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Inhaled lysophosphatidylcholine provokes bronchoconstriction in guinea pigs in vivo

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Abstract

Lysophosphatidylcholine is increased in the airway of bronchial asthma, but its role is not clear. We investigated the role of lysophosphatidylcholine in asthma in anaesthetized, mechanically ventilated guinea pigs. Pressure at the airway opening was measured as an index of bronchial response. Increasing doses of lysophosphatidylcholine (1–10 mg/ml) were inhaled and then bronchoalveolar lavage was carried out. 100 and 200 μg/ml methacholine were inhaled 10 min after inhalation of 2.5 mg/ml lysophosphatidylcholine, 10 mg/ml dipalmitoyl phosphatidylcholine and 10 mg/ml glycerophosphocholine, all of which per se did not change the pressure at the airway opening. Effect of 1.0 μg/kg salbutamol, or 60 mg/kg diphenhydramine on the lysophosphatidylcholine-induced increase in the pressure at the airway opening was investigated. Inhalation of lysophosphatidylcholine dose-dependently increased the pressure at the airway opening and increased bronchial responsiveness to methacholine. On the other hand, inhalation of dipalmitoyl phosphatidylcholine decreased the pressure at the airway opening and decreased bronchial responsiveness to methacholine. Intravenously administered salbutamol, but not diphenhydramine, prevented the lysophosphatidylcholine-induced increase in the pressure at the airway opening. The percentage of leukocytes in bronchoalveolar lavage fluid did not change significantly at least within 20 min after the lysophosphatidylcholine inhalation. Lysophosphatidylcholine causes bronchoconstriction and enhances bronchial responsiveness without inducing leukocyte infiltration in the airway, suggesting that lysophosphatidylcholine may be a new bronchoconstrictor mediator in bronchial asthma.

Keywords: Airway edema; Bronchial asthma; Bronchoconstriction; Lysophosphatidylcholine; (Guinea pig)

1. Introduction

Pulmonary surfactant is a lipid protein complex that is synthesized by the alveolar type II epithelial cells, and it has important function in maintaining alveolar stability (Lewis and Lobe, 1993). Surfactant composition and biophysical activity change in various pulmonary diseases, especially in adult respiratory distress syndrome (Zhou et al., 2000). It consists of 10% protein and 90% lipid and has a high proportion of dipalmitoyl phosphatidylcholine (DPPC) (Lewis and Lobe, 1993). It has been shown that pulmonary surfactant lies not only in the alveoli but also in the conducting airways (Enhorning et al., 1983).

Asthma is a complex disease, characterized by increased bronchial responsiveness and Th2 cells mediated airway inflammation (Barnes, 2001). Liu et al. (1991) showed surfactant inhibition in the acute asthma model of guinea pigs. In the guinea pig asthma model, secretory phospholipase A2, which is produced from alveolar macrophages, plays a major role in the hydrolysis of surfactant phospholipids, producing lysophosphatidylcholine (LPC) derived from the hydrolysis of DPPC (Hidi et al., 1997). Thereafter, LPC is converted to DPPC and glycerophosphocholine by lysophosphatidylcholine-acyltransferase (Erich et al., 1990). Increased levels of secretory phospholipase A2 have been

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found in leukocytes and serum of asthmatics (Mehta et al., 1990; Toyoshima et al., 1995) and the levels of secretory phospholipase A2 have been reported to correlate with the severity of asthma. Bowton et al. (1997) reported that secretory phospholipase A2 and arachidonic acid increased rapidly in bronchoalveolar lavage fluid from allergic asthmatic subjects 4 h after inhaled antigen challenge. Chilton et al. (1996) showed that a significant increase in secretory phospholipase A2 activity was found in bronchoalveolar lavage fluid from allergic subjects challenged with antigen and a lot of lysophospholipid formation including LPC was caused in human airways 20 h after the challenge. In asthmatic patients, the treatment with inhaled beclomethasone dipropionate and oral prednisolone resulted in an improvement in lung function accompanied by a decrease in the plasma LPC level (Chhabra et al., 1999). However, the role of LPC in bronchial asthma is unclear.

In the animal model, sensitization of guinea pigs with ovalbumin resulted in increased secretory phospholipase A2 activity (Taki et al., 1986). Nath et al. (1983) reported that there was a good correlation between airway and plasma LPC levels in guinea pigs.

It is possible that formation and accumulation of LPC may mediate inflammatory and allergic reactions in the lung, or directly induce airway smooth muscle contraction. In this study, we examined the effect of inhaled LPC on airway smooth muscle tone and bronchial responsiveness in anesthetized, mechanically ventilated guinea pigs.

2. Materials and methods

2.1. Animals

Male albino Hartley strain guinea pigs (350–400 g) were obtained from Sankyou Laboratory Service (Toyama, Japan). They were quarantined in the Animal Research Center of Kanazawa University for 1 week before the study. They were allowed food and water ad libitum before experiments.

2.2. Preparation of animals

Guinea pigs were anesthetized with sodium pentobarbitone (75 mg/kg, i.p.). They were placed in the supine position and a polyethylene tube (outside diameter 2.5 mm, inside diameter 2.1 mm) was inserted into the trachea, and then artificially ventilated with a small animal respiratory pump (Model 1680, Harvard Apparatus Co., Inc., South Natick, MA, U.S.A.) adjusted to a tidal volume of 10 ml/kg at a rate of 60 strokes/min. The changes in lung resistance to inflation, the lateral pressure of the tracheal tube (pressure at the airway opening, cmH_2O), were measured using a pressure transducer (Model TP-603T, Nihon Koden Kogyo Co., Ltd., Tokyo, Japan) by the modified method of Konzett and Rossler as described by Jones et al. (1982). Since we confirmed that the change in pressure at the airway opening following inhalation of leukotriene C4 and histamine represented the average of the changes in pulmonary resistance and reciprocal dynamic lung compliance (Fujimura, 1983), pressure at the airway opening was used as an overall index of bronchial response to bronchoactive agents. The lungs were over-inflated by twice the tidal volume for two breaths by clamping the outlet port of the respirator to standardize the volume history of the lungs (Fujimura, 1983). When pressure at the airway opening had been stabilized for 10 min after starting the artificially ventilation, this pressure at the airway opening value was determined as the baseline pressure at the airway opening value. In following studies, we used naive guinea pigs. All the animal procedures in this study complied with the standard set out in the Guideline for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University.

2.3. Bronchial responsiveness to inhaled LPC

Ten minutes after the installation of artificial ventilation the pressure at the airway opening had stabilized and increasing doses of LPC (1, 2.5, 5, and 10 mg/ml), dissolved in doses of dimethyl sulfoxide (DMSO) (1%, 2.5%, 5%, and 10%) respectively, were inhaled to the guinea pigs. The animals in the control group were given an aerosol consisting of only DMSO with the same time schedule as those in the inhaled LPC group. Each inhalation was done for 20 s at intervals of 5 min under the continuous ventilation using an ultrasonic nebulizer. The rate of aerosol production was 15.2 μl/min and 46.4% of the aerosol was deposited in the lungs as measured by the radio-aerosol technique (Minami et al., 1983). The median aerodynamic diameter of the particles of physiologic saline was 3.59±1.96 μm (mean±S.D.), as measured by a laser particle size analyzer (Kurashima et al., 1997).

To examine the effect of inhaled LPC at the early phase, bronchoalveolar lavage was carried out 5 min after an inhalation of 10 mg/ml LPC or 10% DMSO in guinea pigs. A 2 ml/100 g weight of guinea pigs aliquot of normal saline was injected into the lungs through the tracheal cannula and the lavage effluent was allowed to drain into a 50 ml conical tube. The recovered bronchoalveolar lavage fluid was centrifuged at 334×*g* for 5 min. Then, we used a Turk solution for determining the number of the cells as well as cytocentrifugation (Cytospin 2, Shadon, England) for differential counts of the cells. For differential counting, 300 cells were counted after the staining with May–Grunwald–Giemsa stain.

2.4. Bronchial responsiveness to inhaled methacholine

In the result of bronchial responsiveness to inhaled LPC (see Results), 1 mg/ml and 2.5 mg/ml of LPC gave

no change of pressure at the airway opening compared with each vehicle (DMSO). In addition, our preliminary study confirmed that an inhalation of 10 mg/ml of DPPC or the same dose of glycerophosphocholine did not change the pressure at the airway opening. To examine the effect of LPC, DPPC and glycerophosphocholine on non-specific bronchial responsiveness to methacholine, the animals were given 2.5 mg/ml of LPC or vehicle (2.5% DMSO), 10 mg/ml of DPPC or vehicle (10% DMSO), and 10 mg/ml of glycerophosphocholine or vehicle (10% DMSO) using the nebulizer. Ten minutes later, when pressure at the airway opening had been stabilized, ascending doses of methacholine (100 and 200 μg/ml) were inhaled for 20 s via the nebulizer at an interval of 5 min under the continuous ventilation (Fujimura et al., 1997).

2.5. Effect of salbutamol on LPC-induced bronchial response

In order to examine the contribution of airway smooth muscle contraction to the LPC-induced bronchial response a beta-2 agonist, salbutamol (1.0 μg/kg, dissolved in saline), or vehicle (saline) was intravenously given 5 min before the anesthesia, and then 10 mg/ml of LPC was inhaled. The dose of salbutamol administered is enough to present its bronchodilator effect (Fujimura et al., 1999).

2.6. Effect of diphenhydramine on LPC-induced bronchial response

In order to examine the involvement of histamine in the LPC-induced bronchial response, the antihistamine diphenhydramine (60 mg/kg, dissolved in saline), or vehicle (saline) was intraperitoneally administered 5 min before the anesthesia, and ascending doses of LPC were inhaled. The 60 mg/kg of diphenhydramine is a sufficient dose to have its antihistamine activity (Fujimura, 1983).

2.7. Chemicals

The following drugs were used: sodium pentobarbitone (Abbot Laboratories, North Chicago, IL, U.S.A.); salbutamol sulfate (Wako Pure Chemical Ind., Osaka, Japan); diphenhydramine hydrochloride (Sigma); methacholine (Wako Pure Chemical Ind., Osaka, Japan); L-α-lysophosphatidylcholine, from egg yolk (Wako Pure Chemical Ind., Osaka, Japan); L-α-phosphatidylcholine, dipalmitoyl (Sigma); L-α-glycerophosphorylcholine 1:1 cadmium chloride adduct (Sigma).

2.8. Statistical analysis

Bronchial responsiveness to inhaled ascending doses of LPC or methacholine was determined as % increase in pressure at the airway opening compared with the value before the first inhalation of LPC or methacholine.

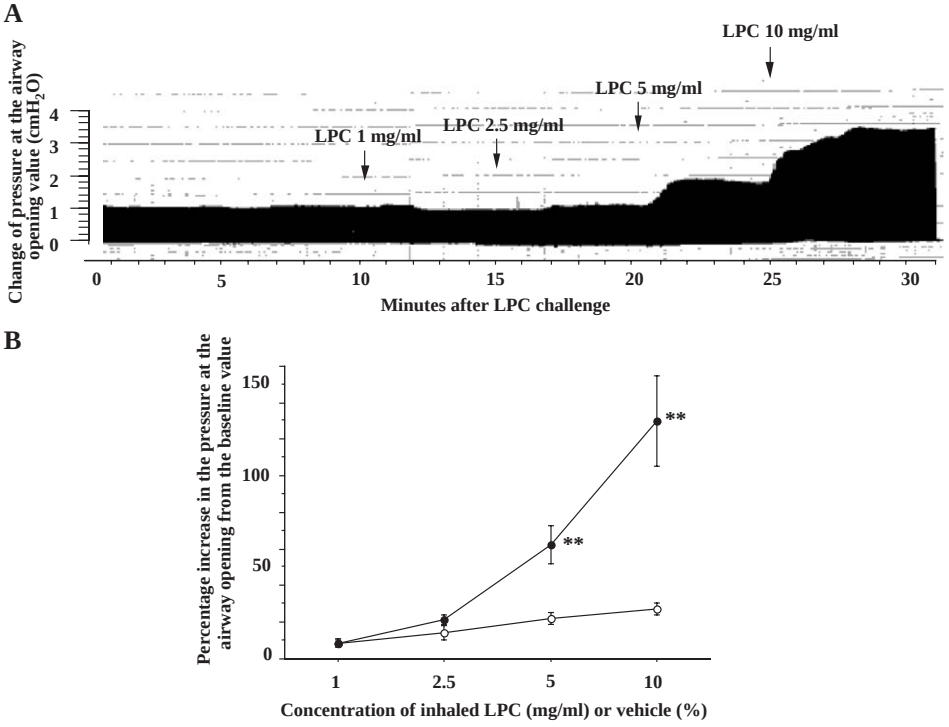

Fig. 1. Bronchial response to inhaled LPC. A sample recording is shown in (A). The data is expressed as peak % increase in pressure at the airway opening from the pre-inhalation value (mean ± standard error of the mean) in the vehicle group (1%, 2.5%, 5%, and 10% of DMSO, open circles) and the LPC group (1, 2.5, 5, and 10 mg/ml of LPC, closed circle) in (B) ($n=8$ in each group). The statistical significance is indicated as $^{**}P<0.01$ compared with the vehicle group (B).

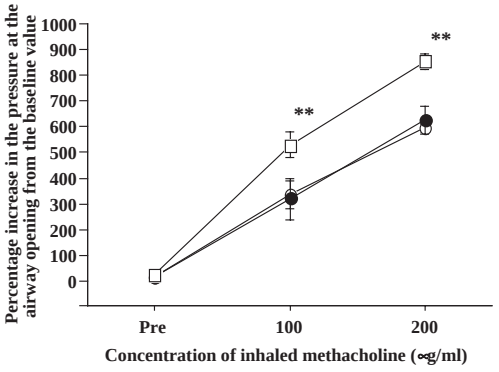

Fig. 2. Effect of inhaled LPC on bronchial responsiveness to methacholine. The data are expressed as the same as Fig. 1B. Open circles: control group (saline), closed circles: the vehicle of LPC group (2.5% DMSO), open squares: 2.5 mg/ml of LPC group as described in Section 2 ($n=5$ in each group). Each tested drug was intravenously administered 10 min before the methacholine challenge. The statistical significance is indicated as $^{**}P<0.01$ compared with control and vehicle. Pre: pre-inhalation of methacholine.

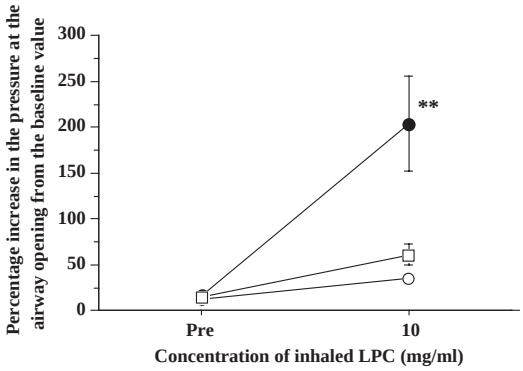

Fig. 4. Effect of salbutamol on inhaled LPC-induced bronchoconstriction. The data are expressed as the same as Fig. 1B. Open circles: control group (saline), closed circles: vehicle of LPC group (10 mg/ml of LPC), open squares: 1.0 μg/kg of salbutamol group (10 mg/ml of LPC and 1.0 μg/kg of salbutamol) as described in Section 2 ($n=7$ in each group). The statistical significance is indicated as $^{**}P<0.01$ compared with vehicle. Pre: pre-inhalation of LPC.

Differences among 3 or more groups were tested for significance by nonparametric analysis of variance (ANOVA), while those between 2 groups were tested by the Mann–Whitney's *U*-test. The significance was based on a 95% confidence level ($P<0.05$) and all measurements were expressed as mean±standard error of the mean.

3. Results

There were no significant differences between the baseline pressure at the airway opening before inhalation of LPC and DMSO. Aerosolized administration of LPC caused increases in pressure at the airway opening, which reached a maximal level at 146.1±22.9 s after the exposure to LPC (Fig. 1A,B).

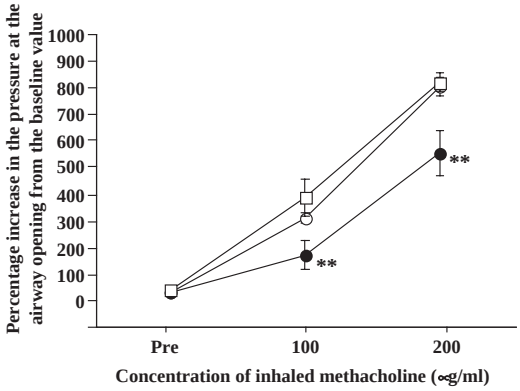

Fig. 3. Effect of inhaled DPPC and glycerophosphocholine on bronchial responsiveness to methacholine. The data are expressed as the same as Fig. 1B. Open circles: vehicle group (10% DMSO), closed circles: 10 mg/ml of DPPC group, open squares: 10 mg/ml of glycerophosphocholine group as described in Section 2 ($n=7$ in each group). Each tested drug was intravenously administered 10 min before the methacholine challenge. The statistical significance is indicated as $^{**}P<0.01$ compared with vehicle. Pre: pre-inhalation of methacholine.

The percentage increase in pressure at the airway opening from the baseline by the inhalation of 10 mg/ml LPC and 10% DMSO were 128.1±24.7% and 25.4±3.0%, respectively. There was significant difference in recovery rate of bronchoalveolar lavage fluid between the LPC group and the vehicle group (3.5±0.5 ml and 4.5±0.5 ml, $P<0.01$). However, bronchoalveolar lavage cell differentials were not different between the two groups: macrophages 57.9±5.1% and 65.9±4.3%, neutrophils 4.4±0.8% and 3.8±0.8%, lymphocytes 16.1±2.8% and 15.6±1.9%, and eosinophils 20.9±4.1% and 16.0±4.7%, respectively).

LPC at a dose of 2.5 mg/ml, that did not increase pressure at the airway opening by itself, significantly altered pressure at the airway opening responses to inhaled methacholine (Fig. 2). The rate of increase in pressure at the airway opening at 200 μg/ml of methacholine was 595.2±18.7% (control), 627.2±53.5% (vehicle; 2.5% DMSO), and 854.8±33.2% (2.5 mg/ml of LPC), respectively. Bronchial responses

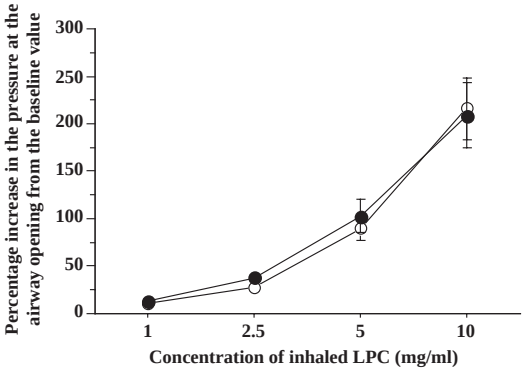

Fig. 5. Effect of diphenhydramine on inhaled LPC-induced bronchoconstriction. The data are expressed as the same as Fig. 1B. Open circles: vehicle group (saline), closed circles: 60 mg/kg of diphenhydramine group as described in Section 2 ($n=8$ in each group). No significant difference was observed between the groups.

to inhaled methacholine 10 min after inhalation of DPPC and glycerophosphocholine are shown in Fig. 3. Baseline pressure at the airway opening value before inhalation of methacholine was not different among animals given DPPC, glycerophosphocholine and vehicle (10% DMSO) inhalation. The methacholine responsiveness was significantly attenuated by DPPC, but not by glycerophosphocholine. The rate of increase in pressure at the airway opening at 200 μg/ml of methacholine was 797.2±34.3% (vehicle), 545.5±80.7% (10 mg/ml of DPPC), and 810.6±32.6% (10 mg/ml of glycerophosphocholine), respectively.

Pretreatment with salbutamol significantly and almost completely diminished the increase in pressure at the airway opening induced by LPC (Fig. 4). The rate of increase in pressure at the airway opening was 35.1±5.3% (10% DMSO), 203.8±52.2% (10 mg/ml of LPC), 61.0±11.6% (10 mg/ml of LPC pretreated with 1.0 μg/kg of salbutamol), respectively.

Finally, to rule out the involvement of histamine in the LPC-induced bronchial response, we examined the effect of diphenhydramine. Pretreatment with diphenhydramine did not change the increase in pressure at the airway opening following inhalation of LPC (Fig. 5).

4. Discussion

The present animal study showed that inhalation of LPC increased pressure at the airway opening with emphatic effect on bronchial responsiveness to methacholine, and intravenously administered salbutamol almost completely prevented the LPC-induced pressure at the airway opening elevation. The percentage of leukocytes in bronchoalveolar lavage fluid did not change significantly at least within 20 min after the LPC inhalation. These results suggest that LPC could cause bronchoconstriction and increased bronchial responsiveness in guinea pigs without inducing leukocyte infiltration in the airway. Bronchial hyperresponsiveness seen in asthmatics consists of specific responsiveness to ultrasonically nebulized distilled water and cold, dry air, and so on, which is investigated in only asthmatics, and increased non-specific responsiveness to methacholine and acetylcholine. Thus, increased bronchial responsiveness may not be equal to inflammation-mediated bronchial hyperresponsiveness of asthmatic airway. At least, increased bronchial responsiveness to methacholine investigated in this study is thought to mean increased non-specific bronchial responsiveness. We will have to determine whether increased non-specific bronchial responsiveness induced by LPC lasts longer time after exposure to LPC like bronchial hyperresponsiveness as seen in asthma and whether LPC could induce specific bronchial hyperresponsiveness.

Although recent investigations indicate that the formation of LPC may be important in cell surface recognition processes and in transmembrane signaling (Lindahl et al., 1988), the role of LPC in airway surface fluid was not previously investigated. Triggiani et al. (2003) showed that incubation of human eosinophils with LPC did not reproduce leukotriene C4. On the other hand, Diana and Linda (1991) reported that LPC induced release of leukotriene C4 as a mast cell mediator. Taking these reports into consideration, in mast cells but not eosinophils, LPC might be of importance for mediating inflammatory and allergic reactions in the lung. These findings are in agreement with our result that bronchoalveolar lavage cell differentials were not different between the LPC group and the vehicle group.

Previous studies showed that higher concentrations of LPC had a range of membrane damaging and toxic effects on various cells and tissues (Lindahl et al., 1988), and LPC had a direct effect on cell membrane permeability (Lindahl et al., 1986). In addition, it is known that beta-2 agonists can potentially inhibit edema formation (Gomez-Del Rio et al., 1986). The 2.5 mg/ml of LPC was the dose that did not change baseline pressure at the airway opening (Fig. 1A,B). This subthreshold dose, which did not affect cell membrane permeability, increased bronchial responsiveness to methacholine (Fig. 2) and there was no involvement of histamine in the LPC-induced bronchial response (Fig. 5). As a result of the above, we might not clearly be able to conclude the mechanism of increase in pressure at the airway opening: bronchoconstriction or airway edema. During the first 10–20 s after airway challenge with allergen, the lamina propria is flooded with the plasma exudation (Persson et al., 1998). Pressure at the airway opening is thought to increase within a few minutes after allergen challenge. However, pretreatment with salbutamol almost completely inhibited the increase in pressure at the airway opening induced by LPC (Fig. 4). Although enough dose of terbutaline significantly reduced exudation into the lumen induced by capsaicin and bradykinin, the rate of inhibition against the increase in luminal plasma was about 50% compared with vehicle (Erjefalt and Persson, 1991).

Nishiyama et al. (2004) reported that increase in resistance in airways and eosinophilic infiltration was caused 4 and 6 h after LPC inhalation, respectively. Until 2 h after LPC inhalation, neither the bronchoalveolar lavage cell differentials nor the resistance in airways was affected. However, in their study, guinea pigs were just placed in a box attached to an ultrasonic nebulizer and exposed to aerosolized 0.5 mg/ml LPC (Nishiyama et al., 2004). In our study, for inhalation of drugs, animals were inserted a polyethylene tube into their trachea and inhaled 1–10 mg/ml LPC directly through the tube. It may be the reason why LPC could cause bronchoconstriction within a few minutes in our study. Nishiyama et al. (2004) showed no edematous changes in the airway wall in the pathological specimen after exposure to LPC and suggested LPC was involved in the inflammatory process in bronchial asthma. Collectively, it is suggested that inhaled LPC causes bronchoconstriction rather than airway edema.

We examined effect of the anti-histamine diphenhydramine. The LPC-induced increase of pressure at the airway

opening was not influenced by the treatment with diphenhydramine. Consequently, LPC has less association with release of histamine. Further studies will be needed to examine possible involvement of other chemical mediators such as leukotriene C4, D4, E4, thromboxane A2, prostaglandin D2, neurokinins and so on in the LPC-induced bronchoconstriction. If LPC has less association with these mediators, LPC, a degradation product of surfactant, may act as a new bronchoconstrictor mediator in bronchial asthma. It is much important that LPC caused bronchoconstriction and increased non-specific bronchial responsiveness not only in late phase (Nishiyama et al., 2004), but also in early phase.

Pre-inhalation of subthreshold dose of LPC, which did not cause bronchoconstriction itself, produced increase in bronchial responsiveness to inhaled methacholine. Inversely, pre-inhalation of DPPC attenuated the bronchial responsiveness to methacholine. This result might show that LPC could increase non-specific bronchial responsiveness, and this increased bronchial responsiveness could be cancelled when LPC turns into DPPC in the airway. It seems possible that the metabolism between DPPC to LPC is deteriorated to increase LPC in asthma, contributing to developing increase in non-specific bronchial responsiveness. Further studies are needed to confirm these hypotheses.

Finally, we discovered acute effect of LPC to cause bronchoconstriction and increase non-specific bronchial responsiveness in the present study. If LPC has effect on cell surface recognition processes and in transmembrane signaling, it would be important to examine the late phase effect of LPC inhalation in future studies.

References

Barnes, P.J., 2001. Th2 cytokines and asthma: an introduction. Respir. Res. 2, 64–65.

Bowton, D.L., Seeds, M.C., Fasano, M.B., Goldsmith, B., Bass, D.A., 1997. Phospholipase A2 and arachidonate increase in bronchoalveolar lavage fluid after inhaled antigen challenge in asthmatics. Am. J. Respir. Crit. Care Med. 155, 421–425.

Chilton, F.H., Averill, F.J., Hubbard, W.C., Fonteh, A.N., Triggiani, M., Liu, M.C., 1996. Antigen-induced generation of lyso-phospholipids in human airways. J. Exp. Med. 183, 2235–2245.

Chhabra, S.K., Khanduja, A., Jain, D., 1999. Decreased sodium–potassium and calcium adenosine triphosphatase activity in asthma: modulation by inhaled and oral corticosteroids. Indian J. Chest Dis. Allied Sci. 41, 15–26.

Diana, L.M., Linda, L.W., 1991. Lysophosphatidylcholine induces mast cell secretion and protein kinase C activation. J. Allergy Clin. Immunol. 88, 721–730.

Enhorning, G., Duffy, L.C., Welliver, R.C., 1983. Pulmonary surfactant maintains patency of conducting airways in the rat. Am. J. Respir. Crit. Care Med. 151, 554–556.

Erich, K., Peter, C., Khaled, Z., 1990. Phospholipase in biology and medicine. Clin. Biochem. 23, 349–370.

Erjefalt, I., Persson, C.G.A., 1991. Pharmacologic control of plasma exudation into tracheobronchial airways. Am. Rev. Respir. Dis. 143, 1008–1014.

Fujimura, M., 1983. Inhibitory effects of steroids on slow-reacting substance of anaphylaxis (SRS-A) mediated bronchoconstriction in the guinea pig in vivo. Jpn. J. Allergy 32, 365–375.

Fujimura, M., Myou, S., Amemiya, T., Tachibana, H., Kita, T., Matsuda, T., 1997. Peptide leukotrienes mediate acetaldehyde-induced bronchial hyper-responsiveness in guinea-pigs. Clin. Exp. Allergy 27, 104–109.

Fujimura, M., Mizuhashi, K., Ishiura, Y., Myou, S., Matsuda, T., 1999. Difference in bronchoprotective effects of bronchodilators on post-allergic propranolol-induced bronchoconstriction. Exp. Lung Res. 25, 467–478.

Gomez-Del Rio, M., Gerbardt, T., Hehre, D., Feller, R., Bancalari, E., 1986. Effect of a beta-agonist nebulization on lung function in neonates with increased pulmonary resistance. Pediatr. Pulmonol. 5, 287–291.

Hidi, R., Vial, D., Havet, N., Berger, A., Vargraftig, B.B., Touqui, L., 1997. Inhibition by pulmonary surfactant Curosurf of secretory phospholipase A2 expression in guinea-pig alveolar macrophages. Biochem. Pharmacol. 54, 1055–1058.

Jones, T., Masson, P., Hamel, R., 1982. Biological activity of leukotriene sulfones on respiratory tissues. Prostaglandins 24, 279–289.

Kurashima, K., Fujimura, M., Tsujiura, M., Matsuda, T., 1997. Effect of surfactant inhalation on allergic bronchoconstriction in guinea-pigs. Clin. Exp. Allergy 27, 337–342.

Lewis, J.F., Lobe, A., 1993. Surfactant and the adult respiratory distress syndrome. Am. Rev. Respir. Dis. 147, 218–233.

Lindahl, M., Hede, A.R., Tagesson, C., 1986. Lysophosphatidylcholine increases airway and capillary permeability in the isolated perfused rat lung. Exp. Lung Res. 11, 1–12.

Lindahl, M., Bruhn, R., Tagesson, C., 1988. Lysophosphatidylcholine and the inflammatory action of neutrophils. Scand. J. Clin. Lab. Invest. 48, 303–311.

Liu, M., Wang, L.M., Li, E., Enhorning, G., 1991. Pulmonary surfactant will secure free airflow through a narrow tube. J. Appl. Physiol. 71, 742–748.

Mehta, D., Gupta, S., Gaur, S.N., Gangal, S.V., Agrawal, K.P., 1990. Increased leukocyte phospholipase A2 activity and plasma lysophosphatidylcholine levels in asthma and rhinitis and their relationship to airway sensitivity to histamine. Am. Rev. Respir. Dis. 142, 157–161.

Minami, S., Okafuji, K., Saga, T., Fujimura, M., Kanamori, K., Miyabo, S., Hattori, K., Kawai, K., 1983. A new quantitative inhalation apparatus for small animals. Jpn. J. Chest Dis. 21, 252–258.

Nath, P., Joshi, A.P., Agrawal, K.P., 1983. Biochemical correlates of airway hyperreactivity in guinea pigs: role of lysophosphatidylcholine. J. Allergy Clin. Immunol. 72, 351–358.

Nishiyama, O., Kume, H., Kondo, M., Ito, Y., Ito, M., Yamaki, K., 2004. Role of lysophosphatidylcholine in eosinophil infiltration and resistance in airways. Clin. Exp. Pharmacol. Physiol. 31, 179–184.

Persson, C.G.A., Erjefalt, J.S., Anderson, M., Greiff, L., Svensson, C., 1998. Microvascular–epithelial exudation of bulk plasma in airway defense, disease, and repair. Inflamm. Mech. Asthma 599–617.

Taki, F., Takagi, K., Satake, T., Sugiyama, S., Ozawa, T., 1986. The role of phospholipase A2 in reduced beta adrenergic responsiveness in experimental asthma. Am. Rev. Respir. Dis. 133, 362–366.

Toyoshima, M., Sato, A., Taniguchi, M., Imokawa, S., Nakazawa, K., Hayakawa, H., Chida, K., 1995. Serum phospholipase A2 activity in patients with aspirin-induced asthma. Nippon Kyobu Shikkan Gakkai Zasshi-Jpn. J. Thorac. Dis. 33, 691–694.

Triggiani, M., Granata, F., Balestrieri, B., Petraroil, A., Scalia, G., Del Vecchio, L., Marone, G., 2003. Secretory phospholipases A2 activate selective functions in human eosinophils. J. Immunol. 170, 3279–3288.

Zhou, Z.H., Sun, B., Lin, K., Zhu, L.W., 2000. Prevention of rabbit acute lung injury by surfactant, inhaled nitric oxide, and pressure support ventilation. Am. J. Respir. Crit. Care Med. 161, 581–588.