Modified reperfusion and ischemia-reperfusion injury in human lung transplantation

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Objective: Ischemia-reperfusion injury remains a major cause of mortality and morbidity in clinical lung transplantation. Interaction of activated leukocytes with injured graft endothelial cells participates in the development of ischemia-reperfusion injury. We sought to determine if modification of the reperfusate (with depletion of leukocytes and alteration of its composition) would decrease the incidence of ischemia-reperfusion injury in human lung transplantation when compared with whole blood reperfusion in a historical group of patients.

Methods: Between June 1999 and July 2001, 23 adult patients undergoing lung transplantation consented to modified reperfusion. After implantation, a catheter was inserted into the main or individual pulmonary arteries, and modified reperfusate was administered at a pressure less than 20 mm Hg. The modified reperfusate was depleted of leukocytes, supplemented with nitroglycerin, adjusted for pH and calcium level, and enriched with aspartate, glutamate, and dextrose. After 10 minutes of modified reperfusion, the removal of pulmonary artery clamp or weaning of cardiopulmonary bypass was performed per usual protocol. Age- and diagnosis-matched historical patients served as the control group. Ischemia-reperfusion injury was defined as PaO₂/FIO₂ < 150 with diffuse infiltrate on the radiograph in absence of other causes.

Results: There was no difference in donor age or oxygenation indices, recipient age, the number of patients requiring cardiopulmonary bypass, ischemia time, and recipient oxygenation indices between the modified reperfusate group and the control group. However, none of the patients in the modified reperfusate group developed ischemia-reperfusion injury in contrast to 5 patients in the control group (P < .05). The early survival in the modified reperfusate group was 96% versus 81% in the control group (P = NS).

Conclusion: This study suggests that modification of the reperfusate content decreases the incidence of ischemia-reperfusion injury in human lung transplantation when compared with whole blood reperfusion in a historical group of patients. Modified reperfusate may allow acceptance of marginal lungs and expansion of the donor pool.

Despite advances in donor management and preservation techniques, ischemia-reperfusion injury (IRI) is estimated to occur in 15% to 20% of lung transplant recipients. It is characterized by patchy pulmonary infiltrates, widening of alveolar-arterial PO₂ gradient, and diminished pulmonary compliance, in the absence of other potential causes. The treatment for this phenomenon in lung transplantation remains largely supportive. Inhaled NO may be beneficial in lung transplant recipients with IRI; however, prophylactic administration of NO...
does not prevent this complication.\(^4\) The mortality associated with IRI among lung transplant recipients is estimated to be 40% to 60%.\(^1,2\) Additionally, the survivors have a protracted recovery course and are often left with residual impairment in lung function.\(^1\) Thus, IRI remains a frequent cause of mortality and morbidity in lung transplantation.

The molecular mechanisms underlying IRI involve the complement system, reactive oxygen species, leukocytes, and endothelium. The complement system, activated by surgical stress and reperfusion of the ischemic organ, stimulates proinflammatory mediators such as tumor necrosis factor-alpha (TNF-\(\alpha\)), interleukin (IL)-1, and IL-6.\(^5\) These mediators promote vascular permeability, activation of leukocytes, and up-regulation of adhesion molecules on endothelium.\(^5\) Reestablishment of blood flow to the ischemic graft also stimulates production of reactive oxygen species (ROS), which can damage cellular membranes via lipid peroxidation and prime endothelial cells for leukocyte interaction.\(^6\)

Although the burst of ROS jump-starts the process, the leukocyte–endothelial interaction is responsible for continuing the injury. Leukocytes, in particular neutrophils, activated by complement and attracted to the endothelium by the increased expression of adhesion molecules, begin the process of adherence and transmigration.\(^7,8\) As the activated neutrophils migrate through the endothelial barrier, they degranulate, releasing proteases, collagenses, lipoxygenases, and myeloperoxidases.\(^9\) These toxins disrupt the integrity of the endothelium and parenchymal tissue, leading to edema, thrombosis, and ultimately ischemia of the tissue and cell death.

Given the importance of interactions between activated leukocytes and graft endothelial cells in the development of IRI, we hypothesized that a modified reperfusion protocol that removes the leukocytes and resuscitates endothelial cells via addition of nutrients will attenuate IRI.

### Methods

#### Patient Population

Between June 1999 and July 2001, 23 adult patients undergoing lung transplantation gave informed consent for modified reperfusion and were prospectively enrolled into this study. The recipient diagnoses were: obstructive lung disease (\(n = 14\)), restrictive lung disease (\(n = 6\)), pulmonary vascular disease (\(n = 1\)), and septic lung disease (\(n = 2\)). Twenty-three lung transplant recipients matched for diagnosis and age (\(\pm 5\) years) immediately preceding this study (September 1997 to June 1999) were used as historical controls. In this group, whole blood reperfusion was administered at a pressure less than 20 mm Hg for the first 10 minutes.

#### Allograft Harvest and Implantation

Donor lungs were harvested using the previously described technique.\(^10\) Donor lung pulmonary artery beds were pretreated with 500 \(\mu\)g of prostaglandin \(E_1\), followed by administration of 4 L of modified Euro-Collins solution under a perfusion pressure not exceeding 20 mm Hg. All allografts were procured in inflation with tidal volume of approximately 10 mL/kg with an FiO\(_2\) of 1.0. Methylprednisolone (500 mg) was given to all recipients preoperatively. Allograft implantation was performed according to the previously described technique.\(^11\) All double lung transplant procedures in this study were performed on cardiopulmonary bypass. The allograft ischemia time for this group of patients refers to the time that both lung allografts were reperfused (with whole blood or modified reperfusate).

### Modified Reperfusion

Following implantation of right or left single lung allografts (off cardiopulmonary bypass), a cannula was inserted into right atrium or descending thoracic aorta, respectively. The recipient blood was removed, depleted of leukocytes via Leukofilter (leukoGuard BC1B, Pall Biomedical Products, East Hills, NY), and then mixed with the modified reperfusion solution in a 1:4 ratio via a roller pump (Table 1). Clinical and experimental studies have demonstrated that this leukofilter can remove in excess of 70% of white blood cells from up to 5.3 L of blood per minute.\(^12,13\) The modified reperfusate was then administered through a DLP retrograde cardioplegia cannula (Minneapolis, Minn) that had been inserted into the clamped pulmonary artery via the suture line (Figure 1). The modified reperfusion flow rate was adjusted to maintain the reperfusion pressure at <20 mm Hg; the reperfusion pressure was continuously monitored through the side port of the DLP retrograde cardioplegia cannula. The effluent during the first 3 minutes of reperfusion was collected by pump suckers (in patients undergoing lung transplantation on cardiopulmonary bypass) or by cell saver system (in patients undergoing lung transplantation without cardiopulmonary bypass). After 3 minutes, the modified reperfusion solution was stopped and leukocyte-depleted blood was continued for an additional 7 minutes. The time intervals of the modified reperfusion protocol were based on prior similar studies in heart transplantation.\(^14\) After the initial 3 minutes of reperfusion and adequate deairing of the allograft, the left atrial clamp was removed to allow return of the leukocyte-depleted blood into the recipient’s circulation. The flow rates during the reperfusion phase ranged from 200 to 500 mL per lung allograft.

In patients undergoing single lung transplant on cardiopulmonary bypass, the modified pump blood was used to reperfuse the allograft. In patients undergoing double lung transplantation on cardiopulmonary bypass, the modified reperfusate was administered into the clamped main pulmonary artery in a similar fashion (Figure 2). The reperfusion pressure was measured with a needle in the distal main pulmonary artery. Following 10 minutes of modified reperfusion, the removal of pulmonary artery (PA) clamp or weaning off cardiopulmonary bypass was performed per usual protocol.

#### Postoperative Care

All patients were mechanically ventilated in a pressure-controlled mode with settings adjusted to maintain \(PaO_2 > 90\) mm Hg. Positive end-expiratory pressure of 3 to 10 mm Hg was used to optimize oxygenation and hemodynamics. All of the patients in the control group and the first 5 patients in the modified reperfusate
group were treated with equine anti-thymocyte globulin (ATGam, Pharmacia, Peapack, NJ), followed by cyclosporine, azathioprine, and prednisone. In January 2001, the immunosuppression regimen was changed to induction therapy with rabbit anti-thymocyte globulin (Thymoglobulin, SangStat, Fremont, Calif), followed by tacrolimus, mycophenolate mofetil, and prednisone. The remaining patients in the modified reperfusate group were treated with this protocol.

**Definition of Terms**

Acute cellular rejection was defined as histologic grade A2 or higher on lung biopsy. In some patients who had not undergone lung biopsy, the diagnosis was made by constellation of clinical findings consistent with rejection and reversal of clinical findings with steroid bolus. Criteria for diagnosis of IRI were (1) hypoxemia characterized by \( \text{PaO}_2/\text{FiO}_2 < 150 \) and (2) radiographic evidence of diffuse alveolar infiltrates in the absence of other potential causes. Early deaths were defined as deaths within 30 days of the operation or during the same hospitalization period.

**Informed Consent**

The protocol for modified reperfusion in lung transplantation was reviewed and approved by the Human Subjects Protection Committee at UCLA.

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**TABLE 1. Composition of modified reperfusate solution**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Volume added (mL)</th>
<th>Component modified</th>
<th>Final concentration*</th>
</tr>
</thead>
<tbody>
<tr>
<td>THAM (0.3 mol/L)</td>
<td>225</td>
<td>pH</td>
<td>pH 7.5–7.6</td>
</tr>
<tr>
<td>CPD</td>
<td>225</td>
<td>( \text{Ca}^{2+} )</td>
<td>0.2–0.3 mmol/L</td>
</tr>
<tr>
<td>Aspartate/glutamate</td>
<td>250</td>
<td>Nutrients</td>
<td>13 mmol/L</td>
</tr>
<tr>
<td>(0.46 mol/L) D5W</td>
<td>235</td>
<td>Osmolarity</td>
<td>380–400 mOsm</td>
</tr>
<tr>
<td>D50</td>
<td>40</td>
<td>Glucose</td>
<td>&gt;400 mg/dL</td>
</tr>
<tr>
<td>Mg SO(_4) (4 mEq/mL)</td>
<td>24</td>
<td>( \text{Mg}^{2+} )</td>
<td>10–12 mEq/L</td>
</tr>
<tr>
<td>Nitroglycerin (5 mg/mL)</td>
<td>1</td>
<td>Vasodilator</td>
<td>5 mg/mL</td>
</tr>
</tbody>
</table>

*THAM, Tromethamine; CPD, citrate-phosphate-dextrose.

*After being mixed with blood in a 4:1 ratio.
TABLE 2. Perioperative characteristics and short-term outcome of the modified reperfusate and the control groups

<table>
<thead>
<tr>
<th></th>
<th>Modified reperfusate group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor age</td>
<td>32 ± 14</td>
<td>26 ± 13</td>
</tr>
<tr>
<td>Donor PaO₂/FI₂O₂ on 100%</td>
<td>440 ± 93</td>
<td>497 ± 103</td>
</tr>
<tr>
<td>Recipient mean pulmonary artery pressure (mm Hg)</td>
<td>31 ± 12</td>
<td>28 ± 9</td>
</tr>
<tr>
<td>Patients requiring CPB</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Ischemia time (min)</td>
<td>268 ± 78</td>
<td>205 ± 39</td>
</tr>
<tr>
<td>PaO₂/FI₂O₂ at 1 hour</td>
<td>272 ± 112</td>
<td>233 ± 96</td>
</tr>
<tr>
<td>PaO₂/FI₂O₂ at 6 hours</td>
<td>314 ± 138</td>
<td>267 ± 100</td>
</tr>
<tr>
<td>Mean hours on ventilator</td>
<td>72 ± 190</td>
<td>418 ± 956</td>
</tr>
<tr>
<td>Mean days in ICU*</td>
<td>6 ± 6</td>
<td>22 ± 39</td>
</tr>
<tr>
<td>Incidence of IRI*</td>
<td>0</td>
<td>5/23</td>
</tr>
<tr>
<td>Early survival</td>
<td>22/23 (96%)</td>
<td>19/23 (81%)</td>
</tr>
</tbody>
</table>

CPB, Cardiopulmonary bypass; ICU, intensive care unit; IRI, ischemia-reperfusion injury.
* P < .05.

Statistical Analysis
All data measurements are presented as the means ± standard deviations. Comparison of the mean values was performed by using repeated measures analysis of variance. Chi-square with 1 degree of freedom was used to compare the episodes of rejection and infection between the modified reperfusate and the control group.

Results
Patient Characteristics
There was no significant difference in donor age, donor oxygenation level, recipient age, or the number of patients undergoing lung transplantation on cardiopulmonary bypass between the modified reperfusate group and the control group (Table 2). Three patients in the modified reperfusate group required intracardiac procedures in addition to lung transplantation: mitral and tricuspid valve repair (n = 1) and tricuspid valve repair and repair of patent foramen ovale (n = 2). The allograft cold ischemia time was also similar in the two groups (Table 2).

Patient Outcome
There were no intraoperative technical complications associated with the modified reperfusate protocol. Two patients in the modified reperfusate group developed acute renal failure due to hemolysis associated with high flows through the leukocyte filters. These 2 patients had not required cardiopulmonary bypass for their lung transplant procedures, and other causes of hemolysis were excluded. The leukocyte filters have subsequently been changed to high-flow filters.

There was no difference in the mean oxygenation indices at 1 and 6 hours posttransplantation or the duration of mechanical ventilation between the modified reperfusate group and the control group (Table 2). Furthermore, the mean initial white blood cell counts (13.6 ± 6.9 vs 14.3 ± 5.1, P = NS), the mean initial platelet counts (203 ± 67 vs 191 ± 71, P = NS), and the mean number of transfused red blood cell units in the first 24 hours after transplantation (2 ± 2.7 vs 1 ± 2.8, P = NS) were also similar in the modified reperfusate and the control groups. However, the mean number of days in intensive care was lower in the modified reperfusate group when compared with the control group (Table 2). We did not detect any difference in the cumulative incidence of infection (pneumonia, urinary tract infection, and sepsis; 0.014 ± 0.004 vs 0.011 ± 0.004 episodes/patient · days, P = NS) or acute cellular rejection in the first 6 months after transplantation (0.009 ± 0.003 vs 0.008 ± 0.004 episodes/patient · days, P = NS) between the modified reperfusate and the control groups. None of the patients in the modified reperfusate group developed IRI, in contrast to 5 patients in the control group (22%) (Table 2). And finally, the early survival was 96% in the modified reperfusate group compared with 81% in the control group (P = NS).

There was 1 early death in the modified reperfusate group due to disseminated intravascular coagulation and multisystem organ failure. The modified reperfusate in this patient was delivered via a low-flow filter; however, the hemolysis workup (free hemoglobin and haptoglobin level) immediately after reperfusion was negative. There were 4 early deaths in the control group; one patient, who had not developed IRI, died of aspiration pneumonia on postoperative day 32. The other 3 deaths occurred in patients with IRI (2 due to nosocomial pneumonia and respiratory failure and 1 due to sepsis of pulmonary versus abdominal source).

As noted above, 5 patients in the control group developed IRI. The diagnoses in this cohort were obstructive lung disease (n = 2) and restrictive lung disease (n = 3). These patients were treated with standard supportive care as well as inhaled NO. The range of intensive care and hospital stay in this subset of patients was 15 to 143 days and 28 to 143 days, respectively. The in-hospital mortality in the cohort of patients with IRI was 60% (3/5).

Discussion
This study demonstrates that modification of the reperfusate by leukodepletion and alteration of its composition decreases the incidence of IRI when compared with whole blood reperfusion in human lung transplantation. The beneficial effects of the modified reperfusate may be due to (1) reduction in leukocyte-mediated injury, (2) resuscitation of allograft vessel wall cells via the changes in composition of the reperfusate, or (3) other factors. Neutrophils are known to play a critical role in the inflammatory cascade that follows the reperfusion of an ischemic organ. Neutrophils can cause tissue injury via (1) elaboration of elastases and
other proteases; (2) direct release of inflammatory cytokine such as TNF-α, IL-1, IL-6, and IL-8; and (3) capillary plugging leading to no-reflow phenomenon, further exacerbating local ischemia. The importance of neutrophils in IRI is further substantiated by multiple experimental studies utilizing leukocyte filters or monoclonal antibodies directed against adhesion molecules on leukocytes and endothelial cells. More recently, lymphocytes have also been shown to directly participate in the development of IRI. Hence, mechanical removal of leukocytes during the initial critical phase of reperfusion may be responsible, at least in part, for the beneficial effect of the current protocol.

Aside from leukocytes, the composition of the initial reperfusate can also affect the functional recovery of ischemic organ. Alteration of the composition of the reperfusion solution has been shown to prevent reperfusion injury in myocardial ischemia. The content of the initial reperfusate in this study was based on similar theoretical grounds and prior experimental studies in lung transplantation. Calcium influx into the cells was prevented by addition of citrate and magnesium to the reperfusate; tissue edema was minimized by adding agents with high oncotic pressure; and acidosis was buffered by addition of tromethamine. Cellular resuscitation was facilitated by enriching the solution with aspartate and glutamate, which have been shown to improve functional recovery of myocytes after a brief period of ischemia. In addition, nitroglycerin was added to ensure the homogeneous delivery of the reperfusate. It is important to note that other factors such as hemodilution may also contribute to the beneficial effects of the modified reperfusate. With better understanding of molecular mechanisms of IRI in lung transplantation and development of more effective agents to counteract the inflammatory process, the content of initial reperfusate is likely to change and result in further protection.

This report, to our knowledge, is the first study comparing modified reperfusion with whole blood reperfusion in human lung transplantation. The hypothesis leading to this study was based on the pioneering work of several groups. Furthermore, the technique of modified reperfusion has been described previously in 5 patients undergoing lung transplantation. Although the content of reperfusate is likely to change in the future, the concept of controlling the composition and condition of initial reperfusate remains the principal message of this report. The ideal duration and pressure of the initial reperfusate administration in lung transplantation is unknown. In an experimental model, Bhabra and colleagues have shown that controlling the reperfusate condition for less than 10 minutes was not as effective and that extending beyond 30 minutes did not confer any additional benefit. In contrast, in myocardial ischemia, 20 minutes of controlled reperfusion has been shown to be superior to 10 minutes. Together, these reports highlight the importance of the initial phase of reperfusion in mediating tissue injury, especially the first 10 minutes. Experimental studies have also shown that lowering the reperfusion pressure to 20 to 30 mm Hg decreases the reperfusion injury and improves allograft function. It is not known if further lowering of PA pressure can confer any additional benefit while ensuring homogeneous distribution of the reperfusate. Additional experimental studies are needed to define the ideal duration and pressure of the reperfusate, as well as the optimal conditions of ventilating the allograft (FiO₂, airway pressure, etc).

A safety concern associated with leukocyte depletion in immunosuppressed transplant recipients is the risk of infection. In this study, the initial white blood cell count was similar in the modified reperfusate and the control groups. Moreover, the cumulative incidence of infections in the first 30 days posttransplantation was also similar. Another safety concern with the use of leukofilters is thrombocytopenia. In addition to leukodepletion, currently available filters also remove platelets and may promote perioperative bleeding. In this study, we could not detect a significant difference in the initial platelet count or the incidence of perioperative bleeding. Thus, this study suggests that administration of modified reperfusate in human lung transplantation is associated with acceptable safety profile. The limitations of this study include the small number of patients in each group and the historical/noncontemporaneous comparison group.

Bronchiolitis obliterans syndrome (BOS) remains the leading cause of late death among lung transplantation recipients. In addition to alloantigen-dependent mechanisms, alloantigen-independent factors such as the impact of donor brain death on the allograft and ischemia-reperfusion injury may participate in the development of BOS. In fact, in kidney transplantation, delayed early allograft function, a manifestation of IRI, has been associated with late graft loss due to chronic rejection. Hence, there is emerging data that minimization of allograft injury in the perioperative period may not only impact early graft function but may also favorably affect long-term graft function. Future comparison of the incidence of BOS in the cohort of patients enrolled in this study may provide insight into the impact of modified reperfusate versus whole blood reperfusion on long-term allograft function.

In conclusion, this study suggests that modification of reperfusate by leukodepletion and alteration of its composition decreases the incidence of reperfusion injury in human lung transplantation. A prospective randomized trial of modified reperfusion versus whole blood reperfusion is necessary to define the role of this technique in lung transplantation. Strategies that minimize the risk of IRI may not only improve patient survival but may also allow accep-
tance of nonstandard lungs and expansion of the donor lung pool.

References

14. Pearl JM, Drinkwater DC, Laks H, Capouya ER, Gates RN. Leuko-