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Effect of methylprednisolone on phospholipase A_2 activity and lung surfactant degradation in acute lung injury in rabbits

Kenji Kuwabara [a,b], Shingo Furue [a,b], Yasuhiko Tomita [a], Masahiko Ueno [a], Takashi Ono [a], Akihiro Matsukawa [c], Masaru Yoshinaga [c], Katsuya Mikawa [b], Kahoru Nishina [b], Makoto Shiga [b], Hidefumi Obara [b], Yozo Hori [a,*]

[a] *Discovery Research Laboratories, Division of Pharmacology, Shionogi & Co., Ltd., 3-1-1, Futaba-cho, Toyonaka, Osaka 561-0825, Japan*
[b] *Department of Anesthesiology, Kobe University School of Medicine, Kusunoki-cho 7, Chuo-ku, Kobe 650-0017, Japan*
[c] *Department of Pathology, Kumamoto University School of Medicine, 2-2-1, Honjo, Kumamoto 860-0811, Japan*

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Abstract

Glucocorticoids are the most potent and widely used anti-inflammatory agents, but they are not particularly effective against early phase of acute respiratory distress syndrome. We investigated whether methylprednisolone, a synthetic glucocorticoid, could inhibit increase of phospholipase A_2 activity in the lung and lead to protection against a model of acute respiratory distress syndrome in rabbits. Infusion of oleic acid (0.1 ml/kg/h, i.v. for 2 h) provoked pulmonary hemorrhage and edema, protein leakage and massive neutrophil infiltration, resulted in severe hypoxemia and impaired lung compliance, accompanying the increase of phospholipase A_2 activity and interleukin-8, and degradation of surfactant in the bronchoalveolar lavage fluid. Infusion of methylprednisolone (60 mg/kg/h, i.v. for 30 min before the oleic acid and then 0.5 mg/kg/h, i.v. for 6 h) did not improve the above described lung injury induced by oleic acid, nor did it suppress phospholipase A_2 activity and degradation of surfactant in bronchoalveolar lavage fluid, while it strongly reduced interleukin-8 levels in both plasma and bronchoalveolar lavage fluid. We conclude that methylprednisolone did not attenuate oleic acid-induced acute lung injury and this can be explained partly by its failure to reduce the increase of phospholipase A_2 activity and the surfactant degradation in the lung, which might also account for its clinical ineffectiveness against early acute respiratory distress syndrome.

Keywords: Lung injury, acute; Methylprednisolone; Oleic acid; Secretory phospholipase A_2; Surfactant

1. Introduction

Acute respiratory distress syndrome is a complex syndrome of acute lung inflammation with high morbidity and mortality, characterized by massive alveolar leukocyte infiltration and a flood of protein-rich exudates that are associated with severe alterations in gas exchange (Kollef and Schuster, 1995). Various inflammatory mediators have been found in the bronchoalveolar lavage fluid and the blood of patients with acute respiratory distress syndrome, which include cytokines/chemokines, platelet activating factor, eicosanoids and phospholipase A_2 (Demling, 1993; Touqui and Arbibe, 1999). These mediators have been suggested to play important roles in the initiation and progression of acute respiratory distress syndrome.

Glucocorticoids are the most potent and widely used nonspecific anti-inflammatory agents, and their functional mode is believed to be due to their suppressive effects on the gene transcription and protein synthesis of inflammatory mediators (Schwiebert et al., 1996; Jantz and Sahn, 1999). Their powerful anti-inflammatory properties have led to their use in the treatment of acute respiratory distress syndrome. Randomized trials of early high-dose glucocorticoids did not benefit patients with this disease (Luce et al., 1988; Bone et al., 1987), while high-dose prolonged trials of glucocorticoids aiming at late fibrosing process showed efficacy in unresolving acute respiratory distress syndrome (Meduri et al., 1995, 1998). Thus, glucocorticoids are now regarded as not being particularly effective against early acute respiratory distress syndrome but may have a role in

* Corresponding author. Tel.: +81-6-6331-8081; fax: +81-6-6332-6385.
E-mail address: yozo.hori@shionogi.co.jp (Y. Hori).

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the late fibroproliferative phase of the disease (Jantz and Sahn, 1999). However, clear reasons for their ineffectiveness in the early phase of acute respiratory distress syndrome have not been addressed so far.

Recently, we have shown that phospholipase A_2 activity was elevated in a rabbit model of acute respiratory distress syndrome induced by oleic acid, and a novel specific group IIA secretory phospholipase A_2 inhibitor S-5920/LY315920Na could attenuate acute lung injury with prophylactic and therapeutic effect (Furue et al., 1999, 2001). At the mechanistic level, we showed that the inhibitor not only suppressed phospholipase A_2 activity but also protected the degradation of surfactant and the production of thromboxane B_2, leukotriene B_4 and interleukin-8 in the lung (Furue et al., 1999), which suggests the critical role of group IIA phospholipase A_2 in the induction of acute lung injury in a rabbit model of acute respiratory distress syndrome.

It is well known that glucocorticoids strongly inhibit the expression and production of group IIA phospholipase A_2 in vitro (Nakano et al., 1990). However, their efficacy in animal model of acute lung injury is controversial (Julien et al., 1986; Shiue and Throll, 1991), and their effects on the changes of phospholipase A_2 activity and the lung surfactant in relation to their efficacy on lung function have not yet been studied. Thus, in the present study, we attempted to find whether methylprednisolone, a synthetic glucocorticoid, could attenuate acute lung injury by inhibiting phospholipase A_2 activity and the lung surfactant degradation in a rabbit model of acute respiratory distress syndrome induced by oleic acid.

2. Materials and methods

2.1. Animals

Male Japanese white rabbits (2.1–2.7 kg) were purchased from Charles River (Kanagawa, Japan). All animals were caged at room temperature and allowed to eat and drink ad libitum. The current study was conducted according to the guidelines of the Animal Care Review Board of the Kobe University School of Medicine.

2.2. Experimental design

A rabbit model of acute lung injury has been described in detail elsewhere (Furue et al., 1999). Briefly, rabbits were initially anesthetized with ketamine hydrochloride (25 mg/kg, i.v.; Sankyo, Tokyo, Japan), and maintained with continuous infusion of ketamine at a rate of 0.5 mg/kg/h. A tracheotomy was performed aseptically and a 3.5-mm uncuffed endotracheal tube was inserted into the trachea. After injection of pancuronium bromide (4 mg/kg, i.v.; Sankyo) for neuromuscular blockade, the rabbits were mechanically ventilated with a pressure-limited ventilator (Model IV-100B; Sechrist, Anaheim, CA, USA). A catheter was placed in the distal aorta via a femoral cut-down to monitor arterial pressure and harvest blood samples, and then baseline measurements of lung mechanics, hemodynamics, peripheral leukocyte counts and arterial blood gas pressure were performed. Twenty-four rabbits were randomly divided into three groups. The oleic acid group (eight rabbits) received 0.1 ml/kg/h, i.v. of oleic acid (Wako, Osaka, Japan) for 2 h. The control group (eight rabbits) received 0.1 ml/kg/h, i.v. of saline for 2 h. The oleic acid + methylprednisolone group (eight rabbits) was treated with methylprednisolone (Upjohn, Tokyo, Japan) for 30 min before oleic acid infusion (methylprednisolone; 60 mg/kg/h, i.v.), followed by continuous infusion of methylprednisolone (0.5 mg/kg/h, i.v.) until the end of the experiments. The dose of methylprednisolone was selected according to a high dose treatment for clinical sepsis (Luce et al., 1988). The oleic acid and control groups were infused with an equivalent volume of saline instead of methylprednisolone. Arterial blood samples were obtained at -0.5, 0, 1, 2, 3, 4, 5 and 6 h after the start of oleic acid to determine blood gas, blood cell counts, plasma phospholipase A_2 activity and plasma level of interleukin-8. All rabbits were killed at 6 h after the start of oleic acid by injection of an overdose of thiamylal.

2.3. Arterial blood gas and cell counts

Arterial blood specimens were analyzed for Pao_2, $Paco_2$, and pH using ABL2 (Radiometer, Copenhagen, Denmark). The numbers of peripheral leukocytes and platelets were measured with a Coulter counter (Coulter Electronics, Harkenden, UK).

2.4. Lung mechanics and lung wet–dry weight ratio

Lung mechanics was examined by the passive expiratory flow-volume technique, as described previously (Furue et al., 1999).

At the end of the experiments, the thorax was opened, and the lungs were removed en bloc by observers unaware of the nature of the experiment. The left upper lobe was weighed and then dried to constant weight at 60 °C for 24 h in an oven. To assess tissue edema, the ratio of wet weight to dry weight (*W*/*D* ratio) was calculated.

2.5. Histopathological examination

The left lower lobe was fixed by instillation of 10% glutaraldehyde solution through the left lower bronchus at 20 cmH_2O. The lungs were embedded in paraffin, and the sections were stained with hematoxylin and eosin. Two observers, unaware of the nature of the experiment, scored the lung injury from 0 (no damage) to 4+ (maximal damage), according to combined assessments of alveolar congestion, hemorrhage, edema, infiltration/aggregation of

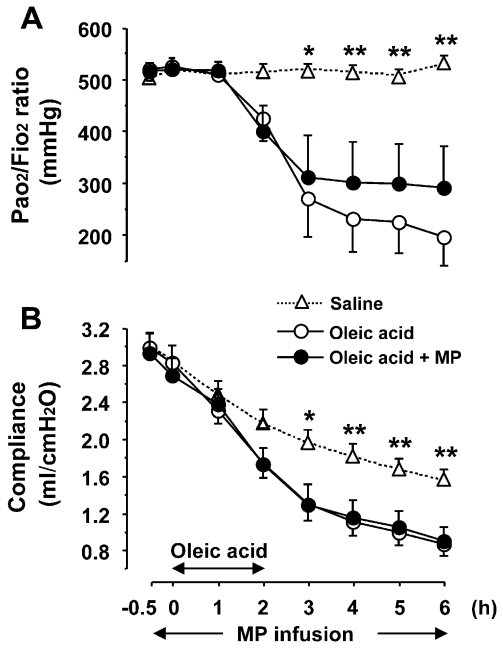

Fig. 1. Effect of methylprednisolone (MP) on changes in blood oxygenation and lung mechanics in oleic acid-induced acute lung injury. The parameters depicted are (A) the ratio of the partial pressure of arterial oxygen (Pao_2) to the fraction of inspired oxygen (Fio_2) (Pao_2/Fio_2 ratio), and (B) lung compliance. Each point represents mean ± S.E.M from eight animals. * $P<0.05$, ** $P<0.01$ vs. time-matched value in saline control group (two-way repeated measures ANOVA followed by Dunnett's test).

neutrophils in the airspace or vessel wall, thickness of the alveolar wall, and hyaline membrane formation.

2.6. Preparation of bronchoalveolar lavage fluid

The bronchoalveolar lavage fluid was harvested from the right lung, as described previously (Furue et al., 1999), and was analyzed for cell count and cell differentiation. A cytospin preparation (Shandon Southern Products, Runcorn, UK) of the bronchoalveolar lavage fluid was stained with Wright–Giemsa for cell differentiation. The numbers of leukocytes and platelets in the bronchoalveolar lavage fluid were counted with a Coulter counter (Coulter Electronics). The fluid was then centrifuged at $250 \times g$ at 4 °C for 10 min. The cell-free supernatant was divided into several aliquots and stored at − 80 °C for measurements of various mediators.

2.7. Measurements of mediators in bronchoalveolar lavage fluid

The secretory phospholipase A_2 activity in bronchoalveolar lavage fluid was measured as described previously (Furue et al., 1999). Briefly, the substrate was used in the form of mixed micelles of sodium cholate/1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (Avanti Polar Lipids, Alabaster, AL). The reactions were initiated by addition of bronchoalveolar lavage fluid samples to the assay mixture. The reaction was carried out at 40 °C for the defined time periods then stopped, and the mixture was subjected to extraction according to Dole's extraction system followed by silicic acid treatment. Phospholipase A_2 activity was determined by measuring 9-anthryldiazomethane-labeled free fatty acid with high pressure liquid chromatography.

The protein concentrations in bronchoalveolar lavage fluid were determined by using protein assay reagent (Pierce, Rockford, IL). Thromboxane A_2 was quantified by enzyme immunoassay (Amersham, Little Chalfont, UK) as thromboxane B_2, the stable metabolite of thromboxane A_2. Leukotriene B_4 was also quantified by enzyme immunoassay (Amersham). Interleukin-8 was determined by enzyme-linked immunosorbent assay (Amersham). The assay kit cross-reacts with rabbit interleukin-8, and rabbit recombinant interleukin-8 was used as the standard.

2.8. Surfactant phospholipid analysis

Lipids were extracted from bronchoalveolar lavage fluid, as described previously (Furue et al., 1999). The individual phospholipids were separated by two-dimensional chromatography on pre-coated activated silica-gel type 60G thin-layer plates. The plates were developed in chloroform/methanol/H_2O (72:25:3, v/v) and chloroform/methanol/acetic acid/H_2O (90:40:12:2, v/v), respectively. The lipid area

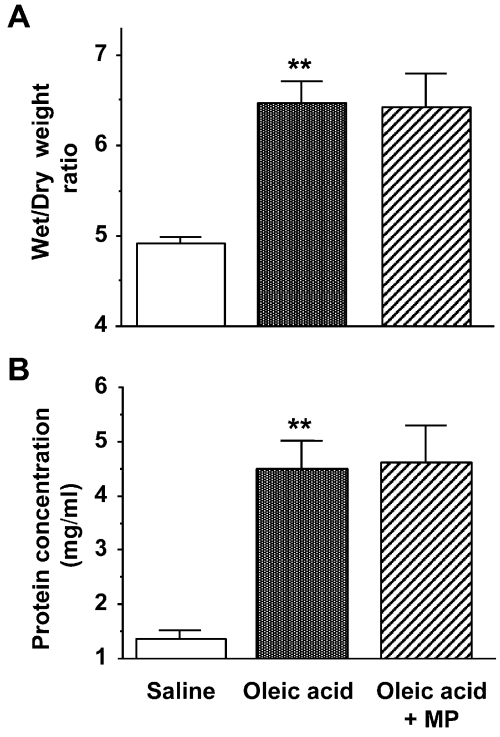

Fig. 2. Effects of methylprednisolone (MP) on increased (A) lung edema and (B) vascular permeability induced by oleic acid. The lungs were harvested at the end of the experiments (6 h). Each value represents mean ± S.E.M. from eight animals. ** $P<0.01$ vs. saline control group (Student's *t*-test). No statistical significance was observed between the oleic acid and the oleic acid + MP groups.

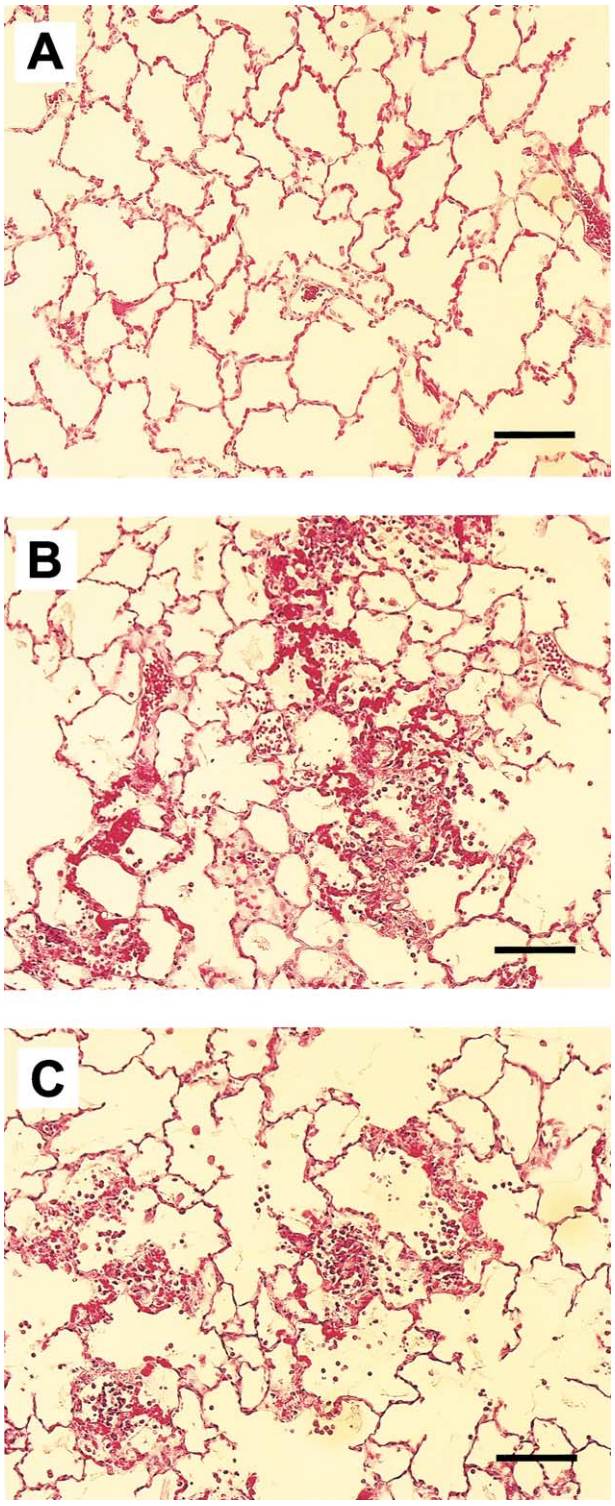

Fig. 3. Effect of methylprednisolone on lung tissue damage in rabbits at 6 h after oleic acid infusion. Representative photomicrographs showing hematoxylin and eosin staining in (A) saline control group, (B) oleic acid control group, and (C) oleic acid + methylprednisolone group. Scale bar = 100 μm. Lung injury score (D): lung injury was scored 0 (no damage) to 4+ (maximal damage) according to the criteria described in Materials and methods. Results are expressed as median (bars) from eight animals. * $P < 0.05$, ** $P < 0.01$ (Wilcoxon U-test).

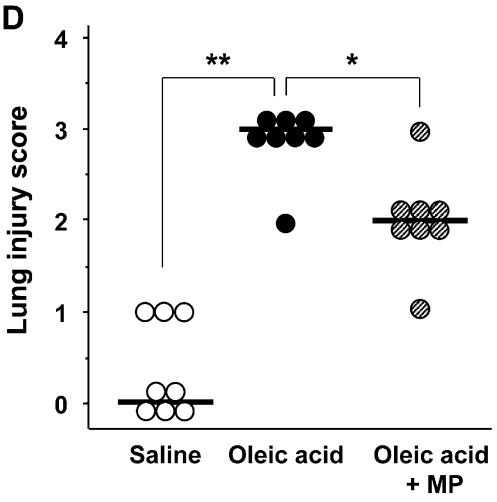

Fig. 3 (*continued*).

of the chromatograms was visually detected by exposure to I_2 vapor. The spots corresponding to individual phospholipids were scraped off for phosphorous determination. The data are expressed as percentages of the total phospholipid recovered.

2.9. Statistical analysis

Data from experiments are expressed as means ± S.E.M., except the lung injury score which is given as a median. Data were statistically analyzed using the following tests for multiple comparisons: two-way repeated measures analysis of variance (ANOVA) followed by Dunnett's test for multiple time points observation, Student's unpaired t-test for single time point observation, and Wilcoxon U-test for histologic data. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Prophylactic effect of methylprednisolone on parameters of acute lung injury

3.1.1. Oxygenation and lung compliance

As shown in Fig. 1, oleic acid infusion caused a marked decrease in the Pao_2/Fio_2 ratio (Fig. 1A) and the lung compliance (Fig. 1B). Methylprednisolone treatment slightly alleviated the deleterious changes in the Pao_2/Fio_2 ratio, but not with statistical significance (Fig. 1A). The decreased lung compliance could not be recovered by methylprednisolone treatment (Fig. 1B).

3.1.2. Increased vascular permeability in the lung

Oleic acid infusion resulted in an increase in the W/D ratio of the lung and protein level in the BALF, as compared to the control, and both were not attenuated by methylprednisolone treatment (Fig. 2).

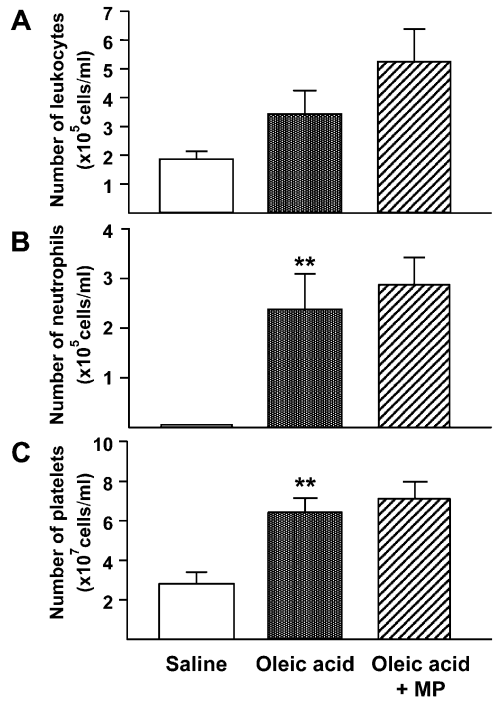

Fig. 4. Effects of methylprednisolone (MP) on the recruitment of leukocytes induced by oleic acid. The numbers of leukocyte populations in the bronchoalveolar lavage fluid were counted at the end of the experiments (6 h). Differential cell analyses were done with Giemsa-staining and the numbers of (A) leukocytes, (B) neutrophils, and (C) platelets were counted. Each value represents mean ± S.E.M. from eight animals. ** $P < 0.01$ vs. saline control group (Student's *t*-test). No statistical significance was observed between the oleic acid and the oleic acid + MP groups.

3.1.3. Histopathology

Light microscopic findings in the lung at 6 h after oleic acid infusion demonstrated a marked lung injury resembling those seen in lung of patients with acute respiratory distress syndrome, represented by hemorrhage, edema, thickened alveolar septum, formation of hyaline membranes and the existence of inflammatory cells in alveolar spaces (Fig. 3A,B). In the oleic acid + methylprednisolone group, these changes were less pronounced (Fig. 3C). The grading score of the lung damage in the oleic acid + methylprednisolone group was significantly lower than that for the oleic acid control (Fig. 3D).

3.2. Analysis of peripheral blood and bronchoalveolar lavage fluid

3.2.1. Leukocytes in peripheral blood and bronchoalveolar lavage fluid

Neutrophil infiltration is a characteristic feature of acute respiratory distress syndrome, and the cells play a role in destroying the lung tissue by secreting many types of mediators and enzymes (Demling, 1993). We next examined the effects of methylprednisolone on the regulation of neutrophil infiltration in the lung (Fig. 4). The number of leukocytes in bronchoalveolar lavage fluid tended to increase at 6 h after oleic acid infusion (Fig. 4A), and the number of neutrophils and platelets increased significantly, as compared to the control (Fig. 4B,C). Methylprednisolone treatment could not prevent increases of neutrophils and platelets (Fig. 4B,C).

3.2.2. Surfactant composition in bronchoalveolar lavage fluid

Surfactant degradation critically affects lung function (Hallman et al., 1977; Casals et al., 1989; Lewis and Jobe, 1993). We next examined the effects of methylprednisolone on the surfactant degradation induced by oleic acid. There was a significant decrease in the percentage of phosphatidylglycerol but a slight increase in the percentage of lyso-phosphatidylcholine in the bronchoalveolar lavage fluid at 6 h after oleic acid infusion (Fig. 5A), and the lyso-phosphatidylcholine/phosphatidylglycerol ratio was significantly increased, as compared to the control (Fig. 5B). Methylprednisolone treatment did not alter these changes in the phospholipid composition (Fig. 5A,B).

3.2.3. Phospholipase A_2 activity and eicosanoid level in bronchoalveolar lavage fluid

The effects of methylprednisolone on the production of inflammatory mediators were next assessed. Plasma phos-

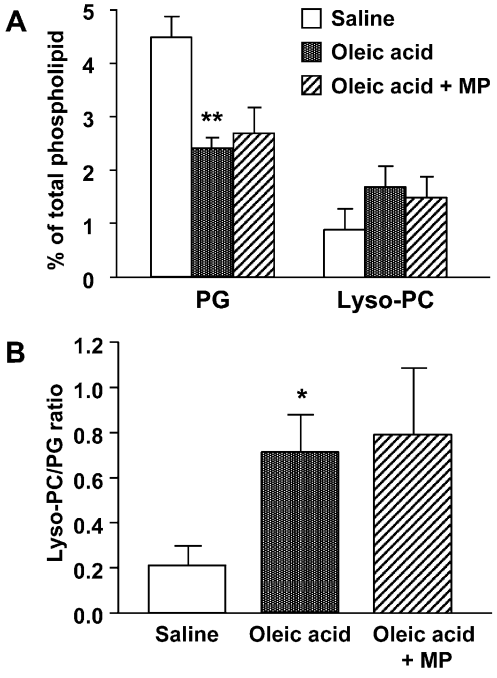

Fig. 5. Effects of methylprednisolone (MP) on the changes of phospholipid composition in bronchoalveolar lavage fluid induced by oleic acid. The fluid was harvested at the end of the experiments (6 h) and the phospholipids were extracted using Bligh and Dyer's methods and quantified by phosphorus determination after two-dimensional TLC. PG, phosphatidylglycerol; Lyso-PC, lyso-phosphatidylcholine. Each value represents mean ± S.E.M. from eight animals. ** $P < 0.01$ vs. the saline control group (Student's *t*-test). No statistical significance was observed between the oleic acid and the oleic acid + MP groups.

pholipase A_2 activity was not changed by oleic acid infusion (data not shown). In contrast, oleic acid induced a dramatic increase in the level of phospholipase A_2 activity in the bronchoalveolar lavage fluid at 6 h, as compared to the control, and this could not be reduced by methylprednisolone treatment (Fig. 6A). The eicosanoid level (thromboxane B_2 and leukotriene B_4) was also increased after oleic acid infusion, but neither the level of thromboxane B_2 nor that of leukotriene B_4 was reduced by methylprednisolone treatment (Fig. 6B,C). The mean leukotriene B_4 level in the oleic acid + methylprednisolone group doubled, as compared to the oleic acid group, although the difference was not statistically significant.

3.2.4. Interleukin-8 level in plasma and bronchoalveolar lavage fluid

As interleukin-8 plays a central role in mediating neutrophil infiltration as well as activation in acute respiratory distress syndrome (Miller et al., 1992), we also assessed the regulation of interleukin-8 production by methylprednisolone. The interleukin-8 level in plasma increased rapidly after oleic acid infusion, peaked at 3 h, and then decreased gradually. The level remained elevated at 6 h, as compared to the control (Fig. 7A). Treatment with methylprednisolone strongly inhibited the increase and the level reverted to the control level (Fig. 7A). The interleukin-8 level in bronchoalveolar lavage fluid obtained at 6 h after oleic acid infusion also increased, as compared to the control, but this was dramatically inhibited by methylprednisolone treatment (Fig. 7B). Thus, unlike the other mediators, interleukin-8 was suppressed by methylprednisolone.

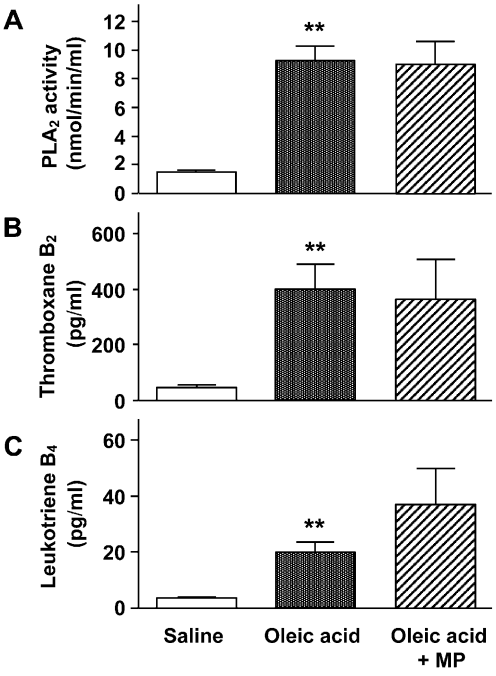

Fig. 6. Effects of methylprednisolone (MP) on the level of phospholipase A_2 (PLA_2), thromboxane B_2 and leukotriene B_4 in bronchoalveolar lavage fluid induced by oleic acid. The fluid was harvested at the end of experiments (6 h), and the levels of (A) PLA_2, (B) thromboxane B_2, and (C) leukotriene B_4 were measured. Each value represents mean ± S.E.M. from eight animals. ** $P < 0.01$ vs. the saline control group (Student's *t*-test). No statistical significance was observed between the oleic acid and the oleic acid + MP groups.

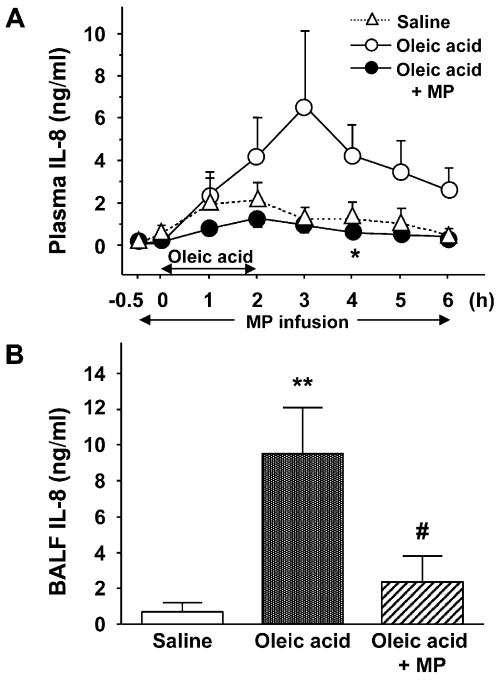

Fig. 7. Effects of methylprednisolone (MP) on interleukin (IL)-8 level in (A) plasma and (B) bronchoalveolar lavage fluid in oleic acid-induced acute lung injury. Plasma samples were collected at the indicated times, and bronchoalveolar lavage fluid samples were harvested at the end of the experiments (6 h). IL-8 concentration was measured by enzyme-linked immunosorbent assay. Each value represents mean ± S.E.M. from eight animals. ** $P < 0.01$ vs. the saline control group; $^{\#}P < 0.05$ vs. the oleic acid group (Student's *t*-test).

4. Discussion

Many investigators have employed oleic acid-induced acute lung injury as an animal model of acute respiratory distress syndrome, since the morphological and cellular changes in the lung in this model are similar to those seen in patients with this disease (Schuster, 1994). Although the therapeutic efficacy of glucocorticoids in this clinically relevant model is controversial (Julien et al., 1986; Shiue and Throll, 1991), our findings demonstrated that prophylactic treatment with methylprednisolone did not alleviate oleic acid-induced acute lung injury nor attenuated the increased level of phospholipase A_2 activity, leukotriene B_4 and thromboxane B_2 in the bronchoalveolar lavage fluid, but greatly reduced that of interleukin-8.

An elevated level of phospholipase A_2 activity was detected in bronchoalveolar lavage fluid from both clinical and experimental acute respiratory distress syndrome, which

correlated well with the severity of the lung injury (Kim et al., 1995; Furue et al., 1999). Surfactant degradation is also frequently seen in the bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome, where %phosphatidylglycerol is decreased while %lyso-phosphatidylcholine is increased (Lewis and Jobe, 1993), suggesting that lyso-phosphatidylcholine/phosphatidylglycerol ratio would be a better and more useful index of the changes of surfactant composition related to lung injury as reported previously (Furue et al., 2001). Indeed, in the present study, a significant increase of lyso-phosphatidylcholine/phosphatidylglycerol ratio was noted in the oleic acid group, and methylprednisolone did not affect this ratio, clearly indicating that methylprednisolone treatment could not prevent the surfactant degradation induced by oleic acid. Since group IIA phospholipase A_2 can induce hydrolysis of surfactant phospholipids in vitro and in vivo (Arbibe et al., 1998; Hite et al., 1998; Furue et al., 2001), it could be a key mediator in inducing lung surfactant degradation, leading to lung dysfunction.

Although glucocorticoids inhibit the production of group IIA phospholipase A_2 in vitro (Nakano et al., 1990), the present data demonstrated that methylprednisolone did not attenuate the phospholipase A_2 activity in the bronchoalveolar lavage fluid. Interestingly, the number of platelets in the bronchoalveolar lavage fluid was not affected by methylprednisolone treatment. Platelets store group IIA phospholipase A_2 in their α-granules and secrete it upon stimulation (Horigome et al., 1987). The facts that methylprednisolone achieves its function in nuclei and platelets lack them suggests that phospholipase A_2 activity in the bronchoalveolar lavage fluid might arise from infiltrated platelets. Thus, failure to modulate phospholipase A_2 activity in the lung appears to cause the surfactant degradation, which leads to lung dysfunction in this model. Recently, Satoh et al. observed that pre-operative treatment with methylprednisolone in esophageal carcinoma patients suppressed increases of interleukin-6 and interleukin-8 but not group IIA phospholipase A_2 levels in serum after surgery (personal communication), suggesting that expression and production of group IIA phospholipase A_2 in vivo could be more refractory to glucocorticoid treatment than those of inflammatory cytokines.

Interleukin-8, a potent neutrophil chemoattractant and activator, has been detected in the bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome, and its level correlates well with development and mortality of the disease (Miller et al., 1992), suggesting that interleukin-8 plays an important role in acute respiratory distress syndrome. Interleukin-8 blockade has been shown to prevent acute lung injury in different types of animal model of acute lung injury (Yokoi et al., 1997; Modelska et al., 1999). In the present study, methylprednisolone dramatically inhibited the level of interleukin-8 in bronchoalveolar lavage fluid and plasma, which clearly indicates that it achieved strong inhibition of expression and production of this chemokine with the current 30 min pre-treatment protocol. However, methylprednisolone had no effect on the recruitment of neutrophils in the bronchoalveolar lavage fluid. This might be explained by the fact that methylprednisolone treatment increased the level of leukotriene B_4, a powerful neutrophil chemoattractant, in the bronchoalveolar lavage fluid (Ford-Hutchinson, 1990). In vitro studies have shown that glucocorticoids up-regulate the mRNA and protein expression of 5-lipoxygenase and 5-lipoxygenase activating protein (Riddick et al., 1997), respectively. The former is an enzyme that catalyzes arachidonic acid to produce leukotriene B_4 and the latter is an essential cofactor for 5-lipoxygenase. In agreement with our data, glucocorticoid treatment increased serum level of leukotriene B_4 in patients with asthma and chronic obstructive pulmonary disease (Seggev et al., 1991) and a recent study also showed that the leukotriene B_4 level was enhanced by glucocorticoids in the brain (Uz et al., 1999). The increased level of leukotriene B_4 in the bronchoalveolar lavage fluid might functionally compensate for the decreased level of interleukin-8.

Thromboxane A_2, which is catalyzed by cyclooxygenase-2 from arachidonic acid, causes edema formation (Rostagno et al., 1990). Although glucocorticoids suppress cyclooxygenase-2 expression (Mitchell et al., 1994), methylprednisolone treatment had no effect on the production of thromboxane B_2 (the stable metabolite of thromboxane A_2) in the bronchoalveolar lavage fluid. As in the case of phospholipase A_2, the lack of methylprednisolone effect on thromboxane A_2 production may be explained by the contribution of platelets infiltrating the lung, as platelets are rich sources of thromboxane A_2 (Shen and Tai, 1998). The failure of methylprednisolone to regulate thromboxane A_2 in the bronchoalveolar lavage fluid is likely to result in its failure to modulate increased vascular permeability in this model.

Histologically, methylprednisolone showed moderate but significant inhibition of oleic acid-induced lung injury score, which might contribute to a tendency to improve Pao_2/Fio_2 ratio (Fig. 1A). Besides inhibition of inflammatory cytokine production, glucocorticoids are known to suppress in vivo expression of matrix metalloproteinases such as collagenases and gelatinases, the enzymes capable of destroying extracellular matrix components (Xu et al., 2001). Thus, this mechanism of action might be responsible for the above mentioned histological improvement.

In conclusion, methylprednisolone did not attenuate oleic-induced acute lung injury, presumably because it could not modulate elevated levels of phospholipase A_2 activity and the degradation of surfactant in the lung, which might also account for its lack of efficacy against early acute respiratory distress syndrome in the clinic. These further support the crucial role of secretory phospholipase A_2 in oleic acid-induced acute lung injury, and suggest the importance of lung surfactant protection for the treatment of early acute respiratory distress syndrome.

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