

Surgical Technique of Experimental Lung Transplantation in Rabbits

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Background: Although rabbits have often been used as an experimental model for the analysis of lung preservation, there are no reports of long-term survival after rabbit lung allotransplantation. The purpose of this study was to establish a lung allotransplantation model for the evaluation of acute lung rejection in the rabbit and to investigate the transplantation techniques in the rabbit.

Methods: Left unilateral lung allotransplantations were performed in 10 pairs of Japanese white male rabbits, weighing from 2.8 to 3.7 kg. Rabbits were divided into two groups. Group A rabbits (n=5) received Cyclosporine A (CsA) (20 mg/kg/day) orally for 5 days postoperatively, while Group B rabbits (n=5) received no CsA. All rabbits were sacrificed at the fifth postoperative day for histological examinations.

Results: Anastomoses of the pulmonary vein were achieved by using the atrial cuff technique. Satisfied blood flow was obtained in all arterial and venous anastomosis sites. Bronchial anastomosis was also well healed and all rabbits could maintain adequate spontaneous ventilation. In Group A, histopathology revealed that three cases were grade A0, one was grade A1 and the other was A2. In Group B, three cases lived for five days, and histopathology showed two cases were grade A2 and one case was grade A3.

Conclusion: We established a left unilateral lung allotransplantation model in the rabbit and observed suppression of acute rejection of the transplanted lung by CsA. This study suggests that the rabbit is also an experimental model suited for the analysis of lung preservation as well as lung allotransplant rejection. (*Ann Thorac Cardiovasc Surg* 2005; 11: 7–11)

Key words: rabbit, lung transplantation, acute lung rejection

Introduction

Rabbits have often been used for the analysis in experimental models of lung preservation and lung injury because of the homogeneous immunoreactions and low costs. However, there are few reports describing lung allotransplantation in the rabbit model. This is due to difficulties in the surgical technique especially in the anastomosis of pulmonary vein for complicated anatomy. In this

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study, since rabbit lung transplantation may become a useful tool for investigating lung preservation as well as lung allotransplant rejection in the future, we attempted to establish a rabbit lung transplantation model.

Materials and Methods

Left unilateral lung allotransplantations were performed in Japanese white male rabbits, weighing between 2.8 to 3.7 kg (mean weight 3.0 kg). All rabbits were cared for in compliance with the 'Guide for the Care and Use of Laboratory Animals' published by the National Institutes of Health (NIH publication No. 85-23, revised 1985).

Twenty rabbits were used for this study. Rabbits were divided into two groups, Group A: rabbits received Cyclosporine A (CsA) (20 mg/kg/day) orally for 5 days

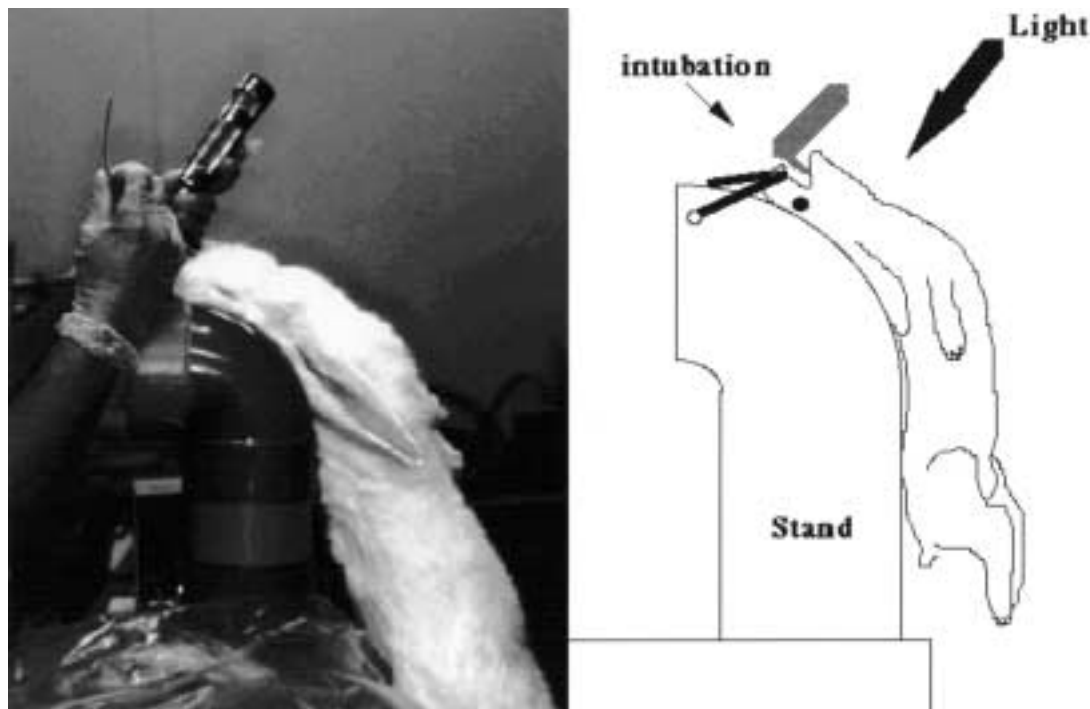


Fig. 1. The rabbit was fixed in a posture in order that the vocal cord could be delineated with a light, and tracheal intubation was performed (I.D. 4.0 mm).

postoperatively, Group B: rabbits received no CsA.

Surgical technique

1) Intubation and anesthesia

All rabbits received ketamine (50 mg/body) and atropine sulfate (0.5 mg/body) intramuscularly. After inhalation of Isoflurane using a mask, rabbits were fixed in a hyper-extensive position that allowed visualization of the vocal cords with a light guidance (Fig. 1). Tracheal intubation was performed using a 4.0 mm (internal diameter) endotracheal tube with no cuff. Anesthesia was maintained with pentobarbital (100 mg I.V.) from the auricular vein in the donor, and with low doses of Isoflurane in the recipient. Ventilation was maintained using a pressure cycle ventilator (AIKA Co., EVM50, Tokyo, Japan), with tidal volumes of 25 ml, respiratory rate 30/min and inspired 100% oxygen with 5 cm H₂O positive end expiratory pressure (PEEP).

2) Heart-lung graft preservation, donor operation and lung preparation

Donor animals were fixed in a supine position, and median sternotomy was performed. The thymic tissue was removed, and the pericardium opened widely. A catheter was inserted via the main pulmonary artery (PA), and was connected to a roller-head pump (RP-NE3, Furukawa

Science Co., Tokyo, Japan) for infusing preservation solution. Blood in the lung was washed out from the left ventricle with 100 ml EP-4 lung preservation solution at 4 degree centigrade in situ. During infusion for five minutes, the pulmonary arterial blood pressure (PAP) was maintained at 5-6 mmHg using by a pressure monitor (Model 78533B, Hewlett Packard Co., Tokyo, Japan).

After infusion of EP-4 solution, superior and inferior vena cavae were transected, the aorta and trachea were separated, and the caudal lobar pulmonary vein was ligated. After donor heart-lung retrieval, the lung was soaked in EP-4 solution for 30 min.

3) Recipient pneumonectomy and anastomoses

Recipient left pneumonectomy was performed using vascular clips and clamp forceps. When the recipient left atrium (LA) was clamped, a part of the left anterior vena cava was involved (Fig. 2).

In transplantation, the LA was firstly anastomosed with a running everted mattress suture using 6-0 monofilament polypropylene (proline, Johnson & Johnson Co., Tokyo, Japan).¹⁾ The PA was anastomosed with an over and over running suture²⁾ using 6-0 monofilament polypropylene, and pulmonary circulation was restarted. Methylpredonisolone (25 mg/kg) and antibiotics (Cefazoline sodium 25 mg/kg) were given immediately

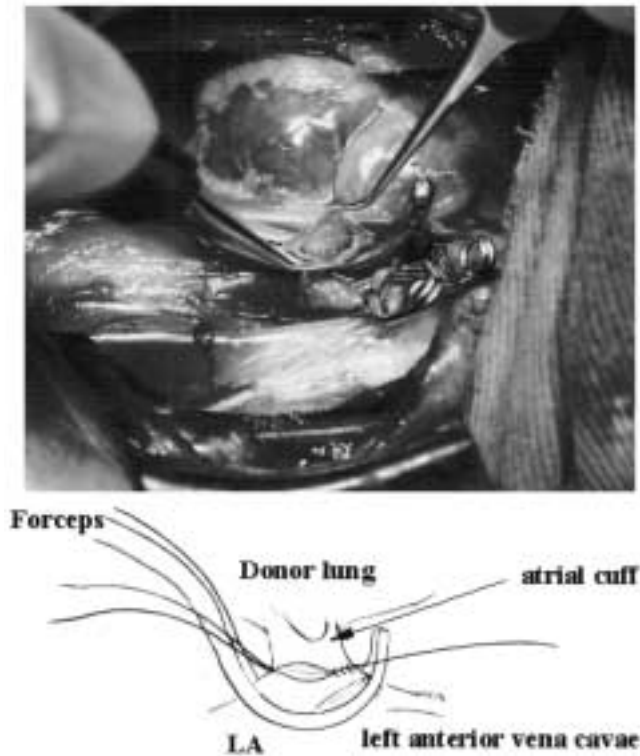


Fig. 2. The recipient left atrium was clamped by a vessel forceps with a part of the left anterior vena cava.

after reperfusion. The bronchus was finally anastomosed using a running everted mattress suture of 5-0 monofilament polypropylene (proline). No transfusion was performed during the operation.

A drainage tube was detained in the left thorax, the lung was inflated and the chest was closed. After the recipient revived, the drainage tube was removed. A chest radiograph was performed to confirm the donor lung expansion, and the tracheal tube was removed.

4) Postoperative care

All rabbits received Cefazoline (25 mg/kg) intramuscularly for 5 days postoperatively. Rabbits in the Group A received CsA (20 mg/kg/day) post operatively daily for 5 days. Weights were measured and chest radiographs were examined at days 1, 3 and 5 postoperatively. All surviving rabbits were sacrificed at day 5 postoperatively.

Evaluation

Acute rejection episodes in the rabbits were evaluated by chest roentgenograms and histological examination at autopsy. Chest roentgenograms were classified into four grades: grade 0, normal; grade 1, mild infiltrates; grade 2, moderate diffuse infiltrates with air bronchograms; grade 3, severe infiltrates or complete opacity according to the reports by Sekine et al.^{3,4)}

Histopathological analysis was performed using hematoxylin and eosin staining and was classified into five grades: grade A0: no significant abnormality, grade A1: minimal acute rejection, grade A2: mild acute rejection, grade A3: moderate acute rejection, grade A4: severe acute rejection, according to the Lung Rejection Study Group.⁵⁾

Results

Anastomoses

All rabbits maintained good blood flow and spontaneous ventilation, and no thrombus was observed.

Examination of X-ray and histological findings

Results of the chest radiological examinations and histological findings are shown in Table 1. In Group A, all rabbits survived until the fifth postoperative day. Histologically, three cases were classified as grade 0, one as

Table 1. Radiological and histological findings after transplantation

No.	CsA	BW (kg)	Outcome	X-ray grade*	Pathological grade**
1	(+)	2.8	Sacrifice	2	1
2	(+)	3.7	Sacrifice	1	0
3	(+)	3.7	Sacrifice	1	0
4	(+)	3.5	Sacrifice	2	2
5	(+)	3.4	Sacrifice	1	0
6	(-)	3.3	Sacrifice	3	3
7	(-)	3.4	Autopsy	3	Pneumonia
8	(-)	3.2	Autopsy	3	Lung edema
9	(-)	3.6	Sacrifice	3	2
10	(-)	3.6	Sacrifice	3	3

* classified by Sekine et al.^{3,4)}

** classified by Lung Rejection Study Group⁵⁾



Fig. 3. Chest radiographs at the fifth postoperative day. The left film (A) is grade 1 (rabbit #2) and the right (B) is grade 3 (rabbit #6).

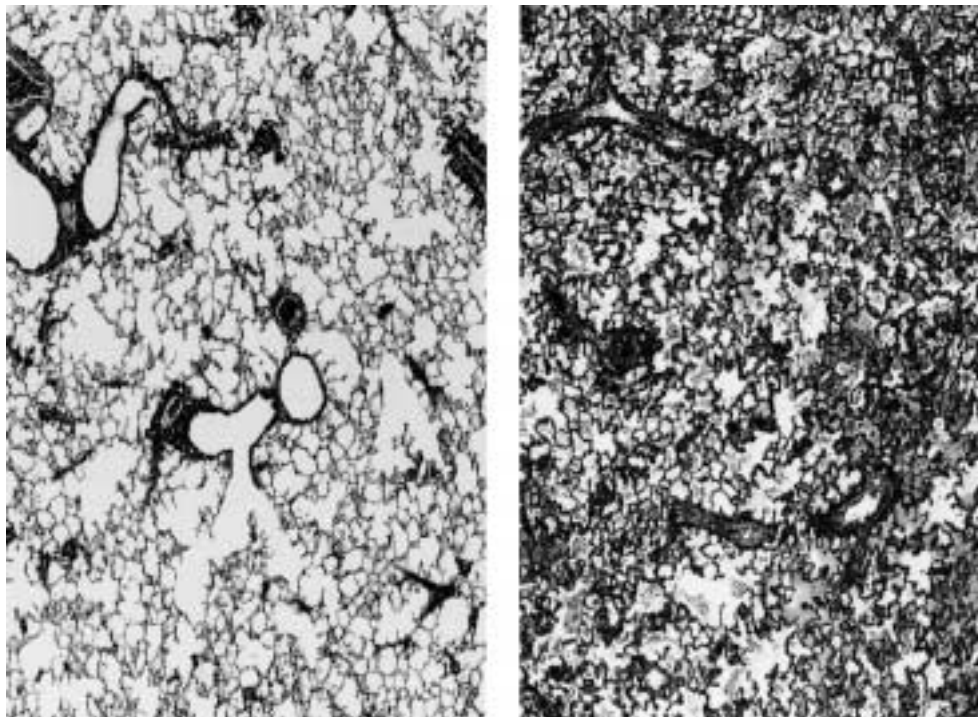


Fig. 4. Histopathological findings. Left (A) shows grade A0 (rabbit #2). Right (B) shows grade A3 (rabbit #6) defined by the Lung Rejection Study Group.⁵⁾

case grade 1 and one case as grade 2. In group B, three cases lived 5 days postoperatively, of which one case was grade 2 and two cases were grade 3. Two cases died before euthanasia. The causes of death were pneumonia and congestive lung edema.

Chest radiographic findings were consistent with histological findings. Group A clearly suppressed acute rejection (Figs. 3, 4).

Discussion

Carrel⁶⁾ first reported the successful heart-lung transplantation in 1907, and the first experimental canine lung transplantation was performed by Demikhov in 1962.⁷⁾ Subsequently, experimental studies of lung transplantation have become widespread. The first unilateral human lung transplantation was performed in 1963 by Hardy et al.⁸⁾

Since Cooper et al.⁹⁾ reported long-term survival of cases in 1986, human lung transplantation has become widespread as a clinical practice. Unilateral lung transplantation has been established in many animal models, including the rat, dog, pig and monkey. Although rabbits have been widely used in lung preservation and functional experiments, little work has been done as a post-transplantation model. Apart from two reports of rabbits that lived for a few hours after lung transplantation supported by a ventilator;^{10,11)} there are no reports describing survival for more than three hours after lung transplantation. With increasing concern over unnecessary cruelty to animals, experiments using large animals except pigs have become more difficult. Because the MHC system is not well described in the rabbit compared to rats or mice, rabbits cannot be used as a substitute for all experimental animals. However, rabbits are less expensive compared to other large animals, and some strains are comparatively similar to human physiology. Therefore, rabbits may be useful for an experimental model of lung transplantation. Functional studies of lung preservation using a rabbit model^{12,13)} have not been considered for survival after transplantation. We felt the necessity of establishing a technique of rabbit lung allotransplantation. According to the establishment of this model, a wide variety of transplantation research can be performed, such as lung mechanics, drug metabolism, the efficacy of new immunosuppressant for transplantation and immunological reaction.

The outline of the technique is essentially the same as that performed in dogs. However, vessels were very fragile, and we had to be very careful to anastomose LA. After transplantation, acute rejection occurred without immunosuppression and CsA suppressed rejection similar to human and dog transplantations. This is the first report of successful left lung allotransplantation and graft acceptance by CsA in rabbits.

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