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α-Adrenergic Blockade in Preventing Posttransplant Edema of Lung Allograft

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Kondo, T., Horikoshi, A., Fujimura, S., Matsumura, Y., Okada, Y. and Shimada, K. *α-Adrenergic Blockade in Preventing Posttransplant Edema of Lung Allograft*. Tohoku J. Exp. Med., 1999, **189** (2), 135-145 —— Effect of α-adrenergic blockers on pulmonary edema in lung transplantation was studied with a rat model of syngeneic left lung transplantation. Prior to harvesting, 0.1 mg of Prazocin or 0.4 mg of Yohimbin was given to the donor. Pulmonary and systemic hemodynamics were measured under the right pulmonary arterial occlusion (RPAO) at different time points after grafting. Wet to dry weight ratio (W/D) of all transplants was also calculated. Same procedure was conducted in rats with normal and ischemic lung and in transplanted animals without any treatments. While RPAO did not increase W/D in normal lung with a significant elevation in pulmonary arterial pressure (PAP), both these values significantly increased in transplanted lung. Transplanted animals could not tolerate RPAO 24 hours after grafting, but were tolerable later than 48 hours with elevated W/D and PAP. On the contrary, animals given Prazocin or Yohimbin were all tolerable at 24 hours postsurgery. Yohimbin significantly improved W/D. Consequently, it was demonstrated that pulmonary edema of the graft reached its peak during first 24 to 48 hours after transplantation and was alleviated by the blockade of α-adrenergic recepter in the graft vessel. ———— lung transplantation; reimplantation response; pulmonary edema; α-adrenergic blockade

Although lung transplantation has been established as an optional treatment for irreversible endstage pulmonary diseases, survival rate is still not satisfactory. According to the analysis of recipient deaths in St. Louis International Lung Transplant Registry in 1997, the primary organ failure is still one of the major causes of the early deaths (14%) after lung transplantation. Since pulmonary edema of the lung allograft in the early stage that has been called reimplantation

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response may be contributory to this category, it is necessary to elucidate the pathogenesis and to establish the method for management of this condition in order to improve the result of lung transplantation, especially in its early stage after surgery. Previous studies on the function of catecholamine receptors in the cardiac allograft (Dorothy et al. 1985; Curz Caturla et al. 1992; von Scheidt et al. 1992) and on the neurogenic pulmonary edema (Malik 1985) revealed the accelerated function of adrenoreceptors in the cardiac allograft and the development of pulmonary edema by the sudden autonomic disturbance. These studies indicate the possibility of the presence of a same kind of event in the pulmonary allograft, which may be contributory to the development of the graft edema after lung transplantation. Based on these hypotheses, we focused on the significance of the adrenergic blockade in the treatment of pulmonary edema after lung transplantation in this study.

Materials and Methods

Lung transplantation

Using inbred strain of 106 male Buffalo/Ma rats weighing 250 g to 330 g, 53 syngeneic orthotopic left lung transplantation were performed with the technique previously reported (Kondo et al. 1986). In brief, recipient rats were anesthetized with the inhalation of Enfluren followed by the intraperitoneal administration of 50 mg/kg of the pentobarbital sodium and 0.01 mg of atropine sulfate. A mechanical ventilation (tidal volume; 8 ml/kg, frequency; 100/minutes, PEEP; 5 cmH_2O, FiO_2; 0.21) was established with a small animal ventilator (Model 683, Harvard Apparatus, Holliston, MA, USA) through an endotracheal tube (ID 19G, OD 16G). Polyethylene tube (ID 0.5 mm, OD 0.9 mm) was placed in the left jugular vein to administer designated agents or physiological saline. Left thoracic cavity was entered through the 4th intercostal space under the right lateral position, pulmonary hilar structures were divided, and the left main bronchus, left pulmonary artery and left pulmonary vein were clamped with surgical clips for neurosurgery to remove the left lung. Donor rats were anesthetized and operated in the same way with the intravenous administration of 300 units of heparin sodium. Pulmonary vein, bronchus and pulmonary artery were all anastomosed with 9-0 nylon (CY 4 m/n, Crownjun, Chiba) by a running suture. Endotrachel tube and the chest tube were removed after the establishment of the spontaneous breathing, and animals which were scheduled to be examined later than 24 hours were recovered from the anesthesia to survive postoperative days. Surgical procedure for lung transplantation was conducted with the aid of surgical microscope. In addition to these animals, 5 and 4 male rats were assigned to normal control group and ischemic group, respectively, to obtain the data without transplantation.

All animals have received humane care in compliance with the guideline prepared by Institute of Laboratory Animal Resources and published by the

National Institute of Health (NIH Publication No. 85-23, revised) (Clark et al. 1985).

Experimental groups

1. Normal control group (NC group): normal rats without any treatment or surgery ($n=5$).

2. Ischemic group (I group): normal rats with a collapsed left lung by clamping both pulmonary artery and left main bronchus for 55 minutes followed by the right pulmonary arterial occlusion (RPAO) by ligation 30 minutes after the start of reperfusion ($n=4$). The duration of ischemia was determined by our previous studies in which the maximum ischemic time for performing the left lung transplantation was 55 minutes.

3. Transplantation control group (TC group): left-lung transplanted rats without any treatment ($n=29$).

4. Prazocin group (T+P group): left-lung transplanted rats from donors treated with 0.1 mg of Prazocin as an α-1 adrenergic blocker prior to harvesting ($n=10$).

5. Yohimbin group (T+Y group): left-lung transplanted rats from donors treated with 0.4 mg of Yohimbine as an α-2 adrenergic blocker prior to harvesting ($n=14$).

Measurement of pulmonary hemodynamics

Pulmonary hemodynamics and blood gases were measured under the median sternotomy 30 minutes ($n=4$), 24 hours ($n=4$), 48 hours ($n=4$), 72 hours ($n=4$), 7 days ($n=4$), 21 days ($n=5$) and 28 days ($n=4$) after surgery in TC group, and 30 minutes and 24 hours after surgery in T+P and T+Y groups. All measurements were performed under both bilateral pulmonary perfusion and RPAO. Five ml an hour of physiological saline was infused with a syringe pump (SP-60, Nipro, Osaka) through an intravenous polyethylene catheter. The trachea was ligated with an endotracheal tube to avoid air leakage around the tube. Arterial line was secured with a catheter (24G, Terumo, Tokyo) placed in the right carotid artery, and the pulmonary arterial pressure (PAP) was monitored through a polyethylene catheter (ID 0.5 mm, OD 0.9 mm) placed in the pulmonary arterial trunk from the outflow portion of the right ventricle (Fig. 1). PAP is expressed as mean value in the following part of this report. Pulmonary and systemic hemodynamics and arterial blood gases were measured 5 minutes after the ligation of right pulmonary artery under the following ventilatory conditions; 8 ml/kg of tidal volume, 140/minutes of frequency, 5 cmH_2O of PEEP, and 100% of Oxygen. After finishing measurements, transplants were transected to determine wet to dry weight ratio (W/D) that was obtained by the wet weight immediately after the removal and the dry weight after the dehydration in the oven at a temperature of 50°C for 48 hours.

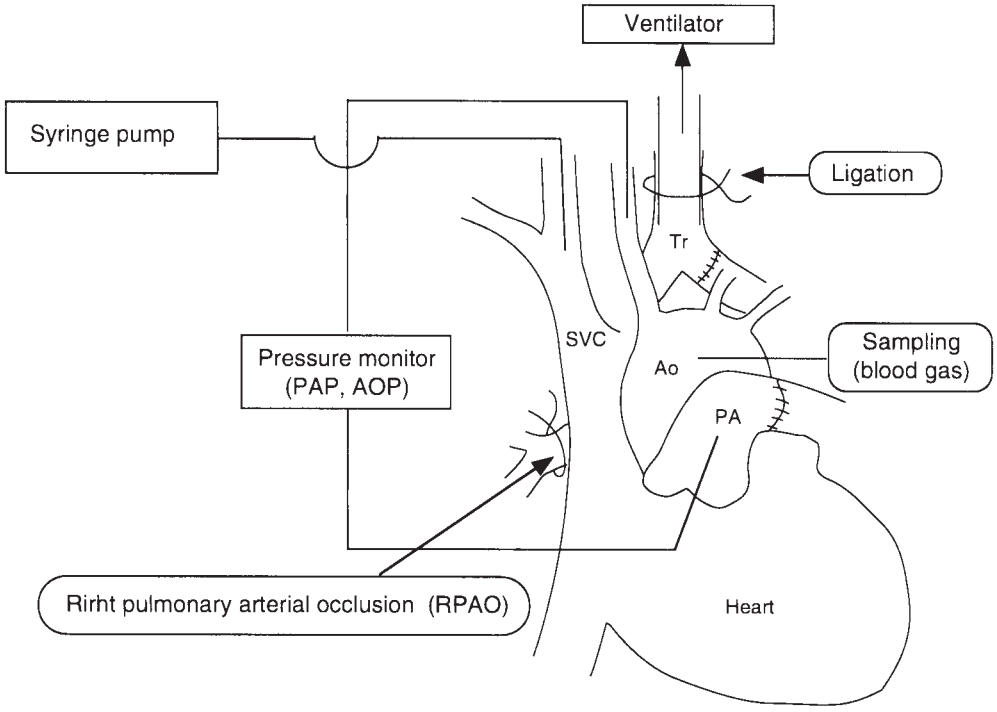

Fig. 1. Schematic illustration of the experimental setting.

Administration of α-adrenergic blockers

α-Adrenergic blockers were given to donor rats before harvesting in order to neutralize the function of α-adrenergic receptors of the pulmonary vasculature in lung transplants, since severe hypotension was encountered when they were given to recipient rats. To determine the sufficient doses of these agents, previous physiological studies on α-blockers were referred (Hyman and Kadowitz 1986), and three- to four-fold amount compared to that used in previous reports was given to obtain the sufficient effects in this study. Preliminary studies showed that the mean systemic pressure fell down to 40 to 50 mmHg immediately after the administration of designated doses of these agents.

Data analysis

All values were expressed as mean ± S.D. and p values less than 5% that were calculated with the analysis of variance were designated as statistically significant.

Results

Pulmonary hemodynamics and W/D in ischemia and transplantation

PAP was significantly elevated under RPAO in normal rats without the increase in W/D (NC group). On the contrary, significant increase in W/D was observed with the same extent of the elevation of PAP when left lungs were exposed to 55-minute ischemia under RPAO (I group). In left-lung transplanted

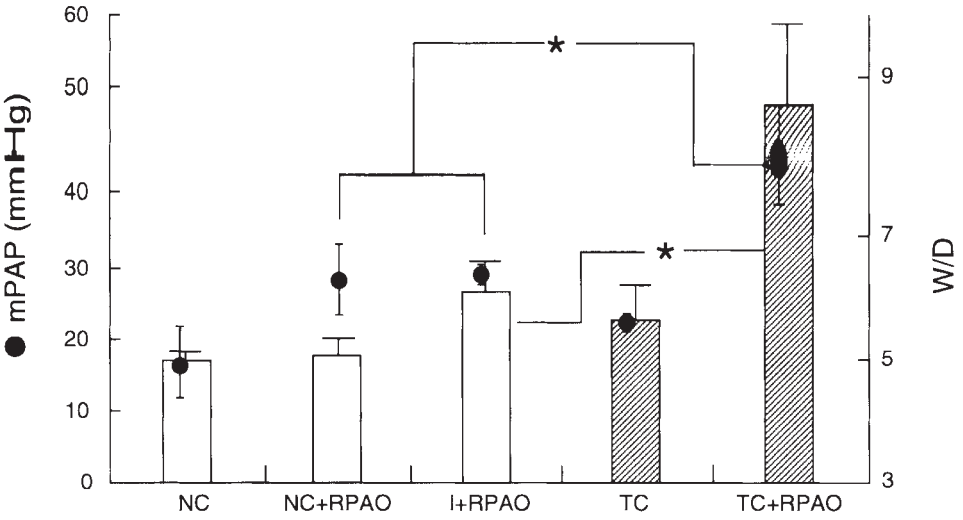

Fig. 2. Effect of RPAO, 55-minute ischemia and transplantation on the mean pulmonary arterial pressure (mPAP) and the wet to dry weight ratio (W/D) of lung transplants. White bar and shaded bar indicate wet to dry weight ratio in the native and the transplanted lung, respectively. $^*p<0.05$.
NC, normal control group; I, ischemic group; TC, transplantation control group; RPAO, right pulmonary artery occlusion.

animals, further increase in values of both PAP and W/D were observed under RPAO at 30 minutes posttransplant (TC group), and these values were significantly higher than normal and ischemic lungs (Fig. 2).

RPAO later than 24 hours after surgery in left-lung transplanted animals (TC group)

Twenty four hours after transplantation, all animals in TC group could not tolerate RPAO and died during the procedure. The most suspicious cause of

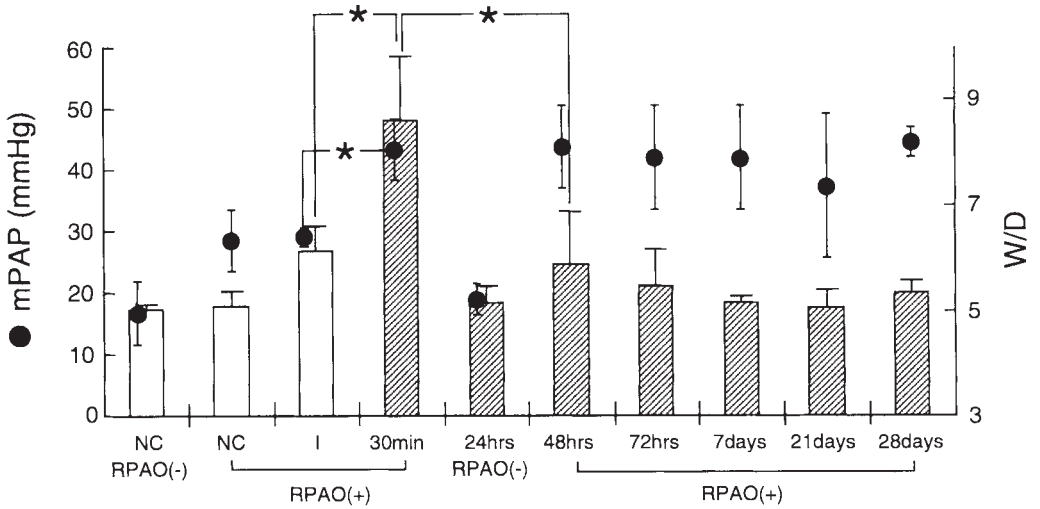

Fig. 3. Mean pulmonary arterial pressure and wet to dry weight ratio (W/D) at different points after lung transplantation. White bar and shaded bar indicate W/D in the native and the transplanted lung, respectively. Animals were all untolerable to RPAO at 24 hours and PAP was measured under bilateral lung perfusion in this group. $^*p<0.05$.

TABLE 1. *Arterial blood gases of NC and TC groups*

	NC	NC+RPAO	TC+RPAO					
			30 min	48 hrs	72 hrs	7 days	21 days	28 days
PaO_2 (torr)	441.8	472.0	417.3	442.4	436.3	444.6	425.8	473.5
$PaCO_2$ (torr)	45.2	44.0	50.2	47.5	62.0	59.8	51.3	55.8

NC, Normal control group; RPAO, Right pulmonary artery occlusion; TC, Transplantation control group.

death was cardiac failure because instantaneous striking elevation of pulmonary arterial pressure was observed when the right pulmonary artery was ligated. However, animals in this group that survived for 48 hours revealed to tolerate RPAO with a significantly high PAP compared to those with normal or ischemic lung. This elevation in PAP during RPAO was also observed on day 3, 7, 21, and 28 after transplantation (Fig. 3). In contrast, W/D of transplanted left lungs did not show any increase in animals died from RPAO 24 hours after surgery. After 48 hours, lung transplants showed slight increase in W/D under RPAO though not significant.

There was no significant difference in PaO_2 under RPAO at 30 minutes, 3, 7, 21, and 28 days after transplantation (Table 1), however, PaO_2 under RPAO after

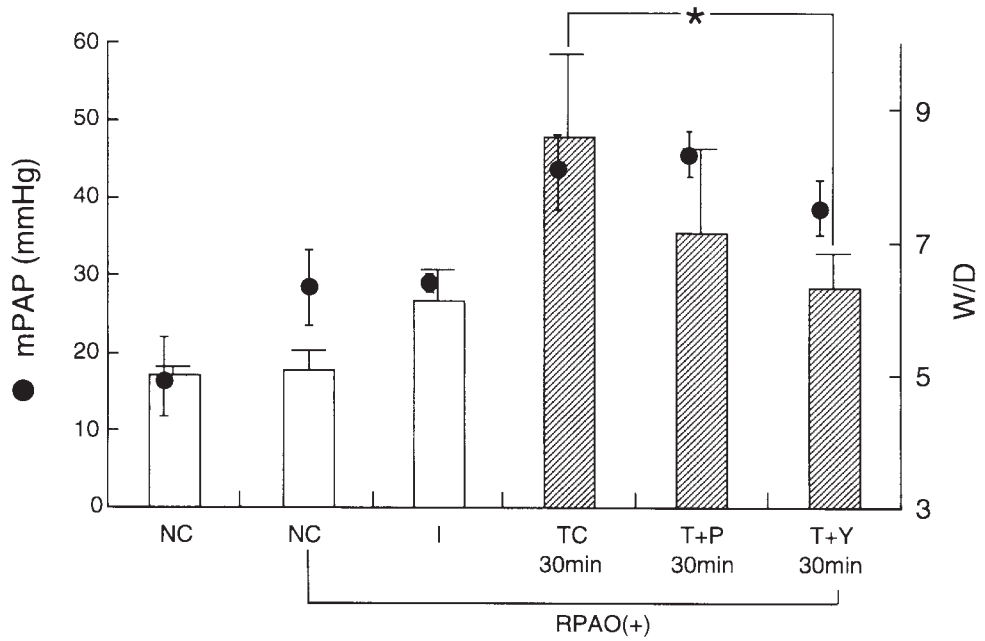

Fig. 4. Effects of Prazocin and Yohimbin on the mean pulmonary arterial pressure and the W/D under RPAO 30 minutes after grafting. White bar and shaded bar indicate wet to dry weight ratio in the native and the transplanted lung, respectively. $^*p<0.05$.
NC, normal control group; I, ischemic group; TC, transplantation control group; T+P, prazocin group; T+Y, yohimbine group; RPAO, right pulmonary artery occlusion.

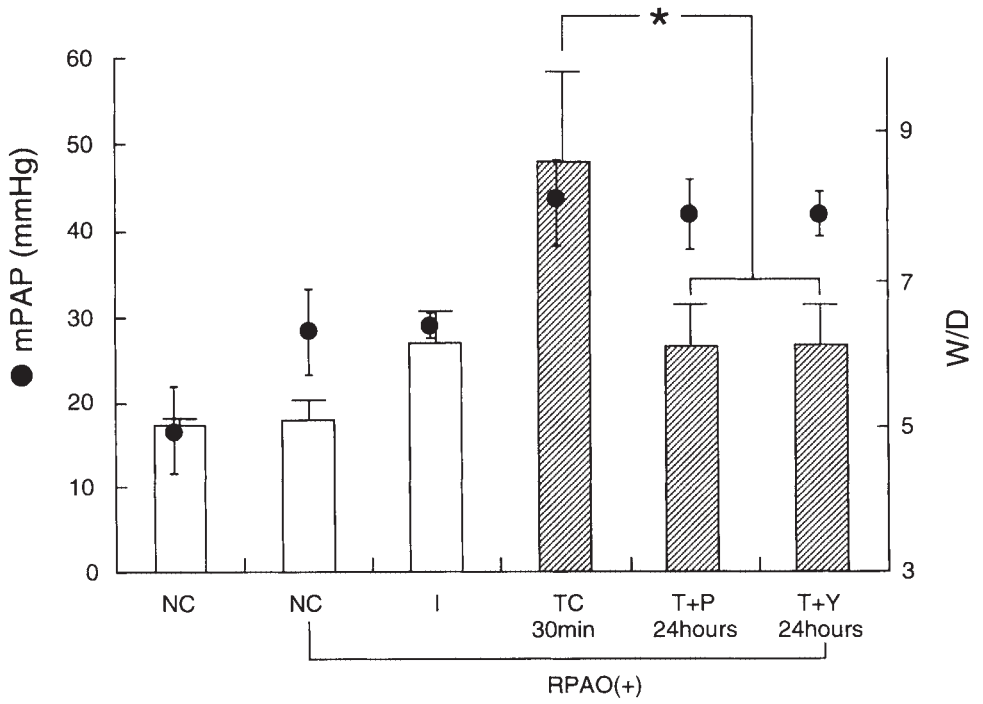

Fig. 5. Effects of Prazocin and Yohimbin on the mean pulmonary arterial pressure and W/D under RPAO 24 hours after grafting. White bar and black bar indicate wet to dry weight ratio in the native and the transplanted lung, respectively. $^*p<0.05$.

24 hours could not be measured because of an intolerable condition to RPAO.

Effects of α-1 adrenergic blocker (T+P group)

Although Prazocin administration to grafts prior to harvesting did not improve PAP and W/D significantly under RPAO at 30 minutes after surgery when compared to animals with transplants without any treatment (Fig. 4), this procedure revealed to enable animals to tolerate RPAO at 24 hours after transplantation. While PAP under RPAO remained as high as that observed at 30 minutes after grafting in TC group, W/D in T+P group significantly decreased (Fig. 5).

Effects of α-2 adrenergic blocker (T+Y group)

Animals with Yohimbin treated grafts showed slightly lowered PAP and significant decrease in W/D compared with those in TC group at 30 minutes after grafting (Fig. 4). At 24 hours postsurgery, animals could tolerate RPAO by Yohimbin treatment, and W/D was significantly lower than those observed at 30 minutes in TC group (Fig. 5).

Discussion

A lot of studies on the effect of autonomic denervation on the function of transplanted organs have been performed to date. In the field of cardiac trans-

plantation, the supersensitivity of β-adrenergic receptor has been noted (Beck et al. 1969; Carleton et al. 1969), and this is considered to enable transplanted heart to be regulated only by circulating catecholamines without an autonomic regulation. This phenomenon is known to be observed until at least several months after transplantation. Although the mechanism of supersensitivity is not clearly understood, it is postulated that the excess amount of catecholamines around adrenergic receptors due to the loss of uptake of catecholamines by the presynaptic nerve endings plays an important role in the development of this phenomenon (Curz Caturla et al. 1992; von Scheidt et al. 1992). In the field of lung transplantation, we have reported that the higher elevation of pulmonary arterial pressure and calculated total pulmonary vascular resistance was noted in the transplanted lung compared to normal lung when contralateral pulmonary artery was occluded in canine model of unilateral lung transplantation (Suzuki et al. 1969). Since catecholamine receptors are known to be also widely distributed in the vascular wall, it is hypothesized that the same kind of supersensitivity of adrenergic receptors in the pulmonary vessel takes place in the lung allograft. Accordingly, this functional alteration of the adrenergic receptor due to the autonomic denervation in lung transplantation can cause elevated tonus of the pulmonary vessel.

It is well known that a transient pulmonary edema called reimplantation response occurs and reaches its peak several days after lung transplantation. Although denervation, the impairment of lymph drainage, ischemia reperfusion, or combination of these has long been paid attention as a cause(s) of this phenomenon, the mechanism of its development is still unclear. In postulating the mechanism of reimplantation response, a pathophysiological study on the neurogenic pulmonary edema is quite suggestive. Severe damage of central nervous system is well known to cause pulmonary edema by the extensive contraction of pulmonary vessels leading enlargement of the pore size of the vessel wall (Malik 1985). Although the region of impairment of the autonomic nerve is different between these two conditions, neurogenic pulmonary edema shows an intimate correlation between the impairment of adrenergic receptor and pulmonary edema, and this mechanism may be able to explain the cause of reimplantation response in lung transplantation.

As a result of this study, the increase in W/D was significantly larger in the ischemic lung than in the normal lung under RPAO in spite of the same extent of elevation of PAP in both groups. This is likely to be caused by the accelerated permeability of the pulmonary vessel in the ischemic lung. When the same procedure was performed in the transplanted lung (TC group), further elevation of PAP and W/D was encountered at 30 minutes after surgery. Although cardiac output was not measured in this study without an available device at hand, the increased vascular resistance rather than the increased cardiac output can explain the elevated PAP. Same extent of elevation of PAP was noted until 28 days after surgery, however, increased W/D was observed only at 30 minutes after

transplantation, suggesting the increased vascular permeability to be transient after lung transplantation. When right pulmonary artery was ligated after 24 hours after surgery, prompt and extreme rise of PAP was observed instantaneously before cardiac arrest, but W/D of the graft proved to be normal, indicating the cause of death to be cardiac failure due to the extremely elevated pulmonary vascular resistance rather than pulmonary edema. Possibly, the shift of the blood flow to the normal right lung may prevent pulmonary edema in the graft at this period. Our previous study on canine lung transplantation (Suzuki et al. 1969) also supports this hypothesis. Consequently, it is postulated that the accelerated pulmonary vascular permeability which develops immediately after grafting followed by the extensively increased vascular resistance probably due to the vasoconstriction by 24 hours after surgery subsides completely by 48 hours after transplantation with the improvement of the vascular tonus. Although persistent elevation of PAP under RPAO in the chronic stage may be caused by the reduced elasticity of arterial wall at the suture line, our previous study have demonstrated sufficient blood flow distribution to the transplanted left lung under bilateral lung in the same rat model (Kondo et al. 1986). On the contrary, vasoconstriction is the most likely to be the cause of the temporary but extensive increase in vascular resistance which was observed 24 hours after surgery. Although micro thrombosis/embolism in the pulmonary vessel can be the cause of the elevated vascular resistance in this situation, it does not seem to be contributory, since animals survived 48 hours after surgery could tolerate RPAO without any anticoagulants. Accordingly, the lung allograft is possibly in critical condition during the first 24 to 48 hours after transplantation. Animals in TC group could not have survived this critical period without normal right lung, possibly supporting the inferior survival rate of the early stage of lung transplantation for patients with pulmonary hypertension in which transient hyperdynamic condition in pulmonary hemodynamics appears immediately after grafting.

When the results of PAP and W/D at 30 minutes postsurgery were compared in T+P and T+Y groups to examine the effect of α-blockers (Prazocin and Yohimbin) on altered pulmonary hemodynamics in lung transplantation, these agents were demonstrated to improve the graft condition. Particularly, Yohimbin (α-2 blocker) proved to improve the W/D of the graft significantly, strongly indicating the participation of altered function of adrenergic receptor on the development of the increased pulmonary vascular permeability that is observed immediately after transplantation. In spite of the dramatic improvement in W/D, PAP did not significantly fall with the use of an α-2 blocker. As previously mentioned, this moderate elevation of PAP under RPAO is possibly caused by the loss of elasticity at the anastomotic site, since the same extent of elevation was observed until 28 days after surgery. Although α-blockers did not dilate the anastomotic site and the moderate elevation of PAP was still observed, blocking of the α-adrenergic receptors in the graft effectively improved the peripheral

pulmonary circulation, since the lethal elevation of the graft vascular resistance 24 hours after surgery was completely ameliorated by the procedure. However, improvement of blood flow to the graft is expected to exacerbate pulmonary edema when the vascular permeability remains to be accelerated. In this study, α-2 blockade is suspected to improve not only vascular resistance but also vascular permeability, since the animals were tolerable to RPAO at 24 hours postsurgery without pulmonary edema. It is not clear whether the fact observed at 24 hours postoperatively is a direct effect of α-blockade or not, since there are no data on how long the effect of α-blockade persists under denervated condition. Accordingly, it cannot be denied that the beneficial effect of α-blockade on the pulmonary vessel immediately after surgery result in the maintenance of vascular function leading to the following better result.

From the results obtained by the present study, it was concluded that both α-1 and α-2 adrenergic blockers are helpful in managing pulmonary edema in lung transplantation. Among them, α-2 adrenergic blocker proved to dramatically improve both vascular permeability and tonus, resulting in the elimination of pulmonary edema immediately after surgery and making animals to be tolerable to RPAO at 24 hours posttransplantation. The mechanism of the different results in the effect of these two agents is not clearly understood from the present study. Previous studies on the response of pulmonary vessels to the adrenergic stimulation (Hyman and Kadowitz 1981), in which the sensitivity of α-2 receptors is accelerated higher than α-1 receptors under the hypertonic condition of pulmonary vessels, may be helpful for further investigations.

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