Model of single left rat lung transplantation. Relation between surgical experience and outcomes

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Abstract

Purpose: The model of unilateral orthotropic left rat lung transplantation is well known and established experimental procedure. The author's personal learning curve of mastery process of this microsurgical procedure is presented.

Material and methods: During 18 months the author has performed 197 single left lung transplantations on the Thoracic Surgery Ward in University Hospital, Berne, Switzerland. There were 147 allogeneic and 50 isogeneic transplantations done. The allogeneic transplantations were carried out from Brown-Norway to Fischer F344 rats whereas isogeneic transplantations were done among Fischer F344 rats solely. Grafted lung was obtained from the intravenously anaesthetised, oxygen-ventilated donor. The implantation was carried out through left postero-lateral thoracotomy on the gas anaesthetised, respirator ventilated recipient. The anastomoses of the vessels were done using the cuff technique, bronchi were sutured using continuous running over-and-over suture. Recipients were sacrificed on day 5 post-transplant. All recipients were divided into four consecutive groups. Warm ischaemia time and presence of perioperative pure technical complications were observed.

Results: We observed time dependent decline of complications number of consecutive recipient groups, respectively 20, 5, 4, 1. The warm ischaemia time in minutes decreased from 35.6±5.4 in group I through 26.7±4.4 in group II, 24.8±2.3 in group III to 22.0±3.1 in group IV.

Conclusions: Continuous training of the procedure shortens the average warm ischaemia time and reduces the number of complications. This tedious microsurgical procedure is possible to master by the surgeon.

Key words: learning curve, rat lung transplantation, warm ischaemia time.

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Introduction

Lung transplantation is an established method to treat some chronic end-stage pulmonal diseases. As it is a difficult, time consuming and extremely expensive treatment, rodent model of lung transplantation seems to be convenient, cheap and reliable survey to observe physiological or pathological changes or to introduce a new treatment to the transplanted lungs.

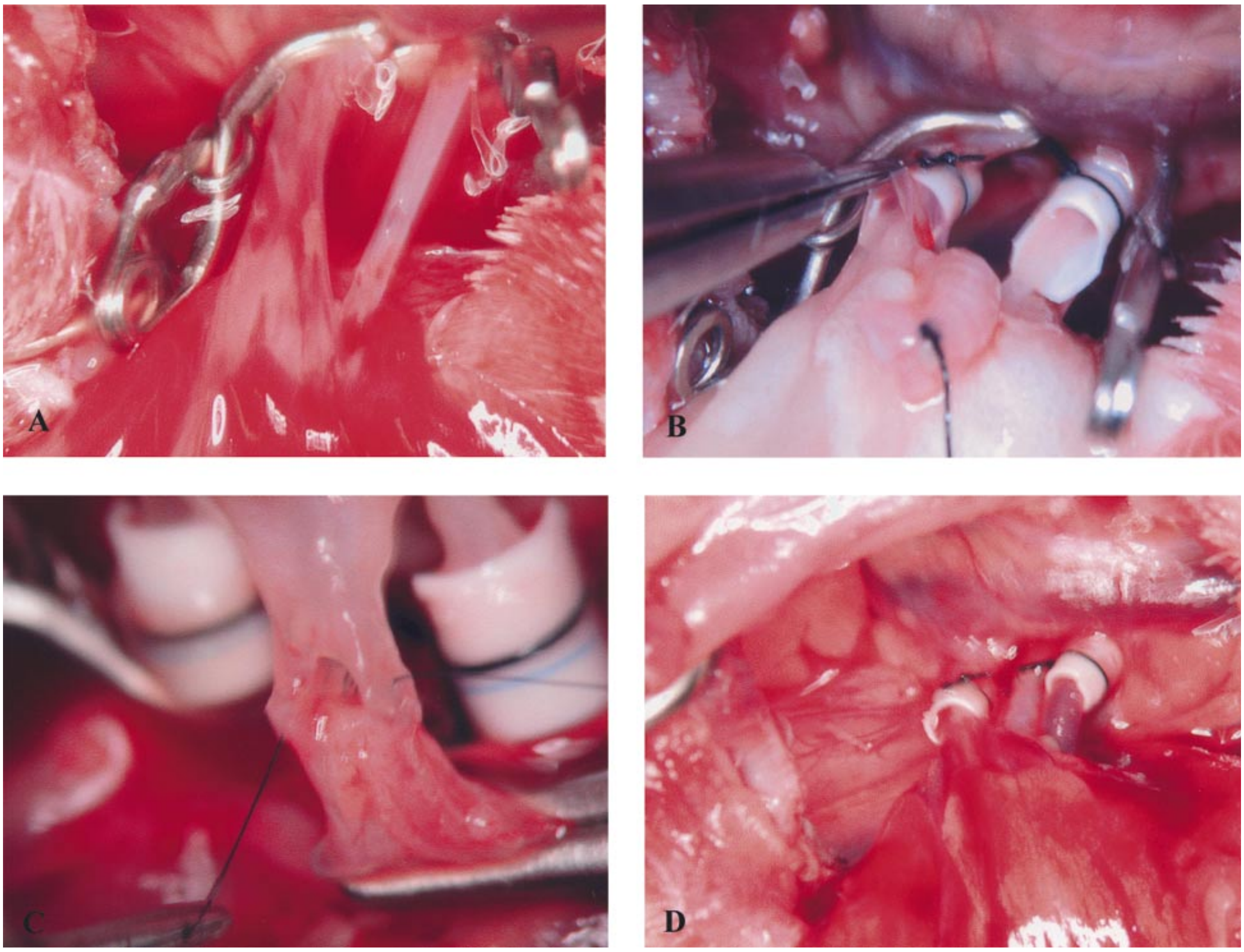
Thanks to the two years of author's scientific fellowship in the General Thoracic Surgery Ward in University – and Kanton--Hospital Insel in Bern, Switzerland, it is possible to describe here the process of mastery the rat lung transplantation.

Material and methods

Between June 2000 and December 2001 the author has performed the total of 197 orthotropic single left rat lung transplantations. The majority of 147 were allotransplantations done from Brown-Norway to Fischer F344 rats. 50 isotransplantations were done among the same bred of Fisher F344 rats. To observe the progress of learning the procedure, recipients were divided into four groups. Group I: 50 recipients grafted between 15.06.2000 and 13.09.2000, Group II: 50 recipients grafted between 14.09.2000 and 7.02.2001, Group III: 50 recipients grafted between 8.02.2001 and 8.09.2001, Group IV: 47 recipients grafted between 9.09.2001 and 17.12.2001.

Operative Procedure: All the microsurgical procedures were done under the magnification between 6 to 20x of the Olympus® SZX12 stereoscopic microscope, Japan. The microsurgical tools of Fehling, Miltex, Pilling – Germany

Figure 1A-D. Model of single left rat lung transplantation.



and Dumont Surgical, Switzerland were used. The donor and recipient procedure took approximately 120 minutes. The method of cuff technique for vessel anastomosis was used [1].

Donor: The animal was preanaestetized in the glass chamber inhaling 4% Halothane (SIGMA®, Buchs, Switzerland). Thiopental (Penthotal, Abbott AG) 50 mg/kg was injected intraperitoneally. Heparin (Liquemin, Roche Pharma, Switzerland) was administered by a peripheral vein (500 IU/kg). A tracheostomy was performed and the animal was ventilated through the tube (14 GA iv. Catheter, Insyte®, Spain) with $FiO_2=1.0$, f=100/min, TV=10 ml/kg by Harvard Rodent Ventilator model 683 (Harvard Apparatus, South Natick, Massachusetts, USA). After cutting the inferior vena cava and left appendix of the heart, a small silicon hose was inserted into the main pulmonary artery via the incision in the right ventricle. Both lungs were flushed with 20 ml of LPD solution (Perfadex®, Medisan Pharmaceutics, Uppsala, Sweden) at a pressure of 20 cm H_2O. The trachea was then tied in end-inspiratory position. The heart-lung block was removed and the left lung was separated ex-vivo from the heart and right lung. 24 gauge cuffs were placed around the pulmonary artery and vein and the vessels were everted and tied onto the cuff [1] and fastened with 8-0 monofilament thread (Surgipro, USSC, USA). The lung was stored in LPD solution at 10^0C until implantation.

Recipient: The recipient was anaesthetised by breathing Halothane (SIGMA®, Buchs, Switzerland) in a glass chamber, intubated, and anesthesia was maintained with 2% Halothane. A left thoracotomy under the 4^{th} rib was performed. The neurovascular clips were put onto the left pulmonal artery (PA) and left pulmonal vein (PV). The left main native bronchus was ligated with 6-0 polifilament thread (Sofsilk, USSC, USA) and cut off. The incision was made in PV and PA) (*Fig.1A*). These vessels were flushed with 0.9% NaCl. The cuffs of the graft were inserted into the recipient's vessels respectively (*Fig.1B*). The 6-0 polifilament ligatures (Sofsilk, USSC, USA) were put around the cuffs. The native PA and PV were cut off beyond the anastomosis and native lung was taken away. The 9-0 Monosof (Tyco Healthcare, Wollerau, Switzerland) running over-and-over continuous suture was performed for bronchial anastomosis (*Fig.1C*). The ventilation and then the perfusion of the graft were restored by removing clips from left bronchus, PV and PA respectively (*Fig.1D*). The chest drain (24Gx3/4" infusion set, Terumo®, Belgium) was inserted to the left haemithorax and the thoracotomy was closed with four layers of continuous suture (4/0 Prolene, Johnson and Johnson®). The thoracic drain was removed after the animal restored spontaneous breathing and the animal was extubated.

Graft Function: On day 5 postransplant the animal was preanaestetized in the glass chamber inhaling 4% Halothane

Tabele 1.

197 transplantations: June 2000 – December 2001	GROUP I 1-50	GROUP II 51-100	GROUP III 101-150	GROUP IV 151-197
Time frame (months)	3	5	7	3
Complications (n)	20	5	4	1
Warm ischaemia time (minutes: average ± standard deviation)	35.6 ± 5.4	26.7 ± 4.4	24.8 ± 2.3	22.0 ± 3.1

(SIGMA®, Buchs, Switzerland). Thiopental (Pentothal, Abbott AG) 50mg/kg was injected intraperitoneally. Animal was ventilated via tracheostomy – Harvard Rodent Ventilator model 683 (Harvard Apparatus, South Natick, Massachusetts) with FiO_2=1.0, f=100/min, TV=10ml/kg. A thoraco-laparotomy in the anterior midline was performed. Neurovascular clips were put on the right main bronchus and right pulmonary artery in order to ventilate and perfuse only the left lung. After five minutes 300μl of blood was aspirated from the aortic arch to the syringe (Pico™ 50 Radiometer-Copenhagen, Denmark) to assess blood gas (Radiometer ABL 700 Serie, Copenhagen, Denmark). After cutting the inferior vena cava and left appendix of the heart, a small silicon hose was inserted into the main pulmonary artery via the incision in the right ventricle. The lungs were then flushed with 20ml of 0.9% NaCl under the pressure of 20cm H_2O. The tracheostomy tube was removed and the trachea was ligated in end-inspiratory position. The heart-and-lung block was cut out, the samples of lungs were put in 10% formaline solution (SIGMA®, Buchs, Switzerland) for histological analysis.

However, the blood gas analysis and formaline fixation are of no interest in this paper.

Results

There is a big difference in both complication rate and warm ischaemia time between the first group I and the other groups II to IV, which show consecutively only slight improvement. The results are shown in the *Tab. 1*.

The perioperative complications include: death, bronchial or vessel stenosis, bronchial fistula, haemorrhage from the anostomosis, anaesthesia overdose, vena cava rupture during chest drain removal, pleural empyema.

The complications due to not technical reasons, like lethal virus instillation, peritonitis due to intraperitoneal injection, expected and physiological rejection of the allograft were not classified in these results.

The warm ischaemia time is defined here as the time between taking the graft from +10^{0}C storage solution and putting it on the recipient till its complete reperfusion and reventilation.

Discussion

The rat lung transplantation technique has many variations; some authors prefer cuffless way to anastomose vessels and bronchi [2,3], though from the author's experience cuffs properly applied for the vessels allow for relatively easy and fast graft circulation restoration. Some others recommend no suture for these anastomoses [4] and that is probably good advice, as the continuous suture for the bronchus anastomosis make great difficulty for the surgeon, especially on the beginning and is the main cause of whole transplantation failure. In our experience we did not meet any serious complications due to reperfusion injury as the others describe [5], as the graft was excised from the living donor and properly stored. As the time for the +10^{0}C storage in this procedure is rather short (approximately 40 minutes), we presume that the disturbances caused by fatty acids metabolism [6] did not have big impact for our outcomes. The temperature indicated is presumed to be optimal for the living lung tissue storage [7]. The reperfusion injury is a cause of the tissue damage and it is proportional to the time the graft storage. Decrease of reperfusion pressure is thought to be of benefit to diminish this complication [8-10]. In our experience the faster this procedure is performed, the lesser influence of the reperfusion injury on the outcomes is.

There are many opinions about how fast one can master given surgical procedure. Basing on this material, completing of 50 transplantations allows the surgeon to feel confident about it, though further practise also improves the outcomes in lesser extent.

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