Early effects of the ex vivo evaluation system on graft function after swine lung transplantation

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Abstract

Objectives: Ex vivo lung evaluation (ex vivo) has been developed as a useful method by which to assess lungs from donation-after-cardiac death (DCD) donors prior to transplant. However, the safety of the ex vivo circulation itself with respect to grafts has not been fully investigated. The aim of this study is to evaluate the effects of the ex vivo circuit using a swine lung transplant model. Methods: Lungs with or without 2-h warm ischemia were used. To assess post-transplant graft function, the left lung was transplanted after 2-h ex vivo or cold preservation; blood gas analysis of the left pulmonary vein (partial pressure of oxygen, \( P_{O2} \)) was performed during the 6-h post-transplant follow-up period. Data were compared between the ex vivo (+) and ex vivo (−) groups. Results: Partial pressure of oxygen/inspired oxygen fraction (\( P_{O2}/Fi_{O2} \)) in the ex vivo (−) group was significantly greater than that in the ex vivo (+) group until 3 h after transplant. The \( P_{O2}/Fi_{O2} \) levels in both groups then increased and became similar at 6 h after transplant, regardless of whether ischemic or non-ischemic lungs (\( p < 0.001 \) and \( p = 0.004 \), respectively) were used. Conclusions: Negative effects of the ex vivo system were limited and seen only in the immediate post-transplant period. Therefore, in DCD swine lung transplantation, the ex vivo system appears to be safe.

Keywords: Lung transplantation; Organ preservation; Machine perfusion

1. Introduction

Lung transplantation as a therapy for end-stage lung disease has been limited by donor shortages. Therefore, strategies that increase the number of available donor organs would be of great benefit to prospective recipients. Lung transplantation using donation-after-cardiac death (DCD) donors has the potential to alleviate such shortages, and transplantation using DCD donors has been performed in several transplant centers worldwide. However, lung grafts from DCD donors may be damaged due to warm ischemic injury or latent lung diseases, and it is difficult to assess graft function after cardiac arrest. Therefore, viability testing using an ex vivo reperfusion and ventilation circuit is considered necessary [1,2]. Several feasibility studies have reported that the ex vivo lung evaluation system seems to be a useful method by which to evaluate DCD or non-acceptable donor lungs [2–7]. Nevertheless, even though lungs evaluated by an ex vivo process have been successfully transplanted in clinical settings, the ex vivo system has the potential to damage the lung graft during mechanical perfusion and limited numbers of study have focused on the adverse effects of the ex vivo process itself [2,8]. Thus, the primary aim of this study is to investigate whether the ex vivo system affects post-transplant graft function in DCD lung transplantation using a large-animal-transplant model.

2. Materials and methods

2.1. Experimental groups

To investigate the transplant suitability of clinical ex vivo lung evaluation, the experiments were conducted under two different scenarios simulating controlled and uncontrolled DCD donors. In the uncontrolled DCD scenario, we used lungs that had been subjected to 2 h of warm ischemia, while in the heart-beating (HB) scenario, warm ischemic lungs were not used. In each scenario, data were compared between the two groups, that is, the ex vivo (+) group that was subjected to 2-h ex vivo circulation was compared with the ex vivo (−) group without ex vivo circulation. In the groups without ex vivo...
circulation, the lungs were subjected to 2 h of cold storage to adjust for total time.

2.2. Surgical procedure

2.2.1. Donor lung retrieval

Landrace domestic pigs (27 ± 2.2 kg) were used for the animal experiments. Donor pigs were premedicated with an intramuscular injection of ketamine hydrochloride (10 mg kg⁻¹) (Ketalar; Daiichi Sankyo, Tokyo, Japan) and atropine sulfate (0.025 mg kg⁻¹) (Fuso, Osaka, Japan). Animals were then anesthetized through inhalation of halothane (Fluothane; Takeda, Osaka, Japan), and intravenous pancuronium bromide (0.2 mg kg⁻¹) (Mioblock; Schering Plough, Osaka, Japan) was given before intubation. In all scenarios, following administration of heparin (10 000 U/ body IV) (heparin sodium; Ajinomoto, Tokyo, Japan), cardiac arrest was induced by electrical stimulation between the trachea and diaphragm without opening the chest. About 1000 ml of blood was withdrawn from donor pigs into sterile blood bags to fill the perfusion circuit after cardiac arrest.

In the DCD scenario, mechanical ventilation was stopped after electrical cardiac arrest was induced. Then, the animals were secured on a table in the supine position and left at room temperature for 2 h. A median sternotomy was performed and the pericardium was opened. The cold perfusion/ventilation system consisted of a hard-shell ex vivo perfusion/ventilation circuit (Pall Guard LG; Pall Biomedical, Tokyo, Japan), a centrifugal pump (Bio-console 520, Minneapolis, MN, USA) was also included. The system was primed with 750 ml of STEEN Solution (Vitrolife, Kungsbacka, Sweden) mixed with donor blood to a hematocrit of 0.12–0.15. Cefazolin, 0.5 g (Cefamezin α; Astellas, Tokyo, Japan); insulin, 20 IU (Humarin R; Eli Lilly, Tokyo, Japan); and 10 000 IU of heparin were also added to the solution. Mixed gas consisting of oxygen, nitrogen, and carbon dioxide was supplied to the membrane oxygenator through a gas mixer (Gas Mixer MX-3S; Yutaka Engineering Corporation, Tokyo, Japan). The flow of the three mixed gases was adjusted to achieve partial pressure of carbon dioxide (PCO₂) levels of perfusate taken from the inflow cannula ranging from 35 to 45 mmHg. No prostaglandins and steroids were used in the perfusate.

The heart—lung block was prepared for ex vivo circulation as follows. The pulmonary artery was cannulated via the right ventricle through the pulmonary valves with a 24-F cannula (femoral cannula; Toyobo, Osaka, Japan). The left atrium was cannulated through the apex of the left ventricle with a 38-F cannula (venous drainage Cannula; Edwards Lifesciences, Irvine, CA, USA). A 19-G catheter for pressure measurement was placed in the left atrium. A 4-Fr catheter for pressure measurement was placed in the pulmonary artery. Another 4-Fr catheter for pressure measurement and a temperature sensor were placed in the left atrium, which had been used as a vent for the cold flush. The pulmonary artery cannula was connected to the corresponding tube of the extracorporeal circuit, and a de-airing maneuver was performed. A low-flow perfusion (100 ml min⁻¹) at 25 °C was initiated through the lungs, and the first 200 ml of effluent was discarded. The cannula was then connected to the circuit. When the temperature reached 32 °C, ventilation (1 l min⁻¹) was started with a positive end-expiratory pressure (PEEP) of 5 cmH₂O and an inspired oxygen fraction (FiO₂) of 0.4. The pump flow was adjusted to keep the pulmonary artery pressure below 20 mmHg or at a flow of 1.0 l min⁻¹. When the temperature of the solution exiting the left atrium was 37 °C, full ventilation (10 ml kg⁻¹ breath⁻¹, 10 breaths min⁻¹) was applied. During the circulation, the left atrial pressure was kept stable between 1 and 5 mmHg. After 2 h of ex vivo perfusion, the heart—lung block was preserved with 1500 ml of cold low-potassium dextran glucose solution and stored in a semi-inflated condition at 4 °C with 100% oxygen.

2.2.2. Ex vivo perfusion/ventilation circuit

The technique of ex vivo lung evaluation was according to the methodology of the Lund group [2]. The ex vivo lung perfusion/ventilation system consisted of a hard-shell reservoir with a membrane oxygenator and a built-in heat exchanger (Mera HP Excelung Prime; Senko Medical Instrument, Tokyo, Japan), a centrifugal pump (Bio-console 520, BioMedicus; Medtronic, Minneapolis, MN, USA), and a leukocyte/arterial filter (Pall Guard LG; Pall Biomedical, Tokyo, Japan). A flow probe (Bio Probe TX50; Medtronic, Minneapolis, MN, USA) was also included. The system was primed with 750 ml of STEEN Solution (Vitrolife, Kungsbacka, Sweden) mixed with donor blood to a hematocrit of 0.12–0.15. Cefazolin, 0.5 g (Cefamezin α; Astellas, Tokyo, Japan); insulin, 20 IU (Humarin R; Eli Lilly, Tokyo, Japan); and 10 000 IU of heparin were also added to the solution. Mixed gas consisting of oxygen, nitrogen, and carbon dioxide was supplied to the membrane oxygenator through a gas mixer (Gas Mixer MX-3S; Yutaka Engineering Corporation, Tokyo, Japan). The flow of the three mixed gases was adjusted to achieve partial pressure of carbon dioxide (PCO₂) levels of

2.2.3. Recipient operation

The recipient pig was sedated in the same manner as the donor pig. General anesthesia was maintained with 100% oxygen and 1.0% halothane. Left pneumonectomy was performed through the left fourth intercostal space. The left allograft was implanted using standard techniques. After the left lung transplantation, both lungs were ventilated with a tidal volume of 10 ml kg⁻¹ breath⁻¹, 10 breaths min⁻¹, and an inspired oxygen concentration of 1.0.

2.3. Analysis of the graft function

A 5-F catheter was inserted into the left pulmonary vein and positioned just at the anastomosis for blood gas analysis of the lung graft. The recipient pigs were observed for 6 h, and measurement of blood gas from the pulmonary venous catheter was performed at specific time points (30 min and hourly until 6 h after transplantation).

2.4. Histology

Interstitial widening was measured in the grafts 6 h after transplantation to investigate histological damage to the grafts. Lung tissue samples were inflation-fixed in 10% buffered formalin and stained with hematoxylin and eosin. The width of the interstitium was measured and compared at 10 alveoli per sample.
2.5. Wet-to-dry weight ratio

The specimen for wet-to-dry weight ratio was obtained from each lobe of the graft after the measurements at 6 h post-transplantation and after 2 h of ex vivo circulation or cold storage for the controls. Specimens were dried in an oven at 60 °C and reweighed to determine the wet-to-dry weight ratio.

2.6. Myeloperoxidase activity assay

The myeloperoxidase (MPO) assay was used to quantitate neutrophil accumulation in the lung. Lung tissue samples were collected from the tips of the lower lobe at 6 h post-transplantation, and immediately frozen in liquid nitrogen and stored at −80 °C. MPO activity was measured as described previously [9]. Enzyme activity is expressed as changes in optical density per milligram of tissue protein per minute (ΔOD min⁻¹ mg⁻¹).

2.7. Animal care

All pigs received humane care in compliance with the European Convention on Animal Care and with the Principles of Laboratory Animal Care, formulated by the National Society for Medical Research as well as the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health, Bethesda, MD, USA (NIH Publication No. 86-23, revised 1996). The study was approved by the institutional ethics committee.

2.8. Statistics

All results are expressed as mean ± standard error of the mean (SEM). Statistical tests were performed using Statcel version 2 (OMS Publishing Inc., Japan) on a PC-compatible computer running Windows Vista. Differences were accepted as significant if the p value was less than 0.05. The Mann—Whitney test was used for comparisons between groups for parametric data. Repeated-measures analysis of variance (ANOVA) was used for analysis of serial measurements.

3. Results

3.1. Baseline characteristics

Baseline donor and recipient characteristics and warm ischemic times were similar between the ex vivo and non-ex vivo groups (Table 1).

Table 1. Baseline parameters in all four study groups (mean ± SEM). Differences are not statistically significant.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Weight (kg)</th>
<th>Donor (\text{PaO}_2/\text{FiO}_2) (mmHg)</th>
<th>Donor PAP (mmHg)</th>
<th>Recipient (\text{PaO}_2/\text{FiO}_2) (mmHg)</th>
<th>Recipient PAP (mmHg)</th>
<th>WIT (min)</th>
<th>Ex vivo time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB ex vivo (−)</td>
<td>4</td>
<td>27 ± 1</td>
<td>524 ± 23</td>
<td>27 ± 3</td>
<td>487 ± 14</td>
<td>24 ± 1</td>
<td>22 ± 1</td>
<td>127 ± 2</td>
</tr>
<tr>
<td>HB ex vivo (+)</td>
<td>5</td>
<td>26 ± 1</td>
<td>556 ± 19</td>
<td>26 ± 1</td>
<td>504 ± 22</td>
<td>23 ± 4</td>
<td>22 ± 1</td>
<td>127 ± 2</td>
</tr>
<tr>
<td>DCD ex vivo (−)</td>
<td>5</td>
<td>27 ± 1</td>
<td>526 ± 20</td>
<td>20 ± 1</td>
<td>525 ± 9</td>
<td>26 ± 7</td>
<td>142 ± 3</td>
<td></td>
</tr>
<tr>
<td>DCD ex vivo (+)</td>
<td>5</td>
<td>27 ± 1</td>
<td>540 ± 15</td>
<td>21 ± 1</td>
<td>506 ± 20</td>
<td>22 ± 2</td>
<td>144 ± 5</td>
<td>128 ± 1</td>
</tr>
</tbody>
</table>

3.2. Post-transplant graft function

The partial pressure of oxygen/inspired oxygen fraction \(\text{PaO}_2/\text{FiO}_2\) within 6 h after transplant is shown in Fig. 1. The \(\text{PaO}_2/\text{FiO}_2\) in the HB scenario was significantly greater than that in the DCD scenario \((p = 0.008, \text{ANOVA})\). In both HB and DCD scenarios, the \(\text{PaO}_2/\text{FiO}_2\) in the ex vivo (−) group was greater than that in the ex vivo (+) group until 3 h after transplant. However, the \(\text{PaO}_2/\text{FiO}_2\) in the ex vivo (+) group then improved and became similar to that in the ex vivo (−) group at 6 h after transplant (HB: \(p = 0.004\); DCD: \(p < 0.001\); ANOVA).

3.3. Histological findings

Histological findings at 6 h after transplantation are shown in Figs. 2 and 3. In the HB scenario, there was no difference in the width of the interstitium (micrometers, \(\mu\)m) between the ex vivo (+) and ex vivo (−) groups \((8.0 ± 0.3 \text{ vs } 7.2 ± 0.2, p = 0.2\)). In the DCD scenario, the width of the interstitium in the ex vivo (+) group was significantly greater than that in the ex vivo (−) group \((9.0 ± 0.2 \text{ vs } 8.0 ± 0.2, p = 0.002\)). Normal lung histology was observed in the HB ex vivo (−) group (Fig. 3(A)), and interstitial widening with a mild inflammatory infiltrate and edema was observed in the other groups (Fig. 3(B)—(D)).

3.4. Wet-to-dry weight ratio

The wet-to-dry weight ratio of lungs before and after transplantation is shown in Fig. 4. In the HB scenario, there was no difference in the wet-to-dry weight ratio of lungs before transplantation between the ex vivo (+) and ex vivo (−) groups \((5.2 ± 0.2 \text{ vs } 5.0 ± 0.2, p = 0.5)\). In the DCD scenario, the wet-to-dry weight ratio tended to be higher in the ex vivo (+) group than in the ex vivo (−) group \((6.4 ± 0.5 \text{ vs } 5.4 ± 0.3, p = 0.08\)). On the other hand, there was no difference in the wet-to-dry weight ratio of grafts 6 h after transplantation between the ex vivo (+) and ex vivo (−) groups \((\text{HB}: 5.6 ± 0.5 \text{ vs } 6.3 ± 1.0, p = 0.8; \text{DCD}: 6.3 ± 0.3 \text{ vs } 6.7 ± 0.9, p = 0.8)\).

3.5. MPO activity

MPO activity of lung grafts after transplantation is shown in Fig. 5. There was no significant difference in the MPO activity 6 h after transplantation between the ex vivo (+) and ex vivo (−) groups \((\text{HB}: 0.319 ± 0.087 \text{ vs } 0.236 ± 0.033, p = 0.51; \text{DCD}: 0.511 ± 0.065 \text{ vs } 0.494 ± 0.105, p = 0.56)\).
4. Discussion

Previous studies have revealed that the lung is the only solid organ that can receive oxygen in the alveoli through ventilation in the absence of blood circulation; research also indicates that the lung is tolerant of warm ischemia for up to 1 h [3,8,10—12]. Moreover, brain death itself can induce inflammatory reaction, a catecholamine storm, and neurological pulmonary edema [13]. Therefore, the use of DCD donors has been reconsidered as an alternative strategy for resolving the shortage of lung transplant donors. In controlled DCD, the incidence of grade 3 primary graft dysfunction (PGD) is approximately 13%, which is comparable to that in brain-dead donor lung transplantation [14]. By contrast, in uncontrolled DCD, the incidence of grade 3 PGD is 29% with a 1-year mortality of 31% [15]. Warm ischemic injury, which potentially damages DCD lungs, may produce a high incidence of PGD. Therefore, objective functional assessment of grafts from DCD donor lungs is a necessity, although it has not yet been established. Two assessment techniques have been reported for clinical DCD lung transplantation. One is the pulmonary artery flush technique using a solution based on the donor’s blood [15—17], and the other is the \textit{ex vivo} reperfusion technique [1,2]. The \textit{ex vivo} evaluation technique is considered promising and is currently performed in several transplant institutions worldwide.

The clinical \textit{ex vivo} reperfusion technique was introduced by the Lund Team in 2001 [1]. \textit{Ex vivo} reperfusion without additional edema formation in the graft was successfully performed with a specially developed high-osmotic solution added to pure red blood cells as a gentle method for rewarming grafts. The feasibility and efficacy of \textit{ex vivo} lung reperfusion has been further investigated by many researchers, and lung grafts evaluated by 2-h \textit{ex vivo} lung perfusion without edema formation have been successfully transplanted [2,6,7]. Moreover, \textit{ex vivo} lung perfusion has been used not only for graft evaluation but also as a strategy for protection and improvement. The Lund group also reported encouraging results of clinical lung transplantation of
marginal donor lungs after reconditioning using an ex vivo circuit within 2 h [18]. The ex vivo reperfusion technique has been further developed by the Toronto group, resulting in the achievement of stable, 12-h normothermic ex vivo lung reperfusion [6,7].

Historically, most experimental extracorporeal systems to perfuse the lung have resulted in edema and degeneration of the graft, suggesting that ex vivo reperfusion has a negative effect on grafts. However, the specific influence of the ex vivo reperfusion system has not been fully investigated in isolation, especially in DCD lungs with warm ischemic damage. The present large-animal study was designed to mimic human lung ex vivo perfusion and ventilation, which can be used to evaluate lungs obtained from controlled and uncontrolled DCD donors for transplant suitability. We compared the effects of ex vivo reperfusion in two different scenarios simulating controlled and uncontrolled donor grafts. In both scenarios, ex vivo reperfusion affected graft function within the first 2 h after transplantation. However, graft function recovered at 6 h after transplantation. Thus, although the adverse effect of ex vivo reperfusion may exist, it seemed reversible in vivo. Oto and colleagues reported that arterial oxygen pressure/inspired oxygen fraction (PaO₂/FiO₂) at 6, rather than 0 h after lung transplantation, was an early and the most significant predictor of post-transplant outcomes [19]. The authors also indicated that PaO₂/FiO₂ could improve within the first 12 h after reperfusion.

There seem to be several possible reasons for the decline in early post-transplant graft function after ex vivo evaluation. Physical factors, such as non-pulsatile mechanical flow by a centrifugal pump, can damage the graft [2,20]. Sensitive inflammatory cells, such as alveolar macrophages and neutrophils, may also cause additional ischemia/reperfusion (I/R) injury to grafts. An acellular solution of ex vivo circulation containing corticosteroids or an administration of prostaglandin E1 for the graft on harvesting could prevent such lung injuries, which is the method of the Toronto team. We have investigated the changes and effects of pro-inflammatory cytokines in the perfusate of ex vivo circuits. However, we were unable to improve the results in porcine lung transplantation by removing these cytokines with an absorbent membrane in the ex vivo circuit [19]. Consequently, inflammatory reaction might not be the only reason for graft injury during ex vivo perfusion.

Interstitial widening because of inflammatory infiltrate and edema is considered to be a sign of I/R injury [21–25]. In the present study, post-transplant histology showed a significant difference in the widening of the interstitium between the ex vivo (+) and the ex vivo (−) group in the uncontrolled DCD scenario. However, the wet-to-dry weight ratio and the MPO activity of the grafts did not reflect the widening of the interstitium. Therefore, the I/R injury after ex vivo circulation seemed to be mild and temporary.

Considering all of the previously mentioned points, this study provides significant evidence that the damage resulting from 2-h ex vivo circulation seems to be reversible and that the period of time needed to recover from such damage is approximately within 3 h after transplantation.

In conclusion, the adverse effect of the ex vivo lung perfusion system was limited. Ex vivo lung evaluation seems to be safe and may potentially contribute to expansion of DCD lung use.

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References


