Can ischemic preconditioning enhance to protection of ischemia-reperfusion injury of the lung?

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Abstract

Background: Ischemic preconditioning (IP) has been focused on as a novel strategy to overcome ischemia-reperfusion injury. Steroid administration has also been routinely employed to reduce ischemia-reperfusion injury in lung transplantation. Therefore, we investigated whether ischemic preconditioning could enhance the effects of steroid on ischemia-reperfusion injury. Methods: Twenty-four Japanese white rabbits were randomly divided into four groups (n=6 in each group). Group I was the warm ischemia group (after 3 hours of warm ischemia, the left lung was reperfused for 2 hours). Group II was the steroid group (10 mg/kg of methyl predonisolone was administered just before reperfusion). Group III was the IP group (3 cycles of IP were performed prior to left lung warm ischemia). Group IV was the combination group (3 cycles of IP and steroid were given before warm ischemia). The hemodynamics, airway pressure and blood gas measurement were assessed during reperfusion period. In addition, histological and immunohistochemical analyses were performed. Results: In group I, warm ischemia led to rapid and severe deterioration PaO₂ after reperfusion. Group IV had significantly higher PaO₂ than the other groups (p<0.05). PaCO₂ was significantly lower in Group IV than the other groups (p<0.05). Histologically, the left lung in group I showed diffuse alveolar damage and interstitial edema. However, groups II, III, and IV showed almost normal structures. Conclusion: This study demonstrates ischemic preconditioning can enhance the effects of steroid on ischemia-reperfusion injury.

Background

Lung transplantation has been accepted as treatment for end-stage pulmonary disease1,2. However, early allograft dysfunction caused by ischemic and reperfusion injury remains an important and unpredictable issue to be solved. Although a number of strategies have evolved to limit lung ischemic injury during storage3-9, most clinical lung transplantation programs do not accept ischemic times in excess of 8 hours for organ storage. Even with short ischemic times, graft dysfunction occasionally occurs10. Inadequate preservation of the donor lung may result in acute ischemia-reperfusion injury, characterized by increased pulmonary capillary, pulmonary edema, impaired gas exchange, and sometimes right heart dysfunction following an acute rise in pulmonary vascular resistance. Ischemic preconditioning (IP) describes the phenomenon by which a brief period of ischemia is used to induce tissue protection against subsequent ischemia and reperfusion injury. This has been intensively investigated with regard to myocardial ischemia11,12. Recently it has been applied
to other organs such as liver, kidney, and lung. It has also been reported effective in preventing apoptosis. Since corticosteroids are routinely employed to reduce acute lung injury in lung transplantation, we investigated whether ischemic preconditioning could enhance the effects of steroid on ischemia-reperfusion injury, using a rabbit lung reperfusion model.

**Materials and method**

**Operative procedure**

All animals received human care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health. (NIH publication No. 86-23, revised 1985). Twenty-eight female Japanese white rabbits (3.80 ± 0.40 kg) were premedicated with subcutaneous atropine sulfate (0.25 mg/kg), ketamine hydrochloride (35 mg/kg). Isovolemia was maintained by intravenous infusion of modified Ringer’s lactate, in which 4 ml of 7% sodium bicarbonate was added to 500 ml normal Ringer’s lactate to maintain the pH of the fluid at 7.4. The infusion was given at a rate of 15 ml/kg/h via a peripheral ear vein. They were anesthetized with intravenous sodium pentobarbital (25 mg/kg) and 0.5 mg/kg of pancuronium bromide. After cervical tracheostomy and placement of an endotracheal tube (interior diameter 4 mm), animals were ventilated with 100% O2, tidal volume was maintained to ensure good inflation (15–25 ml/kg). With a respiratory rate of 30/min and positive end–expiratory pressure 1.0 cm H2O, using a volume–cycled ventilator (Shinano SN-480-5 Tokyo). Core temperature was maintained at 38–40°C with warming blankets. Intravenous anesthesia was maintained by sodium pentobarbital and pancuronium bromide. After median sternotomy and thymectomy, a 20 gauge catheter was placed in the right carotid artery to monitor systemic blood pressure. A 6 F introducer inserted through a 5–0 nylon pursestring suture in the right ventricle to place a 6 F Swan–Ganz catheter in the trunk of the pulmonary artery. After pleurectomy, the pulmonary hila were carefully dissected bilaterally andatraumatically encircled with rubber vascular tapes. Tourniquets were applied loosely over these tapes.

**Experimental groups**

The rabbits were divided into 4 groups of 6 each. In the warm ischemia group, 500 IU/kg of sodium heparin was given via a fluid line before the tourniquet was firmly tightened around the right pulmonary hilum. The ventilator was readjusted for unilateral ventilation. To keep minute ventilation roughly unchanged, tidal volume was reduced the 12–20 ml/kg, and the rate increased to 40/min. After 5 minutes of stabilization, the baseline measurement was obtained, and the

![Fig 1](image-url)
tourniquet around the right hilum released. The left pulmonary hilum was then occluded for 180 minutes at end inspiration to keep the lung at fully inflated tidal volume. Three hours later the tourniquet was released and reperfusion performed for 2 hours, while during this reperfusion time the contralateral side was occluded in the same way. The IP group was preconditioned with 3 cycles of short periods of ischemia, (5 minutes each) by clamping the left hilum with reperfusion four 10 minutes after the cycles before performing ischemia and then reperfusion as in warm ischemia group. In the steroid group, after 3 hours of left lung ischemia 10 mg/kg of methylprednisolone sodium succinate was injected, followed by reperfusion for 2 hours. The combination group was subjected that of the IP group followed by the same treatment as the steroid group (Fig. 1).

Measurement of lung function
Assessments, blood gas analysis, mean systemic blood pressure (AoP), mean pulmonary arterial pressure, heart rate and peak inspiratory pressure were performed before left hilar clamping and after lung ischemia. The first post-ischemia assessment was made after 5 min of reperfusion. Subsequent assessments were made at 20-min intervals during the 120 min of reperfusion. During the 2 hours of reperfusion, the level of anesthesia was maintained with pancuronium bromide and sodium pentobarbital intravenously. Blood pH was normalized as necessary by administration of sodium bicarbonate.

Histological studies
For light microscopic studies, the middle portion of the lower lobe was removed from the left lung. One sample was fixed in 10% neutral buffered formalin for H-E staining and another sample was fixed in 4.0°C of 99% acetone for TUNEL staining of immunohistochemically detect apoptosis. These sections were embedded in paraffin.

In situ detection of cell damage
After deparaffinization, the 5-μm sections were placed in 1× phosphate-buffered saline for 10 minutes at room temperature and digested by proteinase K for 15 minutes at 37°C. Intrinsic peroxidase activity was quenched by the addition of 3% hydrogen peroxide in methanol for 5 minutes. Staining procedures followed the instructions of the apoTACS™-DAB In Situ Apoptosis Detection Kit (Trevigen®).

Fig. 2  Changes in a) PaO₂ and b) PaCO₂ in all experimental group. Each point is mean±SEM. *p<0.01 vs. warm ischemia group, by repeatedly measured ANOVA. †p<0.05 vs. combination group, by repeatedly measured ANOVA.
Statistical analysis
All statistical analysis was performed using StatView® (Abacus Concepts Inc.) A p value of less than 0.05 was considered to indicate statistically significant difference. All data were presented as means±SEM. Continuously recorded data were compared among groups over time using repeated measurement analysis of variance (ANOVA) with a polynomial transformation applied to time.

Results
Hemodynamic, Airway Pressure, and Blood Gas Measurements
In each animal, following reperfusion there was a brief period of hypotension, which in most cases recovered spontaneously within minutes. However, two rabbits died within 40 minutes of reperfusion, one in the IP group due to hilar bleeding, another rabbit in the control group died of cardiac failure of unknown cause. Two rabbits in the control group and one rabbit in the steroid group died in the warm ischemic period due to cardiac failure. Data from these animals were excluded from the analysis.

Arterial blood gas analysis
Among the four groups, there were significant overall differences in the trends of partial arterial oxygen pressure (PaO₂). In the warm ischemia group, 3 hours of warm ischemia led to rapid and severe decrease in gas exchange after reperfusion. In the 3 other groups, PaO₂ at the end of 2 hours reperfusion was significantly higher (p<0.01) than in the warm ischemia group. And there was significant difference in PaO₂ at the end of 2 hours of reperfusion in the combination group and other two treatment groups (p<0.05). A significant difference in the arterial carbon dioxide pressure (PaCO₂) level was seen in the combination group and other three groups at the end of 2 hours of reperfusion (Fig. 2).

Hemodynamics and Airway Pressure
There were no significant differences in pulmonary artery pressure in each groups after 2 hours of reperfusion, but only the warm ischemia group showed a significantly higher level after 2 hours of reperfusion.
compared to before ischemia. No significant difference in systemic pressure or airway pressure was seen in any groups, after 2 hours of reperfusion (Fig. 3).

**Wet to Dry Lung Weight Ratio**

The wet to dry lung weight ratios of the reperfusion lung were 6.00±1.00 in the combination group, which was significantly lower than in the other groups (p<0.05). The ratio in the steroid group and IP group were significantly lower than in the warm ischemia group (p<0.05) (Fig. 4).

**Histological Findings**

After 2-hour assessment, the left lung in the warm ischemia group showed severe interstitial edema and thickening of the alveolar septum, and markedly increased intraalveolar red cells and granulocytes. The warm ischemia group showed diffuse alveolar damage and interstitial edema. The steroid group and the IP group showed mild interstitial edema, while the combination group showed normal structures and no sign of pulmonary edema (Fig. 5).

**TUNEL staining**

The warm ischemia group showed severe cell damage but the other groups had less alveolar cell damage, especially in the combination group (Fig. 6).

**Discussion**

We attempted to determine whether ischemic preconditioning is useful for lung transplantation. Our in situ warm ischemic lung model for this study was based on the report by Qayumi et al. They reported that ischemia–reperfusion injury after warm ischemia resulted in very similar conditions biochemically, functionally and morphologically to those recognized after lung transplantation following cold ischemia[21]. Our model is suitable to investigate the effects of ischemic preconditioning. In lung transplantation, early allograft dysfunction caused by ischemic and reperfusion injury remains an important and unpredictable issue. In IP, solid organs are subjected to repeated periods of short ischemia and reperfusion to protect against ischemia–reperfusion injury following prolonged otherwise lethal ischemia. This phenomenon was first reported in myocardium by Murry and colleagues in 1986[13], has the mechanism of the effects of IP is not clear. In his summary of cardiac preconditioning, Meldrum stated that stress hormones like adenosine, nor epinephrine and high-level of intracellular calcium were propagated as signaling mechanisms in acute preconditioning, leading to activation of protein kinase C, ecto–5′–nucleotidase, and K_ATP channels[12]. The results suggest that it is good strategy for ischemia–reperfusion injury not only in cardiomyocytes but also for other solid organs[13–18]. These reports suggested the possible usefulness of IP for organ transplantation. However, clinically, steroid what may inhibit the production of proinflammatory cytokines and the sequestration of PMNs[22], is given intraoperatively just prior to graft perfusion in lung transplantation. We investigated whether ischemic preconditioning enhances the protective power of steroids against ischemic reperfusion injury of the lung. Histologic findings 2 hours after reperfusion in the warm ischemic group revealed severe alveolar damage. In the steroid group and IP group, the damage was mild and the combination group showed almost no damage. Blood gas analysis data showed similar results with regard to PaO2 2 hours after reperfusion and there were significant differences between the combination group and the steroid group. The combination group showed the best results in comparison to all other groups. Though we found a difference in each group in blood gas analysis and histological assessment by this study, no difference was found in the hemodynamic and airway

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**Fig. 4** Wet to dry weight ratio after 2 hr single left lung perfusion. After assessment, the wet to dry ratio of the reperfusion rabbit left lung in the combination group was greater than in the other group. *p<0.05 vs. combination group, by repeatedly measured ANOVA. †p<0.05 vs. warm ischemia group, by repeatedly measured ANOVA.
Fig. 5 Histological finding in a left lung after 2-hour reperfusion. (a) The warm ischemic group showed severe interstitial edema and thickening of the alveolar septum. (b) The IP group and (c) The steroid group showed almost no abnormal structure and slight thickening of the alveolar septum. (d) The combination group showed almost no abnormal structure and no sign of pulmonary edema. (HE × 400)

Fig. 6 In TUNEL staining, the warm ischemic group showed severe cell damage (a). (b) The IP group and (c) the steroid group showed mild cell damage. (d) The combination group was almost normal. (× 400)
pressure. It is thought that may be these results reflect an injury in peripheral tissue, and the influence did not yet extend at this stage to the general state. Ischemic preconditioning in lung transplantation with reperfusion injury in rabbit lung after a three-hour warm ischemic period was less pronounced when the lung was previously subjected to repetitive periods of short ischemia and reperfusion and IP can also enhance steroid effectiveness. This study demonstrates that IP can enhance the protective effect of steroids against ischemia–reperfusion injury in the rabbit lung.

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虚血前短時間虚血による肺虚血再灌流障害抑制の増強効果に関する検討

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【要旨】肺移植後の肺虚血再灌流障害の対策として、ステロイド投与が、第一に行われているが、虚血前短時間虚血を付加することにより、より効果的に再灌流障害を抑制するとの仮定に基づき以下の実験を行った。【方法】日本白兎を用い、左肺における虚血再灌流障害を以下の4群にて比較した。1 (温血群) 群: 開胸後左肺門3時間遮断、温血群の後右肺門を遮断し、同時に左肺門を開放2時間再灌流せめた。2 (ステロイド投与群) 群: 温血群に準じ開胸後左肺門3時間遮断し、左肺開放直前にステロイドを静脈的に投与した。3 (虚血前短時間虚血群) 群: 開胸し左肺門の5分遮断10分開放 (虚血前短時間虚血) を3回繰り返した後、温血群に準じて左肺門の遮断及び開放を行った。4 (併用群) : 虚血前短時間虚血群に準じ虚血前短時間虚血を行った後にステロイド投与群に準じステロイドを静脈的に投与した。それぞれの群に対して再灌流後経時的に血行動態、気道内圧、血液ガス測定を施行した。また乾燥重量比、病理解剖学的検討を行った。【成績】再灌流2時間後の血液ガス測定では、虚血前短時間虚血群、ステロイド投与群、併用群とともに温血群に対し良好な成績を得た。併用群は虚血前短時間虚血群、ステロイド投与群に対しても良好な成績を得た。温血群に於いても同様の傾向を認めた。気道内圧は併用群において、良好な成績を得た。病理組織像においても温血群に対し虚血前短時間虚血群、ステロイド投与群、併用群では良好な結果が得られた。【結語】虚血前短時間虚血はそれ自体に虚血-再灌流障害軽減効果を持つが、ステロイドによる同様の効果を更に増強させる傾向が示され、臨床的付加価値がある可能性が示唆された。

＜キーワード＞虚血-再灌流障害、ischemic preconditioning、lung transplantation

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