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Changes in levels of arachidonic acid metabolites in blood and bronchoalveolar lavage fluid after warm ischemia-reperfusion of lung.

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Abstract

The purpose of this study was to evaluate the role of arachidonic acid metabolites in the reimplantation response after lung transplantation in mongrel dogs. The left lung was used and two groups were studied. Group I underwent hilar stripping, while Group II underwent hilar stripping plus warm ischemia for 60 min., achieved by clamping the left pulmonary artery and veins. We measured the lung wet to dry weight ratio (W/D ratio), total pulmonary vascular resistance (TPVR), and blood and bronchoalveolar lavage fluid (BALF) levels of leukotriene B₄ and C₄ (LTB₄, C₄) and thromboxane B₂ (TXB₂). These parameters were measured periodically for 7 days after reperfusion. In group II, the W/D ratio and TPVR were significantly increased in comparison with Group I. The blood LTC₄ level was elevated immediately after reperfusion, and BALF level of LTC₄ also rose subsequently. These levels changed concomitantly with the W/D ratio. The above results suggest that arachidonic acid metabolism plays an important role in the reimplantation response, especially in pulmonary edema.

KEYWORDS: lung transplantation, reperfusion, arachidonic acids

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Changes in Levels of Arachidonic Acid Metabolites in Blood and Bronchoalveolar Lavage Fluid after Warm Ischemia-Reperfusion of Lung

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The purpose of this study was to evaluate the role of arachidonic acid metabolites in the reimplantation response after lung transplantation in mongrel dogs. The left lung was used and two groups were studied. Group I underwent hilar stripping, while Group II underwent hilar stripping plus warm ischemia for 60 min., achieved by clamping the left pulmonary artery and veins. We measured the lung wet to dry weight ratio (W/D ratio), total pulmonary vascular resistance (TPVR), and blood and bronchoalveolar lavage fluid (BALF) levels of leukotriene B₄ and C₄ (LTB₄, C₄) and thromboxane B₂ (TXB₂). These parameters were measured periodically for 7 days after reperfusion. In group II, the W/D ratio and TPVR were significantly increased in comparison with Group I. The blood LTC₄ level was elevated immediately after reperfusion, and BALF level of LTC₄ also rose subsequently. These levels changed concomitantly with the W/D ratio. The above results suggest that arachidonic acid metabolism plays an important role in the reimplantation response, especially in pulmonary edema.

Key words : lung transplantation, reperfusion, arachidonic acids

The reimplantation response which is mainly caused by pulmonary edema occurs in the early phase of lung transplantation. As the cause of this response, reperfusion injury after ischemia is considered to be an important factor, rather than the effects of denervation or interruption of lymphatic vessels as have been suggested previously (1). This damage is considered to be related to oxygen radicals or various chemical mediators produced or released during reperfusion (2).

The lungs work as gas exchange organs and at the same time metabolize various substances.

We have been interested in the metabolism of leukotriene (LT) and thromboxane (TX) (arachidonic acid metabolites) which promote vascular permeability and vaso-constriction in the lung, and conducted the following experiments to assess the role of these agents in the reimplantation response.

Materials and Methods

Adult mongrel dogs weighing 8-15 kg were used. After the intramuscular injection of 0.025 mg/kg of atropine sulfate, 10 mg/kg of ketamine hydrochloride and

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0.1mg/kg of pancronium bromide were injected intravenously for anesthesia and, respiration was controlled by a Harvard respirator following endotracheal intubation. Thoracotomy was performed at the left fifth intercostal space and the following two experimental groups were set up.

Group I (Control Group, n = 6)

Hilar stripping was performed (*i.e.*, stripping of the hilum, severing of the left bronchial artery, veins, lymphatics, and denervation), after which the left main bronchus was separated and then sutured. After the administration of 100u/kg of heparin, the lung was collapsed for 60 min.

Group II (Warm Ischemia Group, n = 6)

In addition to the above-described procedures performed in Group I, the left main pulmonary artery and pulmonary veins were occluded for 60 min with clamps.

At thoracotomy and 3h, 3 days, and 7 days after reperfusion, the following parameters were investigated.

Lung wet to dry weight ratio. Lung tissue was cut out, the wet weight (W) measured. After drying for 72h at 37°C, the dry weight (D) was measured, and the W/D ratio was calculated.

Total pulmonary resistance (TPR). After temporary occlusion of the right pulmonary artery, the pulmonary artery pressure (PAP) and cardiac output (CO) were measured, and TPR was calculated from the formula:

$$TPR = PAP/CO \times 79.92 \text{ (dyne/sec/cm}^{-5}\text{)}.$$

A cannula was inserted from the femoral artery to the thoracic aorta and arterial blood samples were collected. Bronchoalveolar lavage fluid (BALF) was collected from the left lower lobe by washing with 40ml of saline. Blood samples and BALF were examined before thoracotomy, immediately, 1h, 3h, 1 day, 3 days and 7 days after reperfusion. BALF from the right lower lobe was also examined only before thoracotomy. The following parameters in the blood and BALF were measured.

Leukotriene B₄ and C₄ (LTB₄, C₄). Levels were measured up to 7 days using high-performance liquid chromatography (HPLC).

To measure the LT level, 10ml of blood of BALF was immediately added to 40ml of ethanol and centrifugated. Then, the supernatant was evaporated and dissolved in 50 % acetonitrile. Distilled water was added to this sample, and this solution was poured into an Amprep C₁₈ column. More distilled water, n-hexane, and 10 % ethanol were added to the column into this order, and finally the column was eluted with methyl formate, and the elute was evaporated. The residue was

dissolved in 50 % acetonitrile to obtain a sample for HPLC. HPLC was performed with Waters Model WISP712 and a TSK-GEL ODS 120T (TOSOH) column. The eluent was prepared by mixing acetonitrile, methanol, distilled water, and acetic acid (33:10:33:0.06) and was adjusted to pH5.6. The flow rate was 1 ml/min, and the detection wave was set at 280nm.

LT was identified by amplification of the same peak wave when standard samples were added to the test samples. The quantity of LT was measured by comparison with the curve obtained from a standard sample.

Thromboxane B₂. Since TXA₂ is unstable and has a half-life of 30–40 sec, its stable metabolite TXB₂ was measured. Blood of BALF (5ml) was placed into a container filled with EDTA-2K plus indomethacin. Centrifugated, and the serum obtained was assayed by radioimmunoassay (PEG method).

Significance of differences was evaluated with Student's *t*-test, statistical significance was defined as $p < 0.05$.

Results

The lung wet/dry weight was compared with the preoperative ratio. At 3h after reperfusion, it was 1.26 ± 0.12 in group I and 1.29 ± 0.19 in group II (mean $\pm$ SD); the ratio was elevated in both groups, but there was no significant difference between them. On the third day, however, it was 1.19 ± 0.14 in group I and 1.50 ± 0.18 in group II; it was thus significantly increased ($p < 0.05$) in group II, and pulmonary edema was noted. At 7 days, the ratio was reduced in both groups (1.20 ± 0.11 in group I and 1.17 ± 0.25 in group II) (Fig. 1).

The total vascular resistance was expressed as a ratio to the preoperative value. At 3 h after reperfusion, it was 1.53 ± 0.25 in group II, which was significantly higher than the 1.03 ± 0.05 seen in group I ($p < 0.01$). At 3 days, the ratio was lower in both groups, but still tended to be slightly higher in group II (Fig. 2).

The blood level of LTC₄ (ng/ml) before the operation was 0.54 ± 0.28 in group I and 0.45 ± 0.25 in group II. Immediately after reperfusion, it was elevated to 1.05 ± 0.48 in group II and

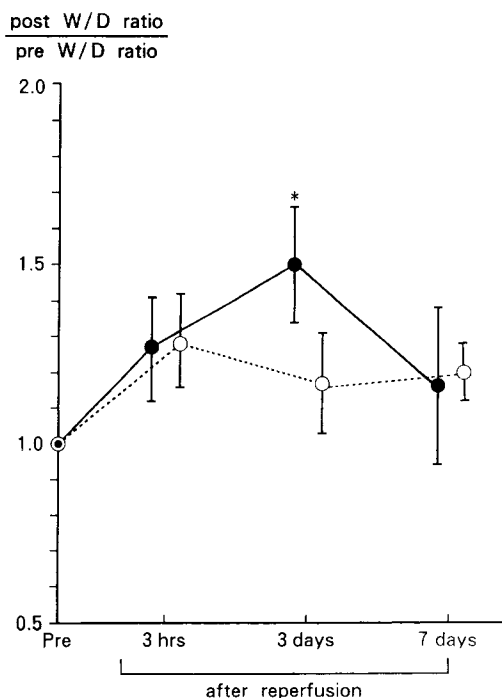


Fig. 1 Change in wet/dry lung weight.
pre-W/D ratio: preoperative wet/dry ratio
(○---○) Group I; (●—●) Group II, Mean ± SD,
* $P < 0.05$

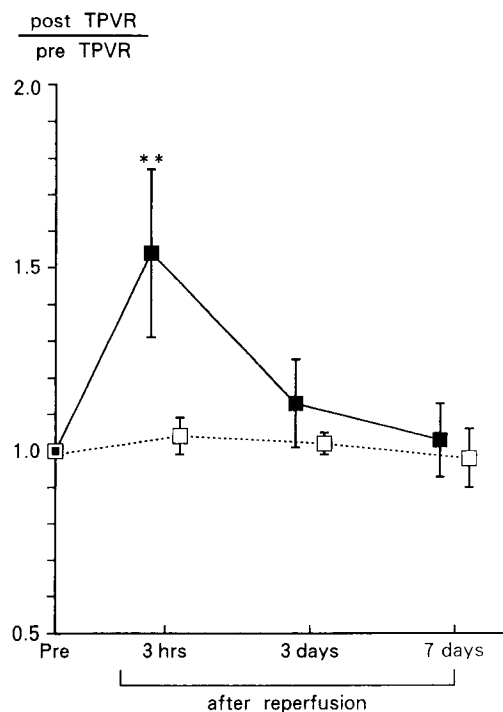


Fig. 2 Change in total pulmonary vascular resistance (TPVR).
pre TPVR: preoperative total pulmonary vascular resistance.
post TPVR: post operative pulmonary total vascular resistance.
(□---□) Group I; (■—■) Group II, Mean ± SD,
** $P < 0.01$

measured to 2.08 ± 0.98 at 3h. The peak level of 2.32 ± 0.94 was seen after 1 day, and then declined gradually at 3 days and 7 days. In group I, however, the level was slightly elevated to 0.93 ± 0.38 at one hour, but thereafter fell to normal and did not change significantly (Fig. 3).

The LTC_4 concentration (ng/ml) of BALF before operation was 0.38 ± 0.33 in group I and 0.42 ± 0.20 in group II. There was no significant change throughout the experiment in group I. In group II, no change was seen at 3 h, but the level was significantly elevated to 3.38 ± 1.13 ($p < 0.01$) at 1 day, remained at 2.79 ± 1.47 at 3 days, and finally fell to 0.91 ± 0.41 after 7 days (Fig. 4).

In contrast, LTB_4 was not detected in either blood or BALF in either group any time during

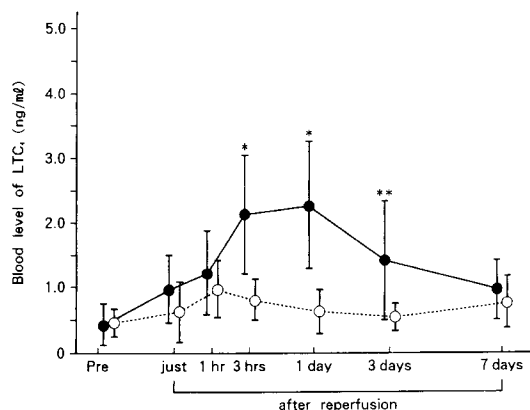


Fig. 3 Change in blood level of LTC_4 .
(○---○) Group I; (●—●) Group II, Mean ± SD,
* $P < 0.01$, ** $P < 0.05$

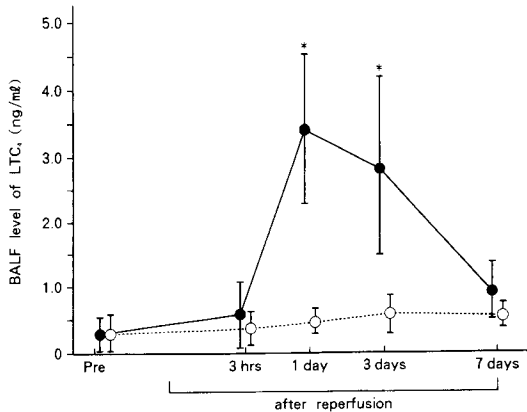


Fig. 4 Change in BALF level of LTC₄.
(○---○) Group I; (●—●) Group II, Mean ± SD,
* P < 0.01

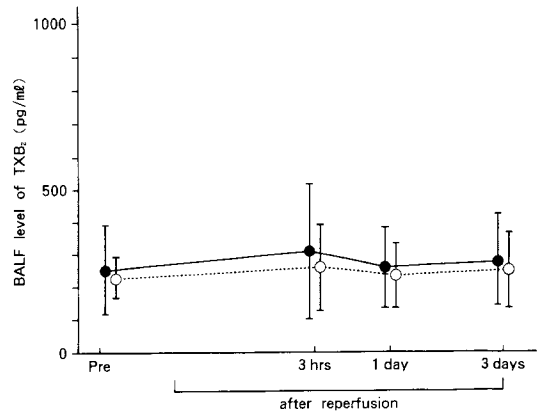


Fig. 6 Change in BALF level of TXB₂.
(○---○) Group I; (●—●) Group II

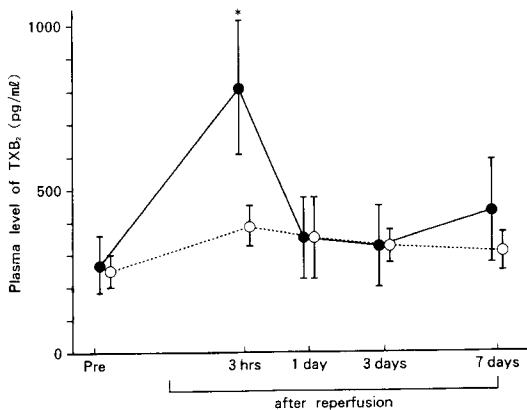


Fig. 5 Change in plasma level of TXB₂.
(○---○) Group I; (●—●) Group II, Mean ± SD,
* P < 0.01

the study.

The serum TXB₂ level (pg/ml) before the operation was 258 ± 37 in group I and 267 ± 81 in group II. After 1 h, it was slightly elevated to 392 ± 64 in group I, but there was no significant change thereafter, (358 ± 117 at 3 h, 338 ± 41 at 1 day, and 306 ± 46 at 3 days). In group II, the level was significantly elevated to 822 ± 191 at 1 h ($p < 0.01$). After 3 h, it dropped to 365 ± 131 , and thereafter did not significantly differ from that in group I (Fig. 5).

The TXB₄ level of BALF did not change significantly in either group I or II, and there was no significant difference between the two groups (Fig. 6).

Discussion

It is known that the lung produces and metabolizes various substances. Arachidonic acid metabolites like LT and TX are found in many organs, but the lung has a large role in the production, release, and inactivation of these substances. Under normal conditions, these substances are not activated. Under conditions of ischemia-reperfusion, an influx of Ca⁺⁺ ions into the cells occurs and phospholipase A₂ is stimulated to activate arachidonic acid metabolism (the calcium paradox (3)). Hence, the effects of the metabolites thus produced on the lung may be considerable.

It is known that LTC₄ causes an intense increase in vascular permeability, as well as pulmonary vaso-constriction and broncho-constriction (4), and that these actions are slow to peak and have a long duration. LTB₄ has a chemotactic effect on leukocytes (5), and TXA₂ causes vaso-constriction and broncho-constriction

besides its potent platelet aggregation effect (6).

Cell damage caused by oxygen radicals following the ischemia-reperfusion process is most important, as the cause of the reimplantation response after lung transplantation. It has also been confirmed histologically that leukocytes invade the capillary vessels of the lung, suggesting that these leukocytes may be associated with reperfusion injury (7).

Denervation and interruption of the lymphatics and bronchial vessels may also be implicated in the reimplantation response (8), but the present study showed that hilar stripping alone had little effect on the lung wet/dry weight ratio or the total pulmonary vascular resistance, while marked changes were noted in the group subjected to warm ischemia.

Pulmonary edema is generally said to reach a peak about 3 days after transplantation, and to subside within 1-3 weeks (8). In our study as well, the warm ischemia group showed a peak on the third day.

In the warm ischemia group, the blood LHC_4 level began to rise immediately after reperfusion, and reached a peak after 3 to 24h, while the BALF LTC_4 level did not change significantly after 3 h and was elevated from day 1 to 3. Thus, in the BALF there was a time lag in the elevation of LTC_4 , which may be attributable to the following.

It is well known that LTC_4 is produced by polymorpho-nuclear cells (neutrophils) and alveolar macrophages and by other lung tissue elements (9-11). Blood LTC_4 levels express the changes in the capillary bed level, while LTC_4 levels in BALF reflect more explicitly the changes in the pulmonary parenchyma and alveoli. Neutrophils trapped in the pulmonary vascular bed at the time of reperfusion may produce LTC_4 and be responsible for the generation of oxygen radicals. This would increase the permeability of the blood vessels injured by oxygen radicals and aggravate pulmonary edema. Pulmonary vessels would become constricted and the pulmonary vascular resistance would rise. Next, the injury

would extent to the pulmonary parenchyma and alveoli, and LTC_4 would be produced by the lung tissue, alveolar macrophages, and polymorpho-nuclear cells. Thus, the LTC_4 levels in BALF would eventually rise and pulmonary edema would be further aggravated.

We found that the elevation of the blood LTC_4 level preceded the LTC_4 in BALF, with the peak coming on the first day in both blood and BALF. However, the level was also elevated on the third day, and since the action of LTC_4 is prolonged it appears that the pulmonary edema reached its peak about the third day.

In the warm ischemia group, the serum TXA_2 level was significantly elevated as compared with the control group for only one hour after reperfusion, and fell again by 3 h after reperfusion. No significant changes were noted in the BALF TXA_2 level following warm ischemia. These results suggest that TXA_2 may be responsible for the elevation of the pulmonary vascular resistance only in the very early phase after reperfusion.

In conclusion, pulmonary edema was significantly worse in the warm ischemia group than in the control group, and LTC_4 levels in the blood and BALF correlated with the degree of pulmonary edema. This finding suggests that LTC_4 may be responsible for the development of pulmonary edema after warm ischemia-reperfusion. The rise of LTC_4 in the blood preceded that in the BALF, suggesting that reperfusion injury first occurs in the pulmonary vascular bed and then progresses to lung parenchyma.

References

1. Farber S: Studies on pulmonary edema: II. The pathogenesis of neuropathic pulmonary edema. *J Exp Med* (1937) **66**, 405-411.
2. McCord JM: Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med* (1985) **312**, 159-163.
3. Zimmerman ANE and Huelsmann WC: Paradoxical influence of calcium ions on the permeability of the cell

- membranes of the isolated rat heart. *Nature* (1966) **211**, 646-647.
4. Voelkel NF, Stenmark KR, Reeves JT, Mathias MM and Murphy RC: Action of lipoxygenase metabolites in isolated rat lungs. *J Appl Physiol* (1984) **57**, 860-867.
 5. Malik AB, Perman MB and Cooper JA: Pulmonary microvascular effects of arachidonic acid metabolites and their role in lung vascular injury. *Fed Proc* (1985) **44**, 36-42.
 6. Higgs GA, Moncada S and Vane JR: Eicosanoids in inflammation. *Ann Clin Res* (1984) **16**, 287-299.
 7. Miyai Y: Reperfusion injury of the rabbit lung after 6 hours preservation and the effects of free radical scavengers. *Jpn J Transplant* (1989) **24**, 337-343.
 8. Siegelmann SS, Sinha SBP and Vieth FJA: Pulmonary reimplantation response. *Ann Surg* (1973) **177**, 30-36.
 9. Hansson G and Radmark O: Leukotriene C₄: Isolation from human polymorpho-nuclear leukocytes. *FEBS (Fed Eur Biochem Soc) Lett* (1980) **122**, 87-90.
 10. Reuser JR, Scott WA, Hamill AL and Cohn ZA: Synthesis of leukotriene C₄ and other arachidonic acid metabolites by mouse pulmonary macrophages. *J Exp Med* (1982) **155**, 720-733.
 11. Robinson C and Holgate ST: Inophore-dependent generation of cicosanoids in dispersed lung cells. *Biochem Pharmacol* (1986) **35**, 1903-1908.

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