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Analysis of Phosphatidylcholine Oxidation Products in Human Plasma Using Quadrupole Time-of-flight Mass Spectrometry

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We report here an application of the previous method for the analysis of phosphatidylcholine (PC) and lysophosphatidylcholine (lysoPC) oxidation products in human plasma using quadrupole time of flight (Q-TOF) mass spectrometry with electrospray ionization. We separated these products using an HPLC C8 column with a gradient of methanol and 10 mM aqueous ammonium acetate. Monohydroperoxides, epoxyhydroxy derivatives, oxo derivatives, and trihydroxides of palmitoyl-linoleoyl (C16:0/C18:2) PC and stearoyl-linoleoyl (C18:0/C18:2) PC were detected mainly as MH^+ and $[M+Na]^+$ ions in the plasma of alcoholic patients. Using standard synthetic PC-OH (C16:0/C18:2-OH), the lipid extract component was identified as (C16:0/C18:2-OH) PC based on the product ions of ESI-MS-MS. Using standard synthetic PCOOH (C16:0/C18:2-OOH) as a reference, the PCOOH concentration in plasma was quantified. Two oxidatively modified lysoPCs were also detected. This is the first report showing the presence of epoxyhydroxy derivatives, monohydroperoxides, oxo derivatives, and trihydroxides of (C16:0/C18:2) PC and (C18:0/C18:2) PC, and PC-OH (C16:0/C18:0-OH) in human plasma.

Increased interest in lipid peroxidation has accelerated the development of techniques for the determination of lipid peroxides in biological samples. Phosphatidylcholine (PC) is a major component of phospholipids in plasma and is easily oxidized to produce phosphatidylcholine hydroperoxide (PCOOH), phosphatidylcholine hydroxide (PC-OH), and other products. Plasma PCOOH has been used as a general indicator of lipid peroxidation in dialysis patients (19) and in patients with hyperlipidemia (14). Previously, we identified a monohydroperoxide (1) and a monohydroxide (2) of palmitoyl-linoleoyl (C16:0/C18:2)PC in rat heart using quadrupole time of flight (Q-TOF) mass spectrometry. We detected monohydroperoxides (1) and monohydroxides (2) of stearoyl-linoleoyl (C18:0/C18:2)PC and oleoyl-linoleoyl (C18:1/C18:2)PC. We also detected epoxyhydroxy derivatives (1), oxo derivatives (2), and trihydroxides (2) of (C16:0/C18:2)PC, (C18:0/C18:2)PC, and (C18:1/C18:2)PC in the rat heart.

The aim of this study was to elucidate the molecular structure of oxidation products of PC and lysoPC in human plasma by Q-TOF mass spectrometry with electrospray ionization.

The proposed molecular species of oxidatively modified PC and protonated molecular ions are shown in Table 1, and the proposed molecular species of lysoPC and oxidatively modified lysoPC are shown in Table 2.

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Table 1. Molecular species of oxidatively modified phosphatidylcholine (PC) and protonated molecular ions

Peak	Molecular species of PC		
	R_1	R_2	MH^+
Standard PCOOH			
1	16:0	Hydroperoxy-18:2	790.6
Epoxyhydroxy-PC			
2a	16:0	Epoxyhydroxy-18:2	790.6
3a	18:0	Epoxyhydroxy-18:2	818.6
Hydroperoxy-PC			
2b	16:0	Hydroperoxy-18:2	790.6
3b	18:0	Hydroperoxy-18:2	818.6
Standard PC-OH			
4	16:0	Hydroxy-18:2	774.6
Hydroxy-PC			
5	16:0	Hydroxy-18:2	774.6
Oxo-PC			
6	16:0	Oxo-18:2	772.6
7	18:0	Oxo-18:2	800.6
Trihydroxy-PC			
8	16:0	Trihydroxy-18:2	808.6
9	18:0	Trihydroxy-18:2	836.6

Table 2. Molecular species of lyso-phosphatidylcholine (lyso-PC) and oxidatively modified lyso-PC

Peak	Molecular species of lyso-PC		
	R_1	R_2	MH^+
Oxidized lyso-PC			
10	H	Hydroperoxy-18:2	552.3
11	H	Oxo-18:2	534.3
Lyso-PC			
12	H	18:2	520.3
13	H	20:4	544.3
14	16:0	H	496.3
15	H	18:1	522.3
16	18:0	H	524.3

MATERIALS AND METHODS

Materials

3, 5-Di-tert-butyl-4-hydroxytoluene (BHT), luminol and cytochrome C (from horse heart, type VI) were purchased from Wako Pure Chemical Co. (Osaka, Japan). 1-Palmitoyl-2-linoleoyl-phosphatidylcholine hydroperoxide (C16:0/C18:2-OOH, PLPC-OOH) was synthesized as described previously (1). In brief, a solution of PLPC in methylene blue solution was irradiated using a tungsten lump (30W) at 15 °C for 8 h. The reaction mixture was then subjected to HPLC. The product obtained was checked by MS, TLC, and HPLC. The concentration of PCOOH was determined photometrically by absorbance at 660 nm after changing phospholipids to inorganic phosphine by perchloric acid and reacting with ammonmolybate (5). The amount of phospholipids was 1.95 mg/ml. MS: 790.6 (MH^+, monoperoxide) and 812.5 ($[M+Na]^+$, monoperoxide). 1-Palmitoyl-2-linoleoyl-phosphatidylcholine hydroxide (C16:0/C18:2-OH, PLPC- OH) was provided by Prof. Yamamoto of Tokyo University of Technology. It was prepared by the aerobic oxidation of PLPC with lipoxygenase as described previously (17). In brief, PLPC (6 μmol) was dissolved in sodium borate buffer (pH 9) containing sodium deoxycholate and mixed with soybean lipoxygenase (Sigma, Type 1B). The solution was stirred for 45 min at room temperature and was passed through a solid phase extraction tube (Supelco LC-18, 1 g, Sigma-Aldrich, Tokyo, Japan) to remove bile acid and other components. The eluant was concentrated and PLPC-OOH was purified by HPLC (Superiorex ODS column; 20 x 250 mm, 5 μm, Shiseido, Tokyo, Japan) using 0.02% triethylamine in methanol as the mobile phase with a flow rate of 8 ml/min. The corresponding alcohol (PLPC-OH) was purified by

solid phase extraction and HPLC separation as described above after the reduction with sodium borohydride. The concentration was determined photometrically by absorbance at 234 nm using the molar absorption coefficient for a conjugated diene, 28,000 $cm^{-}M^{-}$(4). The amount of phospholipid was 2.20 mg/ml. MS: 774.6 (MH^{+}, monooxide) and 796.6 ($[M+Na]^{+}$, monooxide).

Subjects

Five male alcoholic patients, 36-63 years old (mean ± SD: 51.4 ± 10.7 years) were studied. This experiment had the approval of the ethics committee of the National Hospital Organization, Kurihama Alcoholism Center. Blood samples were collected from alcoholic patients at the first medical examination. They were suffering from alcoholic liver disease with high γ-GTP, AST, and ALT activities.

Extraction

Three milliliters of blood was collected in a test tube containing 0.3 mg of EDTA·2Na and centrifuged at 4°C and 800 g for 15 min, and the plasma was fractionated. Total lipid was extracted by adding 0.5 ml of distilled water and 8 ml of ice-cold chloroform-methanol (3:1, v/v) containing 0.005% (v/v) BHT (as antioxidant) to 0.5 ml of plasma. The mixture was spun vigorously for 1 min, and then centrifuged at 800 g for 20 min. The chloroform layer was removed by aspiration, concentrated in a rotary evaporator and then dried under a nitrogen stream. The phospholipid fraction was then isolated from the total lipid sample by solid phase extraction. A silica column (Sep-Pak, Waters Co., Milford, MA) of 3 ml capacity packed with aminopropyl-derivatized silica ($-NH_2$) was used. The total lipid sample was dissolved in a small amount of chloroform and layered on the column, which then was flushed with a mixture of 2 ml of chloroform and 1 ml of *iso*-propanol. The column was next flushed with methanol containing 0.005% BHT, giving an eluate consisting mainly of phospholipid. This was concentrated using a rotary evaporator, dried under a nitrogen stream and then dissolved in 150 μl of methanol. A 1 μl portion was injected into the LC-MS. A 10 μl portion was injected into the HPLC column for quantitative analysis.

LC-MS conditions

An HPLC model 1100 system (Agilent Technologies, Waldbronn, Germany) with a Luna C_8 column (1.0 mm x 150 mm, 5.0 μm; Phenomenex, Torrance, CA) was used. Injections of plasma samples (1 μl (MS) and 2 μl (MS/MS)) were made. The column was maintained at 40°C and eluted at 100 μl/min. The mobile phase consisted of 5% methanol with 10 mM aqueous ammonium acetate (solvent A) and 95% methanol with 10 mM aqueous ammonium acetate (solvent B). Separation was carried out with a linear gradient starting with 90% solvent B followed by ramping up to 100% solvent B at 20 min and then maintaining for 17 min. The total run time was 37 min.

Mass spectrometric analysis was performed on a quadrupole orthogonal acceleration time of flight, Micromass Q-TOF Micro (Waters Corporation, Milford, MA) equipped with an electrospray interface. The instrument was operated in positive ion mode with a capillary voltage of 3200 V and a cone voltage of 40 V. The desolvation gas was set at 600 l/h with a desolvation temperature of 150°C and source temperature of 80°C. Full-scan spectra were recorded in profile mode. The range between *m/z* 100 and 1000 was recorded at a resolution of 5000 (FWHM) and the accumulation time was 1 sec/spectrum. Accurate masses were measured by comparison to a reference compound, Leucine Enkephalin ($[M+H]^{+}$=556. 2771 Da) infused into the Lock Spray reference channel.

HPLC analysis

Phosphatidylcholine hydroperoxide (PCOOH, C16:0/C18:2-OOH) was quantitated by HPLC with chemiluminescence detection (HPLC-CL). An LC-18-DB column (SUPELCO,

250 mm x 4.6 mm i.d.) was isocratically run using a mobile phase consisting of 0.01% triethyamine methanol. The mobile phase and chemiluminescent reagent (cytochrome C and luminol) were delivered at 0.7 ml/min. After the column eluant was passed through a UV detector, it was mixed with luminescent reagent in the post-column mixing joint of the chemiluminescence detector. Individual peak areas were calculated using an integrator (Chromatopac C-R8A, Shimadzu).

RESULTS

Accurate masses of 16 components of PC and lysoPC oxidation products in plasma were measured (in a single run) using the Q-TOF system. The results of the measurements are shown in Table 3.

The following possible products of phosphatidylcholine oxidation are shown in Fig. 1. (A) 13-Hydroperoxy-PC and 9-hydroperoxy-PC; (B) 12, 13-epoxy-9-hydroxy-10-en-PC; (C) 13-hydroxy-PC and 9-hydroxy-PC; (D) 13-oxo-PC and 9-oxo-PC; and (E) 9, 12, 13-trihydroxy-PC.

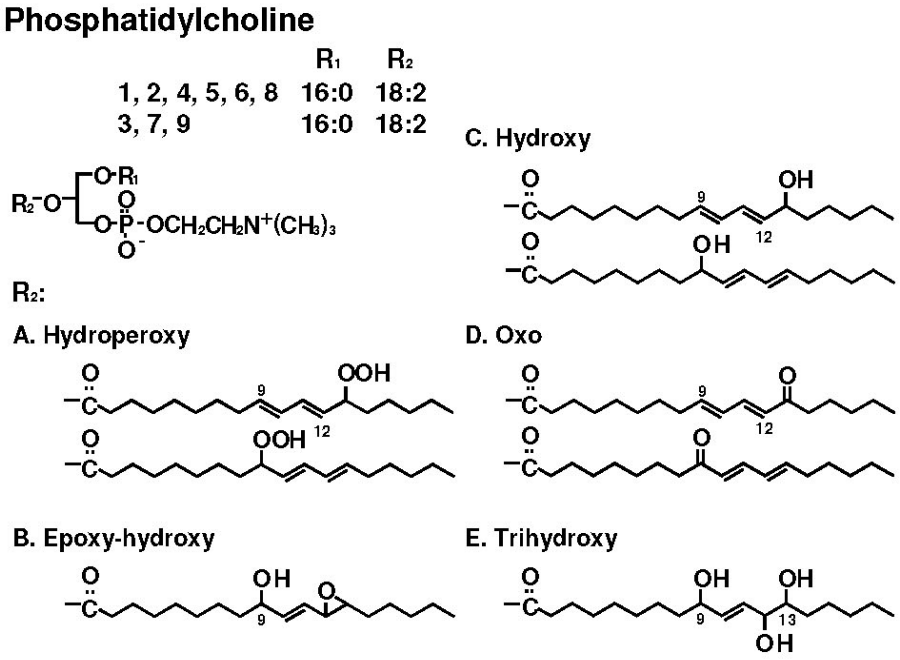

Figure 1: Possible products of phosphatidylcholine oxidation.

Table 3. Accurate mass measurement results for compounds found in plasma

Peak	R.T.	Measured	Calculated	Composition	Differences *
	min	*m/z*	*m/z*		ppm
1**	10.6	790.5564	790.5598	C42H81NO10P	-4.3
2a	7.2	790.5640	790.5598	C42H81NO10P	5.3
2b	10.8	790.5579	790.5598	C42H81NO10P	-2.4
3a	11.3	818.5847	818.5911	C44H85NO10P	-7.9
4***	9.8	774.5657	774.5649	C42H81NO9P	1.0
5	10.6	774.5634	774.5649	C42H81NO9P	-1.9
6	10.0	772.5524	772.5492	C42H79NO9P	4.0
8	4.1	808.5662	808.5704	C42H83NO11P	-5.2
9	5.7	836.6021	836.6017	C44H87NO11P	0.5
10	1.3	552.3283	552.3301	C26H51NO9P	-3.3
11	1.4	534.3209	534.3196	C26H49NO8P	2.5
12	2.5	520.3403	520.3403	C26H51NO7P	0
13	2.6	544.3381	544.3403	C28H51NO7P	-2.2
14	2.8	496.3413	496.3403	C24H51NO7P	2.0
15	3.0	522.3557	522.3560	C26H53NO7P	-0.5
16	4.2	524.3732	524.3716	C26H55NO7P	3.0

*: Differences in values between measured and calculated.

**: Standard PC C16:0/C18:2-OOH.

***: Standard PC C16:0/C18:2-OH

Figure 2 shows mass chromatograms obtained from 5 to 17 min by LC-MS. Mass chromatograms of standard PCOOH (column 1) and plasma extracts (columns 2 and 3) are shown in Figure 2. Coinciding peaks are seen originating from the MH^+ of standard PCOOH (C16:0/C18:2-OOH) at *m/z* 790.6 at a retention time of 10.6 min, from the MH^+ of the hydroperoxy derivative of PC C16:0/C18:2 at *m/z* 790.6 at a retention time of 10.8 min (peak 2b), from the MH^+ of the epoxyhydroxy derivative (estimated) of PC C16:0/C18:2 at *m/z* 790.6 with a retention time of 7.2 min (peak 2a), from the MH^+ of the epoxyhydroxy derivative (estimated) of PC C18:0/C18:2 at *m/z* 818.6 with a retention time of 11.3 min (peak 3a) and mono-hydroperoxide of PC C18:0/C18:2 at *m/z* 818.6 with a retention time of 15.6 min (peak 3b), respectively.

The ESI-mass spectra of peaks 1, 2a, and 2b show MH^+ (*m/z* 790.6) and $[M+Na]^+$ (*m/z* 812.5) ions, as shown in Fig 3. The ESI-mass spectra of peaks 3a and 3b show MH^+ (*m/z* 818.6) and $[M+Na]^+$ (*m/z* 840.6), as also shown in Fig.3.

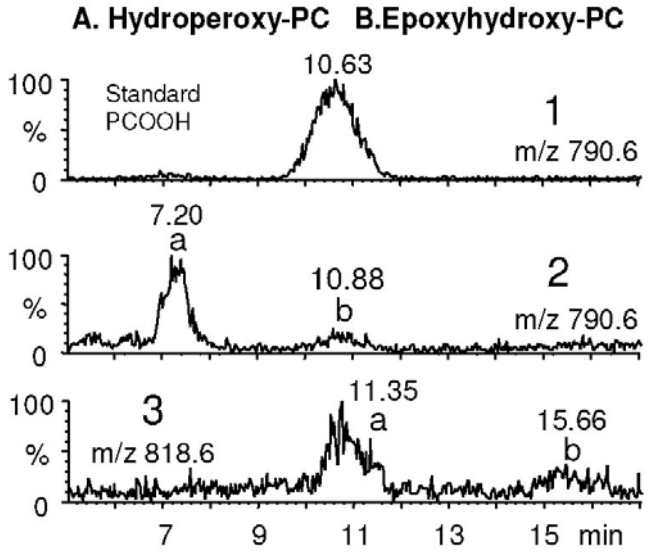

Figure 2: Positive-ion HPLC-ESI-MS analyses of a standard compound (column 1) and extracted human plasma (column 2 and 3) between 5 and 17 min. Mass chromatograms of the standard PC (C16:0/C18:2-OOH) (*m/z* 790.6), PC (C16:0/C18:2-OOH), epoxyhydroxy-PC (C16:0/C18:2) (*m/z* 790.6) in plasma, PC (C18:0/C18:2-OOH) (*m/z* 818.6), and epoxyhydroxy-PC (C18:0/C18:2) (*m/z* 818.6) in plasma.

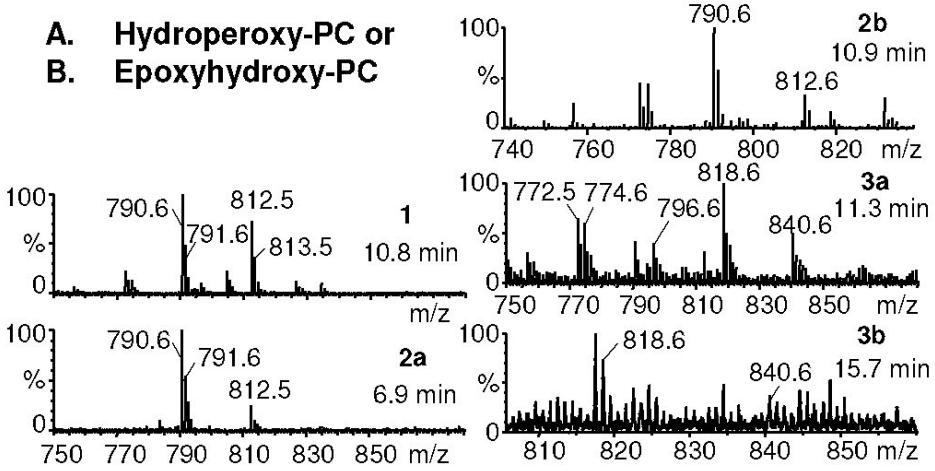

Figure 3: Positive-ion HPLC-ESI-MS analyses of the standard compound (column 1) and extracted human plasma (column 2a, 2b, 3a, and 3b) between 5 and 17 min. Mass spectra of the standard PCOOH (C16:0 /C18:2-OOH) (column 1), epoxyhydroxy-PC (C16:0/C18:2) (column 2a), PCOOH (C16:0/C18:2-OOH) (column 2b), epoxyhydroxy-PC (C18:0/C18:2) (column 3a), and PCOOH (C18:0/C18:2-OOH) (column 3b).

Mass chromatograms of standard PC-OH (column 4) and the plasma extract (column 5) are shown in Fig 4. Coinciding peaks are seen originating from the MH^+ of standard PC-OH (C16:0/C18:2-OH) at *m/z* 774.6 at a retention time of 9.8 min and from the MH^+ of the hydroxy derivative of PC C16:0/C18:2 at *m/z* 774.6 with a retention time of 10.6 min (column 5). The ESI-mass spectra of standard PC-OH (column 4) and peak 5 showed MH^+ (*m/z* 774.6), $[M\text{-}H_2O+H]^+$ (*m/z* 756.6), and $[M+Na]^+$ (*m/z* 796.5) ions, as shown in Fig. 5.

Mass chromatograms of oxo-PC from plasma extracts (columns 6 and 7) are shown in Fig. 4. Coinciding peaks are seen originating from the MH^+ of oxo derivatives of PC C16:0/C18:2 at *m/z* 772.6 with retention time of 10.5 min (column 6), and PC C18:0/C18:2 at *m/z* 800.6 with retention time of 14.0 min (column 7). The ESI-mass spectra of peaks 6 and 7 showed MH^+ and $[M+Na]^+$ ions as follows: 6, 772.6 and 794.5; 7, 800.6 and 822.6 (Fig. 5).

Figure 5 also shows the product ion profiles (MS-MS) for standard PC-OH and peak 5. For standard PC-OH, the base peak was *m/z* 756.6. The breakdown product lost 18 mass units, corresponding to dehydration; a small peak was seen at *m/z* 184.1, corresponding to choline phosphate; this supported the assignment as phosphatidylcholine hydroxide. For peak 5, the base peak was *m/z* 756.6; a small peak attributed to fragment ion appeared at *m/z* 184.1. Thus, peak 5 was identified as PC C16:0/C18:2-OH.

Figure 6 shows mass chromatograms of plasma extracts from 0 to 10 min. Coinciding peaks are seen originating from MH^+ of trihydroxy derivatives of PC C16:0/18:2 at *m/z* 808.6 with a retention time of 4.1 min (column 8) and PC C18:0/C18:2 at *m/z* 836.6 with a retention time of 5.7 min (column 9). The ESI-mass spectra of peaks 8 and 9 showed MH^+ and $[M+Na]^+$ ions as follows: 8, 808.6 and 830.5; 9, 836.6 and 858.6 (Fig. 6 right columns).

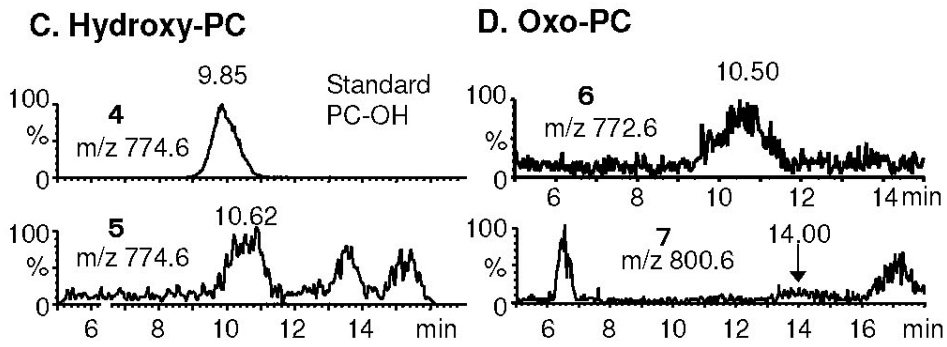

Figure 4: Positive-ion HPLC-ESI-MS analyses of a standard compound (column 4) and extracted human plasma (column 5, 6, and 7) between 5 and 17 min. Mass chromatograms of the standard PC (C16:0/C18:2-OH) (*m/z* 774.6), PC (C16:0/C18:2-OH) (*m/z* 774.6) (column 5), oxo-PC (C16:0/C18:2) (*m/z* 772.6) (column 6), and oxo-PC (C18:0/C18:2) (*m/z* 800.6) (column 7).

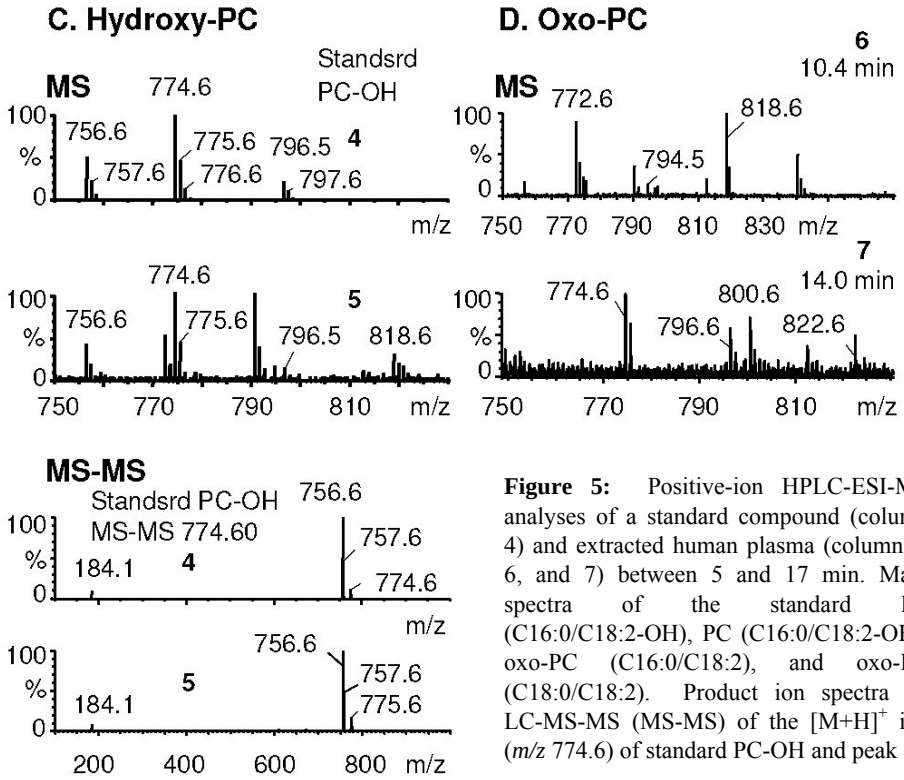

Figure 5: Positive-ion HPLC-ESI-MS analyses of a standard compound (column 4) and extracted human plasma (column 5, 6, and 7) between 5 and 17 min. Mass spectra of the standard PC (C16:0/C18:2-OH), PC (C16:0/C18:2-OH), oxo-PC (C16:0/C18:2), and oxo-PC (C18:0/C18:2). Product ion spectra on LC-MS-MS (MS-MS) of the $[M+H]^+$ ion (*m/z* 774.6) of standard PC-OH and peak 5.

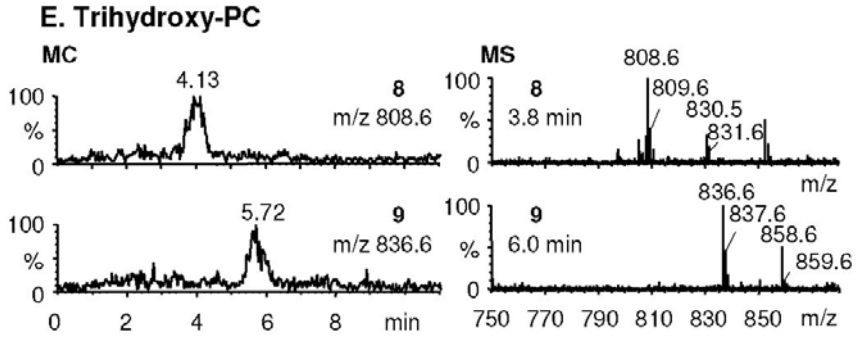

Figure 6: Positive-ion HPLC-ESI-MS analyses of extracted human plasma between 0 and 10 min. Mass chromatograms (MC) and mass spectra (MS) of trihydroxy-PC (C16:0/C18:2) (*m/z* 808.6) (column 8) and trihydroxy-PC (C18:0/C18:2) (*m/z* 836.6) (column 9).

Figure 7 shows mass chromatograms obtained from 0 to 5 min. In Figure 7, coinciding peaks are seen originating from MH^+ of the hydroperoxy derivative of lysoPC C18:2 at *m/z* 552.3 with retention time of 1.3 min (column 10), from MH^+ of the oxo derivative of lysoPC C18:2 at *m/z* 534.4 with retention time of 1.4 min (column 11), from MH^+ of lysoPC C18:2 at *m/z* 520.3 with retention time of 2.5 min (column 12), from MH^+ of lysoPC C20:4 at *m/z* 544.3 with retention time of 2.6 min (column 13), from MH^+ of lysoPC C16:0 at *m/z* 496.3 with retention time of 2.8 min (column 14), from MH^+ of lysoPC C18:1 at *m/z* 522.3 with retention time of 3.0 min (column 15), and from MH^+ of the lysoPC C18:0 at *m/z* 524.3 with retention time of 3.8 min (column 16). Figure 8 shows ESI-mass spectra of peaks 10 to 16. For peak 10, the mass spectrum showed MH^+ (*m/z* 552.3), $[M+Na]^+$ (*m/z* 574.3), and $[M+K]^+$ (*m/z* 590.3), and for peak 11, MH^+ (*m/z* 534.3), $[M+Na]^+$ (*m/z* 556.3). Thus, peaks 10 and 11 were assumed to be the hydroperoxy derivative of lysoPC C18:2 and lysoPC C18:2-oxo, respectively. For peak 12, the mass spectrum showed MH^+ (*m/z* 520.3); for peak 13, MH^+ (*m/z* 544.3); for peak 14, MH^+ (*m/z* 496.3); and for peak 15, MH^+ (*m/z* 552.3), peak 16, MH^+ (*m/z* 524.4), $[M+Na]^+$ (*m/z* 546.3). Thus, peaks 12, 13, 14, 15 and 16 were assumed to be lysoPC C18:2, lysoPC C20:4, lysoPC C16:0, lysoPC C18:1, and lysoPC C18:0, respectively.

The mean concentration of PC C16:0/C18:2-OOH in the plasma of alcoholic patients determined by HPLC was 48.0 ± 18.5 pmol/ml (n=5) .

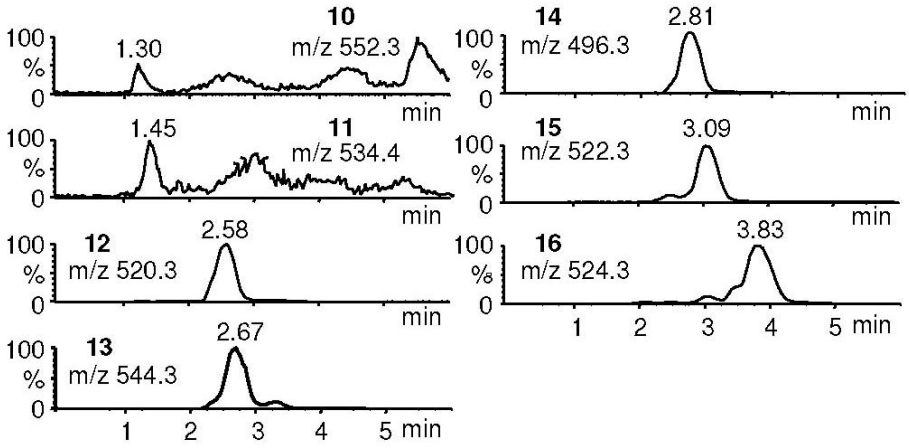

Figure 7: Positive-ion HPLC-ESI-MS analyses of extracted human plasma between 0 and 6 min. Mass chromatograms of hydroperoxy-lysoPC (C18:2) (*m/z* 552.3), oxo-lysoPC (C18:2) (*m/z* 534.4), lysoPC (C18:2) (*m/z* 520.3), lysoPC (C20:4) (*m/z* 544.3), lysoPC (C16:0) (*m/z* 496.3), lysoPC (C18:1) (*m/z* 522.3), and lysoPC (C18:0) (*m/z* 524.3).

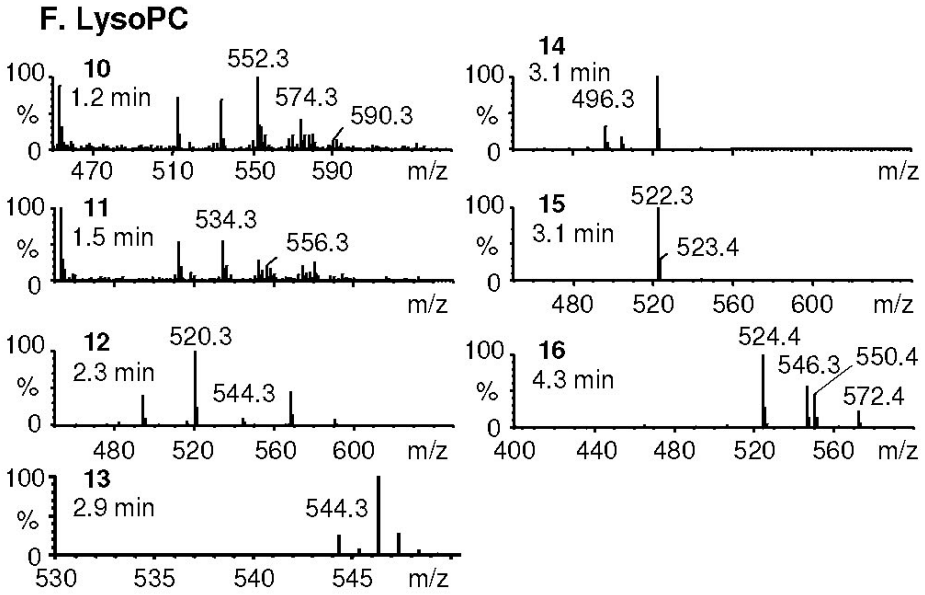

Figure 8: Positive-ion HPLC-ESI-MS analyses of extracted human plasma between 0 and 6 min. Mass spectra of hydroperoxy-lysoPC (C18:2) (column 10), oxo-lysoPC (C18:2) (column 11), lysoPC (C18:2) (column 12), lysoPC (C20:4) (column 13), lysoPC (C16:0) (column 14), lysoPC (C18:1) (column 15), and lysoPC (C18:0) (column 16).

DISCUSSION

In the present study, we applied LC-MS and LC-MS-MS systems to analyze the oxidation products of PC and lyso-PC in human plasma.

First, we detected hydroperoxides of palmitoyl-linoleoyl-PC (PC C16:0/C18:2-OOH) and stearoyl-linoleoyl-PC (PC C18:0/C18:2-OOH) in the plasma of alcoholic patients. By comparison with the standard synthetic compound we confirmed the identification of the former product as PC C16:0/C18:2-OOH. Then, we measured the mean concentration of PC C16:0/C18:2-OOH from five alcoholic patients by HPLC-CL.

Next, we detected hydroxide of palmitoyl-linoleoyl-PC, (hydroxyl derivative of PC) in the plasma of alcoholic patients. By comparison with the standard synthetic compound, we confirmed the identification of this product as PC C16:0/C18:2-OH based on the product ions of MS-MS. We confirm the presence of all discussed oxidation products in human plasma above the limit of detection. When we calculated a signal to noise ratio in the mass chromatogram at *m/z* 774.6 (column 5 in Fig 4) from plasma extract, it was 7.06. In addition, by accurate mass measurement we confirm the elemental composition of hydroxy-PC.

Phosphatidylcholine(PC) is oxidized by soybean lipoxygenase (3), myeloperoxidase (18), and t-butylhydroperoxide (9, 16) to form monohydroperoxides and monohydroxides of PC. The products are 15-hydroperoxyeicosatetraenoate (HPETE) (3, 9, 18), 13-hydroperoxy-octadecadienoate (HPODE) (9, 18), 15-hydroxyeicosatetraenoate (HETE) (3, 9, 18), and 13-hydroxyoctadecadienoate (HODE) (9, 18). Their structures can be identified by LC/ESI/MS/MS (18) and tandem mass spectrometry (9). Accordingly, monohydroxides of PC have been studied following treatment with oxidizing agents.

In contrast, incubation of PC C16:0/C18:2-OOH and PC C18:0/C20:4-OOH aerobically in human plasma gave PC C16:0/C18:2-OH and PC C18:0/C20:4-OH as major products and cholesteryl linoleate hydroperoxide and cholesteryl arachidonate hydroperoxide as minor products, respectively. These cholesteryl esters may be produced by the action of lecithincholesterol acyltransferase (LCAT) present in high density lipoprotein (17, 22). Furthermore, PCOOH is reduced to PC-OH by apolipoprotein A-1, A-2, and B-100 in human plasma. The capacity of reduction by these enzymes is greater than the capacity of plasma glutathione peroxidase (GSHPx) (15). Apolipoprotein B-100 in plasma of pregnant women reduces PCOOH to PC-OH, but GSHPx does not (10).

Thirdly, we detected oxo, epoxyhydroxy, and trihydroxy derivatives of (C16:0/C18:2) PC and (C18:0/C18:2) PC. To our knowledge, they have not hitherto been detected in human plasma, since they may be unstable. Linoleic acid hydroperoxide is converted to hydroxy, keto, epoxyhydroxy, and trihydroxy fatty acids by hematin (6). Keto, hydroxy, trihydroxy, and epoxyhydroxy derivatives of linoleic acid were detected by GC-MS and LC-MS in submitochondrial particles of bovine heart after incubation and hydrolyzed by phospholipase A_2 (12).

Finally, we detected hydroperoxy and oxo derivatives of lysoPC C18:2 in human plasma. To our knowledge, they have not hitherto been detected. In addition, we detected five lysoPCs in human plasma. In the present study, no Na-adduct is present for lysoPC C18:2 (column 12), lysoPC C20:4(column 13), lysoPC C16:0 (column 14) and lysoPC C18:1 (column 15), but for lysoPC C18:0 (column16). As peaks 12, 13, 14, and 15 developed each other at a very close position, it seems that Na-adducts of lysoPC C18:2 (column 12), lysoPC C20:4 (column 13), lysoPC C16:0 (column 14) and lysoPC C18:1 (column 15) were subtracted to show the $[MH]^+$ of the objective peaks clearly.

LysoPC has various stimulatory effects in many types of immune cells in vitro (13), including monocytes (5), macrophages (8), and neutrophils (20). LysoPC can prevent and treat sepsis (23). Lysophospholipids in soy protein isolate are important cell signaling and growth factor molecules experimentaly linked to cardiovascular function and disease (7). Palmitoyl- and stearoyl-lysoPC contents in LDL correlated significantly with monocyte chemoattractant protein-1 mRNA expression and nuclear factor Kappa B activation (21).

CONCLUSION

We were able to measure 16 accurate masses of PC and lysoPC oxidation products and lysoPC in plasma extract using Q-TOF-ESI-mass spectrometry with a lock spray interface. Furthermore, the presence of hydroperoxides, hydroxide, oxo, and trihydroxy derivatives of PC was confirmed.

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