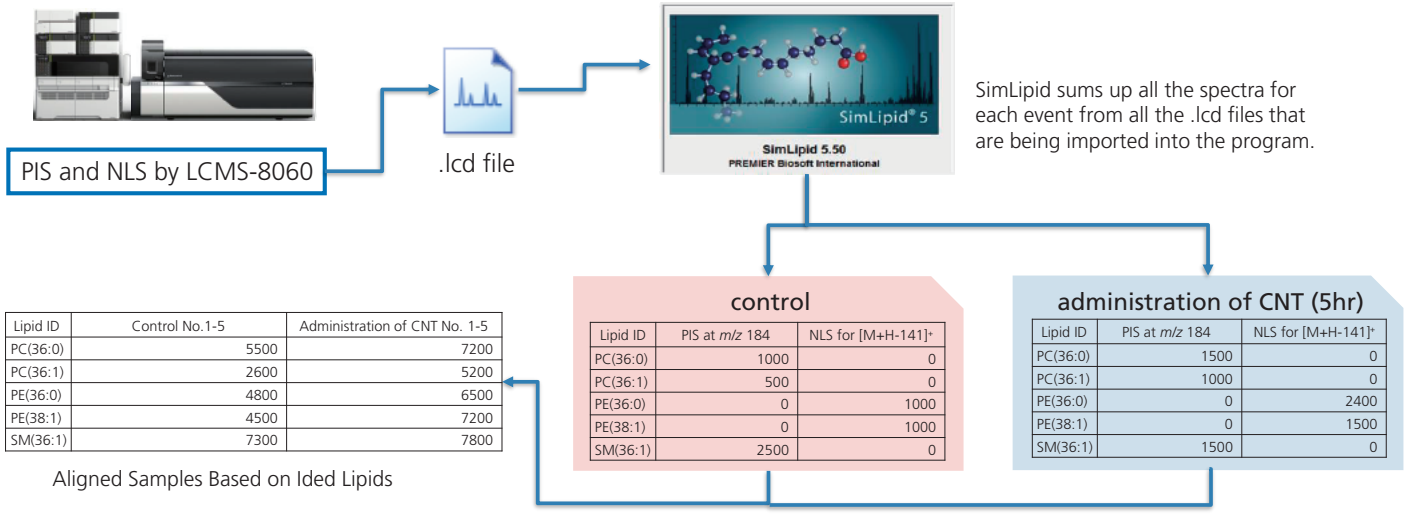

Lipid ID	Control No.1-5	Administration of CNT No. 1-5
PC(36:0)	5500	7200
PC(36:1)	2600	5200
PE(36:0)	4800	6500
PE(38:1)	4500	7200
SM(36:1)	7300	7800

Lipid ID	PIS at *m/z* 184	NLS for [M+H-141]$^+$
PC(36:0)	1000	0
PC(36:1)	500	0
PE(36:0)	0	1000
PE(38:1)	0	1000
SM(36:1)	2500	0

Lipid ID	PIS at *m/z* 184	NLS for [M+H-141]$^+$
PC(36:0)	1500	0
PC(36:1)	1000	0
PE(36:0)	0	2400
PE(38:1)	0	1500
SM(36:1)	1500	0

Scheme 1 Workflow of phospholipid profiling by coupling LCMS-8060 and SimLipid

Phospholipid change in liver tissues by fluorescent probe administration

Figure 3 shows a typical result of the database search for PIS at *m/z* 184 by SimLipid. We can confirm the major PC and SM species as phospholipid candidates in this figure. In this study, PIS for the composition of fatty acids was not measured. Therefore, the candidates of phospholipids were manually narrowed down by considering the information of retention time of phospholipid peaks and the length of carbon chain. To confirm the composition of fatty acids for phospholipid species, this result was also compared with the results of MRM measurements for phospholipid species (data not shown).

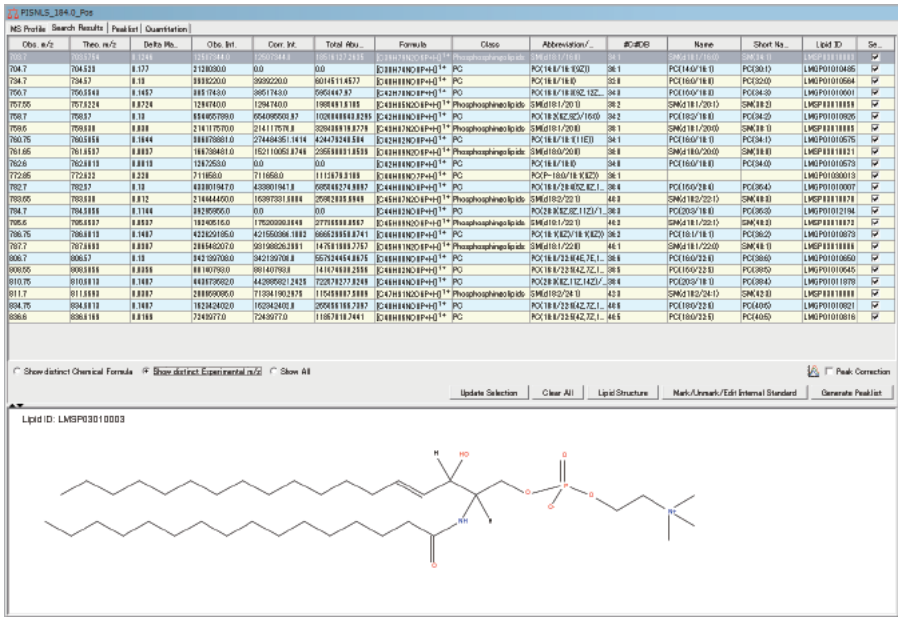

Figure 3 Estimate of phospholipid candidates by SimLipid database search

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Next, Figure 4 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 administration, n=3). Phospholipid species for PC, SM and PE, which have been detected in all samples, are shown in this graph. These peak intensity were normalized by a value of the tissue weight. An increase of phospholipids which was considered to be the influence of probe administration, was confirmed as Figure 4 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.

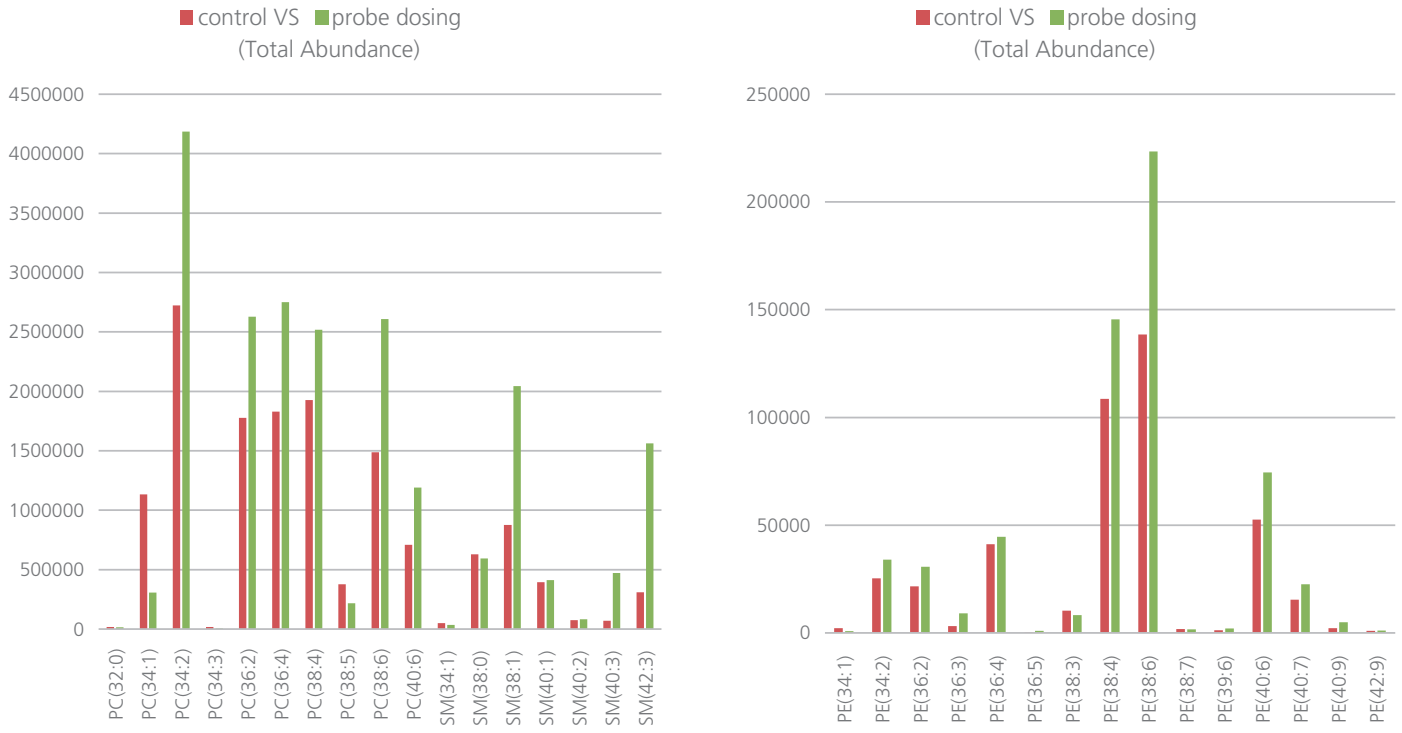

Figure 4 Changes of phospholipids in liver tissues from a normal and a fluorescent probe administration

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Conclusions

- We performed phospholipid profiling by coupling TQ-MS measurement with SimLipid database search.
- To evaluate the phospholipid profiling system, we analyzed the change of phospholipids in liver tissues between control and probe administration.

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