EVALUATING A NOVEL ALBUMIN FREE LUNG PERFUSION SOLUTION IN A SWINE LUNG TRANSPLANT MODEL


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ABSTRACT:

**Purpose:** Ex-vivo lung perfusion (EVLP) has emerged as a new technique that could address the major limitations of cold storage in lung transplantation. To-date EVLP has been performed using only albumin based perfusion solution to perfuse donor lungs ex-vivo. This work focuses on evaluating a new high on-oncotic pressure non-albumin based solution for EVLP.

**Methods and Materials:** Four bilateral adult swine lungs were perfused and ventilated on the OCSTM lung device for 6 hours using OCS perfusion solution supplemented with swine RBCs followed by left lung transplantation into a recipient swine. The Left lung oxygenation capacity was evaluated by clamping the recipient’s native right PA and bronchus and serial blood gases were measured every 30 min. for 4 hours. Bronchoscopy was performed on left lung at 2 & 4 hours after transplantation.

**Results:** Lungs were well maintained on OCS for 6 hours. Post transplant left lung was able to maintain oxygenation of the recipient swine for 4 hours as evidenced by increasing PaO2/FiO2 ratios. Bronchoscopic evaluation revealed normal structures and minimal froth. Histopathological examination revealed normal lung architecture.

**Conclusions:** This study demonstrates the safety and feasibility of perfusing donor lungs with non-albumin based OCS perfusion solution ex-vivo for up to 6 hours with excellent post transplant recovery of function.

Key words: EVLP, Steen Solution, acellular solution, Lung transplantation, Primary Graft Failure (PGD), Ischemia reperfusion injury

INTRODUCTION:

Ex-vivo lung perfusion (EVLP) has emerged as a new technique that could address the major limitations of cold storage in lung transplantation. Steen et al were the first to describe a method to rewarm, perfuse, and ventilate porcine non-heart beating donor (NHBD) lungs ex-vivo, with excellent gas exchange after transplantation. The solution used in this experiment was STEEN Solution. This solution is CE marked and FDA approved for EVLP use. The solution is a high oncotic pressure albumin-based solution. Other EVLP pioneer authors as Havrich, Hanover, Egan, North Carolina, Keshavjee, Toronto and others followed Steen’s EVLP model and used the same perfusate. All of these systems use RBCs free and leukocyte-free perfusate, which may help reduce ischemia–reperfusion injury.
This work focuses on evaluating a new high on-oncotic pressure non-albumin based solution for EVLP.

MATERIALS AND METHODS

- Four studies were planned.
- Six-hours preservation of bilateral swine donor lungs on OCS\textsuperscript{TM}-Lung device using albumin-free OCS perfusion solution [table 1] supplemented with ~ 1000 cc packed red blood cells (pRBCs). After the 6-hours EVLP period, transplantation of the left donor lung in a second recipient animal is done, followed by follow-up of the recipient animal for 4 hours post transplantation.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
<tr>
<td>Dextran 40</td>
<td>50 g/L</td>
</tr>
<tr>
<td>Sodium Chloride (NaCl)</td>
<td>8 g/L</td>
</tr>
<tr>
<td>Potassium Chloride (Kcl)</td>
<td>0.4 g/L</td>
</tr>
<tr>
<td>Disodium Phosphate Dodecahydrate (HNa\textsubscript{2}PO\textsubscript{4} (12H\textsubscript{2}O))</td>
<td>0.117 g/L</td>
</tr>
<tr>
<td>Monopotassium Phosphate</td>
<td>0.063 g/L</td>
</tr>
</tbody>
</table>

EVLP Procedure

- In each experiment, swine lungs are procured following the standard protocol for OCS\textsuperscript{TM}-Lung preservation. This protocol was published elsewhere.
- Lungs are cannulated and instrumented on OCS\textsuperscript{TM}-Lung following the standard protocol for OCS preservation.
- Assessments on the OCS\textsuperscript{TM}-Lung, including blood samples and oncotic pressure samples, are performed at 1-hour and 6-hours preservation following the standard OCS\textsuperscript{TM}-Lung protocol.
- At the end of the 6-hours preservation, lungs are flushed with 3 L ice-cold Perfadx\textsuperscript{®}, and then taken off the OCS\textsuperscript{TM}-Lung device. The block is bisected to left and right lungs, where the left lung was prepared for implantation into a recipient second animal.
The Recipient Animal Protocol:

- Preparation: Tilest (Tiletamine and Zolazepam; Zoletil 15-25 mg/kg body weight
- A peripheral i.v. cannula is placed into an ear vein
- I.V. Injection of propofol at 2-4 mg/kg body weight
- Intubation with 10F endotracheal tube
- Pancuronium is administered at 2 mg per injection for muscle relaxation
- Ventilation is performed at 0.5 Fio2, I:E 1:1, PEEP 5 mbar, 14 breaths/min and the tidal volume is adjusted to achieve a volume per minute of 150 ml/kg body weight
- 500 mg methylprednisolone is administered i.v.
- For surgery heparin is administered at 300 IU/kg
- Epinephrine is prepared as a infusion pump as a 1 mg/10 ml preparation that is needed for infusion beginning 10 min before the right PA is clamped for right ventricular support
- Hemodynamic monitoring:
  - Fixation of a 8.5 F guide cannula into the right external jugular vein together with insertion of a 8.0 F Swan-Ganz thermo-dilution catheter into the PA for cardiac output, PA and left atrial pressure monitoring.
  - Fixation of a small lumen silicone catheter (V. basilica catheter type) into the right carotid artery for invasive blood pressure monitoring
- Equipment:
  Anesthesia monitor for assessing invasive blood pressures of:
  1. Systemic arterial pressure,
  2. PA pressure,
  3. Central venous pressure,
  4. Device for measuring cardiac output via thermo-dilution

Left Lung Transplantation Procedure:

After left-sided lateral thoracotomy the tracheal bifurcation, the left pulmonary artery and the left pulmonary veins are exposed. After clamping of the left main bronchus and the left pulmonary artery, the main stems of left pulmonary veins are ligated. Pneumonectomy is performed. The left atrium is clamped. The donor lung is implanted in a typical manner with an end-to-end anastomosis of the left bronchus, left pulmonary artery followed by the atrial cuff combining the left pulmonary veins. After de-airing the pulmonary artery is de-clamped and the graft is ventilated. After a reperfusion period of 30-60 min the contralateral pulmonary artery is clamped, in order to evaluate only the function of the transplanted lung. If necessary, hemodynamic support is applied by administration of epinephrine or noradrenaline (maximum 0.4 ug/kg/min). Each experiment is terminated after an observation period of 4 hours by an intracardiac magnesium chloride injection.
• Post-Transplant Recorded Parameters:
All parameters are recorded before transplantation and in 30 min intervals after reperfusion of the transplanted left lung for a total of 4 hours

1. Minute volume (l/min)
2. FiO2
3. Compliance (C; ml/mmH₂O)
4. Arterial and venous blood gases parameters
5. SaO₂ (arterial saturation) and SvO₂ (central venous oxygen saturation)
6. Hematocrit (%) and Hemoglobin (g/dl)
7. CVP (central venous pressure; mmHg)
8. LAP (left atrial pressure; mmHg)
9. MPAP (mean pulmonary artery pressure; mmHg)
10. Systolic, diastolic and mean systemic arterial pressure; mmHg)
11. Heart rate
12. CO (cardiac output; l/min)
13. SVR (systemic vascular resistance; dyn/s*cm⁻⁵)
14. PVR (pulmonary vascular resistance; dyn/s*cm⁻⁵)

• Histopathology and wet/dry ratio samples:
Samples are collected from the right lung after OCS™ EVLP preservation and from the left lung after 4 hours post transplantation

RESULTS

Donor Lung Data: Mean donor animal weight was 84±15 kg. Four bilateral Lung blocks were preserved ex-vivo on OCS™ EVLP device for 6 hours. The mean ex-vivo Pretransplant parameters are summarized in table 2.

Table 2: Mean Pretransplant OCS™ parameters

<table>
<thead>
<tr>
<th></th>
<th>1 hour On OCS</th>
<th>6 hours On OCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO₂/FiO₂ ratio</td>
<td>479.8±102.8</td>
<td>510.5±74.8</td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td>8.7±1.6</td>
<td>8.2±2.4</td>
</tr>
<tr>
<td>PAWP (cmH₂O)</td>
<td>14.8±1.5</td>
<td>11.6±0.9</td>
</tr>
<tr>
<td>Compliance</td>
<td>32.3±5.9</td>
<td>37.4±4.5</td>
</tr>
<tr>
<td>Oncotic pressure</td>
<td>22.7±2.6</td>
<td>22.9±2</td>
</tr>
</tbody>
</table>

PaO₂: arterial partial pressure of Oxygen, PAP: pulmonary artery pressure, PAWP: peak airway pressure
Recipient Lung Transplantation Data:

All four animals survived the transplantation procedure and up to the 4 hours post-transplant study time-point. Mean recipient animal weight was 77.7 ± 7.4 kg. Transplanted left lung was able to maintain oxygenation of the recipient swine for 4 hours post-transplant as evidenced by increasing PaO2/FiO2 ratios.

Post-transplantation, all 4 recipient animals maintained good hemodynamics. A period of 1 hour was allowed after implantation of left lung for stabilization and gradual rewarming of the graft. During this time, both lungs, the recipient native right lung and the left transplanted lung, were ventilated and perfused in the recipient animal. After 1-hour, the native right lung bronchus and pulmonary artery were both clamped forcing the transplanted left lung to take-over the oxygenation of the recipient animal. Table 3 shows the mean post-transplant hemodynamics data at 4 hours post-transplantation.

Table 3: Recipient Transplantation Data

<table>
<thead>
<tr>
<th></th>
<th>Pre-Transplant Recipient Baseline (mean±SD)</th>
<th>4-hours Post-transplant (mean±SD)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABP sys (mmHg)</td>
<td>100.3±11.4</td>
<td>100.0±7.5</td>
<td>0.82</td>
</tr>
<tr>
<td>ABP mean</td>
<td>83.3±13.3</td>
<td>51.0±12.5</td>
<td>0.04*</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>5.3±3.6</td>
<td>9.0±2.1</td>
<td>0.07</td>
</tr>
<tr>
<td>LAP (mmHg)</td>
<td>8.9±2.9</td>
<td>10.0±3.4</td>
<td>0.92</td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td>18.1±4.6</td>
<td>46.0±6.6</td>
<td>0.02*</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>5.1±1.1</td>
<td>5.3±0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>SVR (dyn/s*cm5)</td>
<td>1266.6±323</td>
<td>683.7±127</td>
<td>0.02*</td>
</tr>
<tr>
<td>PVR (dyn/s*cm5)</td>
<td>130.2±34</td>
<td>195.0±44.7</td>
<td>0.03*</td>
</tr>
<tr>
<td>Lung Compliance</td>
<td>41.7±16.3</td>
<td>27.17±3.8</td>
<td>0.73</td>
</tr>
</tbody>
</table>

ABP: Arterial blood pressure, CO: Cardiac output, CVP: central venous pressure, LAP: left atrial pressure, PAP: pulmonary artery pressure, SVR: Systemic vascular resistance.
DISCUSSION

Cold flush and static cold storage is the gold standard for preservation of donor lungs in clinical lung transplantation and is considered a successful technique provided that ischemic times are not excessive and the initial quality of the organ is good. Increased interest in ex-vivo lung perfusion has been triggered by the need to develop a method to assess marginal donor lungs (extended criteria donor lungs). This includes those with edema and also lungs from non-heart-beating donors (NHBD). All the normothermic ex-vivo lung perfusion (EVLP) systems that have been described so far are stationary, and thus have restricted potential to reduce cold ischemic time. The Organ Care System (OCS™-Lung) (TransMedics, Andover, MA, USA) is the only fully portable ex-vivo lung perfusion system designed to assess and improve marginal lungs and potentially improve the condition of routine donor lungs. It has several potential advantages compared with conventional systems. OCS Lung can provide immediate and sustained lung recruitment starting at the donor site; substantially reduce cold ischemic time, especially during transport (because it establishes normothermic perfusion); and allow continuous organ assessment and monitoring capability from donor to recipient. The device was CE marked in January 2011.

Steen and colleagues in Sweden were the first to introduce the concept of EVLP in clinical practice in 2003. He proved the EVLP concept by testing his system in a NHBD swine model. Lungs were put on the system for 4 hours, recruited and assessed, and later transplanted in another recipient animal. He then used an identical model to evaluate and transplant lungs from a human NHBD. Aitchison and colleagues used a similar circuit to assess porcine lungs retrieved 2 hours after circulatory arrest by perfusion with deoxygenated blood at 500 mL/min and demonstrated that gas exchange after lung transplantation from these NHBDs was identical to lungs retrieved immediately after arrest. Rega and colleagues used an ex-vivo ventilation and perfusion circuit to show equivalent gas exchange in porcine lungs retrieved from NHBDs after 1 hour of in-situ warm ischemia and 3 hours of cold ischemia compared with lungs flushed with ice-cold Perfadex® immediately after cardiac arrest. These entire EVLP systems used albumin based STEEN Solution for ex-vivo perfusion and assessment [Figure 2]

![Figure 2: STEEN Solution™](image-url)
In this study we have demonstrated a successful strategy for ex-vivo normothermic preservation and assessment of the donor lungs using the OCS™-Lung system. By maintaining the organ at physiologic temperatures and near physiologic conditions, we achieved stable ex-vivo lung function for 6 hours in the circuit. Most importantly, excellent function was demonstrated after these lungs were subjected to the ultimate challenge of lung transplantation. Post-transplant left lung was able to maintain oxygenation of the recipient swine for 4 hours as evidenced by increasing PaO2/FiO2 ratios. Bronchoscopic and histopathological examination revealed preserved lung architecture and minimal post-transplantation edema. We have chosen to implant only the pig’s left lung due to the technical difficulty of implanting the right lung in the swine model given the separate tracheal take-off of the right upper lobe bronchus.

The main objective of this study was to evaluate the perfusate. We used cellular, non-albumin, low-potassium, dextran solution. The colloidal osmotic pressure averaged 22.9±2 mmHg after 6 hours of EVLP due to the high dextran concentration of our perfusate. Most authors have used albumin for maintaining oncotic pressure of the perfusate. Albumin has the draw back of increasing the incidence of the lung edema by crossing the injured alveolar-capillary membrane and thereby drawing water with it in the extravascular space and inside the alveoli.11-12 Dextran has the advantages of being negatively charged, so is repelled by the also negatively charged capillary endothelial cell lining and hence does not cross the capillary membrane. It is also a potent anticoagulant preventing formation of minor thrombi in the lung vasculature. Dextran also has an anti-inflammatory effect.12

We added pRBCs to the perfusate to help achieve a more comprehensive evaluation of the lung functions ex-vivo. Keshavjee et al in Toronto have used a completely acellular solution backed by 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. They believe that the use of red blood cells can be problematic because of the mechanical damage inflicted on the circulating red cells over time.12,13 We have not had this problem because the pump used in our system is a pulsatile pump. The one used in the Toronto model is a centrifugal pump. However, further studies are required to compare acellular versus cellular perfusate, and post-transplant testing in 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.

In the near future, clinical adoption of EVLP will likely to be driven by the promise of donor lung evaluation. Due to the shortage of organs, the transplantation community is more and more shifting towards extended criteria (marginal) donors and NHBD. Thus, a good understanding of physiological parameters of lung injury on the EVLP circuit is necessary. Ultimately, we hope that EVLP physiologic recruitment, resuscitation and assessment, will lead to safe and increased lung transplant volumes.
CONCLUSION:

This work demonstrates the safety and feasibility of perfusing donor lungs with non-albumin based OCS perfusion solution ex vivo for up to 6 hours with excellent post transplant recovery of function.

REFERENCES:


