

Lung Transplantation in Rats: a Viable Experimental Model

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OBJECTIVES: To incorporate a new fast, safe, and reversible anesthetic procedure into the experimental model of lung transplantation (LT) using a cuff technique originally described by Mizuta.

MATERIAL AND METHOD: Eighty-eight Sprague-Dawley rats were used in the experimental model. Thirty left LTs were performed, using 60 rats. The donor heart-lung block was excised by median sternotomy with dissection of the left lung and cuffs (intravenous catheters cut into 3-mm sections) were put in place. The left lung was implanted in the recipient by lateral thoracotomy using the cuffs for anastomoses. The duration of surgery and postoperative complications were recorded. Also noted were signs of ischemia-reperfusion injury, and acute rejection of the transplanted lung.

RESULTS: We discarded lungs excised from 8 animals when developing the experimental model. Transplants could not be completed in 10 rats due to technical problems, despite satisfactory excision. Of the rats who received a transplant, 4 died in the first 24 hours and 26 survived to 48 hours. They were then killed and examined. The state of the anastomoses was good and signs of ischemia-reperfusion injury, as well as acute rejection were observed in the parenchyma of the transplanted lung.

CONCLUSIONS: LT with cuffs in rats is a valid, reliable, reproducible, and cheap model for studying ischemia-reperfusion injury and rejection in LT. The surgical technique is complex, requiring experienced surgeons and a long learning process. Modification of the technique to more closely resemble the surgical procedure in humans is possible, thus facilitating interpretation and allowing more reliable extrapolation to humans.

Key words: Lung transplant. Experimental surgery. Experimental transplant.

Trasplante pulmonar en ratas. Un modelo viable de estudio experimental

OBJETIVOS: Desarrollar el modelo experimental de trasplante pulmonar (TP) con *cuffs* (técnica de manguito), inicialmente descrito por Mizuta, como medio de estudio del TP en nuestro medio incorporando un nuevo protocolo anestésico rápido, seguro y reversible.

MATERIAL Y MÉTODO: Se han utilizado ratas Sprague-Dawley (n = 88) para el desarrollo del modelo experimental y se han logrado realizar 30 TP izquierdos (n = 60). En el animal donante se han efectuado una esternotomía media y extracción del bloque cardiopulmonar con disección del pulmón izquierdo y colocación de los *cuffs* (catéteres de venopunción cortados en cilindros de 3 mm). En el receptor, a través de una toracotomía lateral, se ha llevado a cabo el implante del pulmón izquierdo mediante las anastomosis con *cuffs*. Se ha valorado el tiempo quirúrgico y las complicaciones postoperatorias, así como la presencia de signos de lesión de isquemia-reperusión y rechazo agudo en el pulmón trasplantado.

RESULTADOS: Durante el desarrollo del modelo experimental rechazamos a 8 animales en la extracción. Tras completarla satisfactoriamente, no se pudo realizar el implante en 10 ratas por problemas técnicos. De los animales trasplantados, 4 fallecieron en las primeras 24 h y 26 ratas sobrevivieron hasta las 48 h, momento en que fueron sacrificadas. Hallamos un buen estado de las anastomosis y signos de lesión de isquemia-reperusión y rechazo agudo en el parénquima pulmonar del pulmón trasplantado.

CONCLUSIONES: El TP con *cuffs* en ratas es un modelo válido, fiable, reproducible y económico para el estudio del TP y de los fenómenos de isquemia-reperusión y rechazo. La técnica quirúrgica es compleja, tiene una larga curva de aprendizaje y requiere personal con experiencia quirúrgica. Además, es subsidiaria de modificaciones que hagan que se asemeje más a la técnica quirúrgica utilizada en clínica humana para interpretar mejor y poder extrapolar los resultados al humano con más fiabilidad.

Palabras clave: Trasplante pulmonar. Cirugía experimental. Trasplante experimental.

This project was funded by the Health Research Fund (FIS).

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Manuscript received November 13, 2003. Accepted for publication March 23, 2004.

Introduction

Lung transplantation (LT) is a therapeutic option for certain terminal respiratory diseases. The most important indications are advanced pulmonary emphysema, cystic fibrosis, diffuse interstitial lung disease, and pulmonary

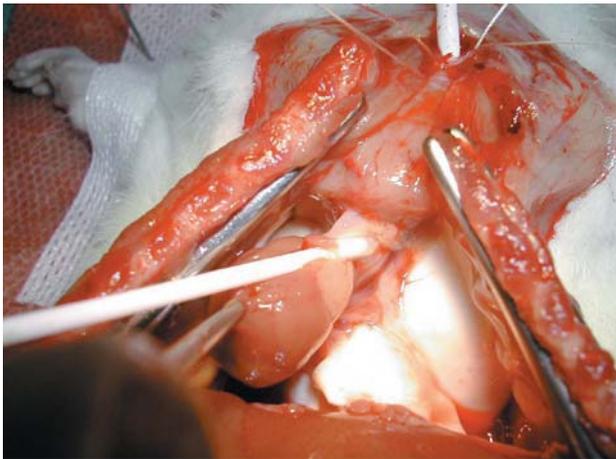


Figure 1. Anterograde perfusion in the donor.

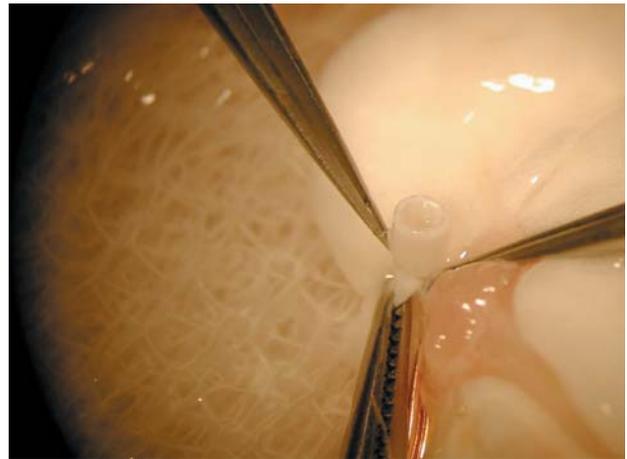


Figure 2. Placement of the cuff on the pulmonary artery during the extraction phase.

hypertension. Currently, double LT is more common than single LT.¹ Experimental LT programs are needed to improve certain aspects of LT, such as preservation, duration of ischemia, and ischemia-reperfusion injury.

Many different experimental models have so far been used. Initially, the canine LT model was preferred.^{2,3} Later, rats were used, first in 1971⁴ and then in 1982.⁵ In 1989, the use of cuffs in LT by Mizuta et al⁶ constituted an improvement in microsurgical technique. In 1995, Reis et al⁷ presented other significant improvements in the surgical technique. Since then, experimental LT with cuffs, with modifications, has been preferred by some groups for studying various aspects of LT.⁸⁻¹¹

In this study, we evaluated a technique based on the original work of Mizuta et al⁶ with the incorporation of modifications by other authors who have studied experimental LT with cuffs and of a new rapid, safe, and reversible anesthesia protocol that had not as yet been applied in this experimental model.

Material and Method

Eighty-eight male Sprague-Dawley rats, housed in optimal conditions and weighing between 300 and 400 g, were used in the experimental model. Thirty LTs were completed. All animals were treated in accordance with international guidelines for animal experiments.

Extraction of the Donor Lung

Anesthesia was induced in the animals with ether before administration of intraperitoneal sodium thiopental (60 mg/kg) to achieve deep general anesthesia. Once anesthetized, the anterior part of the thorax was shaved, and a tracheostomy was performed with ventilation at a tidal volume of 1 mL/100 g body weight.

After median laparotomy with the rat on its back, sodium heparin (100 IU/100 g body weight) was injected in the inferior vena cava. A median sternotomy was then performed and 2 Kocher forceps were used to open up the chest wall.

The thymus was sectioned, followed by the abdominal aorta, the thoracic inferior vena cava, and the left and right atria, and the lungs were perfused with lung preservation solution (physiological saline) at 4°C through a cut at the cone of the pulmonary artery (Figure 1). If perfusion was homogeneous, the trachea was ligated with partial insufflation of the lung and then cut. The heart-lung block was removed, separating it from the esophagus and sectioning the supraaortic trunks, aorta, vena cavae, and pulmonary ligament.

After removal of the block, the left pulmonary artery and vein and left bronchus were dissected under a microscope distally to the hilum of the lung to separate the left lung with the bronchus clamped with a microvascular clamp to maintain partial insufflation. Cuffs (intravenous catheters [Abbotcath®] cut into 3-mm sections) were put in place, with a 1.5 mm sleeve protruding (Figure 2). The lung was stored at 4°C in physiological saline until implantation.

Implantation in the Recipient

In this phase, the recipient animal was anesthetized with subcutaneous medetomidine (0.25 mg/kg), intraperitoneal ketamine (50 mg/kg), and intramuscular atropine (0.7 mg/kg). Once the animals were anesthetized, the left side of the chest was shaved and the rat was intubated orotracheally with an intravenous catheter while ventilating with the same tidal volume as used in the extraction step.

A left posterolateral thoracotomy through the fourth intercostal space was made. The lung was removed from the chest cavity and an arteriole proximal to the pulmonary vein and the peribronchial arteriolar plexus were cauterized.

The hilum of the lung was dissected and the pulmonary artery, pulmonary vein, and bronchus were identified. Ligatures and microvascular clamps were placed on each of these structures next to the heart (Figure 3).

The pulmonary artery and vein were cut close to the lung, and the blood vessels were washed with heparinized saline.

The bronchus was then cut and anastomosed by placing cuffs inside each of the corresponding structures. The anastomoses were fastened by tying ligatures over them.

Finally, reperfusion was carried out slowly by releasing the clamp on the pulmonary vein and then the clamp on the

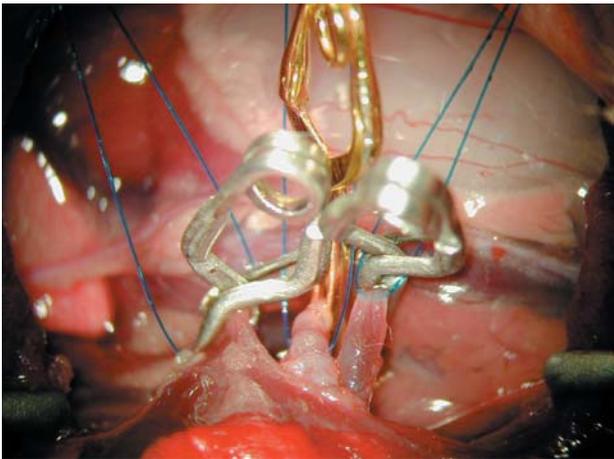


Figure 3. Placement of microvascular clamps and ligatures in the recipient prior to anastomosis (from right to left: artery, bronchus, and pulmonary vein).



Figure 4. Final appearance of implanted lung after reperfusion and anastomoses with the cuffs.



Figure 5. Anteroposterior chest x-ray 48 hours after transplantation, with higher density in left lung related to ischemia-reperfusion injury.

bronchus to ventilate the lung and eliminate atelectasias. Finally, the pulmonary artery was unclamped gradually.

Once the lung was implanted (Figure 4), the native lung of the recipient was excised, and a pleural drainage tube connected to a syringe was introduced. The thoracotomy was then closed. Once the skin was closed, atipamezole—a medetomidine antagonist—and intramuscular buprenorphine were administered. The drainage tube was aspirated to return the pleural cavity to negative pressure and, when the animal was breathing spontaneously, the drainage tube was removed.

Determinations

All animals that survived the intervention were killed after 48 hours. The heart-lung block was removed and perfused with 1% formaldehyde through the trachea. The transplanted lung was embedded in paraffin and longitudinal sections were cut for staining with hematoxylin-eosin. The samples were examined under an optical microscope for signs of ischemia-reperfusion injury such as peribronchial and perivascular edema, vascular congestion, acute inflammatory infiltrate (neutrophils), and bleeding, as well as for the presence of

acute rejection, which was assessed using the international classification of posttransplant lung rejection.

From a clinical point of view, during the postoperative period, the animals were monitored for tachypnea (>100 breaths/min), hemoptysis, and infection of the surgical wound. All animals underwent a chest x-ray (Mobilet II [Siemens, Erlangen, Germany] at 55 KV and 3.2 mAs) immediately after the intervention and after 48 hours (Figure 5) before being killed.

Results

We rejected the lungs of 8 rats during the extraction part of the study and were unable to complete implantation after satisfactory extraction in 10 animals due to the technical problems described in Table 1. The duration of extraction and implantation surgery of the animals is shown in Table 2 for 3 periods that represent different points in the learning curve.

Clinical and radiological complications after transplantation are presented in Table 3. The remaining animals that survived the intervention showed no

TABLE 1
Technical Problems During Extraction and Implantation That Prevented Transplantation

| Extraction | Performed to Completion | (n=40) |
|--------------|--|--------------|
| | Not performed | (n=8) |
| | Hilar tumor | (n=1) |
| | Nonhomogeneous perfusion | (n=5) |
| | Badly sectioned vein during dissection of the heart-lung block | (n=2) |
| Implantation | Performed to Completion | (n=30) |
| | Not performed | (n=10) |
| | Vascular damage during dissection of the hilum | (n=2) |
| | Torn artery during anastomosis | (n=3) |
| | Torn vein during anastomosis | (n=5) |
| | | Total (n=88) |

problems. Of the rats receiving a transplant, 2 died after extubation due to acute lung edema and another 2 died in the first 24 hours due to complete stenosis of the pulmonary vein caused by poor cuff anastomosis.

Twenty-six rats were killed 48 hours after transplantation. Histological examinations showed normal vascular and bronchial lumens, with no signs of stenosis or thrombosis. The evident signs of ischemia-reperfusion injury of moderate and severe intensity in the parenchyma of the transplanted lung and of mild and moderate acute rejection confirmed that there had been reperfusion and ventilation of the implanted lung.

Discussion

Dogs were the preferred animal for experimental LT in its early stages.^{2,3} The ethical and economic problems associated with this model forced investigators to seek other models such as the rabbit and the rat. The rat is the most widely used experimental animal because it is small, resistant, and easily kept and fed.

The first attempts to develop the LT technique in rats came up against the technical complexity of microsurgical vascular and bronchial sutures. Few investigators favored this animal model due to prolonged surgery times and high postoperative mortality.^{4,5} Eight years later, progress was made when Mizuta et al⁶ developed their experimental model of LT in rats using cuffs.

The surgical technique is complex and the investigator is faced with a long learning curve. For the operation, the surgeon has to become familiar with the singular anatomy of the rat and with the use of microsurgical equipment for dissection under the microscope. The cuffs were prepared from small-gauge 16-G (1.7 mm diameter) or 14-G (2.2 mm diameter) catheters. We preferred 16-G cuffs⁷ to minimize the risk of tearing hilar structures—the pulmonary vein in particular. Their patency has been shown to be sufficient; therefore, we consider these the cuffs of choice for animals that weigh between 300 and 400 g.

We extracted the lung from the donor through a median sternotomy,⁷ unlike other authors.⁶ No accidents occurred when we used this technique. The procedure also calls for a laparotomy for easy access to the inferior vena cava below the diaphragm. Heparin can therefore be administered without having to prolong the extraction procedure to obtain additional vascular access. This maneuver requires special care not to damage the vein, and so induce a massive hemorrhage that would destabilize the animal or cause respiratory embolisms.

Perfusion is a crucial step in the extraction of the lung. The volume and flow rate should be sufficient for homogeneous perfusion. Perfusion should take place with the lungs under ventilation to prevent poorly perfused regions arising due to atelectasias.¹² Optimum perfusion was achieved with a 16-G Abbocath[®] with a volume of 20 mL and the upper end of the perfusion tube raised 25 cm. We used physiological saline at 4°C as the only lung preservation solution to lower costs

TABLE 2
Mean Extraction and Implantation Times Over 12 Months*

| | Extraction | Implantation |
|-------------|------------|--------------|
| 1-6 months | 70.2(4.3) | 100.0(3.5) |
| 6-12 months | 65.0(10.0) | 75.0(13.7) |
| At present | 45.0(5.2) | 65.0(7.5) |

*Data expressed in minutes as means (SD).

TABLE 3
Postoperative Clinical and Radiographic Complications in Transplanted Animals

| Complications | No. of Animals |
|----------------------------|----------------|
| Tachypnea | 4 |
| Hemoptysis | 2 |
| Infection | 0 |
| Homolateral pneumothorax | 2 |
| Contralateral pneumothorax | 1 |
| Death | 4 |

and because other authors have worked with this solution in this animal model.⁷ In this study, we used only anterograde perfusion because this perfusion technique had been used in the experimental animal models described previously. We did not use retrograde perfusion, which is more widely used in clinical LT,¹³ therefore we are considering the development of such a perfusion technique in later LT studies.

For removal of the heart-lung block, we ligated the trachea with the lungs partially insufflated,¹⁴ unlike other authors.^{6,7} A microscope should be used for dissection of the structures of the hilum of the lung, particularly the pulmonary vein and the bronchus. The structures should be cut to leave as long a portion as possible to facilitate placement of the cuffs and the implantation. The bronchus has to be clamped below where it is sectioned to keep the lung partially insufflated and to prevent atelectasis that develop in the period prior to implantation.¹⁰

Correct placement of the cuffs is essential. Adson forceps are recommendable for this step to avoid tearing the structures, thus making the lung useless for implantation. It is very important that the sleeve of the cuff faces the posterior edge of the lung so that the structures do not become twisted during implantation.

Orotracheal intubation of the recipient should be performed with extreme care. It is recommendable to use a 14-G Abbocath[®] to prevent air leakage in the region of the trachea and ensure better ventilation of the rat during transplantation.

Unlike other authors who approach through the fifth intercostal space,⁷ we prefer to approach the chest cavity through the fourth.⁶ This gives us a better access to the hilum of the lung for dissection. Dissection itself should be carried out carefully, particularly when sectioning the pulmonary vein, which is easily damaged. The microvascular clamps are placed on each structure of the hilum as close as possible to the heart, and the structures should be sectioned at the anterior face,⁶ as far away as possible to provide long enough

ends for anastomosis. In this respect, our technique differs from previous techniques^{6,7} in which the structures were completely sectioned close to the heart to leave a short end. Particular care should be taken to ensure that the clamp on the vein does not obstruct the left atrium as this would kill the animal.

Heparinization of the vascular structures is useful for eliminating possible thrombi in the ends, but particular care must be taken not to let through small air bubbles which could cause respiratory embolism on reperfusion, killing the animal. During implantation, we irrigate continually with saline at 4°C because it is important to keep the heart hypothermic.

The biggest advantages of this model are its reproducibility, potential to generate ischemia-reperfusion injury and rejection, low cost, and shorter duration of surgical procedures. Its drawbacks are the long learning curve and the need for staff with surgical experience. From a technical point of view, the biggest problems occur with the pulmonary vein; thus, extreme care should be taken when working with this vessel.

We think there is a need for standard experimental animal models of LT. The surgical technique of LT with cuffs in rats can be modified to more closely resemble the surgical procedure in humans, thus facilitating interpretation and allowing more reliable extrapolation to humans.

Acknowledgments

We thank Don Heriberto Grosso, animal technician, and Doña Sandra García, secretary of the Unit of Investigation of the Hospital Universitario Dr. Negrín, for their technical support.

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