

Evaluation of transplanted lung function and immunosuppressive effect of Cyclosporin A

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The effects of immunosuppressive drugs were studied in terms of pulmonary function of canine lung allografts by means of unilateral pulmonary artery occlusion test (UPAO-test) on day 0, 7 and 14 following left allotransplantation in mongrel dogs of which azathioprine (Aza) in 26 dogs and cyclosporin A (CyA) in 15 dogs were given as compared with left lung autografts in 26 dogs.

The results were as follows.

1) In left lung autografts, the mean pulmonary artery pressure (m-PAP) and the pulmonary vascular resistance (PVR) in UPAO-test remained high in relation to the resulting vasospasm from denervation and the stenosis at anastomosis of the pulmonary artery.

2) In most of the autografts, histologic examination of lung grafts revealed a lesion of pneumonitis which seemed to be mainly caused by difficulty in expectorating sputum due to denervation.

3) The results obtained from the pulmonary function test by UPAO-test regarding PaO₂, PaCO₂, and/or m-PAP, PVR and intrapulmonary shunt were much more satisfactory in CyA-administered group rather than in Aza group.

4) The immunosuppressive effects in CyA group were superior to those in Aza group from the standpoint of histologic observation as to perivascular cell infiltration (cuffing).

INTRODUCTION

Since lung transplantation was first applied for clinical use by HARDY¹⁾ in 1963, experience of no more than 50 cases has been accumulated all over the world so far. Nevertheless, long-term two survivors of more than 1 year have been obtained by COOPER, Toronto Canada.

Basic problems regarding lung allotransplantation remained still unsolved. In the present study the pulmonary functions of autografts were evaluated by means of UPAO-test to make it much clear and precise and the immunosuppressive effects were compared in terms of transplanted lung function between Aza and CyA.

MATERIAL AND METHOD

67 Mongrel dogs, weighing 9 to 24kg with an average of 11.5kg were subjected to left lung transplantation, in which 26 dogs received autografts, and 41 allografts utilizing Aza in 26 and CyA in 15. The animals were given pentobarbital sodium of 25mg/kg intravenously and ventilated by Harvard ventilator. In the left uppermost position, left thoracotomy was performed through the 5th intercostal space. The left main bronchus and pulmonary artery were dissected out and separated to divide the branches of the bronchial artery, lumph vessel and vagal nerve. The pulmonary vein also was divided at the left atrial wall attaching the left pulmonary vein. Immediately before taking off the donor of lung, 5000 IU heparin was infused in the state of deflation. The left lung of donor was taken out and transplanted to the recipient in the following order of anastomosis, that is, the left atrial cuff of the pulmonary vein, the left main pulmonary artery and the left main bronchus, the suture material of 5-0 proline was used for vessel anastomoses and 4-0 nylon was for bronchial one using the continuous suture technique.

The immunosuppressive drugs were given from day 0 to the sacrificed day; 26 dogs received Aza at the dosis of 5mg/kg/day and 15 dogs received CyA at the dosis of 20 mg/kg/day. To assess the circulatory function of transplanted lung, UPAO-test was applied on day 0, 7 and 14 following lung transplantation using two Swan-Ganz catheters one of which was used for occlusion of the right pulmonary artery and the other for measurement of the pulmonary artery pressure (PAP) and pulmonary wedge pressure (pwp) before occlusion, at 5min, 10min following occlusion and at 5min after release of occlusion respectively.

Cardiac output was measured by means of thermodilution and calculated by using the cardiac output computer (MODEL 9520, EDWARD LAB. CO.). The hemodynamic study by using UPAO-test was discontinued in case of outbreak of bradycardia and hypotension below 50 mmHg of max systolic pressure. Blood gas analyses of samples taken from both femoral and pulmonary arteries were made with use of IL-Micro 13, and the intrapulmonary shunt was also calculated by the following formula. $Qs/Qt = CcO_2 - CaO_2 / CcO_2 - CvO_2$,

where $PAO_2 = 1.0 \times (760 - 47) - PaCO_2 / 0.8$

$$CcO_2 = SAO_2 \times (Hb \times 1.39) + (PAO_2 \times 0.0031)$$

$$CaO_2 = SaO_2 \times (Hb \times 1.39) + (PaO_2 \times 0.0031)$$

$$CvO_2 = SvO_2 \times (Hb \times 1.39) + (PvO_2 \times 0.0031)$$

The PVR was also calculated as follows,

$$PVR = (m \cdot PAP - LPWP) / CI \times 80 \text{ dyne. sec/cm}^5$$

LPWP: Left pulmonary wedge pressure CI: Cardiac Index

autopsy was carried out in dogs which UPAO-test could be satisfactorily completed and histologic examination was done as to whether the lesion of pneumonitis in autografts or perivascular cuffing (PC) which was one of the rejection signs in allografts, existed or not in comparison with hemodynamic parameters of PaCO₂, PaO₂ and m-PAP values.

RESULTS

The mortality rates in this study were compared between auto- and allo-lung transplantations with respect to the kinds of immunosuppressive drugs of Aza and CyA as shown in Table I. In autografts, the mortality was 31% (8 out of 26 dogs). In allografts given Aza it was 35% (9 out of 26 dogs) and as contrast with the above, in CyA it was 20% (3 out of 15 dogs). These differences were not statistically significant. The tolerance rates of PA-blocking test of healthy lung were 38% (10/26) in autografts, 42% (11/26) in allografts given Aza and 53% (8/15) in allografts given CyA as indicated in Table II. CyA administration allowed UPAO-test to perform much more satisfactorily than Aza.

During UPAO-test, PaCO₂ did not markedly fluctuated in autografts as shown in Fig. 1. It was, however, significantly increased in Aza ($P < 0.05$) and in CyA ($P < 0.01$) on day 0 and 7, but PaCO₂ in auto-grafts ($P < 0.05$) as compared with those in allografts on

Table I. Mortality rate in lung graft of canine

		Autograft	Allograft (Aza)	Allograft (CyA)
graft	(n=67)	26	26	15
death*	(n=20)	8	9	3
mortality rates (%)		31	35	20

*death within 7 day

Table II. Tolerance rate of unilateral pulmonary artery occlusion test of healthy lung

	Postgraft day	Autograft			Allograft (Aza)			Allograft (CyA)		
		0	7	14	0	7	14	0	7	14
graft dog	(n)	26	18	11	26	17	10	15	11	8
UPAO-test	(n)	10	8	7	11	8	8	8	8	7
tolerance rates (%)		38	44	64	42	47	80	53	72	87

n: number, UPAO: unilateral pulmonary artery occlusion

day 14 risen remarkably. On the other hand, Fig. 2 showed that fall in PaO₂ by UPAO-test was significantly noticeable ($P < 0.01$) in autografts on day 0 and day 14 as compared with those in allografts ($P < 0.05$ in Aza, $P < 0.01$ in CyA respectively). The m-PAP values remained high in Aza group on day 0 to 14, although those remained low in CyA group as shown in Fig. 3. The PVR showed low values in autografts and allografts on day 7 as compared with those on day 0 as shown in Fig. 4. However, those in CyA group on day 14 remained significantly low ($P < 0.01$) as compared with those in auto- and allografts given Aza.

The intrapulmonary shunt rate was much more increased on day 0 and reduced on day 14. Those in Aza group kept high on day 7 and 14 ($P < 0.05$) although those in CyA showed low values as compared with those in the other two groups as shown in Fig. 5. The calculated CcO₂-CaO₂ and CcO₂-CvO₂ values were compared. On day 0, CcO₂-CaO₂ and CcO₂-CvO₂ values varied significantly and CcO₂-CvO₂ values were significantly altered in autografts on day 7 and 14 as shown in Fig. 6. The result is a reflection of the fact that changes of the intrapulmonary shunt rates depend mainly upon variation of CaO₂ and CvO₂ values on day 0 in allografts in contrast to pronounced fluctuation of CvO₂ values on day 7 and 14 in autografts.

Autopsy showed a 20 to 30% stenosis at anastomosis of the pulmonary artery which was observed almost in all dogs without thrombus formation. In Aza group of allografts, round cell infiltration was seen in the areas of perivascular space and alveolar septum in

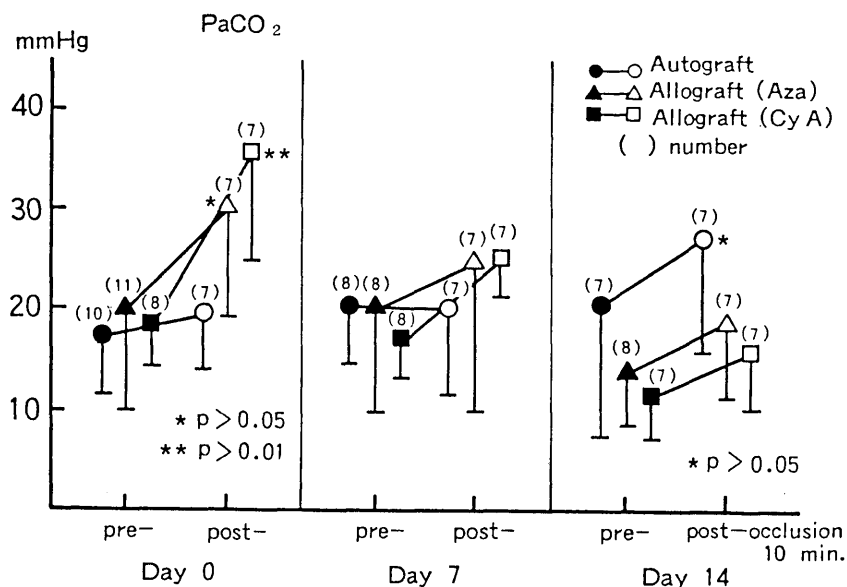


Fig. 1. PaCO₂ values of Auto- and Allograft in UPAO-test on day 0, 7 and 14. PaCO₂ in Allograft (Aza, CyA) on day 0 and in Autograft on day 14 risen remarkably.

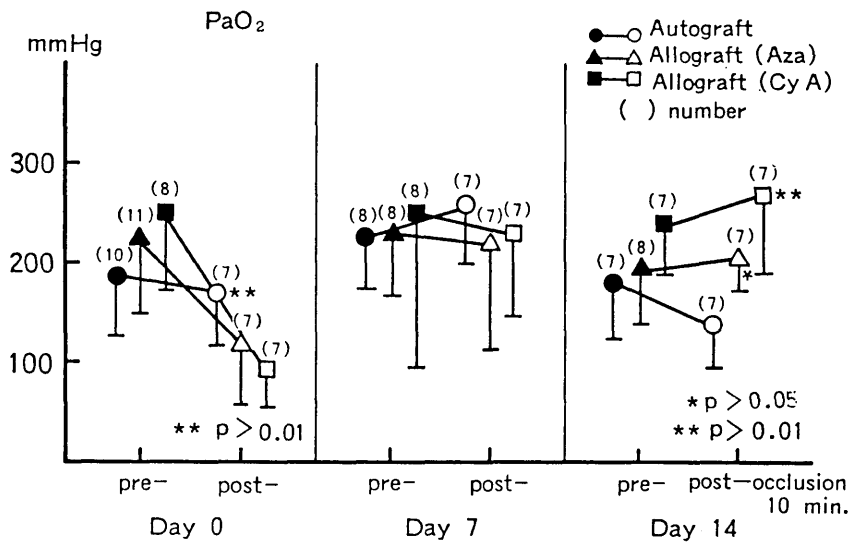


Fig. 2. PaO₂ values of Auto- and Allograft in UPAO-test on day 0, 7 and 14. PaO₂ in Allograft (Aza, CyA) on day 0 and in Autograft on day 14 fell noticeably.

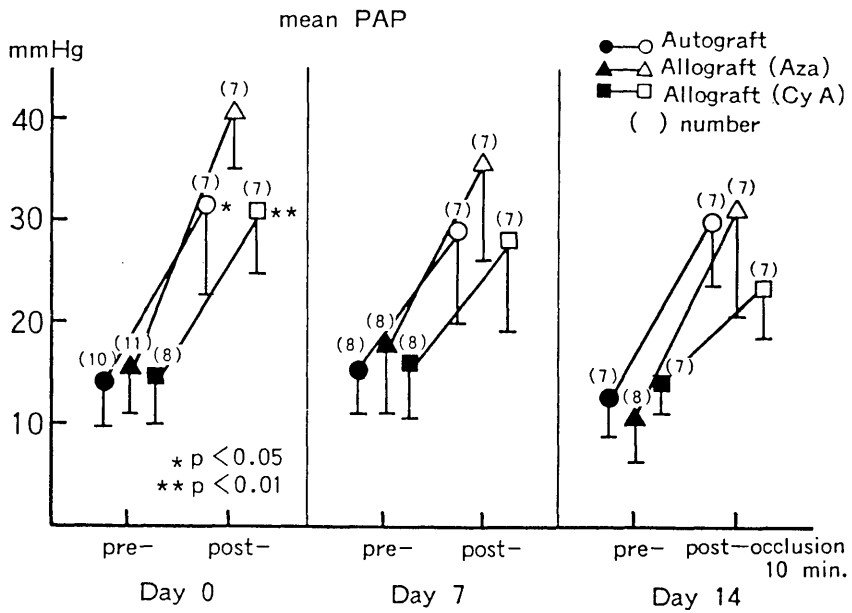


Fig. 3. mean PAP values of Auto- and Allograft in UPAO-test on day 0, 7 and 14. mean PAP remained low in CyA group as compared with those in Aza group.

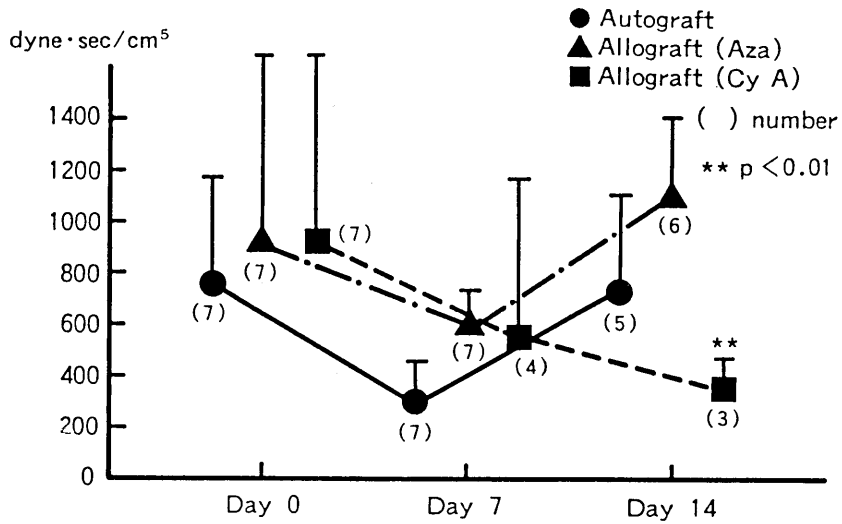


Fig. 4. PVR values of Auto- and Allograft in UPAO-test on day 0, 7 and 14. PVR remained significantly low in CyA group ($P < 0.01$) as compared with those in Auto- and Allograft (Aza group) on day 14.

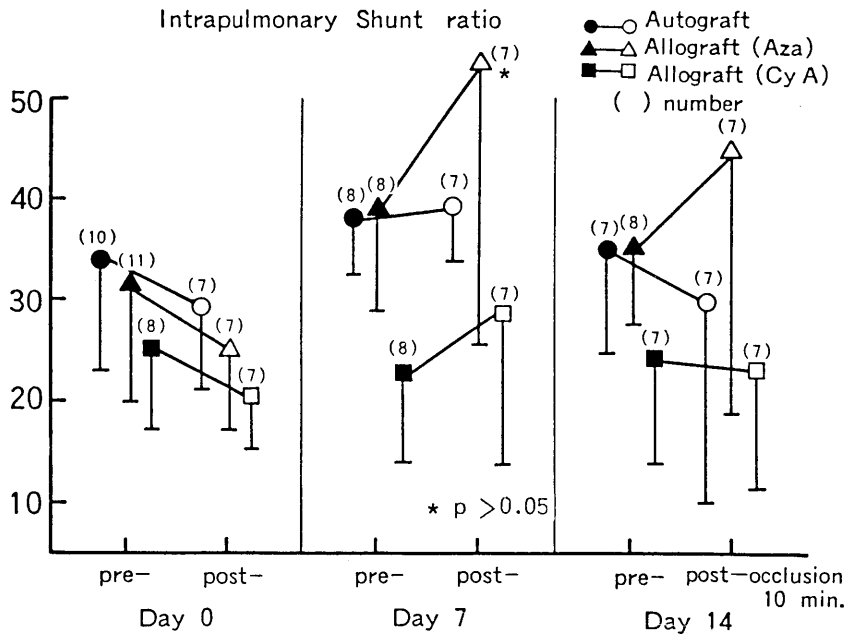


Fig. 5. Intrapulmonary shunt ratio values of Auto- and Allograft in UPAO-test on day 0, 7 and 14. Those in CyA group kept low as compared with those the other two groups.

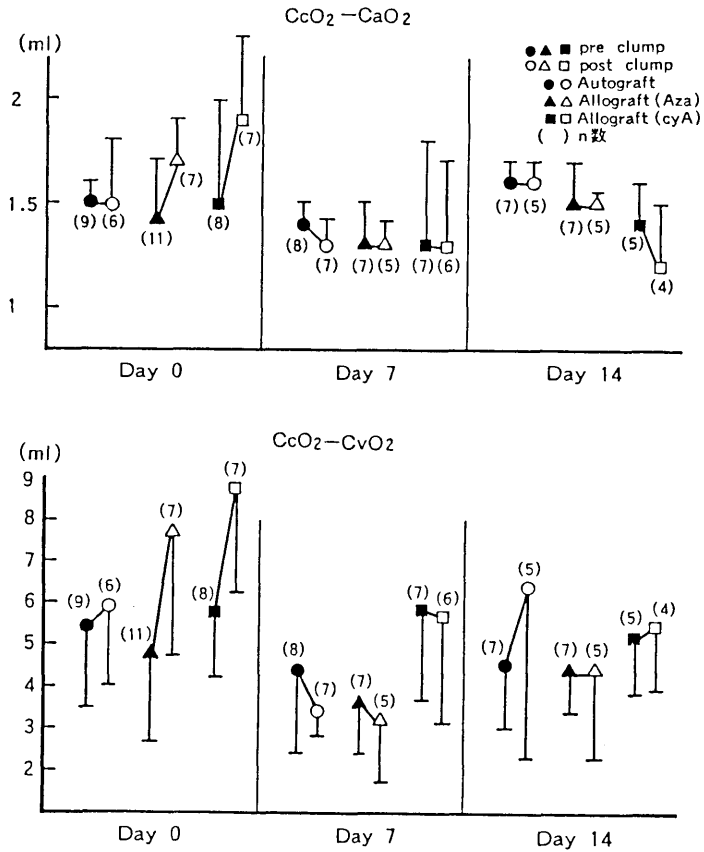


Fig. 6. CcO₂-CaO₂, CcO₂-CvO₂ values of Auto- and Allograft in UPAO-test on day 0, 7 and 14. The calculated CcO₂-CaO₂, CcO₂-CvO₂ varied significantly on day 0 and CcO₂-CvO₂ were significantly altered in Autograft on day 7 and 14.

Table III. Histologic finding of Canine lung.

	Autograft		Allograft(Aza)		Allograft(CyA)	
	Day 7 (n= 2)	Day 14 (n= 8)	Day 7 (n= 8)	Day 14 (n= 7)	Day 7 (n= 8)	Day 14 (n= 7)
Inflammation number	1/2 (50)	4/8 (50)				
Perivascular cuffing number			5/8 (62)	3/7 (43)	3/8 (37)	0/7 (0)

() %

62% on day 7 and in 43% on day 14. In CyA group, Table III indicated that such a histologic finding was observed in 37% on day 7 and day 14, showing much less. The finding of perivascular cuffing (PC) in CyA group as shown in Fig. 7 also was a very few as compared with that in Aza.

A comparison between histologic findings of PC and hemodynamic parameters of PaCO₂, PaO₂ and m-PAP was made during UPAP-test as shown in Fig. 8. Positive PC finding was closely associated with rise in PaCO₂ and fall in PaO₂. However, such was not statistically significant as compared with negative one on day 7, 14. In contrast, m-PAP was significantly greater in positive PC rather than in negative one on day 7 ($P < 0.05$ in Aza, $P < 0.01$ in CyA).

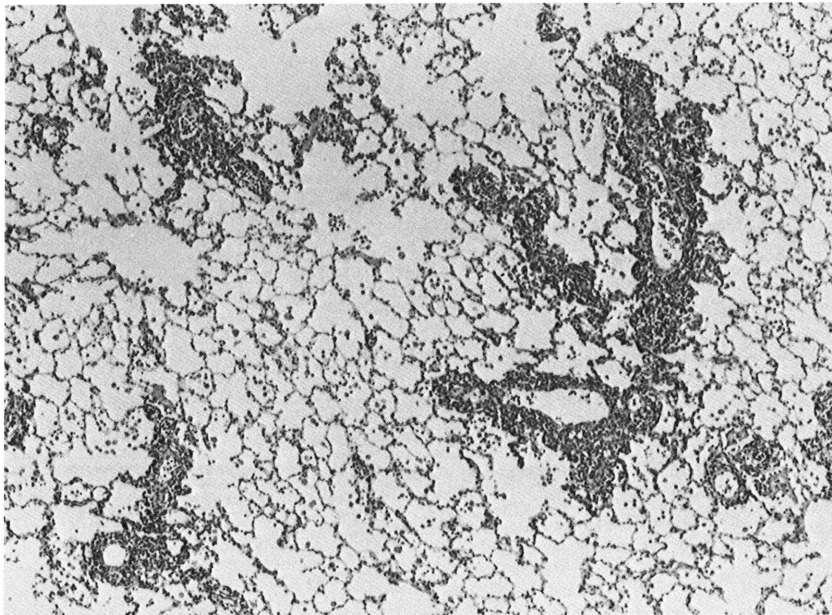


Fig. 7. Photomicrography of Allograft (CyA). This section was obtained 2 weeks of post transplantation and showed mild perivascular cuffing of lung morphology. (HE $\times 20$)

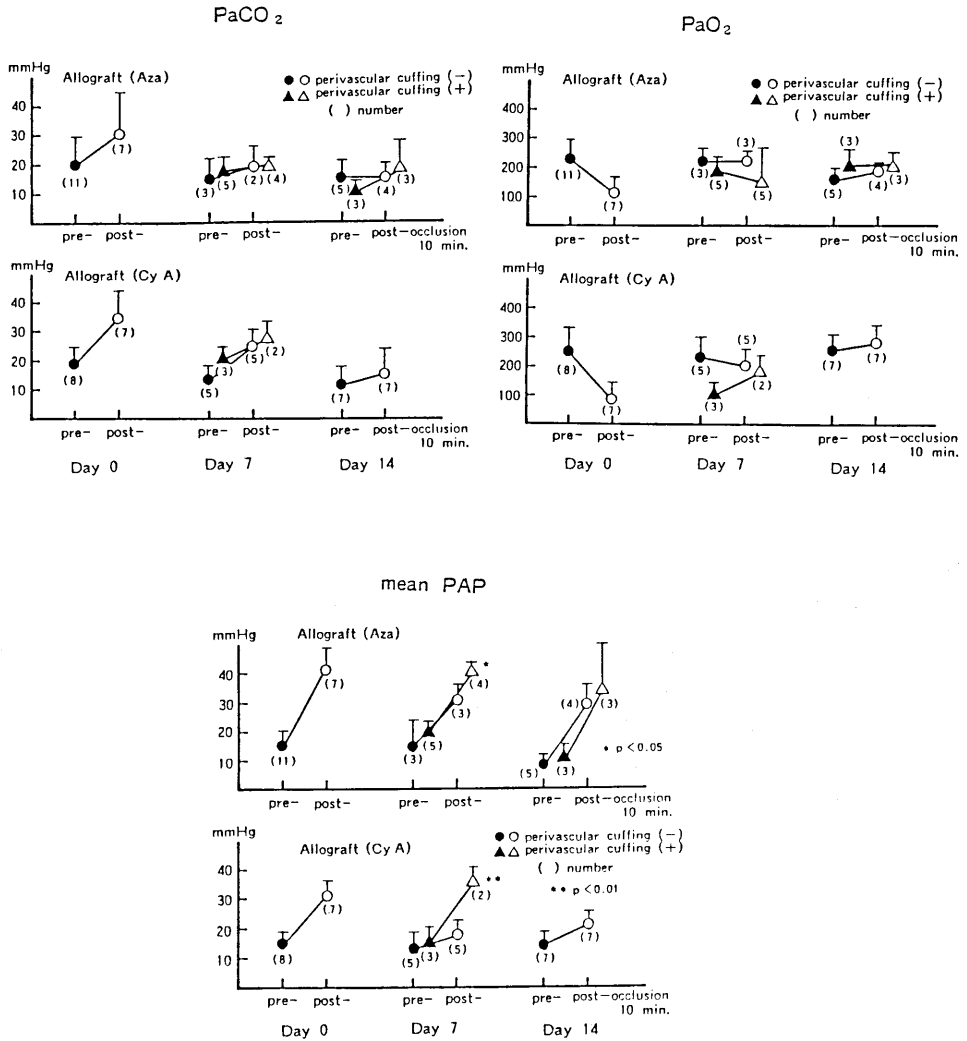


Fig. 8. A comparison between perivascular cuffing and values of PaCO₂, PaO₂ and mean PAP of Allograft in UPAO-test on day 0, 7 and 14.

DISCUSSION

Since lung transplantations in dogs were first reported by JUVENELLE²⁾ in 1951 regarding autografts and then BLUMENSTOCK³⁾ in 1961 regarding allografts, much experimental work concerning auto- and allotransplantation has been accumulated. For all that, allotransplantation of the lung has not yet provided a satisfactory result as compared to other organ transplantations. Recently, it has become recognized that CyA enables the graft survivals to prolong and acts as a potent immunosuppressive drug.

In this study left lung transplantation was applied because of technical problems about anastomosis of the pulmonary vein and preventing the incidence of thrombus formation at atrial cuff anastomosis of the pulmonary vein as reported by TSUJI.⁴⁾ Recent clinical studies have shed some light on the graft rejection problems that CyA could considerably leave the grafts free of rejection on the basis of clinically accumulated results. KAMHOLZ⁵⁾ reported long survival of 155 days. VEITH⁶⁾ and NARVIN⁷⁾ clarified that immunosuppressive effect of CyA was superior to that of Aza.

In CyA group UPAO-test was smoothly performed and could be tolerated better than in Aza. A precise functional evaluation of lung grafts was obtained through the use of two SG catheters as reported by KAWAHARA⁸⁾ instead of performing contralateral pneumonectomy or ligation of the main pulmonary artery on the healthy side for fear of greater surgical load.⁹⁻¹⁰⁾

On day 14 following lung transplantation, a slight rise in PaCO₂ and fall in PaO₂ were observed. It is a reflection of the ensuing phenomenon of alveolar capillary block, demonstrating the impairment of O₂ diffusion and preserving well diffusion capacity for O₂ due to denervation.

In the allografts receiving CyA, there were no significant changes in PaO₂ values during UPAO-test. Moreover, these were much more favorable than those in autografts, reflecting a presence of the slight degree of alveolar-capillary block. It is needless to say that CyA is effectively suppressing an immunoresponse to develop the interstitial alternation. Concerning the hemodynamic changes in lung grafts, we must keep in mind that all the tissues around the bronchus and vessels including the nerve fibers are perfectly separated and divided.

In the present study, m-PAP and PVR were raised by UPAO-test as reported by many researchers.¹¹⁻¹³⁾ The causative mechanism could be accounted for vasospasm due to denervation, some degree of stenosis at anastomosis of the pulmonary artery, thrombus formation at anastomosis of the pulmonary vein.

As for the resulting vasospasm from denervation, it was defined that vasospasm remained unchanged until 3 months or 6 months.¹⁴⁻¹⁵⁾ TOMITA¹⁶⁾ clarified the fact that there was no afferent response of the cervical vagus to the electrical stimulation to the circular muscle of the bronchus and the periphery of the lung. They concluded that interrupted afferent vagal nerve did not regenerate for a long time in the transplanted lung. KOTTMEIER¹⁷⁾ also paid attention to the fact that the vagal nerve surgically divided did not resume its function and regeneration was no longer observed as recipient had grown. Consequently the state of vagal denervation might as well remain prolonged following lung transplantation. It is no doubt that an increase in m-PAP and PVR during UPAO-test on day 14 is in part attributable to anastomosis of the pulmonary artery which is inevitable for lung transplantation and no response to increasing pulmonary blood flow while con-

tralateral pulmonary artery was blocked off by UPAO-test on day 14. On the one hand, SHIRAKUSA¹⁸⁾ clarified that elevation of m-PAP was dependent on the degree of histologic vasculitis which was not infrequently seen in dogs given CyA. In this present study m-PAP and PVR in CyA group were not so much elevated as those in Aza one. KAWAHARA⁸⁾ also pointed out that an increase in m-PAP after allotransplantation using a 6-hours storage donor lung was returned to prior levels to transplantation at 2 weeks when Aza and CyA were used in combination.

It is well known that the intrapulmonary shunt rate represents the function of gas exchange in the lung and the capacity of O₂-uptake. YAMAUCHI¹⁹⁾ clarified that increase in cardiac output led to rise in CvO₂. As a consequence, the intrapulmonary shunt rate became increased on account of increase in reduced Hb content and decrease in O₂-uptake capacity. SAWA²⁰⁾ also cited that increase in O₂ consumption in the tissue at the periphery was associated with reduced SvO₂ and consequently the intrapulmonary shunt rate became reduced, therefore, the venous admixture also was raised. Based on these results, it is assumed that an increase in the intrapulmonary shunt rate on day 7 and 14 following lung allotransplantation may be influenced by varying variety of cardiac output and oxygen consumption of the tissues.

It is of interest to note that the transplanted lung is deprived of cough reflex because of the ensuing denervation and tends to suffer from pneumonitis as shown in Table III.

This present study indicates that 50% of autotransplanted lungs predispose to pneumonitis which was essentially related to a loss of functional reserve of lung grafts. However, in allotransplanted lung, one must take into consideration that histologic findings of round cell infiltration must be differentiated between pneumonia and immunologic response, although it is very difficult to make clear. Moreover, it is not infrequent that Aza occasionally provoked so-called acute rejection within 1 week after allotransplantation in contrast to CyA, which can suppress the immunoresponse much more effectively in spite of allowing a presence of vasculitis though proved otherwise in this study.

SUMMARY

The auto- and allotransplanted lung functions were evaluated in dogs by means of the unilateral pulmonary artery occlusion test (UPAO-test).

In autografts, m-PAP and PVR were raised, reflecting vasospasm due to denervation and the resulting stenosis from anastomosis of the pulmonary artery as well as pneumonitis lesion, probably based on a loss of cough reflex due to denervation.

In allografts, UPAO-test identified that CyA benefited the grafts from suppression of rejection as well as preservation of their functional reserve, which was superior to Aza in view of histologic findings and functional evaluation.

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