Technique for Prolonged Normothermic Ex Vivo Lung Perfusion

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Background: The inhibition of cellular metabolism induced by hypothermia obviates the possibility of substantial reparative processes occurring during organ preservation. The aim of this study was to develop a technique of extended (12-hour) ex vivo lung perfusion (EVLP) at normothermia for assessment and protective maintenance of the donor lung.

Methods: Six double-lung blocks from 35-kg pigs and 5 single human lungs were subjected to 12 hours of normothermic EVLP using acellular Steen Solution. In the animal studies, the left lung was transplanted into recipients at the end of EVLP and reperfused for 4 hours to evaluate the impact of prolonged EVLP on post-transplant lung function. A protective mode of mechanical ventilation with controlled perfusion flows and pressures in the pulmonary vasculature were employed during EVLP. Lung oxygenation capacity (ΔP02), pulmonary vascular resistance and airway pressures were evaluated in the system. Red blood cells were added to the perfusate to a hematocrit of 20% at the end of human lung EVLP to study lung functional assessment with and without cells.

Results: Lung function was stable during 12 hours of EVLP. This stability during prolonged normothermic EVLP translated into excellent post-transplant lung function (PaO2/FIO2: 527 ± 22 mm Hg), low edema formation (wet/dry ratio: 5.24 ± 0.38) and preserved lung histology after transplantation. The acellular perfusion assessment of lung function accurately correlated with post-transplant graft function.

Conclusions: Twelve hours of EVLP at physiologic temperatures using an acellular perfusate is achievable and maintains the donor lungs without inflicting significant added injury. This system can be used to assess, maintain and treat injured donor lungs.

METHODS

Animals

Yorkshire domestic male pigs (25 to 35 kg) were used for the experiments. The Animal Care Committee of the...
Human Lungs

Human lungs from organ donors (n = 5) determined to be unacceptable for transplantation after detailed evaluation by all clinical lung transplant programs, including our own, were used. This protocol was approved by the UHN Research Ethics Board. Donor lung retrieval was carried out according to current clinical practice using Perfadex (Vitrolife, Göteborg, Sweden) flush preservation.16

Experimental Protocol

Six pairs of pig lungs were recovered after Perfadex flush, and subjected to 12 hours of normothermic (37°C) EVLP. After this period, the lungs were cooled to 20°C in the circuit, stored in Perfadex at 4°C for 2 hours (pre-implantation period), and transplanted in order to test the impact of prolonged EVLP on lung function after transplantation.

Donor and Recipient Procedures

The technique for the donor and recipient procedure for lung transplantation in pigs has been described in detail by our group in a separate publication.17 Careful mobilization of the lungs is critical during the retrieval to avoid violation of visceral pleura and subsequent perfusate leaks during EVLP.

The trachea is divided as proximally as possible (divided just below the larynx) to facilitate secure intubation in the ex vivo system.

Extracorporeal Circuit

The components of the EVLP system are detailed in Figure 1.

Priming the Circuit

The circuit is primed with 1.5 liters of Steen Solution (Vitrolife AB). This solution is a buffered dextran-containing extracellular-type solution with an optimized colloid osmotic pressure developed specifically for EVLP. Sodium heparin 10,000 IU (Leo Pharma, Inc., Thornhill, Canada), cefazolin 500 mg (Novopharm, Markham, Canada) and methylprednisolone 500 mg (Pfizer, Quebec, Canada) are added to the perfusate.

Preparation of Lungs for EVLP

After lung retrieval, the heart is excised from the heart–lung block. The left atrial (LA) appendage is trimmed off and a specially designed funnel-shaped cannula with a built-in pressure catheter (Vitrolife) (Figure 2) is sewn to the LA cuff with a 4-0 monofilament suture to create a closed circuit. This splints the LA open to create reliable and consistent outflow drainage. The same cannula is used for pulmonary artery (PA) cannulation (Figure 2). Note that the silicone part of the cannula can be trimmed to fit the LA and PA. A back-table retrograde flush is then performed with 500 ml of Perfadex under gravity drainage at 30
cm. During this procedure, perfusate leaks from the LA and PA cannula anastomoses are checked and secured if necessary. Before transferring the lungs to the XVIVO chamber (Vitrolife), the trachea is opened and bronchial cleaning of secretions is performed. In normal pig lungs, this step is not required and we maintain the lungs in the inflated state until starting ventilation. An endotracheal tube (size: 9 mm inner diameter) is inserted in the trachea and secured circumferentially with an umbilical tape.

**Initiation of EVLP**

The first hour of EVLP strategy is summarized in Table 1. It is very important to follow these stages carefully to avoid injuring the cold-stored lung.

The lungs are transferred from the back-table to the XVIVO chamber (Figure 3) placed on a sterile operating room back-table. First, the LA cannula is connected to the circuit and slow retrograde flow is initiated to de-air the PA cannula (Figure 4A). Once de-airing is complete, the PA cannula is connected to the circuit and anterograde flow is initiated at 150 ml/min with the perfusate at room temperature (Figure 4B). The temperature of the perfusate is then incrementally increased to 37°C over the next 30 minutes. Before increasing flow beyond this level, a careful check of the system is made. At this point, it is important to ensure that PA and LA pressure readings are reliable to avoid hydrostatic damage to the lung. When a temperature of 32°C is reached (usually over 20 minutes), ventilation is started and the perfusate flow rate gradually increased. Then the flow of EVLP gas used to de-oxygenate and provide carbon dioxide to the inflow perfusate via the gas-exchange membrane is started at 0.5 liter/min and titrated to maintain inflow perfusate PCO₂ of between 35 and 45 mm Hg. When the temperature reaches 37°C, the flow is increased stepwise over 30 minutes to a target of 40% of predicted cardiac output (calculated from the size of the lung donor). Recruitment maneuvers to a maximum of 25 cm H₂O are used to recruit regions of lung atelectasis.

**Steady-state Phase of EVLP**

The ventilatory and perfusion parameters during EVLP (maintenance) are summarized in Table 2. A lung-protective strategy of mechanical ventilation is used: tidal volume of 6 to 8 ml/kg; positive end-expiratory pressure (PEEP) of 5 cm H₂O; respiratory rate of 7 beats/min; and fraction of inspired oxygen (FIO₂) of 21%. Recruitment maneuvers are performed every hour to a pulmonary artery wedge pressure (PawP) of 25 cm H₂O.

| Table 1. Strategy for Initiation of Ex Vivo Lung Perfusion |

<table>
<thead>
<tr>
<th>Perfusion time (min)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfusion temp. (°C)</td>
<td>20</td>
<td>30</td>
<td>32–35</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Flow (% calculated flow)</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>50</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>i.e.: 1,500 ml/min (ml)</td>
<td>150</td>
<td>150</td>
<td>300</td>
<td>450</td>
<td>750</td>
<td>1,200</td>
<td>1,500</td>
</tr>
<tr>
<td>Ventilation</td>
<td>None</td>
<td>None</td>
<td>Start</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas exchanger</td>
<td>None</td>
<td>None</td>
<td>Start</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left atrial pressure (mm Hg)</td>
<td>3–5</td>
<td>3–5</td>
<td>3–5</td>
<td>3–5</td>
<td>3–5</td>
<td>3–5</td>
<td>3–5</td>
</tr>
</tbody>
</table>

**Figure 3.** Lungs positioned in the XVIVO chamber. Note the cannulas are positioned at 60° to 75° and slight traction is employed. They are maintained in a stable position by the channels in the XVIVO chamber.
Table 2. EVLP Maintenance Strategy—Settings

<table>
<thead>
<tr>
<th>Measure</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tidal volume</td>
<td>6–8 ml/kg</td>
</tr>
<tr>
<td>PEEP</td>
<td>5 cm H2O</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>7 breaths/min</td>
</tr>
<tr>
<td>FiO2</td>
<td>21%</td>
</tr>
<tr>
<td>Flow rate</td>
<td>40% of estimated cardiac output</td>
</tr>
<tr>
<td>LAP</td>
<td>3–5 mm Hg</td>
</tr>
<tr>
<td>PAP</td>
<td>10–15 mm Hg</td>
</tr>
<tr>
<td>Recruitments</td>
<td>To PawP of 25 cm H2O</td>
</tr>
</tbody>
</table>

PEEP, positive end-expiratory pressure; FiO2, fraction inspired of oxygen; LAP, left atrial pressure; PAP, pulmonary mean artery pressure; Inflow PCO2, partial pressure of carbon dioxide in the inflow perfusate. Recruitments were performed by 2 inspiratory holds of 15 seconds to a PawP (peak airway pressure of carbon dioxide in the inflow perfusate). Recruitments were left atrial pressure; PAP, pulmonary mean artery pressure; Inflow PCO2, partial pressure of carbon dioxide in the inflow perfusate. Recruitments were performed by 2 inspiratory holds of 15 seconds to a PawP (peak airway pressure) of 25 cm H2O every hour.

As a maintenance perfusate flow rate, we use 40% of estimated cardiac output (CO) to perfuse both lungs. Most of the lung can be perfused at this non-pulsatile flow rate while generally maintaining acceptable low pulmonary artery pressures (10 to 15 mm Hg). In pigs, we use an estimated CO of 100 ml/kg. For human lungs, CO is estimated according to the lung donor size as follows: CO = 3 * body surface area (assuming target cardiac index = 3).

Left atrial pressure (LAP) is maintained in the range of 3 to 5 mm Hg. LAP is controlled by adjusting the height of the reservoir.

Every 2 hours we exchange 100 ml of the perfusate to maintain glucose levels and to provide fresh perfusate components, which have been metabolized.

Ex Vivo Lung Functional Evaluation

Lung function was evaluated every hour in the circuit. A recruitment maneuver was performed 10 minutes prior to each evaluation. The following parameters were recorded: oxygenation capacity (ΔPO2/FiO2, PO2 = perfuse LA Po2 - perfuse PA Po2 [mm Hg]), pulmonary vascular resistance (PVR = [PAP - LAP] × 80/pulmonary artery flow [dynes/sec/cm5]), peak airway pressure (PawP [cm H2O]) and airway plateau pressure (Pplat [cm H2O]).

For the human lung perfusion studies, irradiated blood type–compatible red blood cells were obtained from the Toronto General Hospital’s blood services and added to the perfusate at 12 hours of EVLP for correlation of lung function using acellular and cellular lung perfusion.

Termination of EVLP

After the final ex vivo evaluation, the lung block was cooled down in the circuit to 20°C. Thereafter, perfusion and ventilation was stopped (FiO2 was increased to 50% for lung storage) and the trachea clamped to maintain the lungs in an inflated state. The lungs were then stored at 4°C in Perfadex until transplantation in a standard sterile organ bag surrounded by ice.

Evaluation of Lung Function After Transplantation

Blood samples for gas-exchange analysis were collected from the left pulmonary veins every hour after transplantation. At 4 hours after reperfusion, the right PA was occluded with a vascular clamp and, after 15 minutes of exclusion of the right lung, blood gas analysis was performed from femoral artery blood. Lung edema was measured by wet/dry ratio.

Lung Histology

Lung tissue samples collected 4 hours after transplantation were fixed in 10% buffered formalin for 24 hours, embedded in paraffin, sectioned at 5-μm thickness, stained by hematoxylin and eosin (H&E), and examined for pathologic changes under light microscopy.

RESULTS

Ex vivo lung function results are shown in Figure 5. All parameters of lung function were maintained stable during 12 hours of EVLP. The values at 2 hours and 12 hours of EVLP were, respectively: ΔPO2/FiO2 = 434.2 ± 22 and 468.1 ± 13.5 mm Hg (p = 0.23); PVR = 593.8 ± 66.9 and 537.1 ± 56.3 dynes/s/cm5 (p = 0.28); PawP = 10.8 ± 0.5 and 11.1 ± 0.4 cm H2O (p = 0.61); and Pplat = 10.33 ± 0.6 and 11.0 ± 0.6 cm H2O (p = 0.9). More importantly, stable lung function during 12-hour EVLP translated into excellent lung function after transplantation (Figure 6). The PaO2 after 4-hour post-transplant reperfusion and 15 minutes of right lung exclusion was 527 ± 22 mm Hg. Lungs did not gain a significant amount of weight during EVLP or after transplantation and 4 hours of reperfusion. Wet/dry ratios were as follows: before EVLP, 5.12 ± 0.23; after
12 hours of EVLP, 5.25 ± 0.13; and after 4 hours of post-transplant reperfusion, 5.23 ± 0.38.

Lung histology was preserved after 12-hour EVLP plus 4-hour post-transplant reperfusion. There was no evidence of interstitial edema, alveolar hemorrhage or cellular infiltration. Both anterior- and posterior-dependent zones showed excellent preservation of lung histology (Figure 7).

To evaluate the difference between acellular and cellular (red blood cell) EVLP we used unsuitable human donor lungs. We found that the addition of red cells into the perfusate solution to a 20% hematocrit did not significantly alter \( \Delta P_{O_2}/F_{I_2} \) values when compared with an acellular perfusate (Figure 8).

**DISCUSSION**

We have reported herein a successful strategy for ex vivo normothermic maintenance of the donor lung. By maintaining the organ at physiologic temperatures with continuous oxygen and nutrient support, we demonstrated stable ex vivo lung function for 12 hours in the circuit. Most importantly, excellent function was demonstrated after these grafts were subjected to the ultimate challenge of lung transplantation. We considered some technical factors to be key elements for successful prolonged normothermic EVLP.

We routinely excise the heart from the heart–lung block. Others have used the heart to facilitate cannulation; however, in clinical practice, the heart will sometimes be used for transplantation. More importantly, we have found that our funnel-shaped cannula provides a much more reliable drainage of the LA as it tent the atrium open and maintains a straight and patent outflow tract for the pulmonary veins. Once positioned in the XVIVO chamber, the LA cannula should be positioned with slight traction to maintain the pulmonary veins straight and open.

It is important to note that this is a lung maintenance strategy and hence several protective strategies are employed to avoid “stressing” the organ during the...
ex vivo period. These strategies are explained in what follows.

**Perfusate.** The decision to use a completely acellular solution was made using the rationale that the oxygen supply to the lung cells would be derived from the oxygen in the airways provided by the ventilator—an extension of the concept of aerobic lung preservation.18,19 The use of red blood cells can be problematic because of the mechanical damage inflicted on the circulating red cells over time.20,21 However, further studies are required to compare acellular versus cellular perfusates, and post-transplant testing in large animals will shed further light on the pros and cons of having red blood cells in the perfusate in the context of the EVLP maintenance strategy.

**Perfusion flow.** The “low-flow” strategy permits us to perfuse lungs for 12 hours without development of hydrostatic edema. Although the non-dependent areas of the lung are rendered with sub-total perfusion, the “low perfusate flow” strategy remains successful. In fact, after the transplant, those areas exhibited equivalently good histology when compared with the posterior, dependent lung zones that received relatively greater regional flow.

**Ventilation-perfusion interactions during EVLP.** Maintenance of controlled perfusion and ventilation is a key factor in avoiding epithelial and endothelial injury and the subsequent development of lung edema during prolonged EVLP. The ventilatory strategy has an important influence in the “hemodynamics” of the circuit. With very low lung volumes, or in collapsed lungs, the interstitial traction decreases and extra-alveolar vessels decrease in cross-sectional diameter, thereby increasing PVR.22 At the other end of the spectrum, high lung ventilatory volumes translate into increased airway and alveolar pressures that are transmitted to the alveolar perivascular space. The net effect is increased alveolar pressure squeezing the capillaries and increasing PVR.22–24 Therefore, if an inappropriate mode of mechanical ventilation is applied, PVR increases cyclically with each inspiration, causing increased back pressure to the pump, slowing flow and causing collapse of the lung vasculature. This leads to a repetitive vascular collapse and re-opening phenomenon that causes endothelial damage and subsequent lung edema.25 We have found a tidal volume of 6 to 8 ml/kg or a PawP of 10 to 12 cm H2O to be optimal in maintaining a stable PVR in the circuit. A practical way to confirm an optimal ventilatory strategy is to ensure that the pump flow rate has only minor fluctuations with each inspiration. One of the advantages of a centrifugal pump is that it will “back down” in response to increased PVR.

**Pulmonary artery pressure strategy.** We targeted a PAP of between 10 and 15 mm Hg. Technical factors that lead to increases in PAP in the EVLP irrespective of the quality of the lungs are as follows:

1. **Re-warming period.** During the re-warming period of EVLP, high PVRs are expected due to vasoconstriction of the pulmonary vasculature secondary to cold temperature of the lung and the perfusate.26,27 We thus advocate very low perfusate flows during this period (see Table 1).

2. **Low pH in the perfusate.** Perfusate acidosis leading to vasoconstriction of small arteries increasing PVR is a well-described phenomenon in models of isolated lung perfusion.28 Rather than repeated administration of sodium bicarbonate, which tends to lead to increasing sodium levels in the perfusate, adjustment of the gas flow rate is used to effectively titrate PCO2 and pH levels.

3. **Low PCO2 in the perfusate.** High minute-volume ventilation of the lungs quickly eliminates carbon dioxide in the circuit and should be avoided. Whenever the PCO2 decreases to below physiologic levels, the gas supply to the membrane in the circuit should be checked and flow adjusted to achieve PCO2 levels in a range of 35 to 45 mm Hg. It is important to emphasize that maintenance of physiologic levels of PCO2 is critical for prolonged perfusion. A PCO2 level <30 mm Hg in the perfusate leads to endothelial injury and impairs alveolar fluid re-absorption independently of the pH.29

4. **Impaired LA drainage.** Malposition of the LA cannula, kinking of the LA or pulmonary veins, or an airlock in the system will result in outflow blockade increasing PAP. The LAP reading might not change if the obstruction is proximal to the LA pressure monitoring catheter.

5. **High flows.** It is important to re-check the flow meter whenever a rise in PAP is detected.

6. **Pressure measurement catheter problems.** Check position and patency of the catheters. Also, confirm that the height calibrations of the sensors and catheters are correct.

**Left atrial strategy.** We believe that a positive LA pressure of 5 mm Hg is necessary to protect the lung. Maintaining a slightly positive pressure prevents the left atrium and pulmonary veins from collapsing during the decreases in flow observed in inspiration. In fact, it has been demonstrated that the absence of venous afterload results in decreased tension in the pulmonary microcirculation leading to unstable geometry of the alveolar space and decreased lung compliance.23,25 Similarly, it has been demonstrated that a LAP of 6 mm
Hg protects the lung vasculature against cyclic tidal lung stress.\textsuperscript{30} Interestingly, increases in PVR and high rates of vascular failure when using a LAP of 0 to 1 mm Hg occurred independently of lung edema formation, which supports the concept of an underlying mechanism of endothelial damage.\textsuperscript{30}

In conclusion, we achieved a reliable and reproducible EVLP technique that can maintain donor lungs for at least 12 hours at body temperature without inducing lung injury. The application of this donor lung maintenance strategy, which preserves functional metabolic processes (normothermia), will allow for a variety of innovative therapies to be applied to the injured lung, which were previously not possible. Ex vivo repair of organs, along with prognostic testing,\textsuperscript{31} may have a significant impact on lung transplantation, not only by expanding the donor organ pool but also by improving outcomes after transplantation.

The Steen Solution used in this study was provided by Vitrolife AB, Göteborg, Sweden.

REFERENCES