Increased isoprostane levels in oleic acid induced lung injury

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Footnote to the title: Isoprostane and acute lung injury

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ABSTRACT
The present study was performed to examine a role of oxidative stress in oleic acid-induced lung injury model. Fifteen anesthetized sheep were ventilated and instrumented with a lung lymph fistula and vascular catheters for blood gas analysis and measurement of isoprostanes (8-epi prostaglandin F2α). Following stable baseline measurements, oleic acid (0.08 ml/kg) was administered and observed 4 hours. Isoprostane was measured by gas chromatography mass spectrometry with the isotope dilution method. Isoprostane levels in plasma and lung lymph were significantly increased 2 hours after oleic acid administration and then decreased at 4 hours. The percent increases in isoprostane levels in plasma and lung lymph at 2 hours were significantly correlated with deteriorated oxygenation at the same time point, respectively. These findings suggest that oxidative stress is involved in the pathogenesis of the pulmonary fat embolism-induced acute lung injury model in sheep and that the increase relates with the deteriorated oxygenation.

Key words: Oleic acid-induced lung injury, lung lymph flow, increased permeability edema, sheep, oxidant stress
1. Introduction

Isoprostanes are produced during peroxidation of membrane lipids by free radicals and reactive oxygen species, and are currently used as markers of many disease states and experimental conditions in which oxidative stress is a prominent feature[1-3]. A number of studies have shown these compounds to be extremely accurate markers of lipid peroxidation in animal models of acute lung injury (ALI)[4,5] and have revealed the role of oxidant injury in pulmonary diseases [6-9], including pulmonary vascular diseases such as ALI and/or adult respiratory distress syndrome (ARDS) and pulmonary hypertension. The levels of breath condensate or lung fluid isoprostanes are significantly elevated in patients with ALI/ARDS [6-8]. Isoprostane levels in urine are increased in patients with pulmonary hypertension [9]. In addition, the increases are inversely correlated with pulmonary vasoreactivity as measured by decreased pulmonary vascular resistance in response to inhaled nitric oxide. Indeed, isoprostane has been shown to be a modest pulmonary vasoconstrictor [10-12].

Oleic acid-induced lung injury is a well-characterized and clinically relevant experimental model of ALI/ARDS [13-15]. In particular, oleic acid administration is an excellent model of fat embolism-induced ALI [16]. Although the precise mechanisms involved in the development of oleic acid-induced lung injury have not been elucidated, several studies suggested the possible contribution of reactive oxygen species [17-19]. Moriuchi et al. [17] showed that oleic acid activated neutrophils from guinea pigs to release superoxide in an in vitro model. Increased levels of lipid peroxidation products, such as thiobarbituric acid reactive substances, in the bronchoalveolar lavage after intravenous injection of oleic acid were observed [19]. In addition, Mei et al. [19] also demonstrated increased levels of reactive oxygen species by electron spin resonance spectroscopy in rats treated with oleic acid. However, measurements of isoprostanes in this model have been not reported previously.

In the present study, we investigated the effects of oleic acid on the production of isoprostane (8-epi-prostaglandin F2α) in plasma and lung lymph in sheep during the development of oleic acid-induced ALI and compared the results with physiological parameters.

2. Methods
2.1. Materials and Animals

This study protocol was approved by the Institutional Review Board for Animal Experimentation at Shinshu University. Care and handling of animals were performed in accordance with the guidelines of the National Institutes of Health. The results obtained in the present study, including pulmonary and systemic artery pressures and lung lymph flow, were reported in part previously [14].

2.2 Animal preparation

Fifteen sheep weighing 35 – 44 kg were used in this study. We prepared chronic lung lymph fistulae and placed catheters for taking blood samples. Sheep were anesthetized by intravenous injection of pentobarbital sodium at 12.5 mg/kg, then ventilated with 0.5 – 1.0% halothane using positive pressure ventilation. Through two right thoracotomies, the efferent lymphatic channel from the caudal mediastinal node (CMN) was cannulated with a thin silicon tube. The CMN tail was then ligated at the free margin of the inferior pulmonary ligament to block contamination by non-pulmonary lymph. A silicon tube was inserted into the thoracic aorta via the carotid artery. Each animal then underwent tracheostomy. A cuffed airway (ID 8 mm Portex Blue Line Tracheostomy Tube; Smiths Medical Kent, UK) was used in the present study.

2.3 Measurements

Blood samples for blood gas analysis and measurement of isoprostane levels were drawn from systemic artery lines before and every 2 hours after oleic acid administration. Blood gas analysis was performed using a blood gas analyzer (ABL-2; Radiometer, Copenhagen, Denmark). Isoprostane (8-epi-prostaglandin F2α) in plasma and lung lymph was measured by gas chromatography/mass spectrometry-selected ion monitoring as described previously.
2.4 Experimental protocols

After surgical procedures, animals were stabilized in the supine position and stable baselines were observed for at least 1 hour. Animals were received conventional mechanical ventilation (CMV) using a tidal volume of 10 ml/kg and respiratory rate of 20/min with 70% oxygen and positive end-expiratory pressure of 5 cmH$_2$O. Then, sheep received intravenous administration of oleic acid (0.08 ml/kg) to induce acute lung injury and divided into two groups ventilated with different mechanical modes. CMV, \( n=8 \): animals were continuously maintained under the same CMV conditions for 4 hours. High-frequency oscillation ventilation (HFOV, \( n=7 \)): ventilatory mode was switched to HFOV with a mean airway pressure of 15 cmH$_2$O, a stroke volume of 150 ml, and an oscillatory frequency of 15 Hz for 4 hours. Throughout the experiments, animals were anesthetized with an infusion of propofol (3 mg/kg/h) and paralyzed with pancuronium bromide (0.1 mg/kg) initially and as needed to suppress any spontaneous movements; inspiratory oxygen concentration was fixed at 70%.

2.5. Statistical analysis

The data were analyzed by two-way analysis of variance (ANOVA), and the differences were tested by Fisher's exact test. \( P<0.05 \) was accepted as significant. Data are expressed as means ± SD.
3. Results

3.1 Blood gas analysis

The time courses of partial oxygen tension (PO$_2$) are summarized in Table 1. PO$_2$ decreased significantly after oleic acid administration and remained at that level throughout the experiment. The PO$_2$ value in the HFOV group 4 hours after oleic acid administration was slightly higher than that in the CMV group, but the difference was not statistically significant.

3.2 Concentrations of isoprostanes

The time courses of changes in isoprostane concentrations in plasma and lung lymph are summarized in Table 2. In both groups, isoprostanes in plasma and lung lymph were significantly increased 2 hours after oleic acid administration, and then decreased at 4 hours. In the CMV group, a significant increase in isoprostanes in lung lymph was sustained at 4 hours. There were no significant differences in the increases in isoprostanes between HFOV and CMV groups. We examined the correlation between isoprostanes and other physiological parameters during oleic acid-induced lung injury. The percentage increases in levels of isoprostanes in plasma and lung lymph at 2 hours were significantly correlated with the decreases in PO$_2$ at the same time point in all animals treated with oleic acid (Figures 1 and 2).
5. Discussion

In the present study, we focused on the pathological involvement of isoprostanes in the development of oleic acid-induced lung injury. It has been reported that isoprostane is a modest pulmonary vasoconstrictor [10-12] and the levels in urine were increased in patients with pulmonary hypertension [9]. In the present study, mild pulmonary hypertension persisted after oleic acid administration [14]. However, the increases in pulmonary artery pressure were not correlated with the increases in isoprostane (data were not shown). Based on the study by Syrkinia et al. [5], ventilation at a tidal volume of 20 ml/kg increased isoprostane levels in plasma and contributed further to lung injury compared with standard ventilation (7 ml/kg). These finding suggested that oxidant stress could be involved in ventilation-induced lung injury. In the present study, HFOV ameliorated lung injury scores (lung wet/dry ratio and lung lymph protein clearance) induced by oleic acid in comparison with those in CMV at 4 hours [14]. However, there were no significant differences in isoprostane concentrations between the two mechanical ventilation modes. Furthermore, the pattern of increased isoprostane levels was not correlated with the time course of the development of lung injury. At a dose of 0.08 ml/kg of oleic acid, the degree of lung edema and/or injury at 4 hours was more severe than that at 2 hours in sheep model [14]. Thus, it is unlikely that increased isoprostanes at 2 hours after oleic acid administration could be associated with the severity of oleic acid-induced lung injury.

Schuster et al [22] measured plasma isoprostane concentration 2 hours after oleic acid administration in dog. They found that although oleic acid administration failed to show a significant increase in isoprostane and decrease in oxygenation, endotoxin plus oleic acid treatment significantly increased isoprostane and decreased oxygenation compared with those in only oleic acid group. The finding suggested an increased oxidant production with hypoxemia during this acute lung injury model. Indeed, we found that the changes in isoprostanes in plasma and lung lymph were significantly correlated with the severity of hypoxemia 2 hours after oleic acid administration in the present study. Jefferson et al. [23] measured plasma isoprostane concentrations after acute (48 hours) and chronic high altitude exposures in normal subjects and found significantly increased levels of isoprostanes. These findings were consistent with those of another report showing increased levels of plasma thiobarbituric acid reactive substances in normal subjects after exposure to chronic hypoxia [24]. These observations suggest that only hypoxia is related to activation of reactive oxygen species and/or the oxidant stress pathway. However, short-term hypoxic inhalation for 2 hours in normal subjects failed to increase the plasma isoprostane level [24]. Thus, it is unlikely that the increased levels of isoprostane observed in the present study were due solely to hypoxemia. Other contributing factors may have mediated the production of isoprostane after oleic acid administration in the present study.

On the other hand, 8-epi-PGF2α was reported to cause bronchoconstriction, although species differences were observed [10,26]. No information was available about the effects of isoprostane on bronchial smooth muscle in sheep. We speculated that the bronchial constriction by increased isoprostane levels was related to the correlation between hypoxemia and increased isoprostanes. Thus, isoprostanes may not only be markers, but may in fact mediate the effects of free radicals and reactive oxygen species. To our knowledge, this is the first report of increased isoprostanes in an experimental model of acute fat embolism with subsequent development of ALI. Further studies are required to clarify the pathological role of isoprostanes in the development of lung injury.

In conclusion, we demonstrated increased isoprostane production in plasma and lung. The increased levels of isoprostanes were correlated with deteriorated hypoxemia in a large animal model of acute pulmonary thromboembolism. Isoprostane may be involved in the pathophysiology of acute pulmonary thromboembolism.
References


[20] T.Obata, Y.Sakurai, Y.Kase, Y.Tanifuji, T.Horiguchi, Simultaneous determination of endocannabinoids (arachidonylethanolamide and 2-arachidonylglycerol) and isoprostane (8-epiprostaglandin F2alpha) by gas chromatography-mass spectrometry-selected ion
Table 1

<table>
<thead>
<tr>
<th>Time</th>
<th>Partial Oxygen Tension (torr)</th>
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<tbody>
<tr>
<td></td>
<td>baseline</td>
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<tr>
<td>HFOV (n=7)</td>
<td>283.3 ± 30.6</td>
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<tr>
<td>CMV (n=8)</td>
<td>281.4 ± 15.9</td>
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* vs. baseline,  P<0.05
Table 2

<table>
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<tr>
<th></th>
<th>8-epi-prostaglandin F2α (pg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>baseline</td>
</tr>
<tr>
<td>HFO (n=7)</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>155.3 ± 67.2</td>
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<tr>
<td>Lung Lymph</td>
<td>242.9 ± 168.1</td>
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<tr>
<td>CMV (n=8)</td>
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<tr>
<td>Plasma</td>
<td>255.7 ± 137.4</td>
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<tr>
<td>Lung Lymph</td>
<td>299.2 ± 193.7</td>
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* vs. baseline, \( P < 0.05 \)
**Figure Legends:**

**Figure 1**
Relationship between percent increases in 8-epi-prostaglandin F2α concentration in plasma and decreases in partial oxygen tension in sheep 2 hours after oleic acid administration. Closed circles: conventional mechanical ventilation; closed squares: high-frequency oscillation ventilation.

**Figure 2**
Relationship between percent increases in 8-epi-prostaglandin F2α concentration in lung lymph and decreases in partial oxygen tension in sheep 2 hours after oleic acid administration. Closed circles: conventional mechanical ventilation; closed squares: high-frequency oscillation ventilation.
Figure 1

% increase in isoprostane concentrations in plasma

decreases in partial oxygen tension

Figure 2

% increase in isoprostane concentrations in lung lymph flow

decreases in partial oxygen tension

r=0.54

r=0.71