Warm ischemic time tolerance after ventilated non-heart-beating lung donation in piglets

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Abstract

Objective: The availability of lungs for transplantation could be ameliorated with the use of organs retrieved from ventilated non-heart-beating donors (VNHBD). The aim of this work is to determine the limit to tolerable in situ warm ischemia time (WIT) for lung grafts after circulation is stopped.

Methods: Twenty piglets underwent left lung allotransplantation. Animals were randomly allocated based on the donor’s status before lung harvesting into the following study groups: Sham (n = 5), Heart-beating donors – non-warm ischemia; I-30 (n = 5), I-60 (n = 5) and I-90 (n = 5), VNHBD-WIT of 30, 60 and 90 min, respectively. Right pulmonary artery and bronchus were permanently occluded one hour after transplantation. Assessment of pulmonary function was monitored hourly by hemodynamic, oxygenation and pulmonary mechanic measurements during a period of 6 h after reperfusion. Lung grafts were weighed pre- and post-transplantation.

Results: Cold ischemic time was similar for all groups, and averaged 80.1 ± 2.7 min. Final mean lung weight was significantly greater in VNHBD (92.5 ± 3.1 g vs. Sham values 75.6 ± 2.4 g, P < 0.01). After right lung exclusion, hemodynamic changes consisted of a sustained increase in pulmonary vascular resistance and a reduction in cardiac output. Lung mechanics also modified, with a rise in airway resistance and a fall in compliance.

Conclusions: Post-transplantation lung graft function from VNHBD with up to 90 min of WIT, is equivalent to those achieved by grafts harvested after heart-beating donation. This method may be a promising strategy of increasing the pulmonary donor pool. © 1998 Elsevier Science B.V. All rights reserved

Keywords: Lung transplantation; Organ preservation; Heart arrest; Tissue donors; Organ procurement

Lung transplantation is limited by a shortage of suitable donors. This shortage is even more severe for the neonatal and pediatric patient due to the reduced donor pool and the greater need for donor-recipient size matching. Among the strategies to improve the supply of transplantable lungs is the development of methods that use ventilated non-heart-beating donors (VNHBD). Although several transplant centers use kidneys from non-heart-beating donors, there has been reluctance to extend the use of these donors to extrarenal organs [1,2]. A potentially large number of viable lungs could be obtained if cadaveric organs from VNHBD were considered for transplantation. The concept of using lung grafts obtained from VNHBD is not a new idea. In 1963, Dr. James Hardy performed the first successful human lung transplant; the donor lung was removed post-mortem from a patient who had arrested following myocardial infarction [3]. Before the introduction of brain death laws, all transplant procedures were carried out using organs from cadaveric donors. Moreover, transplantation could not have been developed as a therapeutic option without the use of VNHBD.

The ventilated lung is a unique organ that is not dependent on vascular perfusion to meet its oxygen needs. Pulmonary cells obtained from cadavers have been cultured successfully, thus indicating that lung death does not necessarily occur at the time of clinical death [4]. However, little
is known about the duration of the lung viability after death. For the concept of cadaver lung transplantation to be practical, sufficient organ viability time is needed between clinical death and organ harvest.

The present study was undertaken to determine the warm ischemia time (WIT) tolerance after ventilated non-heart-beating lung donation in an acute non-survival model of lung transplantation in piglets.

1. Material and methods

1.1. Experimental design

Twenty size-matched hybrids Large-White and Landrace pigs weighing 6–8 kg underwent orthotopic left lung allotransplantation. The VNHBD condition was achieved by killing the animals with a high potassium intravenous injection and leaving them connected to the ventilator after cardiac arrest. The animals were randomly allocated into the following study groups: Sham (n = 5), Heart-beating donors – non-warm ischemia; I-30 (n = 5), VNHBD-WIT of 30 min; I-60 (n = 5), VNHBD-WIT of 60 min, and I-90 (n = 5), VNHBD-WIT of 90 min. One hour after transplantation, the right pulmonary artery and bronchus were permanently occluded, forcing the animal to be completely dependent on the transplanted lung for gas exchange. Assessment of pulmonary function was monitored hourly by hemodynamic, oxygenation and pulmonary mechanic measurements during a period of 6 h after reperfusion. Lung grafts were weighed pre- and post-transplantation.

1.2. Animal care

All animals received compassionate care in compliance with the ‘Principles of Laboratory Animal Care’ formulated by the National Society for Medical Research and the ‘Guide for the Care and Use of Laboratory Animals’ published by the National Institute of Health (NIH publication No. 85–23, revised 1985). The Research Unit of ‘La Paz’ University Hospital reviewed and approved the research protocols, adhering to the European criteria for the protection of animals used for experimental and other scientific purposes (86/609/EEC).

1.3. Donor procedure and lung harvesting

Twenty pigs served as donors for the heart-lung block. The animals were fasting for 12 h in pre-operative treatment cages before being operated upon. Intramuscular ketamine (20 mg/kg), (Ketolar®, Parke-Davis) and atropine (0.04 mg/kg) were administered. Endotracheal intubation was performed with the animal in a prone decubitus position. The pigs were mechanically ventilated employing a Babylog 1HF ventilator set at 100% inspired oxygen fraction, a peak inspiratory pressure of 20 cmH2O, a tidal volume of 10–15 cc/kg, and a 5 cmH2O of positive end expiratory pressure (PEEP). The ventilator rate was adjusted to 15–20 per min to keep up normal arterial blood gas values (NOVA®, Biomedical Stat-Profile 2). Anesthesia was maintained using a Isoflurane 1.5–2% (Forane®, Abbot Laboratories, S.A., Madrid)-oxygen mixture. Pressure and volume signals were directed to a computerized, portable respiratory monitor (Bicore® Monitoring Systems) that provides real-time display of the pressure-volume (work) loops and calculation of the imposed work. Catheters to monitor arterial and central venous pressure and blood sampling were placed into the right carotid artery and the right external jugular vein, respectively. All animals received heparin (Heparina Leo® 1%) at the rate of 3 mg/kg body weight. Control donors (Sham, n = 5) underwent lung harvest using a standard heart-beating technique. Animals belonging to VNHBD groups were sacrificed with an intravenous injection of potassium chloride and they were then secured to the table in the supine position and left at room temperature. After circulation ceased, ventilation was maintained until harvest time.

1.4. Lung harvest

Through a median sternotomy, an extensive ‘L’ shape pericardiotomy was performed, and both pleural spaces were opened. The main pulmonary artery was cannulated with a #10 fr Bard® cannula (Bard and William Harvey, Santa Ana, CA). In the control group, prostaglandin El 500 mg (Alprostadyl®, Upjohn, Belgium) diluted in 10 cc of saline was administrated into the right ventricular outflow. In the VNHBD groups, prostaglandin El was added to a modified EuroCollins solution (Laboratorios Esteve, Barcelona). Simultaneously, with the injection of modified Euro-Collins solution (60 cc/kg) at 4°C into the main pulmonary artery, the left atrial appendage was incised. The pressure in the pulmonary artery should not exceed 20 mmHg during the injection of the solution. After completion of the flush, the heart-lung block was harvested. The entire block was placed in a plastic bag and immersed in modified EuroCollins solution and stored at 4°C, whilst the recipient animal was prepared.

1.5. Recipient preparation and transplantation procedure

Anesthesia on the twenty recipient animals was accomplished in an identical fashion to that of the donors. After induction, 100 mg/kg of Kurgan® (sodium cephalozin, Normon, S.A., Laboratorios, Madrid) was administered. The recipients were antiaggregated with aspirin two days before operation. Under fluoroscopic control, a Swan-Ganz catheter (5.5F) was advanced into the pulmonary artery and connected to an Edwards Critical-Care Explorer® Multiple Parameter Hemodynamic Monitor (Baxter Health-Care Corporation, Irvine, CA).

The heart-lung block was removed from storage and the
lung graft preparation was made ex vivo simultaneously with the recipient left pneumonectomy. The left lung of the donor was dissected from the heart-lung block. The left main pulmonary artery was divided at its origin and the pulmonary veins were sectioned, leaving a large tissue cuff. The left main bronchus was sectioned near the carina and closed with a clamp. The lung was weighed and wrapped in a cold towel.

With the animals in a right decubitus position, a left thoracotomy was performed through the fifth intercostal space. The recipient’s left lung was mobilized. The left pulmonary artery and veins were dissected, clamped and divided distally. The left main bronchus was then stapled using a TA-30 automatic stapler (United States Surgical, Norwalk, CT) and divided proximal to the bifurcation. The pericardium was dissected off the left pulmonary veins and opened to expose the left atrial appendage.

Vascular tourniquets were placed around the right pulmonary artery and right bronchus for later clamping. Orthotopic left lung allotransplantation was performed in a similar way to that described previously [5]. Briefly, the bronchial anastomosis was achieved by a continuous suture of 5–0 polypropylene. The anastomosis of the donor atrial cuff to the left atrium was carried out with a 6–0 polypropylene running suture. The pulmonary artery was trimmed to the appropriate length and an end to end anastomosis was performed with a 7–0 polypropylene suture. Clamps were released, the graft de-aired and reperfusion started. The chest was closed loosely with towel clips.

1.6. Definitions

Warm ischemic time is defined as the time between cardiac arrest and pulmonary artery flushing. Cold ischemic time (storage time) is defined as the time from starting the lung retrieval until restoration of the graft blood flow.

Cold ischemia (min) 73.4
Total ischemia (min) 120
Implantation time (min) 47.2
Lung weight (Pre-Tx) 35.6

Sham, Heart beating donors; I-30, I-60, I-90, 30, 60, and 90 min of ventilated asystolic warm ischemia, respectively; Pre-Tx, Pre transplantation; Post-Tx, Post transplantation; NS, Non-statistically significant difference.

Asterisks denote statistically significant difference between lung weight after transplantation; *P < 0.04, I-30 versus Sham; **P < 0.03, I-60 versus Sham; ***P < 0.01, I-90 versus Sham.

1.7. Measurements

One hour after transplantation, the right pulmonary artery and bronchus were ligated, forcing the recipient to be completely dependent on the ventilated lung. The tidal volume was decreased by half and the respiratory rate was doubled to maintain adequate ventilation. The inspired oxygen fraction was kept constant at 100% during the 6 h of right lung exclusion. Hourly recordings were made of cardiac output, heart rate, aortic pressure, mean pulmonary artery, right and left atrium pressures, for the 6 h follow up period. In addition, the transplanted lung was assessed by hourly measurement of lung mechanics, gas exchange, and pulmonary shunt fraction. Body temperature was measured with the temperature probe in the Swan-Ganz catheter. The ventilatory rate was adjusted as needed to keep the PCO₂ at 30–45 torr. Ringer’s lactate was used for i.v. fluid. Fluid restriction was instituted, with the left atrial pressure maintained at 5–10 mmHg.

1.8. Statistical analysis

Data are presented as the mean ± SEM. Two way analysis of variance (ANOVA) for repeated measures was used to determine whether an overall difference existed in graft function between the four groups during the lung assessment. The remaining data were analyzed with ANOVA factorial. A P-value ≤0.05 was used to indicate a significant difference between measurements.

2. Results

The characteristics and comparisons of experimental groups are summarized in Table 1. There were no statistically significant differences between the four groups with regard to donor and recipient animals weight (7.37 ± 0.13 kg).

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham (mean ± SD)</th>
<th>I-30 (mean ± SD)</th>
<th>I-60 (mean ± SD)</th>
<th>I-90 (mean ± SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor weight (kg)</td>
<td>7.46 ± .39</td>
<td>7.64 ± .23</td>
<td>7.24 ± .20</td>
<td>7.16 ± .23</td>
<td>NS</td>
</tr>
<tr>
<td>Recipient weight (kg)</td>
<td>7.30 ± .19</td>
<td>7.44 ± .24</td>
<td>7.34 ± .27</td>
<td>7.46 ± .39</td>
<td>NS</td>
</tr>
<tr>
<td>Warm ischemia (min)</td>
<td>0.00 ± .00</td>
<td>38.6 ± 68</td>
<td>68.2 ± 1.4</td>
<td>95.6 ± 1.29</td>
<td>–</td>
</tr>
<tr>
<td>Cold ischemia (min)</td>
<td>73.4 ± 4.7</td>
<td>86.8 ± 4.4</td>
<td>80.6 ± 6.6</td>
<td>79.8 ± 5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Implantation time (min)</td>
<td>47.2 ± 3.6</td>
<td>43.0 ± 4.9</td>
<td>48.4 ± 5.6</td>
<td>39.4 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td>Total ischemia (min)</td>
<td>120 ± 5.8</td>
<td>168 ± 6.9</td>
<td>197 ± 11.2</td>
<td>214 ± 7.3</td>
<td>–</td>
</tr>
<tr>
<td>Lung weight (Pre-Tx)</td>
<td>35.6 ± 1.7</td>
<td>35.2 ± 1.2</td>
<td>34.8 ± 2.0</td>
<td>36.2 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Lung weight (Post-Tx)</td>
<td>75.6 ± 2.4</td>
<td>91.6 ± 4.7</td>
<td>92.2 ± 6.1</td>
<td>93.8 ± 6.4</td>
<td>***</td>
</tr>
</tbody>
</table>

Sham, Heart beating donors; I-30, I-60, I-90, 30, 60, and 90 min of ventilated asystolic warm ischemia, respectively; Pre-Tx, Pre transplantation; Post-Tx, Post transplantation; NS, Non-statistically significant difference.

Asterisks denote statistically significant difference between lung weight after transplantation; *P < 0.04, I-30 versus Sham; **P < 0.03, I-60 versus Sham; ***P < 0.01, I-90 versus Sham.
and 7.38 ± 0.13). Lung weight post-transplantation (Fig. 1) at the end of the experiment was significantly higher in all the VNHB groups (I-30, 91.6 ± 4.7 g, \( P < 0.05 \)), (I-60, 92.2 ± 6.1 g, \( P < 0.05 \)), (I-90, 93.8 ± 6.4 g, \( P < 0.05 \)) than in the Sham group (75.6 ± 2.4 g). During left pneumonectomy (that is unilateral lung perfusion) there was a temporary increase in the values of mean pulmonary artery pressure and pulmonary vascular resistance. The mean pulmonary artery pressure increased significantly after ligation of the right pulmonary artery in all groups and did not differ significantly between them. A remarkable decrease in cardiac output followed right pulmonary artery clamping. Values obtained represent hardly half baseline values. This change affected all study groups without statistically significant differences between them. Figs. 2–4.

The inspired oxygen fraction was maintained at 50% before right lung occlusion and at 100% during the whole reperfusion period. No statistically significant differences in arterial oxygen tension were seen between groups during this period. Fig. 5.

Lung mechanics also altered, with a gradual rise in peak inspiratory pressure and a fall in compliance. No significant differences were obtained in compliance and peak inspiratory pressure between Sham and the three warm ischemia groups. Figs. 6 and 7.

3. Discussion

Currently, lung transplantation offers a realistic therapeutic option and has become an effective method for patients with end stage parenchymal or vascular pulmonary disease. The number of suitable donors among the current pool of multiple organ donors is insufficient to meet the growing demand for lung grafts and this is becoming an increasingly serious problem.

All clinical lung transplant programs currently harvest lungs from brain-dead donors with an intact circulation. The use of cadaveric organs has been proposed to increase the size donor pool for transplantation [2,6] and the toler-
ance of the transplanted lung to different periods of warm ischemia has been studied in a large number of experimental investigations.

The lung is unique in that blood perfusion is not required to maintain aerobic metabolism [7,8]. Using a murine model, D’Armini and Alessandrini showed viable pulmonary cells, both histochemically and at the ultrastructural level, several hours after circulatory arrest [4,9].

Several authors [10–12] have investigated the tolerance of the lung grafts to warm ischemia in different animals. These studies demonstrated that 2–3 h is a safe period of ischemia for the lung. This period increases to 5 h when the lung is inflated or ventilated during the ischemic period [13].

Our findings correlate with previous studies in different animals showing that cadaveric lungs can remain viable for several hours after circulatory arrest [14–17].

Donors were heparinized before sacrifice to exclude intravascular thrombosis as a cause of poor lung function. Anticoagulation of the potential donor raises some ethical questions that need to be managed carefully [6]. Although in this model heparin was infused pre-mortem, it could be administered during cardiac massage once clinical death is declared, achieving good titration levels as demonstrated by Steen and co-workers [18]. Donors lungs in this experiment were maintained ventilated prior to harvest and were then flushed with EuroCollins. The addition of prostaglandin E1 was a constant in all groups. There is considerable experimental and clinical evidence indicating a beneficial role of prostaglandin E1. Besides the potent vasodilator effect and inhibition of platelet aggregation, Alprostadyl has been shown to have a cytoprotective effect, related to properties of cell membrane stabilization [19,20].

The state of donor lung inflation has long been known to have a very important role on pulmonary preservation during warm ischemia. Homatas reported that continuous positive pressure ventilation significantly improved the warm ischemic tolerance of canine lungs [21]. Fonkalsrud demonstrated that static inflation or continuous ventilation with positive end-expiratory pressure (PEEP) provided superior canine pulmonary preservation compared with atelectatic storage or ventilation without PEEP [22]. These findings strongly suggest that donor lung inflation may be critical in lung preservation when a significant period of ischemia is expected. In order to avoid atelectasis formation, the use of PEEP during the warm ischemia period was very important. Atelectasis causes maldistribution of lung preserving solutions.

Lung weight post-transplantation was significantly greater in VNHBD than in the Sham group. This finding probably indicates the presence of a moderate amount of edema by the conclusion of the experiment.

Fig. 5. Arterial oxygen tension versus time before and after lung transplantation. Data are shown as the mean ± SEM. PaO2, Arterial oxygen tension; Bas, Baseline; Imp, Implant; Rep, Reperfusion; 1–6, Hours of right lung exclusion.

Fig. 6. Peak inspiratory pressure versus time before and after lung transplantation. Data are shown as the mean ± SEM. PIP, Peak inspiratory pressure; Bas, Baseline; Imp, Implant; Rep, Reperfusion; 1–6, Hours of right lung exclusion.

Fig. 7. Dynamic lung compliance versus time before and after lung transplantation. Data are shown as the mean ± SEM. CDyn, Dynamic lung compliance; Bas, Baseline; Imp, Implant; Rep, Reperfusion; 1–6, Hours of right lung exclusion.
elevation in pulmonary artery pressure and pulmonary vascular resistance was not caused by pulmonary artery anatomic stricture or elevated left atrial pressure. It is more likely a manifestation of an ischemia-reperfusion injury. We believe that either immaturity or denervation may be responsible for the rise in pulmonary vascular resistance when blood flow increases through the transplanted lung. However, in many experimental models of adult lung transplantation pulmonary vascular resistance has been found to be normal [24–26].

As a group, all the transplanted lungs displayed decreased compliance and a rise of the peak inspiratory pressure compared to findings during combined ventilation of both native and transplanted lungs.

We have evaluated the warm ischemia tolerance in an acute immature animal model of lung transplantation. The ligation of the right pulmonary artery and bronchus creates an overperfused state in the transplanted lung, which correlates with the clinical situation of single lung transplant for pulmonary hypertension. This model permits assessment of allograft function uniquely. The duration of the experiment is considered appropriate based on previous studies, in which significant alterations in pulmonary function had occurred within this period.

In conclusion, these data suggest the following: (a) posttransplantation lung function, using organs obtained from VNHBBD with up to 90 min of warm ischemia, during a 6-h reperfusion period is equivalent to lung function after heart-beating harvest; (b) lungs are surprisingly resistant to warm ischemia under ventilated conditions; (c) retrieval of lungs from VNHBBD may prove to be a safe and effective method that could increase the volume of the pulmonary donor pool.

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References


Appendix A. Conference discussion

Dr A. Haverich (Hannover, Germany): Unless I have overlooked something in the literature, I think this is the first paper that presents non-heart-beating lungs in the growing lung situation, and I think it is most interesting.

Dr F. Venuta (Rome, Italy): I think that heparinization of the donor could play a major role in setting the experiment. I think that in the clinical setting it could be difficult to heparinize a possible donor employable in this way. Did you do any experiments without heparinizing the donor?

I also have to report a personal experience. We had to harvest lungs 15 min after the cardiac surgeon had already taken the heart because we arrived too late and the donor was deteriorating; we took the lungs and, after retrograde perfusion at the back table a lot of clots came out, and the lungs worked very well. It was just a 15-min delay from the heart harvesting, but it worked.

Dr Greco: Well, this model represents a type II non-heart-beating donors of the Maastricht classification. It means controlled heart arrest.

The use of heparin raises some ethical problems because there is no relation between the treatment of the heart attack of the patient and the need to use heparin. However, after you decided to finish all the maneuvers of resuscitation, you can (under cardiac massage) inject heparin, and, after 8–10 min of cardiac massage, you can titrate anticoagulation levels with Hemochrome (ACT) and you will obtain good levels of heparinization. There is some work done in pigs by a Nordic group (Dr. Steen) regarding 6 h of cold ischemic time, and they proved that they can heparinize and titrate good levels of heparin after 10 min of cardiac massage. It is not a big problem.

Dr Haverich: I would not think so either, because the fibrinolytic potential of the lung is extremely high, so I would not be worried.

May I ask if there is an ongoing, or a starting, program of non-heart-beating donor utilization in your country at this point in time, not thinking maybe about the lungs or the hearts, of course, but thinking about renal transplantation.

Dr Greco: Yes. There are some people getting organs, abdominal organs mainly renal and some liver, but when it comes to thoracic organs, no work is being done in this area yet.

Dr D. Van Raemdonck (Leuven, Belgium): I saw that the cold ischemic time in your experiments was rather short, only 80 min. Also, Tom Egan’s group from the University of North Carolina has already shown in dogs that the warm ischemic tolerance in ventilated no-heart-beating donors is easily possible up to 4 h. Why did you choose such short periods of ischemia?

Dr Greco: Well, you are talking about a group with a different model, who did their experiment on dogs. These are immature pigs with weights that oscillate between 6 and 8 kg. The cold ischemia time is short because this is the time you need to prepare the graft with bench surgery, do the pneumonectomy, and then do the implantation.