

Single Lung Transplantation for the Treatment of Monocrotaline-Induced Pulmonary Hypertension in the Rat

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Hemodynamic effects and plasma endothelin concentration following single lung transplantation for pulmonary hypertension were studied in rats with monocrotaline-induced pulmonary hypertension. Inbred male Lewis rats (weight 200-300 g) were divided into two groups. In Group 1 (control group), rats underwent syngeneic left lung transplantation. In Group 2, rats received intravenous administration of monocrotaline (80 mg/kg) and 3 weeks later underwent syngeneic left single lung transplantation. Hemodynamic evaluations were performed prior to transplantation, and one hour, 3 and 7 days after transplantation. The rats were then killed and the heart and lungs were removed to measure the ventricular weight ratio (RV/LV+S), extravascular lung water volume (ELWV : dry/wet ratio), and for histologic examination. Mean pulmonary arterial pressure (m-PAP) rapidly declined after transplantation in Group 2, from 39.3 ± 8.7 mmHg to 18.5 ± 3.0 mmHg 1 hour after transplantation, similar to the control (Group 1) animals, and remained stable through day 7. There was no significant difference in the mean ELWV of grafted lungs between the two groups (0.84 ± 0.03 vs. 0.86 ± 0.04). The mean ELWV in the native lung of Group 2 animals significantly increased on day 3 (0.86 ± 0.02) ($p < 0.01$), but subsequently decreased to control values by day 7 (0.83 ± 0.02). There was no significant difference in peripheral plasma endothelin levels between groups. These data demonstrate that monocrotaline-induced rat pulmonary hypertension does not affect plasma endothelin levels, and hemodynamic improvement can be obtained via single lung transplantation. We conclude that single lung transplantation is an effective modality for the treatment of pulmonary hypertension in the rat, and may eventually be useful in patients with advanced pulmonary hypertension.

Abbreviations : ELWV, extravascular lung water volume
m-PAP, mean pulmonary arterial pressure
s-AoP, systolic aortic pressure
POD, postoperative day

Key terms : monocrotaline
single lung transplantation
pulmonary hypertension
endothelin

Introduction

Single lung transplantation is a viable therapeutic modality for end-stage pulmonary diseases, including interstitial pulmonary disease, obstructive lung disease and various forms of pulmonary hypertension. Previous reports have shown that single lung transplantation can result in decreased pulmonary arterial pressure and pulmonary vascular resistance, and increased cardiac output with improvement of right ventricular function in patients with pulmonary hypertension.¹⁻⁴⁾

Endothelin is a newly isolated peptide derived from vascular endothelial cells.⁵⁾ Plasma endothelin concentrations are elevated in patients with pulmonary hypertension when compared to patients with other pulmonary disease.⁶⁾

In this study we evaluated the efficacy of single lung transplantation in rats with pulmonary hypertension, examined the transplanted lung for histologic evidence of damage, and measured plasma endothelin concentrations before and after transplantation in pulmonary hypertension.

Materials and Methods

Inbred male Lewis rats, weighing 200-300 g, were distributed into two groups. In Group 1 (control group) ($n = 16$, 11 weeks of age), rats underwent isogenic left single lung transplantation using a cuff technique.⁷⁾ In Group 2 ($n = 15$, 8 weeks of age), rats were treated with a single intravenous injection of 80 mg/kg monocrotaline, and underwent isogenic left lung transplantation three weeks later. Hemodynamic measurements were performed before transplantation and one hour and 3 and 7 days after the operation. Animals were killed after each hemodynamic measurement and the heart and lungs were removed. The right ventricle (RV) was isolated from the left ventricle (LV) and septum (S). The ventricular weight ratio was calculated by the formula $RV/LV+S$. Half-cut of the each right and left lungs were stained with hematoxylin-eosin, and examined by light microscopy. The remained half of

the each right and left lungs were weighed (wet weight : W), then stored in a heated chamber at 160°C for 48 hrs and weighed (dry weight : D). The extra-vascular lung water volume (ELWV) was determined after calculation of the wet/dry weight ratio (W-D/W).

Hemodynamic measurement

Animals were anesthetized with pentobarbital (25 mg/kg) and ketalar (12 mg/kg), and intubated, placed in the supine position, and a combined thoracotomy-laparotomy was performed. The left iliac artery was cannulated with a polyethylene tube (0.6 mm diameter) connected with transducer and polygraph (NIHON CODEN Co. Tokyo, Japan) and systolic aortic pressure (s-AoP) and blood gas concentrations were measured. Pulmonary artery pressure (PAP) was measured through a 27 gauge needle directly inserted into the pulmonary artery. This investigation conforms to the Guidelines for the care and use of laboratory animals published by the US National Institutes of Health (NIH publication No 85-23, revised 1985).

Radioimmunoassay of endothelin

Five milliliters of arterial blood were sampled from the left iliac artery, placed into a polypropylene tube containing heparin, and centrifuged at 2000×g for 10 minutes at 4°C. The plasma was collected and stored below -20°C prior to performing the radioimmunoassay.

Extraction of plasma endothelin was done using Amprep C2 columns (Amersham International plc, Buckinghamshire, UK). The column was equilibrated by washing with 2 ml methanol followed by 2 ml water. Each 1 ml plasma sample was acidified using 0.25 ml of 2 M HCl and then centrifuged at 10000×g for 5 minutes at room temperature prior to loading onto the column. After the columns were washed with 5 ml 0.1% trifluoroacetic acid, the absorbed endothelin-like substances were slowly eluted with 2 ml 80% methanol in 0.1% trifluoroacetic acid and collected in a polypropylene tube. The eluate was completely evaporated using a centrifugal evaporator (centrifugal freeze dryer Model RC-11, Yamato Chemical Co, Tokyo, Japan). Plasma endothelin levels were measured using a radioimmunoassay kit (endothelin 1-21 specific assay system ; Amersham International plc). Endothelin concentrations were determined by applying antibody to synthetic endothelin and iodine 125-labeled endothelin. This antibody cross-reacts 100% with endothelin-1, 144% with endothelin-2, 52% with endothelin-3, and does not cross-reacts with big endothelin (human) or atrial natriuretic peptide. Samples and standards (endothelin-1 ; Amersham International plc) were dissolved in assay buffer and incubated for 4 hours with rabbit anti-endothelin serum (Amersham International plc) at 4°C. Iodine 125-labeled endothelin was then added to each tube

and the mixture further incubate for 24 hours at 4°C. Donkey anti-rabbit serum coated onto magnetizable polymer particles (Amerlex-M second antibody reagent ; Amersham International plc) were added on the second day. The contents of each tube were vortex-mixed and incubated at room temperature of 15 minutes. After centrifugation at 1500×g for 10 minutes, the supernatant was removed, and the pellet was counted for iodine 125 with a gamma counter (auto well gamma system APC-300, Aloka. Co, LTD, Tokyo, Japan).

Statistical analysis

Results are presented as mean±standard deviation. The unpaired Wilcoxon test was used for all assays and measurements. Statistical significance was determined when $p<0.01$.

Results

Hemodynamic parameters and arterial blood gas data are shown in Table 1. There was no significant difference in arterial oxygen or carbon dioxide partial pressure between the both groups at any point. Mean PAP in Group 2 was 39.3 ± 8.7 mmHg before transplantation indicating successful induction of pulmonary hypertension. Mean PAP rapidly decreased to 18.5 ± 3.0 mmHg 1 hour after transplantation and remained at that level until POD 7. This was not significantly different from the mean PAP in Group 1 (Figure 1). The mean PAP standardized to the systolic aortic pressure (Table 1) was virtually identical to the mean PAP alone.

Before transplantation, the mean RV/LV+S weight ratio was 0.70 ± 0.08 in Group 2 and 0.31 ± 0.03 in the control group ($p<0.01$). The RV/LV+S weight ratio gradually decreased after transplantation, and there was no significant difference between the two groups on POD 7 (0.33 ± 0.04 vs. 0.40 ± 0.02) (Figure 2).

Mean ELWV of the graft lung was 0.82 ± 0.02 prior to transplant, 0.88 ± 0.03 1 hour after surgery, 0.84 ± 0.03 on POD 3, and 0.84 ± 0.02 on POD 7 in Group 1. Mean ELWV of the graft lung in Group 2 was 0.86 ± 0.01 prior to transplant, 0.87 ± 0.01 1 hour after surgery, 0.86 ± 0.04 on POD 3, and 0.84 ± 0.02 on POD 7. There was no significant difference in mean ELWV of the graft lung between the two groups. Mean ELWV of the right lung was 0.83 ± 0.02 1 hour after surgery, 0.82 ± 0.01 on POD 3, and 0.83 ± 0.01 on POD 7 in Group 1. Mean ELWV of the right lung in Group 2 was 0.84 ± 0.01 before the transplant, 0.85 ± 0.02 1 hour after surgery, 0.86 ± 0.02 on POD 3, and 0.83 ± 0.02 on POD 7. The mean ELWV of the right lung in Group 2 was higher than in Group 1, reaching significance on POD 3 ($p<0.01$) (Figure 3). The plasma endothelin levels were 24.5 ± 11.3 pg/ml before transplantation, 14.7 ± 0.9 pg/ml

Table 1. Hemodynamic parameters, arterial blood gas data and extravascular lung water content of the right and left lungs

	m-PAP (mmHg)	s-AoP (mmHg)	m-PAP/s-AoP	PaO ₂ (mmHg)	PaCO ₂ (mmHg)
Group 1					
pretransplantation (n = 5)	17.6±0.8	83.8±6.5	0.21±0.02	414.3±25.4	13.5±2.0
1 hour after transplantation (n = 4)	15.8±4.0	78.3±14.6	0.20±0.02	439.0±55.9	14.8±0.8
3 days after transplantation (n = 3)	21.0±2.2	76.7±9.4	0.28±0.03	454.5±54.4	13.1±1.5
7 days after transplantation (n = 4)	23.3±4.6	75.3±3.6	0.31±0.06	492.0±24.7	13.3±1.7
Group 2					
pretransplantation (n = 5)	39.3±8.4	96.0±14.3	0.39±0.13	409.4±49.2	14.3±1.7
1 hour after transplantation (n = 4)	18.5±3.0*	78.8±16.7	0.24±0.03*	428.2±88.7	14.2±5.2
3 days after transplantation (n = 3)	25.7±1.2	78.3±11.8	0.33±0.04	453.4±28.8	24.4±7.7
7 days after transplantation (n = 3)	26.0±2.2	83.3±4.2	0.31±0.04	506.5±26.7	20.2±8.8

All values are expressed as mean ± standard deviation. * p<0.01.
m-PAP, mean pulmonary arterial pressure ; s-AoP, systolic aortic pressure

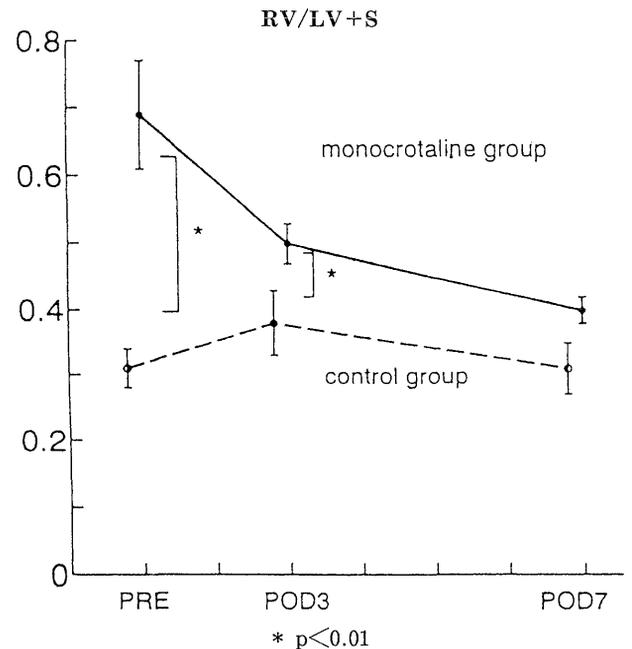
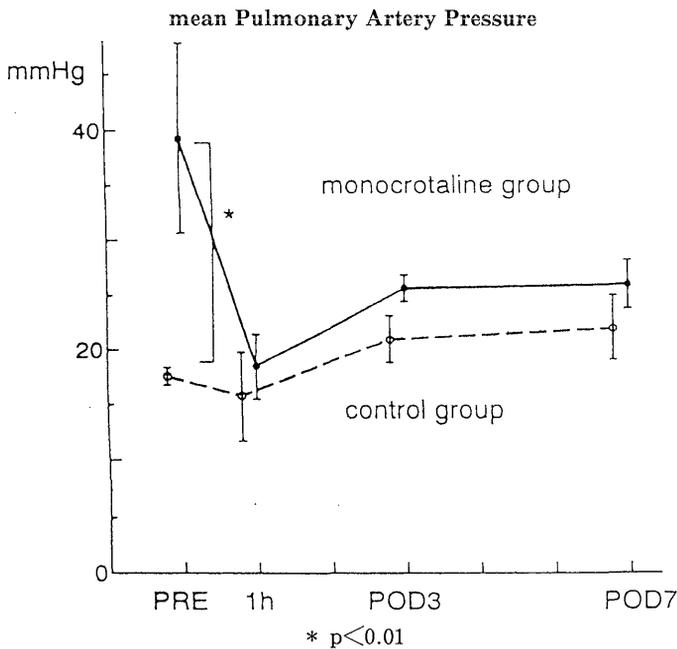


Figure 1. Mean pulmonary arterial pressure pre- and post-transplantation. Group 2 (closed circles), Group 1 (open circles). POD : postoperative day

Figure 2. Mean ventricular weight ratio (RV/LV+S) at 3 different time points. The values preoperatively, and on postoperative day 3 were significantly different between Group 2 (closed circles) and Group 1 (open circles) (p<0.01)

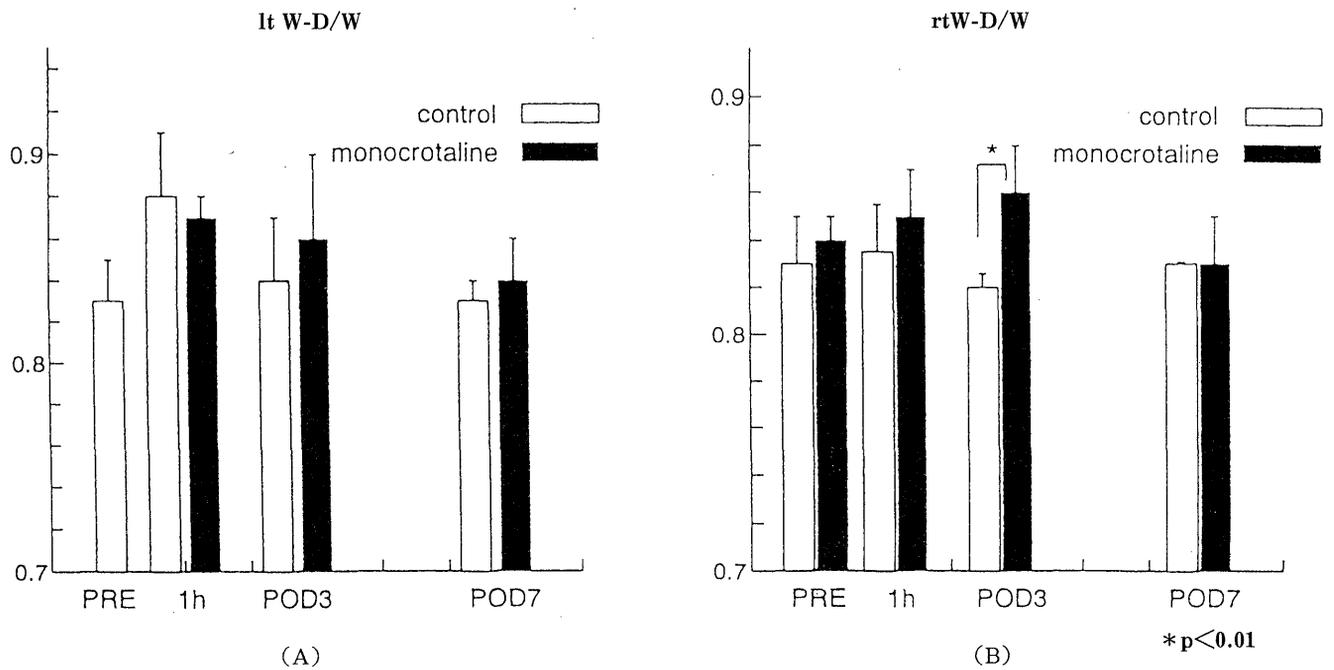


Figure 3. Extravascular lung water volume of the grafted lung (lt W-D/W) (A) and right lung (rt W-D/W) (B)

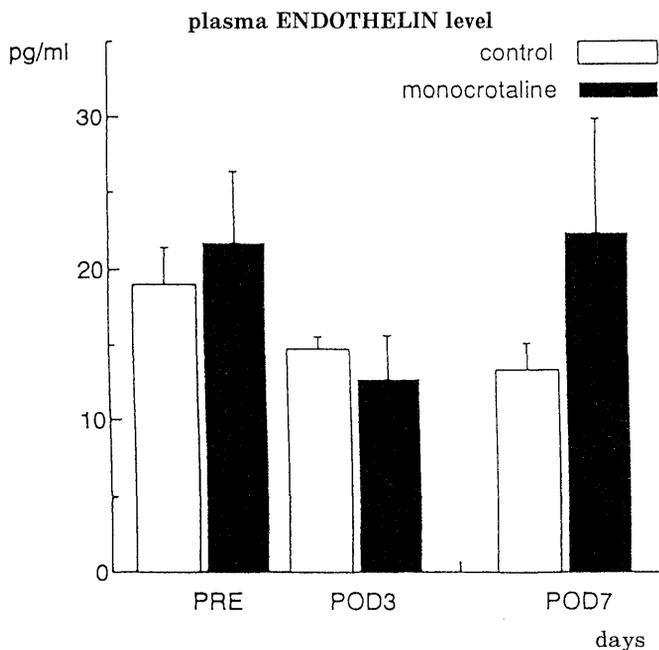


Figure 4. Mean plasma endothelin levels before and after single lung transplantation

on POD 3, and 13.3 ± 1.8 pg/ml on POD 7 in Group 1, and 21.6 ± 4.7 pg/ml before transplant, 12.8 ± 3.5 pg/ml on POD 3, and 22.4 ± 7.5 pg/ml on POD 7 in Group 2. There were no significant differences in peripheral plasma endothelin levels between the groups at any time point (Figure 4).

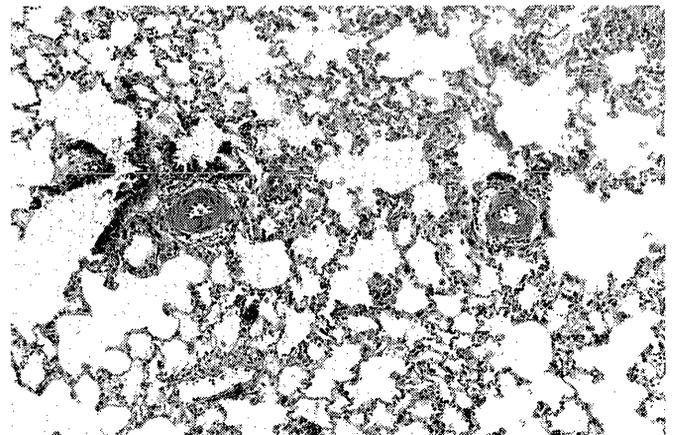


Figure 5. Photomicrograph of the lung 3 weeks after administration of monocrotaline (Hematoxylin-eosin stain $\times 40$)

Histologic examination revealed arterial wall thickness and interstitial cell proliferation in the lung 3 weeks after administration of monocrotaline (Figure 5). In the grafted lungs, massive repletion of red blood cells in alveolar space and edematous thickening of an alveolar wall were observed on day 3 after transplantation in Group 2 (monocrotaline+transplant). On day 7 in Group 2, histological findings were almost normal except minimal interstitial infiltration mainly composed of polymorphonuclear cells. Alveolar and interstitial edema was observed on day 3 after transplantation in Group 1 (transplant only), but congestion was not observed. On day 7 in Group 1,

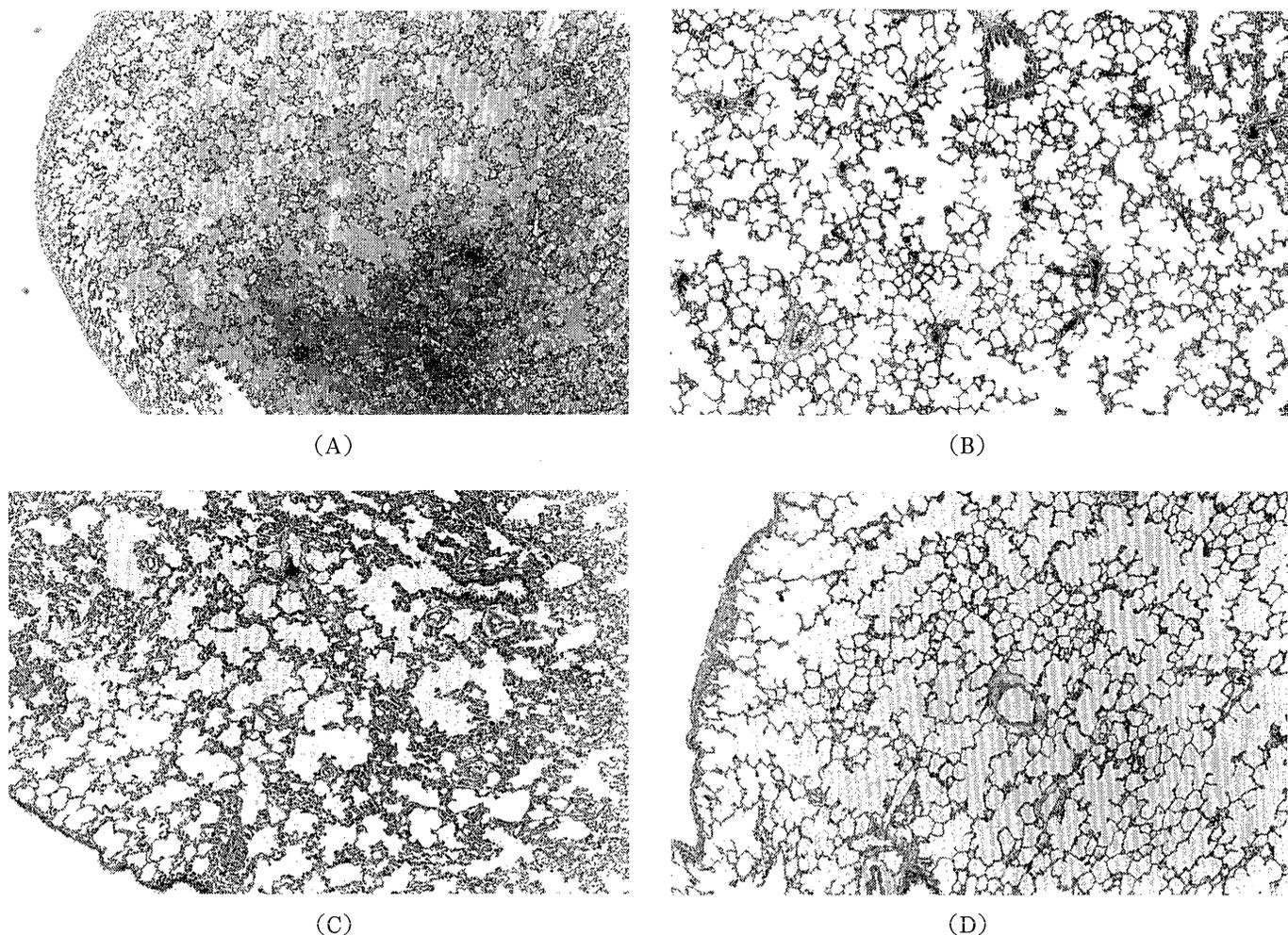


Figure 6. Photomicrographs of the grafted lung in Group 2 (monocrotaline-induced PH) on day 3 (A), and day 7 (B) after transplantation and the grafted lung in Group 1 (control group) on day 3 (C), and day 7 (D) (Hematoxylin-eosin stain $\times 20$)

histological findings were almost normal (Figure 6).

Discussion

Since its establishment in 1981 as the first successful surgical procedure for pulmonary hypertension,⁸⁾ heart-lung transplantation has been accepted as an effective treatment of patients with end-stage primary pulmonary hypertension. Few heart-lung transplant procedures are performed today. Heart-lung transplantation has rapidly been replaced by double lung and single lung transplants due to serious problems, such as peri-operative bleeding and bronchiolitis obliterans, and the scarcity of donors.

Animal studies support the hypothesis that a single transplanted lung can accommodate the total pulmonary blood flow,⁹⁾ and single lung transplantation is easier to perform than heart-lung transplantation.¹⁰⁻¹⁴⁾ After single lung transplantation in patients with pulmonary hyper-

tension, 70-90% of the total perfusion is distributed to the transplanted lung. Severe pulmonary edema often occurs in the postoperative course. In our study, ELWV of grafted lungs was elevated and histologic evidence of pulmonary edema and congestion was more remarkable in the lung recipient rats with monocrotaline-induced pulmonary hypertension than in lungs of control rats, in accord with the clinical course.

Monocrotaline is a pyrrolizidine alkaloid found in species of *Crotalaria* that has been shown to induce pulmonary vascular disease in the rat and has been used as a laboratory model of pulmonary hypertension.¹⁵⁾⁻¹⁷⁾ Pulmonary hypertension is induced in rats following a single dose of monocrotaline, but requires 2-3 weeks for full development. (In our study, the mean PAP was 39.3 ± 8.7 mmHg and the RV/LV+S weight ratio was 0.70 ± 0.08). Although the mechanism of pulmonary toxicity is unknown, it is generally accepted that monocrotaline requires metabolic activation by the hepatic mixed

function oxidase enzymes, and that a toxic metabolite is transported to the lung.¹⁸⁾⁻²²⁾

Endothelin, a newly isolated peptide from vascular endothelial cells⁴⁾, has vasoconstricting activity⁵⁾²³⁾ and induces vascular smooth muscle cell proliferation²⁴⁾. In patients with pulmonary hypertension, endothelin-1-like immunoreactivity is abundant, predominantly in patients with endothelial cell intimal fibrosis. Likewise, endothelin-1 messenger RNA is increased in the patients with pulmonary hypertension and was expressed primarily at sites of endothelin-1-like immunoreactivity.²⁵⁾ Plasma endothelin concentration in patients with primary or secondary pulmonary hypertension is greater than in patients with normotensive lung disease.²⁶⁾ The cause of the elevated plasma endothelin levels has not been known yet. In the present study, there was no significant difference in plasma endothelin concentration between rats with monocrotaline-induced pulmonary hypertension and control rats. Therefore, monocrotaline may not affect the production of this potent vasoconstrictor.

In summary, rat single lung transplantation as treatment of monocrotaline-induced pulmonary hypertension was shown to decrease PAP and improved right heart failure. However, the transplanted lungs in rats with pulmonary hypertension developed more marked pulmonary edema and congestion than lungs of control animals. Further investigation of the long-term function of grafted lung will better define the overall efficacy of single lung transplantation for the treatment of pulmonary hypertension.

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