Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 838 (2006) 31–36
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Quantification of lysophosphatidylcholines and phosphatidylcholines using liquid chromatography–tandem mass spectrometry in neonatal serum☆

Akihiro Takatera[a,*], Atsuko Takeuchi[b], Kayoko Saiki[b], Takeshi Morisawa[a], Naoki Yokoyama[a], Masafumi Matsuo[a]

[a] *Department of Pediatrics, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Japan*
[b] *Kobe Pharmaceutical University, Kobe, Japan*

Received 30 September 2005; accepted 5 March 2006
Available online 17 April 2006

Abstract

We established an improved method for quantification of phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) molecular species in neonatal serum using high-performance liquid chromatography coupled tandem mass spectrometry (LC–MS/MS). A multiple reaction monitoring (MRM) mode of positive ionization for MS/MS was used. The method involved purification of phospholipids by solid phase extraction (SPE) from a 20-μl minimum specimen of serum. The assayed values of authentic 16:0-LPC and 18:0-LPC showed a linear response, and our quantitative results showed high precision for the all species of PC and LPC. Then, we quantified PC and LPC in adult and neonatal serum and compared them. Day 0–1 neonatal serum 16:0-, 18:0-, 18:1-, 18:2-LPC levels were significantly lower than adult ones. All species LPC levels in the day 0–1 neonates were significantly lower than day 4–8 neonates. Day 0–1 neonatal serum 16:0/18:2-, 18:0/18:2-PC levels were significantly lower than adult ones. Our method is advantageous for precise assessments of the relationships between PCs/LPCs levels and neonatal infectious diseases.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Lysophosphatidylcholine; Phosphatidylcholine; Neonate; Liquid chromatography–tandem mass spectrometry

1. Introduction

Lysophospholipids are biologically active lipids regulating a variety of cellular functions [1,2]. Lysophosphatidylcholine (LPC), a lysophospholipid, is a component of human plasma, and is produced from phosphatidylcholine (PC) by lecithin-cholesterol acyltransferase [3], or phospholipase A_2 (PLA_2) [4]. LPC has been suggested to play a functional role in various diseases [5–7], and data indicate a potential use of LPC as a diagnostic marker. However, increasing evidence suggests that LPC also exerts direct biological effects, especially on immune cells. LPC has various stimulatory effects in many types of immune cells [1,8–13].

Recently, it was reported that the plasma concentrations of the main LPC species, i.e., 16:0-, 18:0-, 18:1-, and 18:2-LPCs, were markedly reduced in patients with sepsis. In addition, the molar ratio of LPC to its precursor molecule, PC, was also decreased in these patients, which reflects the enzymatic reaction responsible formation [14]. Moreover, it was shown that administration of 18:0- or 18:1-LPC to mouse models of sepsis protected them against lethality [15]. These compounds may play an important role in neonates as well. However, neonatal blood concentrations of LPC, besides PC, have not been quantified before.

High-performance liquid chromatography coupled tandem mass spectrometry (LC–MS/MS) is becoming the preferred method for routine determinations in highly selective and sensitive analyses. Hence, we utilized LC–MS/MS methods using turbo electrospray ionization (ESI) for the selective analysis of molecular species of phospholipid. This study aimed to establish a method of quantification of neonatal serum PC and LPC, distinguishing molecular species, with a small amount of blood by LC–MS/MS.

☆ This paper was presented at the 30th Annual Meeting of the Japanese Society for Biomedical Mass Spectrometry, Osaka, Japan, 8–9 September 2005.

* Corresponding author. Tel.: +81 78 382 6090; fax: +81 78 382 6099.
E-mail address: taka5510@med.kobe-u.ac.jp (A. Takatera).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.03.006

2. Experimental

2.1. Chemicals and reagents

L-α-Lysophosphatidylcholines, stearoyl (18:0-LPC), palmitoyl (16:0-LPC), and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (14:0/14:0-PC) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) as an internal standard (I.S.) for PCs. L-α-Lysophosphatidylcholine, stearoyl-D35 (18:0-D35-LPC) was purchased from Larodan AB (Malmo, Sweden) as an I.S. for LPCs. HPLC grade methanol was purchased from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals and reagents were of commercially available analytical grades.

2.2. Biological samples

Serum samples of neonates with body weights more than 1500 g were obtained from NICU of Kobe University Hospital, Kobe, Japan. Adult serum samples were obtained from healthy volunteers. Samples were stored at −20 °C until assay.

2.3. Sample preparation procedures

To 20 μl of serum sample, we added 100 μl of methanol solution with internal standards (I.S.) 14:0/14:0-PC (2 μg) and 18:0-D35 LPC (2 μg). Lipids were extracted by Folch's method [16], and the eluate evaporated to dryness using a gentle stream of nitrogen. The residue was dissolved in 200 μl of chloroform and loaded onto an aminopropyl-bounded silica column (Chromabond NH_2 column, 500 mg, 3 ml, Macherey-Nagel, Duren) preconditioned with *n*-hexane. Following, rinsing with 3 ml of chloroform–propanol (2:1, v/v) to eliminate neutral lipids, 3 ml of 2% acetic acid in diethyl ether to eliminate fatty acids, and then, 3 ml of diethyl ether to eliminate acetic acid, were added. Analytes containing phospholipids were eluted with 6 ml of methanol. Entire elute analytes were dried using a gentle stream of nitrogen. The residue was dissolved in 1 ml of methanol, and a 15-μl aliquot was injected into the LC–MS/MS system.

2.4. Instrumentation and chromatographic conditions

A Shimadzu 10ADvp LC system (Shimadzu, Kyoto, Japan) was used to inject sample solutions on a Cosmosil 5NH_2-MS column (4.6 mm i.d. × 50 mm, 5.0 μm) from Nacalai Tesque Inc. (Kyoto, Japan). The isocratic mobile phase, a mixture of 0.2% (v/v) formic acid in methanol, was delivered at 0.2 ml/min into the mass spectrometer ionization chamber. Quantitation was achieved by MRM positive ion mode using an API 3000 mass spectrometer (Applied Biosystems/MDS Sciex, Toronto, Canada), equipped with a Turboionspray™ interface at 400 °C. The ion spray voltage was set at 5000 V. The common parameters, viz., nebulizer, curtain, and collision gases were set at 14, 10, and 4, respectively. Analytical data were processed by Analyst software 1.3.1.

2.5. Assay validations

Calibration standards were prepared at concentrations of 5, 10, 25, 50, and 100 μg/ml for 16:0-LPC or 18:0-LPC. Calibration curves were acquired by plotting the peak area ratio of the analyte to the respective I.S. Dilution linearity was assessed by serially dilution with serum samples. The undiluted serum sample values of 16:0-, 18:0-, 18:1-, 18:2-, and 20:4-LPCs and 16:0/18:1-, 16:0/18:2-, 16:0/20:4-, 16:0/20:3 + 18:1/18:2-, and 18:0-/18:2-PCs, as determined in the assay, were used to establish the expected values for subsequent dilutions but were not used in the construction of the regression plot. The results were fitted to linear regression analysis.

Intra-day precisions were estimated by analyzing six replicates of the same neonatal serum in one day, while inter-day precisions were determined analyzing serum on 6 different days. The mean concentrations and coefficients of variation, CV (%) were calculated.

The absolute recovery was assessed at high and low concentrations, 10 and 50 μg/ ml for 16:0-LPC and 5 and 20 μg/ ml for 18:0-LPC.

2.6. Statistical analysis

Results are expressed as mean value ± standard deviation (S.D.). Data were analyzed using Student's *t*-test, and performed using Microsoft® Excel 2001. Statistical significance was defined at a *p*-value of less than 0.05.

3. Results

3.1. Method development, specificity and selectivity

Electrospray MS/MS in MRM mode was used for simultaneous determination of PCs and LPCs. Best signals and good ionization were achieved with 0.2%(v/v) formic acid in methanol at 0.2 ml/min flow rate. Under the ESI optimized conditions, $[M+H]^+$ ions of LPCs and PCs generated ions at *m*/z 184.2 as the major product ion. The monitored transition pairs are summarized in Table 1. Fig. 1 shows typical chromatograms of PCs, and Fig. 2 shows typical chromatograms of LPCs in neonatal serum including I.S. A single peak, but no interference peaks were observed in each chromatogram.

3.2. Assay validations

Linearity of the respective calibration curves for 16:0- and 18:0-LPCs was observed from 5 to 100 μg/ml. The regression equations of the calibration lines are $y = 0.0224x + 0.016$ ($R^2 = 0.999$) for 16:0-LPC and $y = 0.0247x + 0.008$ ($R^2 = 1.000$) for 18:0-LPC. Serum samples were serially diluted, assessed with PCs and LPCs, and analyzed by linear regression of the expected concentration versus the observed concentration. The R^2 values were 0.999–1.000 for all species of PCs and LPCs.

Intra-day and inter-day precisions of PC assay, expressed as CV (%) were given in Table 2. As shown, the values ranged from 2.0 to 5.4% and from 3.7 to 8.9%, respectively. The intra-

Table 1
Transition pairs monitored for the LC–MS/MS method

Q1 $[M+H]^+$ (*m/z*)	Q3 (*m/z*)	Molecular species
PC[a]		
760.7	184.2	16:0/18:1
758.7	184.2	16:0/18:2
782.7	184.2	16:0/20:4
784.7	184.2	16:0/20:3, 18:1/18:2
786.7	184.2	18:0/18:2
LPC		
496.5	184.2	16:0
524.6	184.2	18:0
522.6	184.2	18:1
520.6	184.2	18:2
544.6	184.2	20:4

Molecular species of PC and LPC from serum were determined by positive ion LC–MS/MS.
[a] Molecular species of PC means the major species.

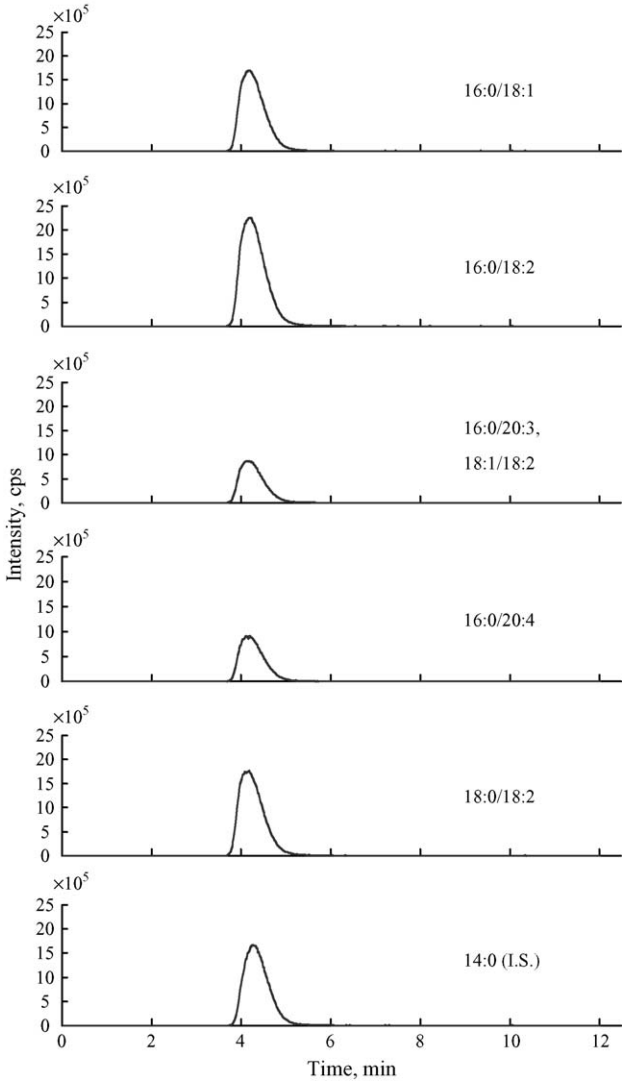

Fig. 1. Typical MRM chromatograms of the PC molecular species including internal standard (I.S.). cps is counts per second.

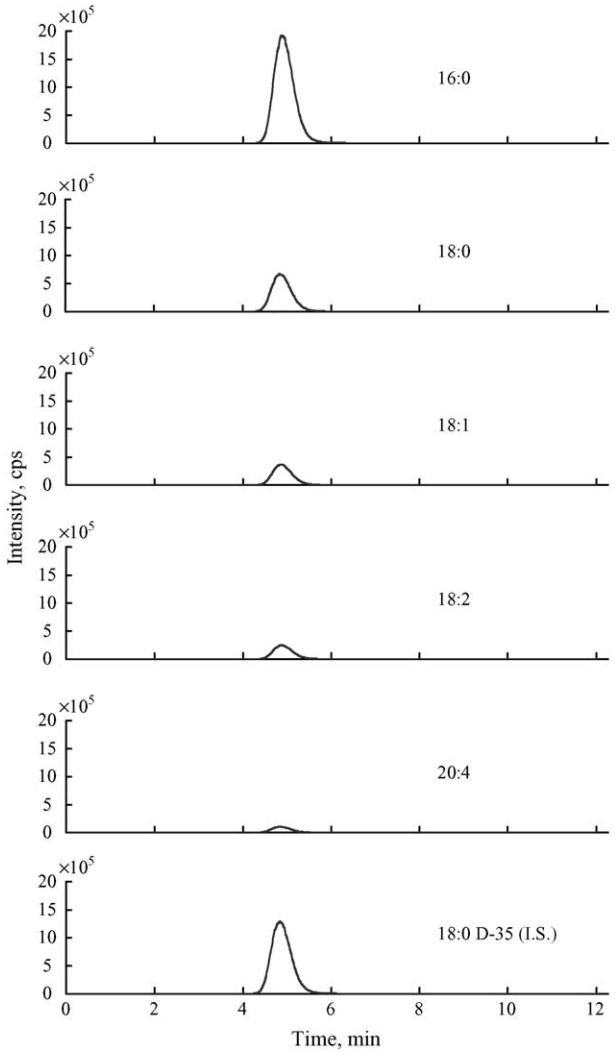

Fig. 2. Typical MRM chromatograms of the LPC molecular species including internal standard (I.S.). cps is counts per second.

day and inter-day precisions of LPC assay, expressed as CV (%) were given in Table 2. As shown, the values ranged from 4.7 to 7.4% and from 3.3 to 6.4%, respectively.

The recovery for high and low levels of 16:0- and 18:0-LPCs was 92.5–97.2 and 92.9–98.6%, respectively.

Since serum samples were stored at −20 °C and the extracted samples in the autosampler were at 4 °C until assay, no assayed values of PCs and LPCs changed (data not shown). Moreover, repeated freeze–thaw cycles had no significant effect on concentrations of PCs and LPCs.

3.3. Assayed values of PCs and LPCs in serum of healthy adults and neonates

Serum samples of healthy adults and day 0–1 and day 4–8 neonates were subjected to the complete procedure. Table 3(b) shows the serum ratio of each LPC species to I.S. in serum and *p*-values. Mean values of the 16:0-, 18:0-, 18:1-, and 18:2-LPCs in the day 0–1 neonate serum were significantly lower than those in both healthy adults and the day 4–8 neonates. Sig-

Table 2
Precision data for quantification of PC and LPC molecular species from serum

Molecular species	Intra-assay ($n=6$)		Inter-assay ($n=6$)	
	Each species/I.S.[a]	CV (%)[b]	Each species/I.S.[a]	CV (%)[b]
PC				
16:0/18:1	1.207 ± 0.024	2.0	1.348 ± 0.120	8.9
16:0/18:2	0.802 ± 0.043	5.3	0.925 ± 0.062	6.7
16:0/20:4	1.043 ± 0.035	3.4	1.135 ± 0.062	5.5
16:0/20:3,18:1/18:2	0.565 ± 0.020	3.5	0.619 ± 0.033	5.3
18:0/18:2	0.579 ± 0.031	5.4	0.650 ± 0.024	3.7
LPC				
16:0	1.317 ± 0.085	6.5	1.318 ± 0.070	5.3
18:0	0.381 ± 0.024	6.3	0.398 ± 0.023	5.8
18:1	0.192 ± 0.009	4.7	0.218 ± 0.010	4.6
18:2	0.081 ± 0.006	7.4	0.094 ± 0.006	6.4
20:4	0.078 ± 0.005	6.4	0.090 ± 0.003	3.3

[a] Each species/I.S. means the ratio of each molecular species to the internal standard; data are expressed as mean ± S.D.
[b] Imprecision is expressed as a coefficient of variation (CV).

nificant differences of 20:4-LPC levels were observed between day 0–1 and day 4–8 neonates, but not between the day 0–1 neonates and adults. There were no significant differences between LPC levels except for 18:2-LPCs in healthy adults and day 4–8 neonates. Table 3(a) shows the serum ratio of each PC species to I.S. in serum and *p*-values. Mean values of the 16:0/18:2- and 18:0/18:2-PC in the day 0–1 neonates were significantly lower than those in both healthy adults and day 4–8 neonates. There were no significant differences in levels between 16:0/18:1-, 16:0/20:4-, and 16:0/20:3- plus 18:1/18:2-PC levels in the day 0–1 and day 4–8 neonates, but 16:0/18:1-, 16:0/20:4-, and 16:0/20:3- plus 18:1/18:2-PC levels in neonates seems to be higher than those in adults. Using the calibration curves of 16:0- and 18:0-LPCs, concentrations were calculated. The values (mean ± S.D.) of 16:0-LPC in healthy adults, and day 0–1 and day 4–8 neonates were 55.4 ± 15.3, 31.3 ± 9.3, and 57.9 ± 18.7 μg/ml, respectively. The values of 18:0-LPC in healthy adults, and of day 0–1 and day 4–8 neonates were 16.5 ± 4.1, 9.53 ± 3.7, and 18.6 ± 6.8 μg/ml, respectively (Table 3).

4. Discussion

In this study, we describe a method for the quantification of the major molecular species of PC and LPC in neonatal serum by an improved LC–MS/MS method. Various methods for LPC measurement in human adult or animal blood samples have been reported; the most common method involves separation by thin-layer chromatography (TLC) [17,18] or HPLC [19–21]. Either TLC separation followed by gas chromatographic analysis [22] or HPLC coupled to electrospray ionization mass spectrometry (ESI-MS) [23] was used for analyzing the fatty acid composition of LPC. A different quantification method for lysophospholipids using ESI-tandem MS (ESI-MS/MS) after TLC separation has been reported [24]. However, these methods are complicated, time-consuming, insensitive to some degree,

Table 3
Comparisons of PC and LPC levels of day 0–1, day 4–8 neonates and adults, for each molecular species

Molecular species	Each species/I.S.[a]			*p*-value		
	Neonates day 0–1 ($n=46$)	Neonates day 4–8 ($n=18$)	Healthy adults ($n=10$)	Day 0–1 vs. day 4–8	Adults vs. day 0–1	Adults vs. day 4–8
(a) PC						
16:0/18:1	1.74 ± 0.65	1.95 ± 2.06	1.34 ± 0.28	N.S.	N.S.	<0.05
16:0/18:2	1.24 ± 0.50	2.06 ± 0.81	2.45 ± 0.44	<0.05	<0.05	N.S.
16:0/20:4	1.44 ± 0.42	1.39 ± 0.38	0.95 ± 0.18	N.S.	<0.05	<0.05
16:0/20:3, 18:1/18:2	0.88 ± 0.30	0.96 ± 0.25	0.77 ± 0.13	N.S.	N.S.	<0.05
18:0/18:2	0.91 ± 0.46	1.63 ± 0.60	1.77 ± 0.33	<0.05	<0.05	N.S.
(b) LPC						
16:0	0.71 ± 0.21	1.31 ± 0.42	1.25 ± 0.35	<0.05	<0.05	N.S.
18:0	0.24 ± 0.09	0.47 ± 0.17	0.42 ± 0.10	<0.05	<0.05	N.S.
18:1	0.17 ± 0.08	0.34 ± 0.11	0.30 ± 0.07	<0.05	<0.05	N.S.
18:2	0.08 ± 0.04	0.26 ± 0.16	0.58 ± 0.16	<0.05	<0.05	<0.05
20:4	0.07 ± 0.03	0.09 ± 0.03	0.08 ± 0.02	<0.05	N.S.	N.S.

There is no tendency among serum PC levels. The mean values of the 16:0-, 18:0-, 18:1-, and 18:2-LPCs in day 0–1 neonates were significantly lower than those in both healthy adults and the day 4–8 neonates. The mean values of all species LPCs in day 0–1 neonates were significantly lower than those in day 4–8 neonates.
[a] Each species/I.S. means the ratio of each molecular species to the internal standard, and data are expressed as mean ± S.D.

unselective, and imprecise. Recently, Wolfgang et al. quantified PC and LPC molecular species in adult blood plasma by LC–MS/MS [14]. Their method was able to resolve many problems; LC–MS/MS is a very useful approach to improve quantitative specificity of molecular species of phospholipids [25]. For the high-throughput quantification, they used precursor-ion scan mode of *m/z* 184, which is specific for phosphocholine-containing phospholipids.

In recent years, there have been many successful quantifications using LC–MS/MS in MRM mode, an important capability of tandem mass spectrometers. The selection and quantification of compound-specific ion-pairs enable a reduction in interference by co-eluting substances and allow for a considerable improvement in assay selectivity. Since the mode is a powerful analytical technique for higher selectivity, sensitivity and specificity, we used the MRM mode, not the precursor-ion scan mode, as the MS/MS condition. Neonates easily become anemic and respiration and circulation conditions easily worsen if a lot of blood is collected. However, the MRM mode was able to resolve the problem of being unable to collect a large amount of blood from neonates. We were able to quantify with high precision PCs and LPCs from 20 μl specimens of serum. A 20-μl specimen of serum is small enough to be collected from neonates. Another key to our success is that we used NH_2 column as SPE and HPLC columns; in this way, we were able to efficiently separate and extract PC and LPC from the serum.

Best signals and good ionization were achieved with 0.2 % (v/v) formic acid in methanol as the mobile phase of HPLC. Sodium adduct ions $[M+Na]^+$ were partially produced using only methanol and disturbed accurate quantification. However, the addition of 0.2% formic acid prevented production of the adduct ions.

We chose 16:0-, 18:0-, 18:1-, and 18:2-LPCs as the molecular species for LPC. There are many PC molecular species containing possible kinds of fatty acids. From published data [26,27], 16:0/18:1-, 16:0/18:2-, 16:0/20:4-, 16:0/20:3-, 18:0/18:2-, and 18:1/18:2-PC were chosen as the major molecular species for PC. The transition pairs monitored are shown in Table 1. Since no interference peak was observed on the each of the chromatograms, we considered that the assay of LPCs and PCs in neonatal serum was successfully performed by this method (Figs. 1 and 2).

Linearity of the respective calibration curves was observed for 16:0- and 18:0-LPCs, which are available as standards. Dilution linearity was also demonstrated for all LPCs and PCs analytes, comparing expected versus measured values. The intra-day and the inter-day precisions as measured by CV (%) were less than 10% for all PCs and LPCs analytes. The respective recovery was higher than 90%. From these results, we confirmed that our method had high precision for quantification of serum PCs and LPCs. Using the calibration curves of 16:0- and 18:0-LPCs, the mean calculated values of 16:0-LPC and 18:0-LPC in healthy adults were 55.4 μg/ml (111.7 μmol/l) and 16.5 μg/ml (31.5 μmol/l), respectively. These data were similar to that of Wolfgang et al. [14]. The mean values of 16:0-LPC in day 0–1 and day 4–8 neonates were 31.3 (63.1) and 57.9 (116.7) μg/ml (μmol/l), respectively. The values of 18:0-LPC in day 0–1 and day 4–8 neonates were 9.53 (18.2), 18.6 (35.5) μg/ml (μmol/l), respectively.

The mean values of LPCs of all molecular species in the day 0–1 neonates were significantly lower than those for day 4–8 neonates. There were no significant differences between LPC levels except for 18:2-LPCs in healthy adults and day 4–8 neonates. These data indicate that serum LPC levels are lower at birth and increase to adult levels in about one week. The results suggest that 0–1 day neonates might be easily infected because of their low serum LPC. Only day 4–8 neonatal 18:2-LPC was lower than the adult level. This result might be caused because 18:2-LPC was consumed for esterification of cholesterol. On the other hand, there was no such tendency among serum PC levels; this result might be because PC dynamics involve various factors.

Sepsis is a major cause of death in neonatal intensive care units (NICU); thus, infectious signs need to be detected as soon as possible to prevent infections in neonates. Since blood LPC levels may become biochemical markers for infection, the quantification of these compounds helps prevention and treatment of infections, even in neonates. The neonatal serum PC and LPC could be important factors when elucidating neonatal infections. If the relationship between neonatal infections and blood PC/LPC level becomes clearer, we will be able to contribute a new approach aimed towards prevention and treatment of infections, and so decrease neonatal mortality. We are sure that our method is useful in research of the infections that present major clinical problems for neonates; we will continue to use it to examine neonatal infections to refine its development.

5. Conclusions

We established an improved LC–MS/MS method for quantification of neonatal serum PC and LPC with high selectivity and precision, using purification of the phospholipids by SPE coupled to LC–MS/MS. Our method is advantageous for precise assessments of the relationships between PCs/LPCs levels and neonatal infectious diseases.

References

[1] J.H.S. Kabarowski, Y. Xu, O.N. Witte, Biochem. Phamacol. 64 (2002) 161.
[2] E.J. Goetzl, Prostaglandins Other Lipid Mediat. 64 (2001) 11.
[3] P.V. Subbaiah, M. Liu, P.J. Bolan, F. Paltauf, Biochim. Biophys. Acta 1128 (1992) 83.
[4] T. Kita, N. Kume, K. Ishii, H. Horiuchi, H. Arai, M. Yokode, Diabetes Res. Clin. Pract. 45 (1999) 123.
[5] A.J. Lusis, Nature 407 (2000) 233.
[6] A.H. Shi, M. Yoshinari, M. Wakisaka, M. Iwase, M. Fujishima, Horm. Metab. Res. 31 (1999) 283.
[7] R. Wu, E. Svenungsson, I. Gunnarsson, B. Andersson, I. Lundberg, E.L. Schafer, et al., Lupus 8 (1999) 142.
[8] F. Coutant, L. Perrin-Cocon, S. Agaugue, T. Delair, P. Andre, V. Lotteau, J. Immunol. 169 (2002) 1688.
[9] B.Z. Ngwenya, N. Yamamoto, Cancer Immunol. Immunother. 21 (1986) 174.
[10] A. Gomez-Munoz, L. O'Brien, R. Hundal, U.P. Steinbrecher, J. Lipid Res. 40 (1999) 988.

[11] Y. Asaoka, M. Oka, K. Yoshida, Y. Sasaki, Y. Nishizuka, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 6447.
[12] E. Nishi, N. Kume, Y. Ueno, H. Ochi, H. Moriwaki, T. Kita, Circ. Res. 83 (1998) 508.
[13] C.C. Silliman, D.J. Elzi, D.R. Ambruso, R.J. Musters, C. Hamiel, R.J. Harbeck, et al., J. Leukoc. Biol. 73 (2003) 511.
[14] D. Wolfgang, L. Gerhard, A. Franz-Xaver, F. Dieter, G. Thomas, V. Peter, et al., J. Lipid Res. 44 (2003) 754.
[15] J.J. Yan, J.S. Jung, J.E. Lee, J. Lee, S.O. Huh, H.S. Kim, et al., Nat. Med. 10 (2004) 161.
[16] J. Folch, M. Lees, G.H. Sloane-Stanley, J. Biol. Chem. 226 (1957) 497.
[17] W.Q. Wang, A. Gustafson, J. Chromatogr. 581 (1992) 139.
[18] K. Yokoyama, F. Shimizu, M. Setaka, J. Lipid Res. 41 (2000) 142.
[19] W. Bernhard, M. Linck, H. Creutzburg, A.D. Postle, A. Arning, I. Martin-Carrera, et al., Anal. Biochem. 220 (1994) 172.
[20] E.J. Lesnefsky, M.S. Stoll, P.E. Minkler, C.L. Hoppel, Anal. Biochem. 285 (2000) 246.
[21] Z. Guan, J. Grunler, S. Piao, P.J. Sindelar, Anal. Biochem. 297 (2001) 137.
[22] M. Croset, N. Brossard, A. Polette, M. Lagarde, Biochem. J. 345 (2000) 61.
[23] W. Pruzanski, E. Stefanski, F.C. Beer, M.C. Beer, A. Ravandi, A. Kuksis, J. Lipid Res. 41 (2000) 1035.
[24] Y. Xiao, Y. Chen, A.W. Kennedy, J. Belinson, Y. Xu, Ann. N.Y. Acad. Sci. 905 (2000) 242.
[25] L. Gerhard, D. Wolfgang, L. Bernd, S. Gerd, Clin. Chem. 48 (2002) 2217.
[26] P.V. Subbaiah, M. Hossain, Biochim. Biophys. Acta 963 (1988) 445.
[27] P. Deborah, M. Marco, B. Fabio, B. Emanuele, G.F. Natale, Rapid Commun. Mass Spectrom. 18 (2004) 2395.