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Attenuation of ischemia reperfusion-induced lung edema by prostaglandin I_2 analogue OP-2507 in the isolated perfused rat lung

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Abstract: Using the physiological salt solution (PSS)/Ficoll-perfused rat lung, we studied the effect of prostaglandin I_2 (PGI_2) analogue, OP-2507, on ischemia-reperfusion lung injury. Ischemia was induced by stopping perfusion and ventilation. Reperfusion after 90 min of normothermic ischemia increased mean pulmonary artery perfusion pressure (Ppa) and produced significant lung edema. Pretreatment with OP-2507 (200 ng·ml^{-1} and 1000 ng·ml^{-1}) equally attenuated the increase in Ppa and lung edema after reperfusion. Lactate dehydrogenase release from the OP-2507-treated lungs of both doses were significantly lower than the untreated lungs. Thus, OP-2507 seems to be a useful agent for preventing ischemia-reperfusion lung injury.

Key words: Ischemia reperfusion injury—Lung—Rat—Prostaglandin I_2 analogue—OP-2507

Introduction

Prostaglandin I_2 (PGI_2) has been shown to be beneficial in the treatment of traumatic shock [1] and ischemia-reperfusion tissue injury in various organs [2,3]. The major effects of PGI_2 are considered to be the prevention of platelet aggregation and the vasodilatory action. However, Araki and Lefer [4] demonstrated that PGI_2 might also protect the isolated heart perfused with platelet-free buffer solution at doses below those which affect vessel tone. This protective effect of PGI_2 in vitro might be related to stabilization of cell membranes. Recently, a stable PGI_2 analogue OP-2507 has been developed and shown to protect the heart and brain from ischemia-reperfusion injury [5,6] and hypoxic hepatocyte damage [7].

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In heart-lung transplantation and cardiac surgery, the prevention of ischemia-reperfusion injury of the lung is a major concern for cardiac surgeons and anesthesiologists. We therefore examined the effect of the stable PGI_2 analogue OP-2507 on ischemia-reperfusion lung injury.

Materials and methods

Isolated lung perfusion

Male Wistar rats (200–230 g) were anesthetized with pentobarbital (50 mg/kg, i.p.) and the lungs were isolated for extracorporeal perfusion as described by McMurtry et al. [8]. Lungs were mechanically ventilated through a tracheal cannula with a small animal respirator at 55 breaths·min^{-1} with 2.5 cmH_2O positive end-expiratory pressure. Inspired gas contained 21% O_2, 5% CO_2, and 74% N_2. A median sternotomy was then performed. After injection of 500 U/kg of heparin into the right ventricle, the pulmonary artery and left atrium were cannulated and the rest of the heart was resected. A double-lumen catheter was placed in the pulmonary artery to facilitate monitoring of the pulmonary artery perfusion pressure (Ppa). Lungs were perfused with physiological salt solution (PSS) containing 4 g·100 ml^{-1} Ficoll (FW = 70 000; Sigma Chemical, St. Louis, MO, USA). The composition of PSS was (in mmol·l^{-1}): 119 NaCl, 4.7 KCl, 1.17 $MgSO_4$, 22.6 $NaHCO_3$, 1.18 KH_2PO_4, 1.6 $CaCl_2$, 5.5 glucose, 50 sucrose. To achieve blood-free lung perfusion, the initial 25 ml of the perfusate was discarded to remove the blood cells [9]. Further, to avoid blood cell contamination in the circulating perfusate, a glass fiber filter was placed in line after the lung and the filter was exchanged several times during lung perfusion. The recirculating perfusion system consisted of an in-line pH electrode, glass fiber filter, air bubble trap, and heat exchanger. The total recirculated

perfusate volume was 50 ml. Perfusate was kept at 36°–37°C throughout the study. The pH was maintained between 7.3 and 7.4 by periodic addition of diluted-HCl or $NaHCO_3$ to the perfusate. Perfusion was accomplished using a peristaltic pump (Master flex, Cole Parmer, Chicago, IL, USA) at a constant flow rate of 0.03 ml·g body wt^{-1}·min^{-1}. Lungs were placed in a humidified heated chamber and suspended by the tracheal cannula on a force displacement transducer (TB-611T, Nihon Koden, Tokyo, Japan) for continuous measurement of lung weight gains. The system was sensitive to a weight change of 0.1 g. The perfusion chamber was maintained at 36°–37°C throughout the study. Ppa was continuously measured by a P-23 Statham transducer (OMEDA, Oxnard, CA, USA).

Experimental protocols

To check the validity of the lung perfusion, we initially perfused the rat lung ($n = 4$) for 180 min and measured the changes in Ppa, lung weight gain and lactate dehydrogenase (LDH) leakage. These lungs served as controls (control group).

Three separate ischemia-reperfusion experimental protocols were put into practice as outlined below. All lungs were allowed to stabilized for 20 min.The first group of lungs (untreated group: $n = 5$) were subjected to 90 min of normothermic ischemia without the test drug. The second group of lungs ($n = 5$) were treated with OP-2507 (200 ng·ml^{-1}) prior to 90 min of normothermic ischemia. The third group of lungs ($n = 5$) were treated with OP-2507 (1000 ng·ml^{-1}) prior to 90 min of normothermic ischemia. These two groups were served as OP-2507-treated groups. OP-2507 was dissolved in distilled water and the required does was put into the reservoir 10 min before the induction of ischemia.

Lung ischemia and reperfusion were conducted in accordance with the method previously described [9–11]. Namely, lungs were subjected to normothermic ischemia by stopping ventilation and perfusion for 90 min. Lungs were inflated with 3 ml of room air at the onset of ischemia to facilitate reperfusion. Lungs were maintained at 36°–37°C throughout the ischemia. After ischemia, lung ventilation was restored and the lungs were reperfused from a reservoir containing PSS/Ficoll at 37°C. The flow rate was gradually increased to baseline preischemic rates. The perfusion chamber was filled with air throughout the study.

LDH activity in the perfusate was determined using Sigma diagnostic kit DG 1340-KI (Sigma Chemical). Sample was obtained from the reservoir at specific time intervals.

At the end of experiments, all lungs were removed for the measurement of the lung wet/dry weight ratio. The lungs were weighed before and after drying at 110°C for 3 days.

Statistical analysis

All data were expressed as mean ± SD. Intra- and inter-group comparisons were performed by analysis of variance (ANOVA) and differences were identified using the Fisher protected least significant difference test. Significance was accepted for $P < 0.05$ unless otherwise indicated.

Results

Changes in Ppa and lung weight gain

Lung perfusion for 180 min did not induce any changes in Ppa and lung weight gain (Figs. 1 and 2). In the untreated group, lung weight decreased by 0.8 ± 0.2 g during 90 min of ischemia. When the ventilation and perfusion were restored after 90 min of ischemia, Ppa and lung weight increased progressively (Figs. 1, 2). The mean Ppa during reperfusion was significantly higher than the preischemic values. Perfusion and ventilation became impossible and was stopped after 15 min of reperfusion because of massive lung edema.

In the OP-2507-treated groups, the initial mean Ppa was not different from the control and untreated groups. In the control group, Ppa did not change with perfusion. In the lungs treated with OP-2507 (200 ng·ml^{-1}), lung

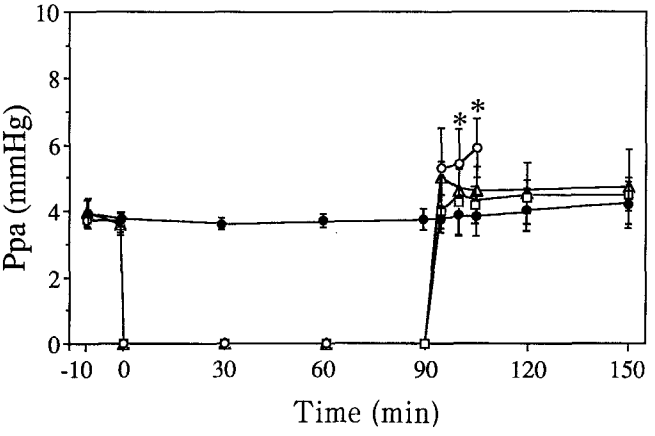

Fig. 1. Effects of OP-2507 on mean pulmonary artery perfusion pressure (Ppa) in the ischemia-reperfused rat lungs. After 20 min of equilibration, ischemia was induced for 90 min by stopping the perfusion and ventilation. With reperfusion, each lung was ventilated with 21% O_2, 5% CO_2, and 74% N_2 gas mixture. *Closed circles*, control group (without ischemia and reperfusion); *open circles*, untreated group; *open triangles*, OP-2507 (200 ng·ml^{-1})-treated group; *open squares*, OP-2507 (1000 ng·ml^{-1})-treated group. Data are given as mean ± SD. *$P < 0.05$ *versus* preischemic values.

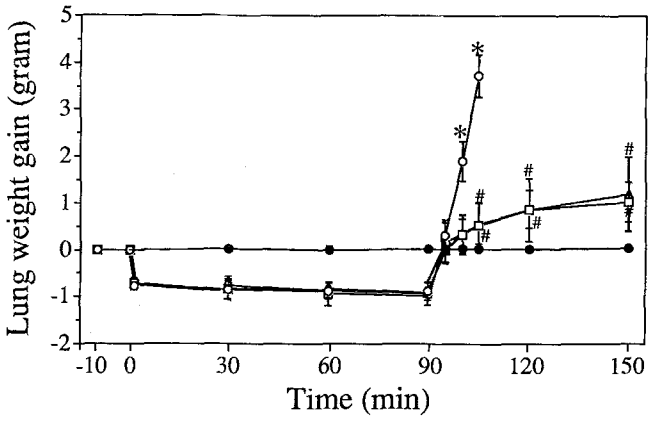

Fig. 2. Effects of OP-2507 on lung weight gains in the ischemia-reperfused rat lungs. After 20 min of equilibration, ischemia was induced for 90 min by stopping the perfusion and ventilation. With reperfusion, each lung was ventilated with 21% O_2, 5% CO_2, and 74% N_2 gas mixture. *Closed circles*, control group (without ischemia and reperfusion); *open circles*, untreated group; *open triangles*, OP-2507 (200 ng·ml^{-1})-treated group; *open squares*, OP-2507 (1000 ng·ml^{-1})-treated group. Data are given as mean ± SD. $^*P < 0.05$ *vs* preischemic values; $^\#P < 0.05$ *vs* the control group

weight decreased by 0.9 ± 0.1 g during 90 min of ischemia (Fig. 2). With reperfusion, the lung weight increased gradually and reached plateau at 15 min of reperfusion. The mean increase in weight at 60 min of reperfusion was 0.8 ± 0.2 g. The mean Ppa increased initially after initiation of reperfusion and then decreased gradually but did not reach its basal levels (Fig. 1). During the reperfusion period, the mean Ppa was significantly higher than the preischemic values. In the lungs treated with OP-2507 (1000 ng·ml^{-1}), the changes in mean Ppa and lung weight gains during ischemia and reperfusion were not different from the lungs treated with OP-2507 (200 ng·ml^{-1}) (Figs. 1 and 2).

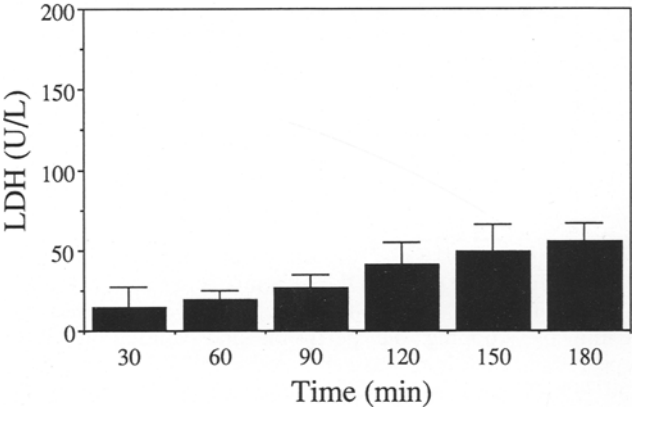

Fig. 3. Lactate dehydrogenase (LDH) activity during 180 min of lung perfusion. LDH activity increased gradually with time over 180 min

LDH activity in perfusate

During 180 min of lung perfusion, LDH activity in the perfusate increased gradually with time (Fig. 3). When the lung was made ischemic for 90 min and reperfused, LDH activity at the end of experiment (15 min of reperfusion) was significantly higher than control lungs (compare Figs. 3 and 4). As shown in Fig. 4, LDH activity was significantly attenuated by both doses of OP-2507 (200 and 1000 ng·ml^{-1}) as compared to the untreated lungs. At the end of experiment LDH activity of OP-2507-treated groups was not different from that of the control group (compare Figs. 3 and 4). There were no significant differences between OP-2507 200 and 1000 ng·ml^{-1}.

Water balance

Figure 5 shows the lung wet/dry weight ratio in the four groups of lungs. In the untreated group, 90 min of normothermic ischemia resulted in significant water accumulation as compared to both of the control and OP-2507-treated groups. However, wet/dry weight ratio in OP-2507-treated group was significantly higher than the control group. There were no significant differences between OP-2507 200 and 1000 ng·ml^{-1}.

Discussion

The present study clearly demonstrated that the PGI_2 analogue OP-2507 prevented the ischemia/reperfusion related lung edema in the PSS-Ficoll perfused rat lung. Our results are compatible with the previous reports

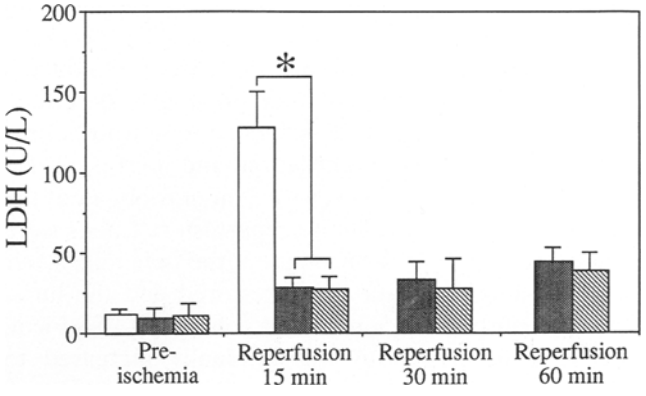

Fig. 4. LDH activity in the untreated and OP-2507-treated groups. After 20 min of equilibration, ischemia was induced for 90 min by stopping the perfusion and ventilation. With reperfusion, each lung was ventilated with 21% O_2, 5% CO_2, and 74% N_2 gas mixture. *Open bars*, untreated group; *shaded bars*, OP-2507 (200 ng·ml^{-1})-treated group; *hatched bars*, OP-2507 (1000 ng·ml^{-1})-treated group. Data are given as mean ± SD. $^*P < 0.05$

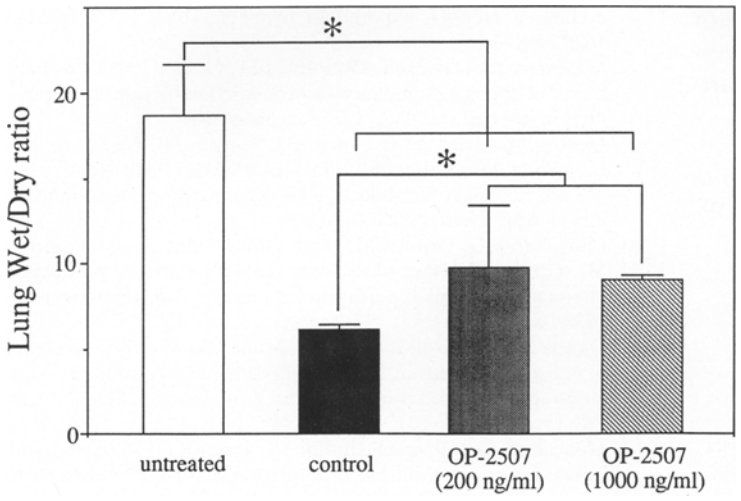

Fig. 5. The lung wet/dry weight ratio at the end of experiment in the four lung groups. *Open bars*, untreated group; *closed bars*, control group; *shaded bars*, OP-2507 (200 $ng \cdot ml^{-1}$)-treated group; *hatched bars*, OP-2507 (1000 $ng \cdot ml^{-1}$)-treated group. Reperfusion after 90 min of ischemia caused significant water accumulation. OP-2507 200 $ng \cdot ml^{-1}$ and 1000 $ng \cdot ml^{-1}$ equally attenuated the water accumulation but failed to inhibit it totally. Data are given as mean ± SD. $^*P < 0.05$

which showed that OP-2507 inhibits ischemia reperfusion-induced myocardial injury [5], ischemic brain injury [6], and hypoxic hepatocyte damage [7].

The increase in lung weight has been taken as an indicator of tissue edema [9, 11]. Previously, Deeb et al. [9] demonstrated that 45 min ischemia caused lung injury in the blood free-PSS/Ficoll perfused rat lung. In the present study, we carried out a more severe ischemia (90 min) and severe lung edema occurred after reperfusion. Perfusion and ventilation became impossible due to massive lung edema which, in turn, resulted in an increase in Ppa and lung weight. We, therefore, think that the marked damage of pulmonary vasculature and alveoli was induced by 90 min of ischemia and reperfusion. This is supported by the large increase in LDH release from the lung. OP-2507 strongly inhibited the increase in Ppa and lung weight gain. Furthermore lung wet/dry weight ratio was significantly lower than the untreated group but significantly higher than control group, which indicates that OP-2507 partially attenuated lung edema. These results indicate that OP-2507 prevents cell injury and attenuates the increase in vascular permeability.

Prostaglandin I_2 has been shown to be produced primarily by endothelial cells [12] and not cleared through the lungs [13]. However, PGI_2 is chemically unstable as it is rapidly hydrolyzed at neutral or acidic pH to a much less active compound, 6-keto prostaglandin $F_{1\alpha}$. The half life of PGI_2 is 5 min in aqueous solution. On the other hand, PGI_2 analogue OP-2507 is stable over 24 h in an aqueous solution [6]. It has been reported that OP-2507 has a less potent hypotensive effect than prostacyclin (about 1/20) and has a less potent in vitro inhibitory activity on ADP-induced platelet aggregation in platelet-rich plasma than prostacyclin (about 1/180) [6]. In the present study, the perfusate we used did not contain blood cell components. Therefore, the beneficial effect of OP-2507 in the isolated perfused rat lung does not appear to depend on the inhibitory effect on platelet aggregation. Whatever the precise mechanism of action of OP-2507, its beneficial effect is manifested during the ischemic period, as evidenced by attenuation of LDH leakage and water accumulation. During reperfusion, Ppa was significantly higher than preischemic levels. Thus, the beneficial effect of OP-2507 on ischemia/reperfusion-related lung injury is independent of its antiaggregation effect in platelets and very likely independent of its vasodilator action. Recently, several reports demonstrated that active oxygen species are involved in the ischemia/reperfusion lung injury in which superoxide dismutase and catalase are beneficial to prevent this injury [9,14]. Therefore, OP-2507 may modulate oxygen radical generation during ischemia and reperfusion. Further studies are necessary to clarify this point.

Prostaglandin I_2 has several biological activities such as prevention of platelet aggregation [12], vasodilatation [15], stabilization of lysozomal membranes and inhibition of thromboxane generation [2]. PGI_2 has also been reported to be a potent stabilizer of lysosomes in the liver [4] and myocardium [16] and the lysozomal membrane stabilizing effect of PGI_2 is much higher than glucocorticoids [2]. Furthermore, Irita et al. [7] demonstrated that OP-2507 has a direct effect on ameliorating mitochondrial dysfunction in Hep G_2 exposed to hypoxia. Although mechanisms for the cytoprotective effect of OP-2507 are unclear, it is conceivable that the strong inhibitory effect of OP-2507 on ischemia reperfusion-related lung edema is due to stabilization of lysozomal membranes and a direct cytoprotective effect.

In conclusion, OP-2507 attenuates the LDH leakage and water accumulation during reperfusion after ischemia in the isolated perfused rat lung. OP-2507 seems to have a direct cytoprotective effects on ischemia/reperfused lung tissue independent of its in-

hibitory effect on platelet aggregation and vasodilator action. OP-2507 appears to be beneficial for lung preservation in a variety of circumstances, such as heart-lung transplantation and cardiopulmonary bypass.

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