

Lysophosphatidylcholine Containing Docosaehaenoic Acid at the *sn*-1 Position is Anti-inflammatory

Long Shuang Huang · Nguyen Dang Hung ·
Dai-Eun Sok · Mee Ree Kim

Received: 20 October 2009 / Accepted: 26 January 2010 / Published online: 18 February 2010
© AOCS 2010

Abstract Lysophosphatidylcholine is known to be a lipid mediator in various cellular responses. In this study, we examined the anti-inflammatory actions of lysophosphatidylcholine containing docosaehaenoic acid esterified at the *sn*-1 position. First, in RAW 264.7 cells, DHA-lysoPtdCho suppressed the LPS-induced formation of NO concentration-dependently. However, ARA-lysoPtdCho showed a partial suppression, and LNA-lysoPtdCho had no significant effect. Additionally, DHA-lysoPtdCho also reduced the level of TNF- α or IL-6, but not PGE₂. In animal experiments, the i.v. administration of ARA-lysoPtdCho (150 or 500 μ g/kg) prevented zymosan A-induced plasma leakage remarkably with a maximal efficacy (E_{max}) of 50%, in contrast to no effect with LNA-lysoPtdCho. Remarkably, DHA-lysoPtdCho suppressed zymosan A-induced plasma leakage with an ED₅₀ value of 46 μ g/kg and an E_{max} value of around 95%. Additionally, mechanistic studies indicated that the anti-inflammatory action of DHA-lysoPtdCho was partially related to the reduced formation of LTC₄, TNF- α , and IL-6. When the interval time between lysoPtdCho administration and zymosan A challenge was extended up to 2 h, such a suppressive action of DHA-lysoPtdCho was augmented, suggesting that a DHA-lysoPtdCho metabolite is important

for anti-inflammatory action. In support of this, 17-HPDHA-lysoPtdCho showed a greater anti-inflammatory action than DHA-lysoPtdCho. Furthermore, a similar anti-inflammatory action was also observed with i.p. administration of DHA-lysoPtdCho or a 17(*S*)-hydroperoxy derivative. Additionally, oral administration of DHA-lysoPtdCho also expressed a significant anti-inflammatory action. Taken together, it is proposed that DHA-lysoPtdCho and its metabolites may be anti-inflammatory lipids in vivo systems.

Keywords Anti-inflammatory · Nitric oxide · DHA-lysoPtdCho · 17-HPDHA-lysoPtdCho · Docosaehaenoic · LPS · Zymosan A · RAW 264.7 cell

Abbreviations

lysoPtdCho	Lysophosphatidylcholine
LNA	Linoleic acid
ARA	Arachidonic acid
DHA	Docosaehaenoic acid
LNA-lysoPtdCho	1-Linoleoyl-2-lyso- <i>sn</i> -glycerol-3-phosphocholine
ARA-lysoPtdCho	1-Arachidonoyl-2-lyso- <i>sn</i> -glycerol-3-phosphocholine
DHA-lysoPtdCho	1-Docosaehaenoyl-2-lyso- <i>sn</i> -glycerol-3-phosphocholine
14-HPDHA-lysoPtdCho	1-14(<i>S</i>)-hydroperoxy-4,7,10,13,15,19-docosaehaenoyl-2-lyso- <i>sn</i> -glycerol-3-phosphocholine
17-HPDHA-lysoPtdCho	1-17(<i>S</i>)-hydroperoxy-4,7,10,13,15,19-docosaehaenoyl-2-lyso- <i>sn</i> -glycerol-3-phosphocholine

L. S. Huang and N. D. Hung contributed equally.

L. S. Huang · N. D. Hung · D.-E. Sok
College of Pharmacy, Chungnam National University,
Gung-Dong 220, Yuseong-Gu, Taejeon 305-764, Korea

M. R. Kim (✉)
Department of Food and Nutrition,
Chungnam National University, Gung-Dong 220,
Yuseong-Gu, Taejeon 305-764, Korea
e-mail: mrkim@cnu.ac.kr

LOX	Lipoxygenase
ED ₅₀	50% effective dose
IC ₅₀	50% inhibitory concentration
PLA ₂	Phospholipase A ₂
LTC ₄	Leukotriene C ₄
PGE ₂	Prostaglandin E ₂
IL-6	Interleukin-6
TNF- α	Tumor necrosis factor alpha
NO	Nitric oxide

Introduction

Previously, certain families of lipid-derived mediators were reported to be implicated in inflammatory diseases such as asthma, rheumatoid arthritis, or inflammatory bowel disease [1–6]. Although much is known about the molecular basis of initiating signals and pro-inflammatory chemical mediators in inflammation, it has recently become apparent that endogenous lipid mediators participate in events of inflammatory or anti-inflammatory events [7, 8]. Arachidonic acid (ARA), released from PLA₂-catalyzed hydrolysis of phosphatidylcholine, is converted to leukotrienes or lipoxins via the lipoxygenase enzymatic pathway, and to prostaglandins via the cyclooxygenase enzymatic pathway. Especially, prostaglandin E₂ (PGE₂) and leukotriene B₄ (LTB₄) are well known to be representative pro-inflammatory lipid mediators [9], whereas lipoxins have recently been reported to show an anti-inflammatory action [7]. Remarkably, arachidonate-derived eicosanoids in inflammatory exudates include lipoxins in addition to prostaglandins and leukotrienes. 5-Lipoxygenase (5-LOX) is crucial for the formation of leukotrienes and lipoxins, while 12/15-lipoxygenase is important for the formation of lipoxin [7, 9–12]. Furthermore, resolvins, which are generated from the oxygenation of eicosapentenoic acid at C-15, and protectins, derived from oxygenation of docosahexaenoic acid (DHA) at C-17 have also been reported to be potent anti-inflammatory lipid molecules.

Meanwhile, lysophosphatidylcholine (lysoPtdCho), another product from PLA₂-catalyzed hydrolysis of phosphatidylcholine, has been reported to be pro-inflammatory [1–3, 6, 13]. The pro-inflammatory action of lysoPtdChos, saturated or monounsaturated, may be due to the generation of reactive oxygen species or nitric oxide in various types of cells [14–17]. Additionally, our recent study also indicated that LNA-lysoPtdCho showed a cytotoxic effect, accompanied by ROS formation [18]. Previous studies reported that lysoPtdChos with a polyunsaturated acyl group were present in animal sources. In plasma, LNA-lysoPtdCho, ARA-lysoPtdCho, and DHA-lysoPtdCho existed at a

substantial level in plasma [19–21]. In addition, DHA-lysoPtdCho was one of the major lipid components in shark liver extract [22]. Noteworthy, our recent studies demonstrated that polyunsaturated-lysoPtdChos containing linoleoyl, arachidonoyl, or docosahexaenoyl group was efficiently oxygenated by reticulocyte 15-LOX or leukocyte 12/15-LOX [23–25]. Therefore, it was supposed that lysoPtdChos with polyunsaturated acyl groups could affect the formation of lipid mediators such as lipoxin [7]. Separately, in our study, ARA-lysoPtdCho and DHA-lysoPtdCho were found to strongly inhibit mammalian 5-LOX activity [26], responsible for the generation of pro-inflammatory leukotrienes [9]. Meanwhile, DHA, a hydrolysis product of DHA-lysoPtdCho, is known to show anti-inflammatory action [27] while ARA, the hydrolysis product of ARA-lysoPtdCho, is converted to lipid metabolites, pro-inflammatory or anti-inflammatory. Nonetheless, there has been no knowledge about the effect of polyunsaturated-lysoPCs on inflammation in vivo system. In this regard, we examined anti-inflammatory effects of lysoPtdChos containing polyunsaturated acyl group in vitro as well as in vivo system.

Materials and Methods

Materials

Dilinoleoyl phosphatidylcholine, diarachidonoyl phosphatidylcholine, and didocosahexaenoyl phosphatidylcholine (purity, 99%) were from Avanti Polar Lipid (Alabaster, AL, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), soybean lipoxygenase-1 (Type I-B), phospholipase A₂ (PLA₂) (honey bee venom), lipopolysaccharide (LPS) (from *Escherichia coli* serotype 0111:B4; purity >99%) and zymosan A (from *Saccharomyces cerevisiae*) were purchased from Sigma-Aldrich Corp (St. Louis, MO, USA). PGE₂ and LTC₄ EIA kit were procured from Cayman Chemical (Ann Arbor, MI, USA). TNF- α ELISA kit was from Invitrogen Corp (Camarillo, CA, USA) and IL-6 ELISA kit was obtained from Ebioscience Inc Co (California, USA). LNA-lysoPtdCho, ARA-lysoPtdCho, DHA-lysoPtdCho were prepared from PLA₂-catalyzed hydrolysis of the corresponding phosphatidylcholines as described previously with a slight modification [23–26]. In brief, didocosahexaenoyl-phosphatidylcholine (2.5 mg), dissolved in chloroform, was dried under N₂, and then rapidly dispersed in 10 ml of 50 mM borax buffer (pH 9.0) containing 10 mM CaCl₂. The hydrolysis was started by adding PLA₂ (100 units), and allowed to continue under N₂ with constant stirring for 2 h at 25 °C. The reaction mixture was partially purified by a Sep-pack column (2 × 1 cm) and the lysophospholipid

product was further purified by silica gel TLC in the solvent system (chloroform: methanol: water: 65:25:4). Finally, the spot containing DHA-lysoPtdCho was scraped off, extracted with methanol, dried under nitrogen and kept at -80°C until use and 17-HPDHA-lysoPtdCho was prepared as described previously [26]; briefly, soybean LOX-1 (200 units/ml) was incubated with DHA-lysoPtdCho (200 μM) in borax buffer (50 mM, pH 9.0) for 30 min. Subsequently, the mixture was passed through a C_{18} column (2×1 cm), and the products were eluted with methanol and concentrated under N_2 gas.

Measurement of Polyunsaturated LysoPtdCho Induced Cytotoxicity in RAW 264.7 cells

RAW 264.7 cells were cultured in DMEM supplemented with penicillin (100 units/ml), streptomycin (100 $\mu\text{g}/\text{ml}$) and heat-inactivated FBS (10% v/v) at 37°C in 5% CO_2 (19). Cells with passages from 6 to 13 were harvested, and seeded in 96-well plate (5×10^4 cells/well), followed by 24 h incubation. Then, cells were pre-incubated with each polyunsaturated lysoPtdCho (0–60 μM) in the medium for 2 h, and then incubated in the presence or absence of LPS (1 $\mu\text{g}/\text{ml}$) at 37°C for 20 h. Cell viability was examined by the assay using MTT [18]; briefly, RAW cells (5×10^4 cells/well) were exposed to MTT reagent (500 $\mu\text{g}/\text{ml}$) at 37°C for 4 h. After the removal of the medium, the formazan was extracted from cells by disrupting the cell membranes with dimethylsulfoxide. The cell viability was measured by determining the absorbance at 570 nm with a reference at 690 nm.

Preventive Effect of LysoPtdCho on LPS-Induced NO Production in RAW 264.7 Cells

RAW 264.7 cells (5×10^4 cells/well) were preincubated with polyunsaturated lysoPtdCho (0–60 μM) at 37°C for 2 h, and then the production of NO (nitric oxide) was stimulated with LPS (1 $\mu\text{g}/\text{ml}$) during further incubation for 20 h. The production of NO was determined by using the Griess reagent consisting of 1% sulfanilamide and 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% H_3PO_4 [18].

Effect of DHA-lysoPtdCho on LPS-Induced TNF- α , IL-6, and PGE₂ Formation in RAW 264.7 Cells

To investigate the effect of DHA-lysoPtdCho on TNF- α , IL-6, and PGE₂ formation in LPS-treated cells, RAW 264.7 cells (5×10^4), seeded on 96-well plate, were pre-incubated with DHA-lysoPtdCho (0–40 μM) 2 h prior to treatment with LPS (1 $\mu\text{g}/\text{ml}$) for 20 h at 37°C , in a 5% CO_2 incubator. The cell-free supernatants were collected

for the determination of TNF- α , and IL-6 by ELISA kit and PGE₂ by EIA kit according to the manufacturers' manuals.

Effect of Polyunsaturated LysoPtdCho on Zymosan A-Induced Peritonitis in Mice

ICR mice (male, 6 weeks) were housed under a 12 h: 12 h light–dark cycle and fed with unlimited commercial food and water. Peritoneal inflammation was induced in ICR mice according to a previously reported method [28, 29] with some modifications. For the measurement of plasma leakage, polyunsaturated PtdChos, polyunsaturated lysoPtdChos (0–500 $\mu\text{g}/\text{kg}$) or their derivatives were administered intravenously, intraperitoneally or orally 30–60 min prior to intravenous injection of 0.5% Evans blue dye (200 μl), dissolved in PBS, and intraperitoneal injection of zymosan A (100 mg/kg), prepared freshly in PBS. Then 30 min later, mice lightly anesthetized were decapitated and blood was collected to obtain serum as described previously [30]. Meanwhile, peritoneal lavages were performed with 4 ml of ice cold PBS followed by the brief centrifugation to get supernatant. Subsequently, the concentration of Evans blue dye in peritoneal lavage fluid and in serum was determined by measuring absorbance at 620 nm, and calculating the amount to ($\mu\text{g}/\mu\text{l}$) using an Evan Blue standard curve. The volume of plasma leakage (μl) for 30 min after zymosan A i.p. injection was calculated by dividing the amount of Evans blue dye in the peritoneal lavage fluid (μg) by the concentration of Evans blue dye in the serum ($\mu\text{g}/\mu\text{l}$) [31].

For the measurement of leukocyte infiltration, lysoPtdChos and their derivatives were administered i.v. 30 min prior to i.p. administration of zymosan A as described above. Total cell counting were performed for lavage fluid collected at the 90 min time point using light microscopy together with trypan blue staining [31, 32].

Determination of LTC₄, TNF- α , and IL-6 Levels in Peritoneal Lavage Fluid

In order to determine the level of LTC₄, TNF- α , and IL-6 in exudates, 1 ml of peritoneal lavage fluid was transferred to microcentrifuge tubes, and centrifuged (15,000 rpm, 5 min). The supernatant was used directly for the analysis of TNF- α and IL-6 by ELISA kit, and analysis of LTC₄ by enzyme immunoassay (EIA) kit according to the manufacturer's instructions [31–33].

Effect of DHA-LysoPtdCho on Leukotriene C₄-Induced Plasma Leakage

DHA-lysoPtdCho (0–150 $\mu\text{g}/\text{kg}$) was injected into the tail vein of mice, and 60 min later, 0.2 ml of 0.5% Evans blue

dye dissolved in saline was intravenously injected just before intraperitoneal (i.p.) administration of LTC₄ (100 µg/kg). Samples of blood and peritoneal fluid were collected as described above [34].

Statistical Analysis

Results were expressed as means ± SD. Statistical significance was evaluated using ANOVA and Student's *t* test, and *P* < 0.05 was considered statistically significant.

Results

Our previous study indicated that polyunsaturated lysoPtdChos strongly inhibited mammalian 5-LOX activity [9], an initial enzyme in the biosynthesis of inflammatory LTC₄ [26]. In the present study, we evaluated the anti-inflammatory actions of polyunsaturated lysoPtdChos by determining the effect of lysoPtdChos on LPS-induced formation of inflammatory mediators such as NO, PGE₂, TNF-α, or IL-6.

Effects of Polyunsaturated LysoPtdChos on LPS-Induced Production of Mediators in RAW 264.7 Cells

It is well known that the stimulation of macrophages by LPS results in high-level production of nitric oxide [35] through the expression of inducible nitric oxide synthase (iNOS) in some cells such as macrophages [36]. First, to see the effect of lysoPtdCho on LPS-induced NO formation, each lysoPtdCho (3–60 µM) was pre-incubated with RAW 264.7 cells for 2 h, and then LPS (1 µg/ml) was included in the same incubation. As shown in Fig. 1b, LNA-lysoPtdCho had no significant suppressive effect on LPS-induced NO formation up to 60 µM. Additionally, ARA-lysoPtdCho also showed no significant inhibition of LPS-induced NO formation at lower concentrations (1–20 µM), although it had some suppressive effect at 60 µM. In contrast, DHA-lysoPtdCho diminished the production of NO in a concentration-dependent manner (12–200 µM), and the 50% effective concentration (EC₅₀) of DHA-lysoPtdCho was estimated to be 18.2 ± 2.1 µM (Fig. 1b). Meanwhile, DHA at 20 µM suppressed LPS-induced NO production by approximately 18% (data not shown), much smaller than the suppression (~50%) achieved with DHA-lysoPtdCho at 20 µM. In a separate experiment to see whether the suppressive effect of DHA-lysoPtdCho on LPS-induced NO production was related to its cytotoxic action, the effect of DHA-lysoPtdCho on the viability of RAW 264.7 cells was measured, based on the MTT assay. Figure 1a indicates that DHA-lysoPtdCho and DHA-lysoPtdCho had no remarkable cytotoxic effect within the

concentrations used, whereas linoleoyl-lysoPC showed a significant reduction of viability at 6 µM or higher concentrations. Thus, the suppressive effect of DHA-lysoPtdCho on LPS-induced NO production was expressed at non-cytotoxic concentrations. Subsequently, we turned to the effect of DHA-lysoPtdCho on the level of TNF-α or IL-6 in RAW 264.7 cells stimulated with LPS. Again, DHA-lysoPtdCho expressed a dose-dependent suppression of TNF-α or IL-6 formation (Fig. 2). Next, we examined the effect of DHA-lysoPtdCho on PGE₂ formation in RAW 264.7 cells stimulated with LPS. However, DHA-lysoPtdCho up to 200 µM failed to diminish the level of PGE₂ (779 pg/ml) in RAW 264.7 cells stimulated with LPS (1 µg/ml) only (data not shown).

Effects of Polyunsaturated LysoPtdChos on Zymosan A-Induced Plasma Leakage

Since DHA-lysoPtdCho exhibited a remarkable anti-inflammatory action in vitro models, we examined whether

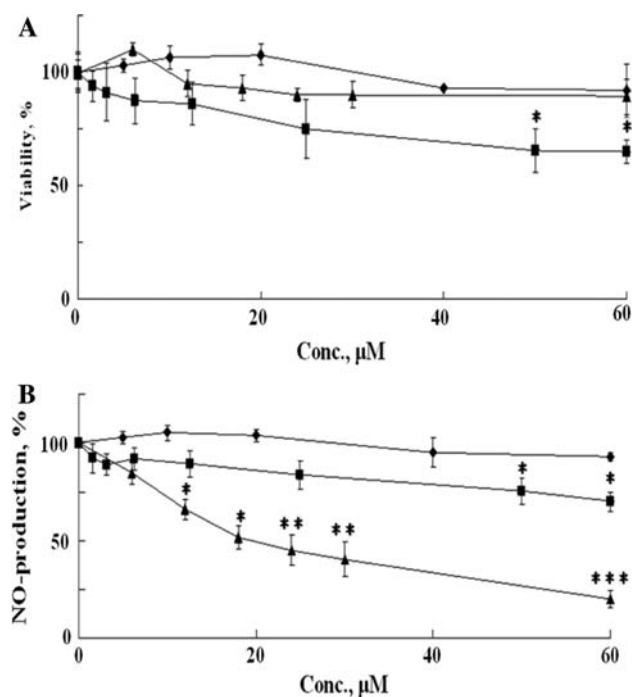


Fig. 1 Effect of polyunsaturated lysoPtdChos on cell viability or LPS-induced NO production. RAW 264.7 cells were preincubated with each polyunsaturated lysoPtdCho (LNA-lysoPtdCho, filled squares; ARA-lysoPtdCho, filled diamonds; DHA-lysoPtdCho, filled triangles) at various concentration (0–60 µM) for 2 h, and then stimulated with LPS (1 µg/ml) for another 20 h. Cell viability (a) and LPS-induced NO production (b) were determined as described in “Materials and Methods”. Data were expressed as a percentile value (%) of the LPS-treated group, which were pre-incubated with LPS (1 µg/ml) in the absence of polyunsaturated-lysoPCs, and were displayed as mean ± SE values of triplicate determinations. **P* < 0.05 and ***P* < 0.01 versus LPS-treated group

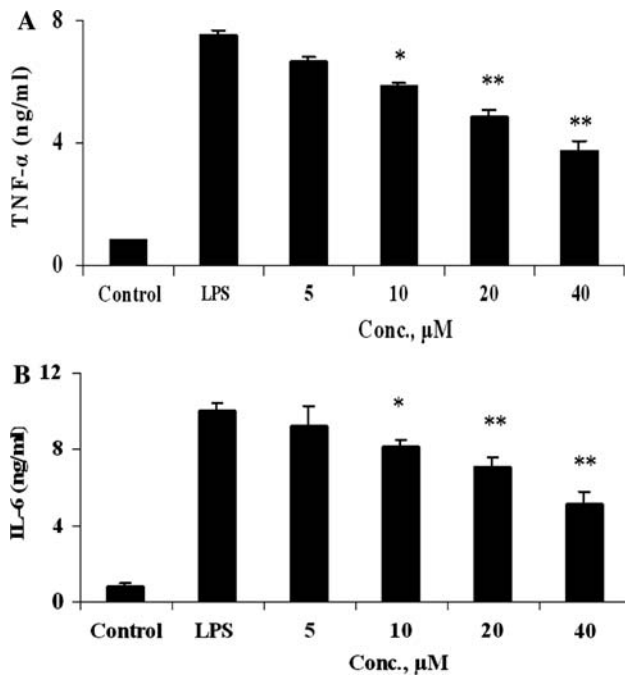


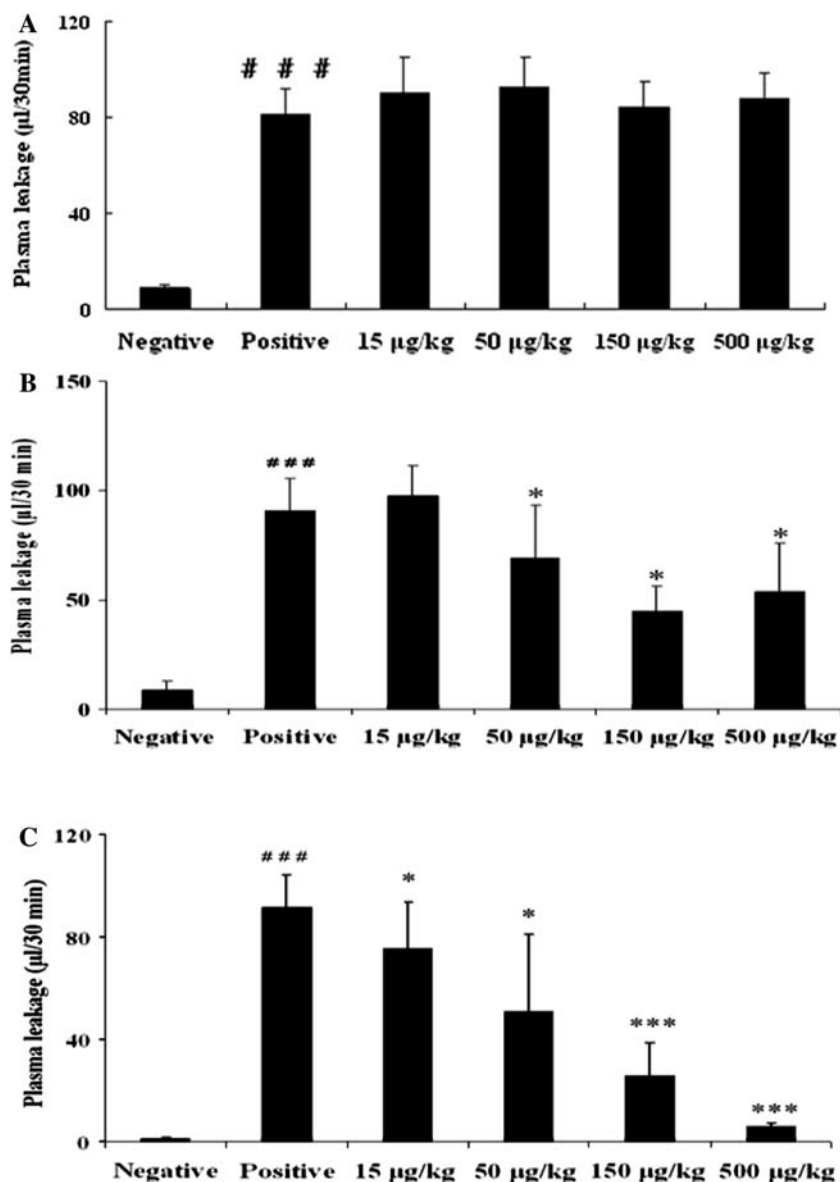
Fig. 2 Effects of DHA-lysoPtdCho on LPS-induced TNF- α (a) and IL-6 (b) production in RAW 264.7 cells. RAW 264.7 cells (5×10^4 cells/well) were incubated with DHA-lysoPtdCho (5–40 μ M) for 2 h and then LPS (1 μ g/ml) was treated for another 20 h. TNF- α and IL-6 in the cell-free cultured supernatant were measured by an ELISA kit. Data were expressed as mean \pm SEM values of triplicate determinations. * $P < 0.05$ and ** $P < 0.01$ versus LPS-treated group

polyunsaturated lysoPtdChos might attenuate zymosan A-induced peritonitis in an animal model. In this study, each lysoPtdCho was tested for the suppression of zymosan A-induced plasma leakage into the peritoneum in mice, based on extravasation of Evans blue dye indicative of vascular permeability. As shown in Fig. 3a, the i.v. administration of LNA-lysoPtdCho up to 500 μ g/kg did not reduce the level of exudate infiltration induced by zymosan A, when it was administered 30 min before i.p. administration of zymosan A (100 mg/kg). Meanwhile, ARA-lysoPtdCho (150 μ g/kg) was observed to decrease the level of exudate infiltration induced by zymosan A, but the elevation of ARA-lysoPtdCho dose to 500 μ g/kg failed to further enhance the suppressive effect (Fig. 3b). Furthermore (Fig. 3c), DHA-lysoPtdCho expressed a remarkable suppression of zymosan A-induced plasma leakage in a dose-dependent way up to 500 μ g/kg; a significant decline of zymosan A-induced plasma leakage into the peritoneum was observed with DHA-lysoPtdCho at a dose as small as 15 μ g/kg ($P < 0.05$). Further, the administration of DHA-lysoPtdCho at a higher dose (500 μ g/kg) almost completely suppressed the zymosan A-induced plasma leakage into the peritoneum, and the ED₅₀ value was estimated to be approximately 46 μ g/kg. However, zymosan A-induced

plasma leakage was not suppressed significantly or remarkably (<10%) by didocosahexaenoyl-phosphatidylcholine (15–50 μ g/kg) (data not shown). From these data, it was found that DHA-lysoPtdCho was the most efficient among phospholipids tested in preventing zymosan A-induced plasma leakage. To prove the anti-inflammatory action of DHA-lysoPtdCho in vivo, we examined the effect of DHA-lysoPtdCho on inflammatory mediators such as interleukin-6 or TNF- α in peritoneal exudates. As indicated in Fig. 4, DHA-lysoPtdCho, administered i.v. significantly inhibited zymosan A-induced formation of IL-6 and TNF- α in dose-dependent manner, supporting anti-inflammatory role of DHA-lysoPtdCho. In further study, the effect of DHA-lysoPtdCho on infiltration of leukocytes into the peritoneum was evaluated. Figure 5 showed that DHA-lysoPtdCho prohibited the infiltration of leukocytes into the peritoneum, providing further support for the anti-inflammatory action of DHA-lysoPtdCho.

In the subsequent study, a time-dependent effect of DHA-lysoPtdCho on zymosan A-induced plasma leakage was examined. For this study, DHA-lysoPtdCho (50 μ g/kg) was administered 10, 30, 60, or 120 min before i.p. administration of zymosan A, and a time-dependent suppression of plasma leakage was evaluated. As exhibited in Fig. 6, the 10-min interval between administration of DHA-lysoPtdCho and zymosan A challenge was not sufficient to express a remarkable suppression of plasma leakage. Meanwhile, both the 30-min interval and the 60-min interval were sufficient to inhibit zymosan A-induced plasma leakage, although there was no significant difference between two experimental conditions. This may suggest that the same mechanism may apply for anti-inflammatory action of DHA-lysoPtdCho between 30- and 60-min intervals. Meanwhile, the extension of interval time to 120 min augmented the inhibitory effect to some extent. Taken together, it is supposed that anti-inflammatory action of DHA-lysoPtdCho may involve multiple mechanisms. Previously, DHA-lysoPtdCho was observed to inhibit 5-lipoxygenase, an initial enzyme in the biosynthetic pathway responsible for the formation of LTC₄ [9]. Therefore, the accumulation of DHA-lysoPtdCho in the peritoneum may lead to the reduction of LTC₄ formation in the peritoneum. To test this possibility, the effect of DHA-lysoPtdCho administration on the formation of LTC₄ in the peritoneum was investigated [34]. As indicated in Fig. 7, the formation of LTC₄ in peritoneal exudates was suppressed by DHA-lysoPtdCho, i.v. administered in a dose-dependent manner. However, the suppression of leukotriene C₄ formation by DHA-lysoPtdCho at 150 μ g/kg was approximately 50%. From this, it is suggested that anti-inflammatory action of DHA-lysoPtdCho may be ascribed at least partially to the inhibition of 5-lipoxygenase. However, the suppressive effect of DHA-lysoPtdCho on

Fig. 3 Effects of polyunsaturated lysoPtdCho, administered i.v. on Zymosan A-induced plasma leakage in mice. LNA-lysoPtdCho (a), ARA-lysoPtdCho (b) DHA-lysoPtdCho (c) was administered i.v. to mice (0–500 $\mu\text{g}/\text{kg}$) 30 min prior to i.p. administration of zymosan A (100 mg/kg), and the plasma leakage was determined as described in Materials and Methods. The column and bar represent the means \pm SEM of results from each group of >10 mice. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus positive group (zymosan A); ### $P < 0.001$ versus negative group (PBS)



zymosan A-induced LTC_4 formation was smaller than zymosan A-induced plasma leakage (Fig. 3c). This led to the assumption that a process responsible for the change of vascular permeability might be directly affected by DHA-lysoPtdCho or its metabolites. To test this possibility, we examined the effect of DHA-lysoPtdCho on plasma leakage caused by LTC_4 , an inflammatory mediator. Figure 8 demonstrates that LTC_4 -induced inflammation was suppressed remarkably by DHA-lysoPtdCho. This may support a notion that a mechanism other than the inhibition of LTC_4 formation may also be responsible for the anti-inflammatory action of DHA-lysoPtdCho. In support of this, a time-dependent action of DHA-lysoPtdCho indicates that the metabolism of DHA-lysoPtdCho may be crucial for its anti-inflammatory action. One metabolic pathway

is the hydrolysis of DHA-lysoPtdCho to produce DHA. And the other may be a lipoxygenation process to give rise to HPDHA-lysoPtdCho. With this in mind, DHA and 17-HPDHA-lysoPtdCho were examined for their anti-inflammatory action. As demonstrated in Fig. 9, 17-HPDHA-lysoPC, a product from the oxygenation of DHA-lysoPtdCho by 15-lipoxygenase, showed a remarkable anti-inflammatory effect, greater than that of DHA-lysoPtdCho. From this, it is suggested that the anti-inflammatory action of DHA-lysoPtdCho may involve the formation of 17-HPDHA-lysoPtdCho as an intermediate in the metabolic activation of DHA-lysoPtdCho. Meanwhile, the treatment with DHA or 17(*S*)-hydroperoxy-4,7,10,13,15,19-DHA at 50 $\mu\text{g}/\text{kg}$ did not show any significant anti-inflammatory action (Fig. 9).

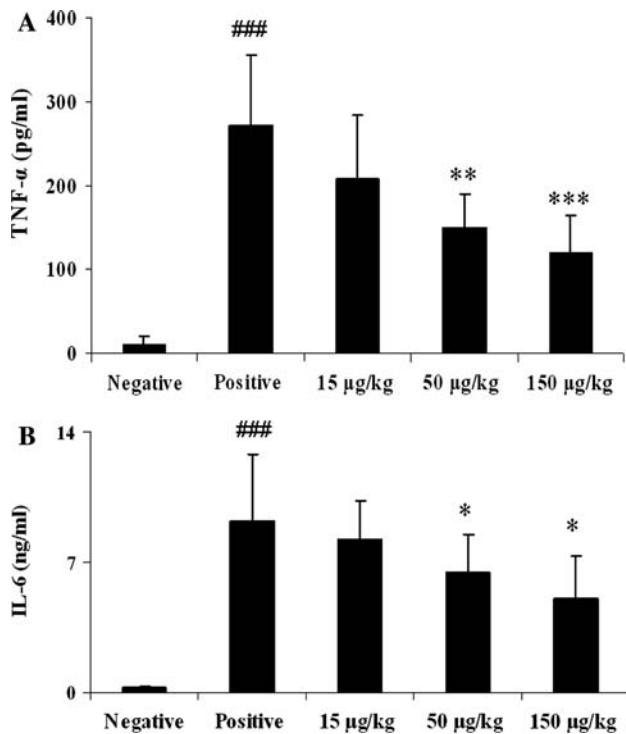


Fig. 4 Effect of DHA-lysoPtdCho, administered i.v. on zymosan A-induced TNF- α and IL-6 formation in mice. DHA-lysoPtdCho was administered i.v. to mice (0–150 $\mu\text{g}/\text{kg}$) 30 min prior to i.p. administration of zymosan A (100 mg/kg). Plasma lavage was performed with 4 ml PBS followed by brief centrifugation (15,000 rpm, 5 min), and cell-free lavage supernatant was used directly for determination of TNF- α and IL-6 levels by ELISA kit. The column and bar represent the means \pm SEM of results from each group of ten mice. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus positive group (zymosan A); $###P < 0.001$ versus negative group (PBS)

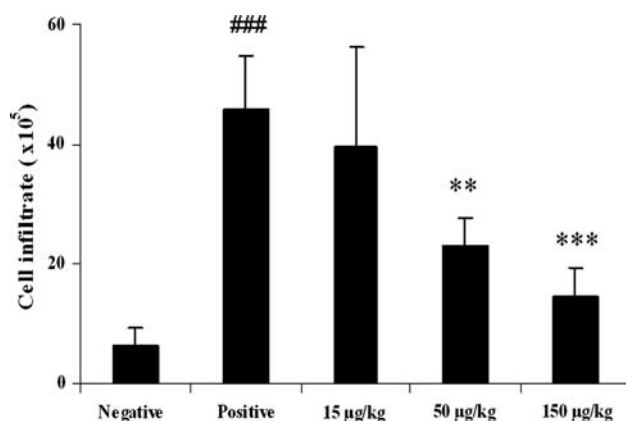


Fig. 5 Effect of DHA-lysoPtdCho, administered i.v. on zymosan A-induced leukocyte infiltration in mice. DHA-lysoPtdCho was administered i.v. 30 min prior to i.p. administration of zymosan A (100 mg/kg). Total cell counts were performed for the lavage fluid collected at the 90-min time point using light microscopy together with trypan blue staining

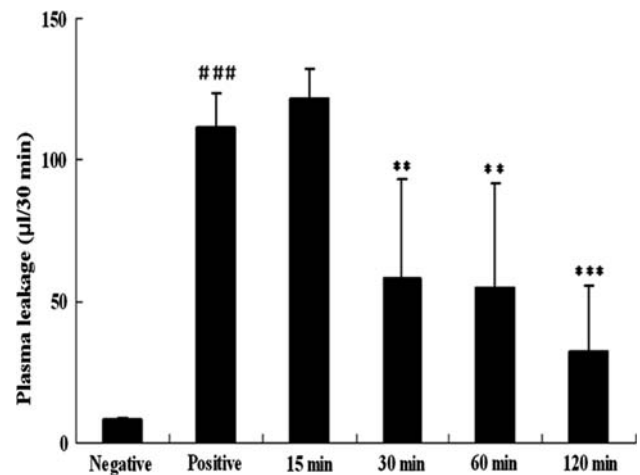


Fig. 6 Time-dependent suppressive effect of DHA-lysoPtdCho, administered i.v. on Zymosan A-induced plasma leakage in mice. DHA-lysoPtdCho was administered i.v. to mice (50 $\mu\text{g}/\text{kg}$) 15 min, 30 min, 60 min or 120 min prior to i.p. administration of zymosan A (100 mg/kg), and the plasma leakage was determined as described in “Materials and Methods”. The column and bar represent the means \pm SEM of results from each group of >10 mice. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus positive group (zymosan A); $###P < 0.001$ versus negative group (PBS)

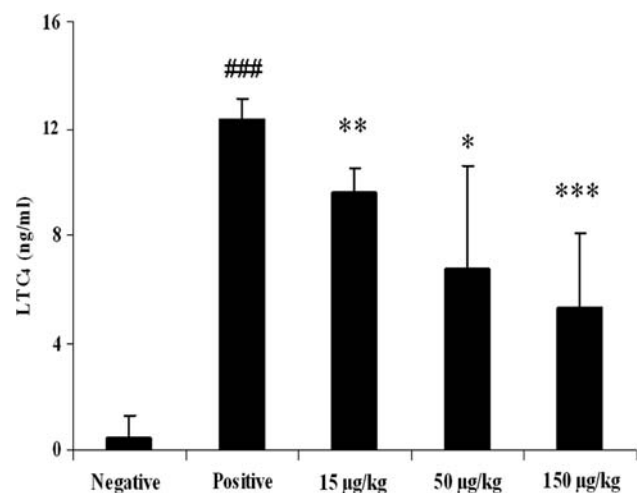


Fig. 7 Effect of DHA-lysoPtdCho, administered i.v. on zymosan A-induced LTC₄ formation in mice. DHA-lysoPtdCho was administered i.v. to mice (0–150 $\mu\text{g}/\text{kg}$) 30 min prior to i.p. administration of zymosan A (100 mg/kg). Plasma lavage was performed with 4 ml PBS followed by brief centrifugation (15,000 rpm, 5 min), and the cell-free lavage supernatant was used directly for determination of LTC₄ level by EIA kit. The column and bar represent the mean \pm SEM of results from each group of 10 mice. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ versus positive group (zymosan A); $###P < 0.001$ versus negative group (PBS)

Effect of DHA-LysoPtdCho, Administered i.p. on Zymosan A-Induced Plasma Leakage

In a separate experiment to see anti-inflammatory action of lysoPtdCho administered by other administration routes,

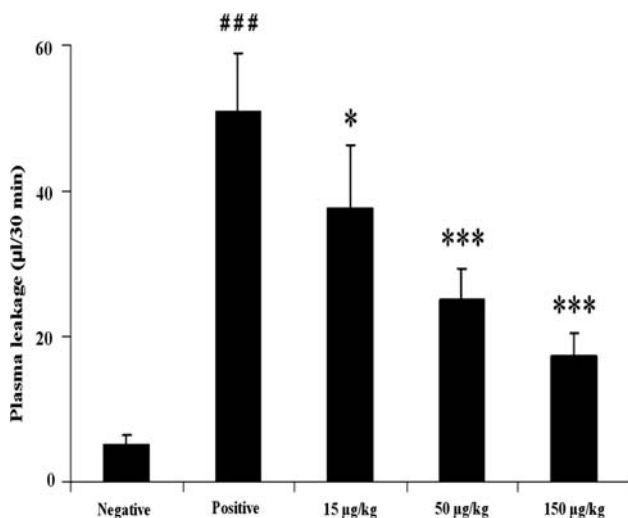


Fig. 8 Effect of DHA-lysoPtdCho, administered i.v. on LTC₄-induced plasma leakage in mice. DHA-lysoPtdCho (0–150 µg/kg) was administered i.v. into tail vein of mice 60 min before i.p. administration injection of LTC₄ (100 µg/kg), and the plasma leakage was determined as described in “Materials and methods”. The column and bar represent the mean ± SEM of results from each group of 10 mice. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus positive group (LTC₄); ###*P* < 0.001 versus negative group (PBS)

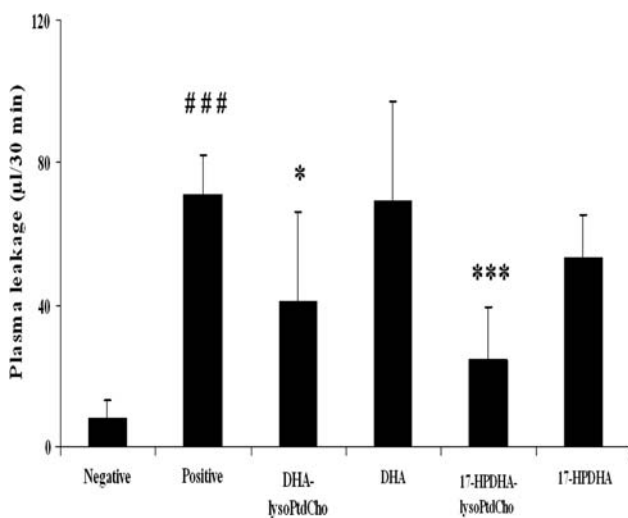


Fig. 9 Effects of DHA, 17-HPDHA, DHA-lysoPtdCho or 17-HPDHA-lysoPtdCho, administered i.v. on zymosan A-induced plasma leakage in mice. Lipids were administered i.v. to mice (50 µg/kg) 30 min before i.p. administration injection of zymosan A (100 mg/kg), and the plasma leakage was determined as described in “Materials and Methods”. The column and bar represent the mean ± SEM of results from each group of >10 mice. **P* < 0.05, ***P* < 0.01 versus positive group (zymosan A); ###*P* < 0.001 versus negative group (PBS)

DHA-lysoPtdCho, and 17-HPDHA-lysoPtdCho were administered i.p. 60 min prior to zymosan A challenge, and its suppressive effect was evaluated. As demonstrated in Fig. 10, the i.p. administration of DHA-lysoPtdCho or

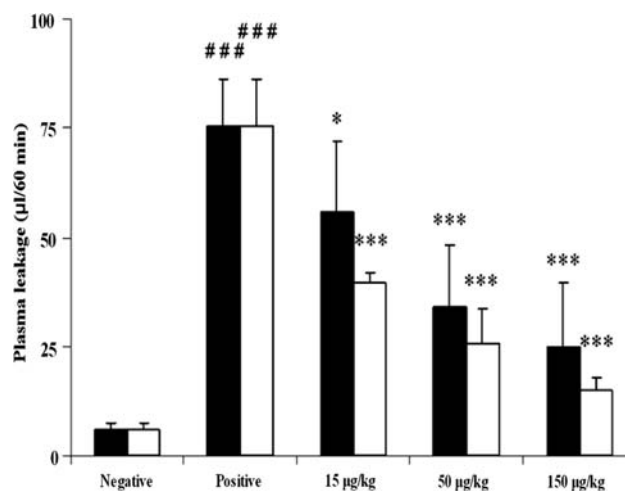


Fig. 10 Effect of DHA-lysoPtdCho or 17-HPDHA-lysoPtdCho, administered i.p. on zymosan A-induced plasma leakage in mice. DHA-lysoPtdCho (filled bars) or 17-HPDHA-lysoPtdCho (open bars) was administered i.p. (50 µg/kg) to mice 60 min before an i.p. injection of Zymosan A (100 mg/kg), and the plasma leakage was determined as described in “Materials and Methods”. The column and bar represent the mean ± SEM of results from each group of >10 mice. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus positive group (zymosan A); ###*P* < 0.001 versus negative group (PBS)

17-HPDHA-lysoPtdCho produced a dose-dependent anti-inflammatory action. In comparison, 17-HPDHA-lysoPtdCho was more efficacious than DHA-lysoPtdCho, similar to the finding with the i.v. administration (Fig. 9). Thus, DHA-lysoPtdCho was suggested to be transformed to 17-HPDHA-lysoPtdCho, more bioactive.

Effect of DHA-LysoPtdCho, Administered Orally on Zymosan A-Induced Plasma Leakage

In a further experiment, the anti-inflammatory effect of DHA or DHA-lysoPtdCho, orally administered, was examined. As shown in Fig. 11, oral administration of DHA-lysoPtdCho showed an apparent suppressive effect on zymosan A-induced plasma leakage, although oral administration was less effective than i.p. or i.v. administration. In contrast, the anti-inflammatory action of docosahexaenoic acid, orally administered, was not significant up to 150 µg/kg. Thus, the structure of DHA-lysoPtdCho is suggested to be important for anti-inflammatory action *in vivo*.

Discussion

Although there have been reports on the anti-inflammatory actions of DHA metabolites *in vitro* and *in vivo* [37–39], there has been no report on the anti-inflammatory action of DHA-lysophospholipids. Recently, our previous study

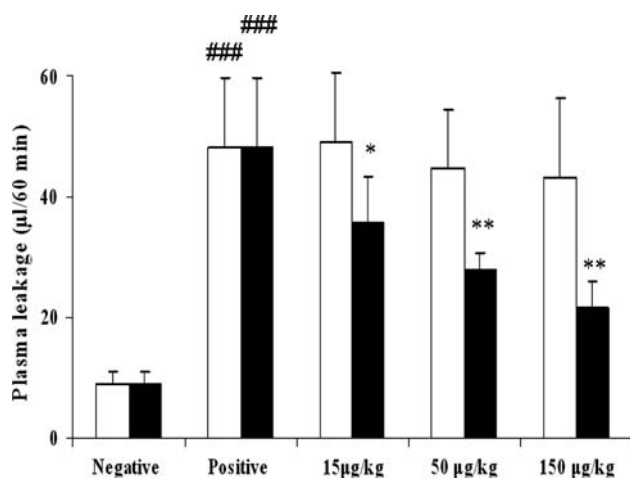


Fig. 11 Effect of DHA-lysoPtdCho or DHA, administered orally, on zymosan A-induced plasma leakage in mice. DHA (open bars) or 17-HPDHA-lysoPtdCho (filled bars) was administered orally (50 µg/kg) to mice 60 min before i.p. injection of zymosan A (100 mg/kg), and the plasma leakage was determined as described in “Materials and Methods”. The column and bar represent the mean \pm SEM of results from each group of >10 mice. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus positive group (zymosan A); #### $P < 0.001$ versus negative group (PBS)

showed that DHA-lysoPtdCho and its oxygenation products strongly inhibited mammalian 5-LOX activity [26], responsible for the generation of inflammatory lipid mediators such as leukotriene B or cysteinyl-leukotrienes. Very recently, we observed that ARA-lysoPtdCho and its 15-hydroperoxy derivative expressed an anti-inflammatory action in zymosan A-induced peritonitis [40]. Present studies indicate that DHA-lysoPtdCho is more efficient than ARA-lysoPtdCho in LPS-induced production of NO in RAW cells. Moreover, the effective concentrations (5–10 µM) of DHA-lysoPtdCho in inhibiting 5-LOX activity [26] as well as LPS-induced NO production are relatively low. Noteworthy, such an action seems to be peculiar to DHA-lysoPtdCho, since other lysoPtdCho species such as ARA-lysoPtdCho or LNA-lysoPtdCho had no remarkable reduction of NO level. Furthermore, DHA-lysoPtdCho also decreased the level of inflammatory mediators such as TNF- α or IL-6 induced by LPS, supporting the anti-inflammatory action of docosahexaenoyl-lysoPC.

Consistent with its anti-inflammatory action in vitro, DHA-lysoPtdCho, i.v. administered, suppressed zymosan A-induced peritonitis in mice at relatively low doses (15–50 µg/kg). In comparison, DHA-lysoPtdCho, i.v. or i.p. administered, was much more effective than ARA-lysoPtdCho in suppressing zymosan A-induced peritonitis. Even oral administration of DHA-lysoPtdCho exhibited some suppressive effect on zymosan A-induced peritonitis, although it was less efficient than i.v. or i.p. administration. Thus, DHA-lysoPtdCho is supposed to be one of

endogenous anti-inflammatory lipids. In support of this, DHA-lysoPtdCho was observed to reduce the levels of TNF- α or IL-6 as well as infiltrated leukocytes in vivo system. In the time course of anti-inflammatory action, a prior administration of DHA-lysoPtdCho seemed to be important for a maximal anti-inflammatory action; the administration of DHA-lysoPtdCho at least 30 min before the zymosan A challenge expressed a remarkable inhibition, but the administration 10 min before the zymosan A challenge did not succeed in showing a noticeable inhibition. From this, it is suggested that the anti-inflammatory action of DHA-lysoPtdCho in the peritoneum may require some delay process. Thus, the appearance of DHA-lysoPtdCho or its metabolites in peritoneal cells, where the mediators responsible for plasma leakage could be generated, may require a transport process. This might be consistent with the previous observation [41] that the appearance of labeled DHA, administered i.v. in peritoneal exudates required approximately a 1-h to 2-h delay for a maximal level. Earlier it had been reported that early vascular permeability, followed by subsequent infiltration of neutrophils into the peritoneum, depended largely on cysteinyl-leukotrienes, derived from 5-lipoxygenation of ARA, released by resident peritoneal macrophages, and to a lesser extent, on mast cell histamine and prostaglandin E₂ of multiple cellular origins [42, 43]. In this respect, the suppressive effect of DHA-lysoPtdCho on zymosan A-induced peritonitis might be partially related to the direct inhibition of 5-LOX [26], an initial enzyme implicated in the biosynthetic pathway of cysteinyl-leukotrienes responsible for vascular permeability [43]. Support for this comes from the finding that the i.v. administration of DHA-lysoPtdCho partially reduced the formation of LTC₄ in peritoneal exudates, reaffirming the notion that one mechanism, responsible for the anti-inflammatory effect of DHA-lysoPtdCho on zymosan A-induced peritonitis, may be related to the reduction of LTC₄ through the inhibition of 5-lipoxygenase. However, a complete inhibition of LTC₄ formation was not achieved using DHA-lysoPtdCho under the conditions used. This led us to the assumption that a process responsible for the change of vascular permeability might be directly affected by DHA-lysoPtdCho or its metabolites. In support of this assumption, the plasma leakage caused by LTC₄ was successfully prevented by DHA-lysoPtdCho administration, confirming that DHA-lysoPtdCho or its metabolites may exert an anti-inflammatory action. Concerned with this, the requirement of the time interval between lysoPC administration and zymosan A challenge may suggest that a metabolism of DHA-lysoPtdCho may be crucial for the maximal expression of anti-inflammatory action at the inflammation site, i.e. the peritoneum. There may be two possible metabolic pathways of DHA-lysoPtdCho; one is the formation of DHA,

and the other, the formation of HPDHA-lysoPtdCho [24]. In the former process, DHA is released from the enzymatic hydrolysis of DHA-lysoPtdCho *in vivo*. However, this hydrolytic metabolic process may not be directly related to the anti-inflammatory action of DHA-lysoPtdCho in peritoneal cells as suggested from no remarkable effect of DHA administered in this experiment. Rather, HPDHA-lysoPtdCho may more likely be a metabolic intermediate accountable for anti-inflammatory action. In support of this, 17-HPDHA-lysoPtdCho showed greater anti-inflammatory effect than DHA-lysoPtdCho in *i.v.* administration as well as *i.p.* injection. The similarity of DHA-lysoPtdCho action between two administration routes may support the idea that the metabolic activation of DHA-lysoPtdCho may occur commonly *in vivo*. This could be explained by the notion that DHA-lysoPtdCho might be primarily subjected to lipoxygenation by 15-lipoxygenase to generate 17-HPDHA-lysoPtdCho, which could be further utilized for the formation of potent anti-inflammatory mediators such as protectin D type derivative [38]. Alternatively, secondary metabolites such as maresin type derivative [37], derived from 14-HPDHA-lysoPtdCho, may be implicated in the anti-inflammatory action. Taken together, it is likely that the primary metabolites such as 17-HPDHA-lysoPtdCho or 14-HPDHA-lysoPtdCho may be finally converted to bioactive products directly responsible for anti-inflammatory action. This might be well supported by the previous finding [24] that DHA-lysoPtdCho was enzymatically converted to 14-HPDHA-lysoPtdCho derivative or 17-HPDHA-lysoPtdCho derivative. In turn, 14-hydroperoxydocosahexaenoyl derivative and 17-hydroperoxydocosahexaenoyl derivative could be transformed finally to maresin [37] or protectin D [38]. Thus, it is supposed that another mechanism for anti-inflammatory action of DHA-lysoPtdCho may involve a series of metabolic activation pathways. Previous studies showed that in zymosan A-induced peritonitis, protectin D significantly reduced PMN infiltration at very low doses, showing an apparent maximal response approximately in the 50% range [9]. In our present study, meanwhile, an almost complete inhibition of zymosan A peritonitis was achieved with DHA-lysoPtdCho at a dose of 500 $\mu\text{g}/\text{kg}$, indicating that DHA-lysoPtdCho might be more efficient in showing a maximal efficacy. This could be explained by the assumption that DHA-lysoPtdCho might express anti-inflammatory activity through multiple actions, such as suppression of the formation of nitric oxide or TNF- α , inhibition of 5-lipoxygenase activity as well as formation of maresin or protectin D.

Once the *sn*-2 position of phosphatidylcholine is hydrolyzed by PLA₂, unsaturated fatty acids are released, and then metabolized to form bioactive lipid mediators, inflammatory or anti-inflammatory [44]. Meanwhile, the

remaining lysoPtdChos with saturated acyl chains or oleoyl group have been found to have cytotoxic effects on various cell systems [14–18]. As a part of the inflammatory actions of lysoPtdChos, lysoPtdChos brought about the superoxide production in neutrophils [17] and nonphagocytic cells [16, 17], and LNA-lysoPtdCho caused oxidative stress in RAW 264.7 cells [18]. In addition, lysoPtdChos elicited the production of pro-inflammatory cytokines in human monocytes and rat aortic smooth muscle cells [45], and played a role as a chemotactic factor for monocytes and T cells [3, 46]. The diverse properties of lysoPtdChos suggest that the functions of this class of lipids are highly dependent on the type of lysoPtdChos. A recent report showed that transcellular secretion of group V phospholipase A₂ from the epithelium induced the synthesis of leukotriene C₄ through the generation of lysoPtdChos in eosinophils [47]. In this regard, it will be of great interest whether or how DHA-lysoPtdCho affects the secretory group V PLA₂-induced formation of LTC₄ in eosinophils.

An apparent advantage with DHA-lysoPtdCho, compared to other types of lysoPtdCho, is that it exhibits anti-inflammatory actions at non-cytotoxic concentrations. Moreover, DHA-lysoPtdCho still exhibits a significant anti-inflammatory action after oral administration. In this respect, it is conceivable that administration of DHA-lysoPtdCho will help ameliorate the pro-inflammatory state, caused by various inflammatory agents including pro-inflammatory lysoPtdChos.

In this respect, DHA-lysoPtdCho therapy is possible if these lipids are introduced through appropriate administration routes in the inflammation progress. For this purpose, the upper limit of DHA-lysoPtdCho dosage, showing anti-inflammatory action selectively, is to be established in further studies.

Acknowledgments This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF 2009-0069242).

References

1. Daleau P (1999) Lysophosphatidylcholine, a metabolite which accumulates early in myocardium during ischemia, reduces gap junctional coupling in cardiac cells. *J Mol Cell Cardiol* 31:1391–1401
2. Fuchs B, Schiller J, Wagner U, Häntzschel H, Arnold K (2005) The phosphatidylcholine/lysophosphatidylcholine ratio in human plasma is an indicator of the severity of rheumatoid arthritis: investigations by 31P NMR and MALDI-TOF MS. *Clin Biochem* 38:925–933
3. Muralikrishna Adibhatla R, Hatcher JF (2006) Phospholipase A₂, reactive oxygen species, and lipid peroxidation in cerebral ischemia. *Free Radic Biol Med* 40:376–387

4. Triggiani M, Granata F, Frattini A, Marone G (2006) Activation of human inflammatory cells by secreted phospholipase A2. *Biochim Biophys Acta* 1761:1289–1300
5. Matsumoto T, Kobayashi T, Kamata K (2007) Role of lysophosphatidylcholine (LPC) in atherosclerosis. *Curr Med Chem* 14:3209–3220
6. Shi Y, Zhang P, Zhang L, Osman H, Mohler ER 3rd, Macphee C, Zalewski A, Postle A, Wilensky RL (2007) Role of lipoprotein-associated phospholipase A2 in leukocyte activation and inflammatory responses. *Atherosclerosis* 191:54–62
7. Serhan CN, Savill J (2005) Resolution of inflammation: the beginning programs the end. *Nat Immunol* 6:1191–1197
8. Farooqui AA, Horrocks LA, Farooqui T (2007) Modulation of inflammation in brain: a matter of fat. *J Neurochem* 101:577–599
9. Funk CD (2001) Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 294:1871–1875
10. O'Meara SJ, Rodgers K, Godson C (2008) Lipoxins: update and impact of endogenous pro-resolution lipid mediators. *Rev Physiol Biochem Pharmacol* 160:47–70
11. Schwab JM, Serhan CN (2006) Lipoxins and new lipid mediators in the resolution of inflammation. *Curr Opin Pharmacol* 6:414–420
12. Kühn H, O'Donnell VB (2006) Inflammation and immune regulation by 12/15-lipoxygenases. *Prog Lipid Res* 45:334–356
13. Fuentes L, Hernández M, Fernández-Avilés FJ, Crespo MS, Nieto ML (2002) Cooperation between secretory phospholipase A2 and TNF-receptor superfamily signaling: implications for the inflammatory response in atherogenesis. *Circ Res* 91:681–688
14. Colles SM, Chisolm GM (2000) Lysophosphatidylcholine-induced cellular injury in cultured fibroblasts involves oxidative events. *J Lipid Res* 41:1188–1198
15. Matsubara M, Hasegawa K (2005) Benidipine, a dihydropyridine-calcium channel blocker, prevents lysophosphatidylcholine-induced injury and reactive oxygen species production in human aortic endothelial cells. *Atherosclerosis* 178:57–66
16. Takeshita S, Inoue N, Gao D, Rikitake Y, Kawashima S, Tawa R, Sakurai H, Yokoyama M (2000) Lysophosphatidylcholine enhances superoxide anions production via endothelial NADH/NADPH oxidase. *J Atheroscler Thromb* 7:238–246
17. Silliman CC, Elzi DJ, Ambruso DR, Musters RJ, Hamiel C, Harbeck RJ, Paterson AJ, Bjornsen AJ, Wyman TH, Kelher M, England KM, McLaughlin-Malaxecheberria N, Barnett CC, Aiboshi J, Bannerjee A (2003) Lysophosphatidylcholines prime the NADPH oxidase and stimulate multiple neutrophil functions through changes in cytosolic calcium. *J Leukoc Biol* 73:511–524
18. Park CH, Kim MR, Han JM, Jeong TS, Sok DE (2009) Lysophosphatidylcholine exhibits a selective cytotoxicity, accompanied by ROS formation, in RAW 264.7 macrophages. *Lipids* 44:425–435
19. Okita M, Gaudette DC, Mills GB, Holub BJ (1997) Elevated levels and altered fatty acid composition of plasma lysophosphatidylcholine (lysoPC) in ovarian cancer patients. *Int J Cancer* 71:31–34
20. Croset M, Brossard N, Polette A, Lagarde M (2000) Characterization of plasma unsaturated lysophosphatidylcholines in human and rat. *Biochem J* 345:61–67
21. Adachi J, Asano M, Yoshioka N, Nushida H, Ueno Y (2006) Analysis of phosphatidylcholine oxidation products in human plasma using quadrupole time-of-flight mass spectrometry. *Kobe J Med Sci* 52:127–140
22. Chen S, Li KW (2007) Mass spectrometric identification of molecular species of phosphatidylcholine and lysophosphatidylcholine extracted from shark liver. *J Agric Food Chem* 55:9670–9677
23. Huang LS, Kim MR, Sok DE (2006) Linoleoyl lysophosphatidylcholine is an efficient substrate for soybean lipoxygenase-1. *Arch Biochem Biophys* 455:119–126
24. Huang LS, Kim MR, Sok DE (2007) Oxygenation of 1-docosahexaenoyl lysophosphatidylcholine by lipoxygenases; conjugated hydroperoxydiene and dihydroxytriene derivatives. *Lipids* 42:981–990
25. Huang LS, Kim MR, Sok DE (2008) Oxygenation of arachidonoyl lysophospholipids by lipoxygenases from soybean, porcine leukocyte, or rabbit reticulocyte. *J Agric Food Chem* 56:1224–1232
26. Huang LS, Kim MR, Sok DE (2008) Regulation of lipoxygenase activity by polyunsaturated lysophosphatidylcholines or their oxygenation derivatives. *J Agric Food Chem* 56:7808–7814
27. Dimitrow PP, Jawien M (2009) Pleiotropic, cardioprotective effects of omega-3 polyunsaturated fatty acids. *Mini Rev Med Chem* 9(9):1030–1039
28. Doherty NS, Poubelle P, Borgeat P, Beaver TH, Westrich GL, Schrader NL (1985) Intraperitoneal injection of zymosan in mice induces pain, inflammation and the synthesis of peptidoleukotrienes and prostaglandin E₂. *Prostaglandins* 30:769–789
29. Rao TS, Currie JL, Shaffer AF, Isakson PC (1994) In vivo characterization of zymosan-induced mouse peritoneal inflammation. *J Pharmacol Exp Ther* 269:917–925
30. Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, Yang RM, Petasis NA, Serhan CN (2005) Stereochemical assignment, anti-inflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. *J Exp Med* 20:713–722
31. Leite DF, Echevarria-Lima J, Ferreira SC, Calixto JB, Rumjanek VM (2007) ABCC transporter inhibition reduces zymosan-induced peritonitis. *J Leukoc Biol* 82(3):630–637
32. Rao NL, Dunford PJ, Xue X (2007) Anti-inflammatory activity of a potent, selective leukotriene A4 hydrolase inhibitor in comparison with the 5-Lipoxygenase inhibitor zileuton. *J Pharmacol Exp Ther* 321:1154–1160
33. Dimitrova P, Ivanovska N (2008) Tyrphostin AG-490 inhibited the acute phase of zymosan-induced inflammation. *Int Immunopharmacol* 8(11):1567–1577
34. Griswold DE, Webb EF, Hillegass LM (1991) Induction of plasma exudation and inflammatory cell infiltration by leukotriene C4 and leukotriene B4 in mouse peritonitis. *Inflammation* 15:251–258
35. Xie QW, Whisnant R, Nathan C (1993) Promoter of the mouse gene encoding calcium-independent nitric oxide synthase confers inducibility by interferon gamma and bacterial lipopolysaccharide. *J Exp Med* 177:1779–1784
36. Petros A, Bennett D, Vallance P (1991) Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet* 338:1557–1558
37. Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M (2009) Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J Exp Med* 206:15–23
38. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL (2002) Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med* 196:1025–1037
39. Hassan IR, Gronert K (2009) Acute changes in dietary {omega}-3 and {omega}-6 polyunsaturated fatty acids have a pronounced impact on survival following ischemic renal injury and formation of renoprotective docosahexaenoic acid-derived protectin D1. *J Immunol* 182:3223–3232
40. Hung ND, Kim MR, Sok DE (2009) Anti-inflammatory action of arachidonoyl lysophosphatidylcholine or 15-hydroperoxy

- derivative in zymosan A-induced peritonitis. *Prostaglandins Other Lipid Mediat* 90(3–4):105–111
41. Lundy SR, Dowling RL, Stevens TM, Kerr JS, Mackin WM, Gans KR (1990) Kinetics of phospholipase A2, arachidonic acid, and eicosanoid appearance in mouse zymosan peritonitis. *J Immunol* 144:2671–2677
 42. Kolaczowska E, Arnold B, Opdenakker G (2008) Gelatinase B/MMP-9 as an inflammatory marker enzyme in mouse zymosan peritonitis: comparison of phase -specific production by mast cells, macrophages, and neutrophils. *Immunobiology* 213:109–124
 43. Kolaczowska E, Shahzidi S, Seljelid R, Van Rooijen N, Plytycz B (2002) Early vascular permeability in murine experimental peritonitis is mediated by residential macrophages and mast cells: crucial involvement of macrophage-derived cysteinyl-leukotrienes. *Inflammation* 26:61–71
 44. Huwiler A, Pfeilschifter J (2009) Lipids as targets for novel anti-inflammatory therapies. *Pharmacol Ther* 124(1):96–112
 45. Roberts JR, Shaw CF 3rd (1998) Inhibition of erythrocyte selenium-glutathione peroxidase by auranofin analogues and metabolites. *Biochem Pharmacol* 55:1291–1299
 46. Maddipati KR, Marnett LJ (1987) Characterization of the major hydroperoxide-reducing activity of human plasma. Purification and properties of a selenium-dependent glutathione peroxidase. *J Biol Chem* 262:17398–17403
 47. Muñoz NM, Meliton AY, Lambertino A, Boetticher E, Learoyd J, Sultan F, Zhu X, Cho W, Leff AR (2006) Transcellular secretion of group V phospholipase A2 from epithelium induces beta 2-integrin-mediated adhesion and synthesis of leukotriene C4 in eosinophils. *J Immunol* 177(1):574–582