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Effecs of cyclosporine on bronchial anastomotic healing in canine lung allograft

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ABSTRACT : Wound healing at bronchial anastomosis of the lung allograft in canine was compared between allografts prescribed with cyclosporine and/or azathioprine and autografts in terms of recanalization of bronchial artery, breaking strength and histologic findings.

1) Restoration of interrupted bronchial artery completed at the 14 day following transplantation in autografts and allografts with cyclosporine, although it has been retarded until day 21 when azathioparine was given.

2) Satisfactory breaking strength at anastomosis was equally measured in autografts and allografts with cyclosporine. In allografts with azathioprine, weakened breaking strength was noticed on day 14 with significant difference ($p<0.05$).

3) From the standpoint of histologic finding, cyclosporine is of benefit to eliminate inflammatory response and to promote collagen production which was essential to good wound healing.

INTRODUCTION

For the purpose of advances in organ transplantation, potent immunosuppressive drugs and/or procedures are required. Much advances in new immunosuppressants may contribute to improvement of the results of organ transplantations more and more as would be expected.

Recently it has become evident that cyclosporine A (CsA) is a potent and efficient drug to supress the immunoresponse following various organ transportations.

In the present series, the effect of cyclosporine on supressing immunoresponse to lung allotransplantation is to clarify in terms of bronchial healing at anastomosis.

MATERIAL AND METHOD

Mongrel dogs, weighing 15 to 20kg were prepared. These dogs were selected at random for a pair of donor and recipient in whom body weight was matched as far as possible.

The donor lung was taken from a donor under left thoracotomy and it was transplanted to the recipient which was prepared by left thoracotomy after removal of the left lung at the same time.

Left lung transplantation was based on conventional method in which cuff anastomosis of

the left atrial wall was used for anastomosis between the pulmonary veins.

The dogs eligible to this study were divided into three groups. Group 1 (G1) comprised 15 autotransplantations which was transplanted immediately after removal. Group 2 (G2) was 18 allotransplantations with azathioprine as an immunosuppressive drug and Group 3 (G3) was 18 allotransplantations with cyclosporine. In G2, azathioprine was given at a dosis of 4-5 mg/kg/day as an immunosuppressive drug. Meanwhile in G3, cyclosporine A was used at a dosis of 20mg/kg/day.

The recanalization of bronchial artery was evaluated by the use of microangiogram as already reported [4] and graded at the 7th, 14th and the 21 or 28th day after lung auto- and allotransplantations.

The tensile strength at bronchial anastomosis was also compared with each group by measurement of breaking-down point against pulling force to a 1cm bronchial strip specimen at anastomosis by using DSC 500 (SHIMAZU CO) as described [5] on day 7, 14, 21 and and 28 respectively following lung transplantation at sacrifice.

Furthermore, the process of wound healing at bronchial anstomosis was histologically observed. Histologic inflammation was graded by inflammatory score as shown in Table 1.

Table 1. Inflammatory response score

1) inflammatory cell type	
migration of neutrophiles	3
neutrophiles, lymphocyte	2
lymphocyte	1
2) bleeding	1
3) edema	1
4) focal degeneration necrosis	1
5) ulcer	1
6) proliferation of collagen fiber	
slight	3
moderate	2
marked	1

RESULT

The degree of the arterial recanalization that we could assess on microangiogram was indicated in Fig. 1.

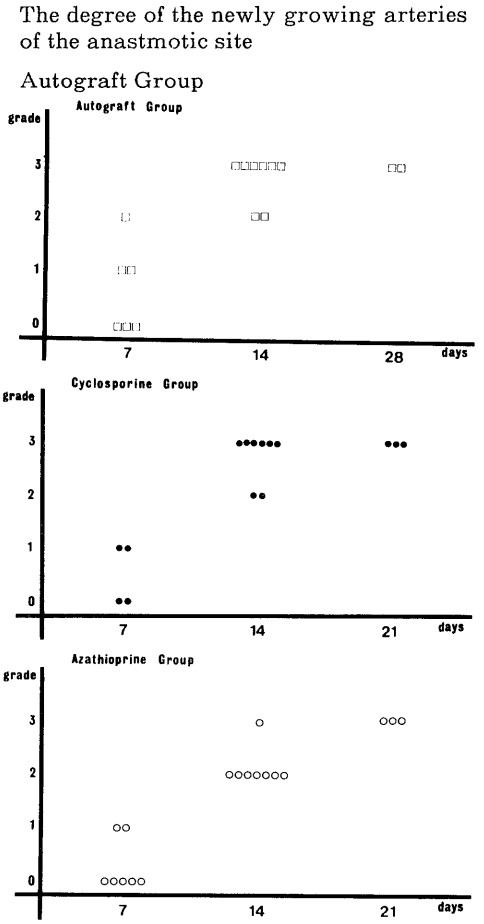

Fig. 1. The degree of recanalization of the bronchial artery at anastomosis on day 7, 14 and 21-28 following lung transplantation in each group.

In autograft, the arterial recanalization at least was starting on day seven and almost completed on day 14.

In the cyclosporine A-adminstered group(G3), the start of the arterial recanalization was somewhat delayed but on day 14 it was almost completed. On the contrary, in the azathioprine-administered group (G2), both begining and completion of arterial recanalization were apparently retarded. Breaking strength was compared in each group as shown in Fig. 2. In the autograft it was increasing with time but even in the allografts that cyclosporine A was given,

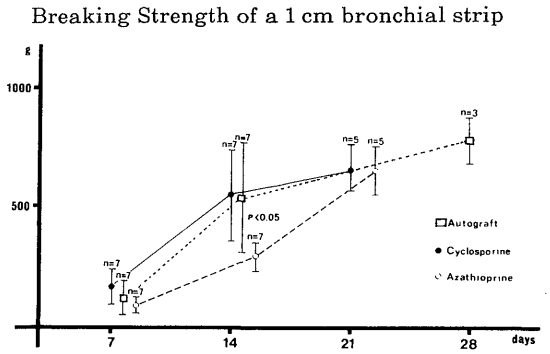

Fig. 2. Breaking strength using a 1cm bronchial strip specimen with time after lung transplantation in autografts and allografts prescribed azathioprine and cyclosporine.

it was increased with a similar fashion to that of the autograft. On the other hand, in the allografts with azathioprine as an immunosuppressive drug, bronchial wound healing in terms of obtaining breaking strength was mostly impaired on day seven with significant difference ($p<0.05$). However, it became increased to the same as the other groups 21 days later.

Histologic finding showed that inflammation at anastomosis was almost the same pattern between the autograft and the allograft with c yclosporine, although the allograft with azathioprine had demostrated pronounced inflammatory response as summarized in Table 2. According to cell types, neutrophile and lymphocyte migration was remarkable finding. Production of collagen fiber was also pronounced in the autograft and the allograft with cyclosporine. In contrast, in case that azathioprine was used, collagen fiber histologically noted was scanty in spite of exclusion of rejection response three weeks later following transplantation.

DISCUSSION

Bronchial anastmosis trouble following lung transplantation is a major concern. It is a major concern. It is attributable to the direct or

Table 2. Histologic finding at bronchial anastomosis

	after transpl.	1W	2W	3W
Autograft	inflamm. score	9.1 ± 1.5	4.8 ± 1.3	3.0 ± 0
	neutro	50	7	7
	%neut-Lym	1	30	1
	Lym	1	70	100
	no cell	50	1	1
		S. mod. mark	S. mod. mark	S. mod. mark
	collagen	83.4 16.6 1	20 50 30	0 100 1
Allograft	inflamm. score	8.8 ± 0.8	7.2 ± 1.6	4.0 ± 1
Aza	neutro	87.5	87.5	1
	%neut-Lym	13.5	1	1
	Lym	1	12.5	100
	no cell	1	1	1
		S. mod. mark	S. mod. mark	S. mod. mark
	collagen	37.5 67.5 0	12.5 50 37.5	0 100 0
Allograft	inflamm. score	8.0 ± 0	5.1 ± 1.2	3.2 ± 109
CyA	neutro	100		
	%neut-Lym		25	
	Lym		25	50
	no cell		50	50
		S. mod. mark	S. mod. mark	S. mod. mark
	collagen	0 100 0	37.5 37.5 25.0	0 50 50

inflamma. score : inflammatory score, S : slight
mod. : moderate, mark : marked, neutro : neutrophile
Lym : Lymphocyte

indirect action of immunosuppressive drugs, poor blood supply to the anastomotic site, clumsy surgical technique and rejection response as cited by VEITH [6].

It was apparently recognized that wound healing at bronchial anastomosis in lung allografts had been impaired as compared with that in autografts with respect to development of recannalization of the bronchial artery and acquirement of tensile strength at anastomosis. GOLEBERG reported that cyclosporine causes promotion of wound healing at bronchial anastomosis in contrast with azathioprine. In the present study, the development of dense bronchial recanalization was not delayed by giving cyclospporine and also weakened breaking strength at anastomosis was not observed. On the contrary, in the group of giving azathioprine, the start and completion of development of bronchial recanalization delayed until on day 21. Furthermore, breaking strength at bronchial anastomosis in the allograft with azathioprine was much less rather than that with cyclosporine during a period of day seven to 21. It is a reflection of delayed and impaired wound healing induced by azathioprine at bronchial anastomosis as reported by GOLEBERG [7].

Needless to say, good blood perfusion at bronchial anastomosis is required for expecting good wound healing. At bronchial anastomosis, it is noted that regeneration of interrupted bronchial artery starts on day 12 and it completes on day 14 to 21 following bronchial anastomosis [8]. Meanwhile, PEASON [9] reported that bronchial recanalization has been achieved at the third or fourth week after canine lung transplantation. It follows that interruption of the bronchial artery is inevitable with bronchial anastomosis and it leads to poor blood supply at anastomosis.

Until restoring recanalization of the bronchial artery, azathioprine used for immunosuppression tends to impair the wound healing at bronchial anastomosis due partly to a delay in regeneration of the bronchial artery. In general, it is believed that cyclosporine A promotes wound healing in the bronchial anastomotic site [5]. The reasons are that cyclosporine causes activation of the macrophage [10], promotes phagocytic activity of the monocyte [11] and increases monocyte population [12].

On the other hand, azathioprine impairs wound healing process, in particular, it takes at least 21 days to approach the normal level.

In the lung allograft wound healing was much more retarded than that in the autograft.

However, use of cyclosporine for the lung allograft results in favorable wound healing as seen in the autograft although azathioprine apparently prevent it until day 21 following lung transplantation.

In conclusion, cyclosporine facilitates wound healing at bronchial anastomosis enough to prevent bronchial complications in the lung allograft as well as in the autograft. It is a main cause that outcome of lung allotransplantation is improving.

According to histologic finding, cyclosporine is of use to eliminate inflammatory response at anastomosis during a period from the second to third week after transplantation as compared with azathioprine. At least with respect to histologic healing at anastomosis the same healing process as the autograft was observed in the allograft that cyclosporine was prescribed.

NEMLANDER and AHONEN [13] reported that cyclosporine does not impaire collagen production and neovascularity which is essential to good wound healing.

It is well known that activated macrophage and monocyte and in proliferation of fibroblast in the wound healing process and cyclosporine plays a key role in making macrophage and monocyte active [13]. PINSKER [14] also noted that the bronchial anastomotic site had become adematous but never brought about anastomosis insufficiency or peribronchial abscess in help of cyclosporine at rejection.

In this present study, it was confirmed that the use of cyclosporine was effective not only to suppress the immunoresponse but also to allow satisfactory wound healing at bronchial anastomosis.

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