When does the lung die? \( K_{fc} \), cell viability, and adenine nucleotide changes in the circulation-arrested rat lung

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The lung, unlike other solid organs, does not rely on perfusion for cellular respiration. Respiration can occur directly across the alveolar wall and is entirely passive. Although cessation of the circulation with death leads to ischemia and cell death in other organs, successful cell culture of lungs retrieved from morgue specimens implies that lung tissue may remain viable long after death of the organism (12). We previously showed 90% parenchymal cell viability in rat lungs ventilated with \( O_2 \) 4 h postmortem (3). In addition, we demonstrated preservation of ultrastructure (1) and a marked attenuation in the time-dependent decrement of lung high-energy phosphate stores in \( O_2 \)-ventilated cadaveric rat lung (4). These studies all attest that the lung is viable for intervals after circulatory arrest.

Despite improved preservation techniques, the ischemia-reperfusion (IR) insult to the transplanted lung remains a significant problem in the early postoperative period. The ischemia-induced hypoxia results in increased pulmonary endothelial cell permeability, which has been shown to be related not only to the absolute level of \( O_2 \) deprivation but also to the duration of ischemia (15). Although we demonstrated postmortem pulmonary parenchymal cell viability before reperfusion, little is known about the integrity of the pulmonary endothelial cell surface and viability of the pulmonary parenchymal cell after reperfusion in the postmortem lung.

The present study was designed to determine the degree and time course of IR-induced microvascular injury as measured by the capillary filtration coefficient \( K_{fc} \) in in situ circulation-arrested cadaveric rat lungs. In addition, we sought to investigate the relationship between \( O_2 \) ventilation and \( K_{fc} \), pulmonary vasculature hemodynamics, parenchymal cell viability, and adenine nucleotide levels in the cadaveric rat lung.

METHODS

Isolated perfused lung. Male Sprague-Dawley rats weighing 250–450 g were anesthetized intraperitoneally with pentobarbital sodium (20 mg/kg) (Abbott Laboratories, Chicago, IL). A small laparotomy incision was made, and 600 U of heparin (Elkins-Sinn, Cherry Hill, NJ) were injected intravenously. The heart was cannulated through a right ventriculotomy, and the left atrium was cannulated via a left ventriculotomy. The catheters, flared at the tip, were sutured in place. The heart-lung block was left in situ in an effort to simulate the cadaveric donor as closely as possible. After varying intervals following death, a median sternotomy was performed, the main pulmonary artery was cannulated through a right ventriculotomy, and the left atrium was cannulated via a left ventriculotomy. The catheters, flared at the tip, were sutured in place. The heart-lung block was left in situ in an effort to simulate the cadaveric donor as closely as possible. After varying intervals following death, a median sternotomy was performed, the main pulmonary artery was cannulated through a right ventriculotomy, and the left atrium was cannulated via a left ventriculotomy. The catheters, flared at the tip, were sutured in place. The heart-lung block was left in situ in an effort to simulate the cadaveric donor as closely as possible.

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0.4 MgSO₄ (anhydrous), 5.4 KCl, 116 NaCl, 0.88 NaH₂PO₄ (anhydrous), 5.5 d-glucose, and 0.3 phenol red) containing 0.21% NaHCO₃ and 4% bovine serum albumin (Sigma Chemical, St. Louis, MO). The initial 75 ml of perfusate, which contain residual red blood cells and plasma, were discarded. An additional 40 ml of perfusate placed in a water-jacketed reservoir were used for recirculation. The perfusate temperature was maintained between 35 and 38.5°C, and the perfusate pH was continuously monitored with a pH probe (Accumet: Fisher Scientific, Pittsburgh, PA) placed in the venous reservoir. The pH was maintained near 7.40 by adding dilute HCl or NaHCO₃ as necessary.

Pressure transducers (Cope Laboratories, Lakewood, CO) were positioned at the hilae of the lungs, zeroed to atmospheric pressure, and calibrated with a mercury manometer. Pulmonary arterial (Ppa) and pulmonary venous (Ppv) pressures were measured continuously. In addition, peak inspiratory airway pressure (Paw) was continuously measured by positioning a T-tube on the inspiratory limb of the respiration plumbing. Peak inspiratory pressure was adjusted to ~7.0 mmHg for the control group, since previous studies have shown that pressures <8.0 mmHg permitted adequate ventilation without altering transvascular fluid flux (13). Zone 3 conditions (arterial > venous > alveolar pressures) were maintained throughout all experiments. All pressure measurements and changes in weight gain were amplified (Hewlett-Packard 8805D, Mountain View, CA) and then analyzed with a special computer software package developed for our laboratory. Data were recorded and displayed on a Macintosh II Fx computer.

Measurement of pulmonary capillary pressure (Ppc). Ppc was estimated by using the double-occlusion technique as described by Townsley (18). Simultaneous occlusion of arterial and venous catheters results in equilibration of Ppa and Ppv to the same pressure. This equilibration equals the Ppc and also reflects the capillary pressure when the lung is not isogravimetric. The pulmonary arterial (Ra) and venous (Rv) resistances were calculated from the following equations:

\[
Ra = (Ppa - Ppc)/Q,
\]

where Q is flow, and

\[
Rv = (Ppc - Ppv)/Q.
\]

Ktc. The Ktc was measured as described previously (5, 8). Briefly, after the lungs reached an isogravimetric state, the venous reservoir was rapidly elevated to increase Ppv by 6–8 cmH₂O for 15 min. The increase in lung weight was recorded over time (ΔW/Δt). The initial 3- to 5-min period of weight gain represents vascular distention and recruitment and is not a reflection of capillary permeability. The ΔW/Δt between minutes 6 and 15 represents increased transvascular fluid flux secondary to increased capillary permeability. This later ΔW/Δt was analyzed by using linear regression of the log₁₀ − weight changes per minute. The initial rate of weight gain was calculated by extrapolation of ΔW/Δt to time 0.

The Starling equation describes the role of Ktc in transvascular fluid flux (14):

\[
J_v = Ktc [(Pc - Pi) - \sigma (Ic - Iv)],
\]

where Jv is transvascular fluid flux; Pc and Pi are hydrostatic pressures in the capillary and interstitium, respectively; Ic and Iv are osmotic pressures in the capillary and interstitium, respectively; and σ is the osmotic reflection coefficient. At the extrapolated time 0, both Pc and Jv are elevated to new steady states before the remaining factors can be affected.

Therefore, Ktc can be calculated by using the equation

\[
Ktc = J_v/Pc
\]

Ktc was calculated by dividing ΔWt/Δt at time 0 by the change in Ppc that occurred after Ppv elevation. It was normalized using baseline wet lung weight and expressed as ml·min⁻¹·100 g lung tissue⁻¹.

Wet-to-dry (W/D) weight ratios. At the completion of the experiment, the upper lobe of the right lung was excised and immediately weighed. It was then dried in a 60°C oven for 48 h and reweighed.

Lung parenchymal cell viability. After excision of the right upper lobe, the right hilum was suture ligated. Right lung pieces were flash-frozen in liquid nitrogen and stored at −70°C. Thirty milliliters of a 500 mM trypan blue solution (Sigma Chemical), dissolved in Krebs-Henseleit buffer (pH 7.4), were infused into the left pulmonary artery via the existing catheter. Trypan blue stains the nuclei of nonviable cells (10). The infusion reservoir was positioned 30 cm above the heart. After infusion of the trypan blue, 30 ml of fixative consisting of 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M Sorenson's buffer was infused from the same reservoir. During both infusions, mechanical ventilation with 100% O₂ was performed. The left lung was then excised, placed in the same fixative, and stored at 4°C.

The left lung tissue was prepared for histological analysis by using standard techniques. Briefly, the tissue was dehydrated in ethanol, washed in xylene, and embedded in paraffin. Five-micrometer sections were cut, mounted on slides, and counterstained with eosin only.

Cell viability was determined microscopically (Nikon, Melville, NY) using >1,000 magnification with oil immersion and an ocular grid. The microscopist was blinded to the experimental group analyzed. Twenty-five parenchymal cell nuclei were identified in each quadrant and recorded as either viable (pink) or nonviable (blue). Each slide was counted twice at different intervals. If a >10% difference existed between counts, a third and final count was performed. Lung parenchymal cell viability was reported as the percentage of viable cells.

High-performance liquid chromatography (HPLC). Tissue samples previously retrieved from the right lung for HPLC were pulverized by using a liquid nitrogen-cooled Bessman pulverizer and then homogenized with ice-cold 0.6 N perchloric acid (2–8 ml/g tissue) using a tissue tearer (Biospec Products, Bartlesville, OK) at 30,000 revolutions/min (rpm) for 30 s. After centrifugation for 2 min at 10,000 rpm, the supernatant was removed and neutralized with cold 1 M potassium phosphate dibasic (pH 12) to achieve a pH of 6.8. The supernatant was separated from precipitated salt by repeating centrifugation for 2 min at 10,000 rpm. The remaining solution was passed through a 0.45-mm acrodisc filter.

ATP, ADP, AMP, xanthine, and hypoxanthine concentrations were determined by HPLC using an LKB Bromma apparatus (LKB-Produkter, Bromma, Sweden). A partisil 10 SAX column (Whatman, Clifton, NJ) in 0.25 potassium phosphate monobasic (pH 6.5) at a flow rate of 1.5 ml/min was used to separate and quantify ATP and ADP levels. An EQC 5u S C18 column (Whatman) in 0.25 M ammonium phosphate monobasic (pH 4.5) at a flow rate of 2.0 ml/min was used to measure AMP, xanthine, and hypoxanthine. For each assay, 50–100 ml of solution were injected into the HPLC system. Chromatograms were analyzed on an IBM 486 DX 33-MHz computer with Peak Simple software.
Table 1. \(K_{fc}\), viability, and wet-to-dry ratios in \(O_2\)-ventilated and nonventilated lungs

<table>
<thead>
<tr>
<th>Time Postmortem, min</th>
<th>No Ventilation</th>
<th>(K_{fc})</th>
<th>Viability, %</th>
<th>W/D</th>
<th>O2 Ventilation</th>
<th>(K_{fc})</th>
<th>Viability, %</th>
<th>W/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>0.24 ± 0.02</td>
<td>80.66 ± 2.60</td>
<td>8.33 ± 0.86</td>
<td></td>
<td>0.44 ± 0.08</td>
<td>74.83 ± 3.52</td>
<td>8.45 ± 0.69</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.84 ± 0.08†</td>
<td>67.83 ± 1.35</td>
<td>8.16 ± 0.54</td>
<td></td>
<td>0.53 ± 0.10</td>
<td>70.17 ± 1.89</td>
<td>8.93 ± 0.71</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>1.60 ± 0.39‡</td>
<td>72.67 ± 1.69</td>
<td>13.45 ± 2.16‡</td>
<td></td>
<td>1.75 ± 0.33‡</td>
<td>66.75 ± 1.44</td>
<td>12.37 ± 1.20‡</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>1.81 ± 0.16‡</td>
<td>68.00 ± 2.17‡</td>
<td>14.81 ± 2.24‡</td>
<td></td>
<td>2.34 ± 0.41‡</td>
<td>57.50 ± 3.66‡</td>
<td>17.05 ± 1.20‡</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 lungs/group: for both no ventilation and \(O_2\) ventilation. \(K_{fc}\), capillary filtration coefficient; W/D, wet-to-dry weight ratio. *\(K_{fc}\) in ml·min\(^{-1}\)·cmH\(_2\)O \(^{-1}\)·100 g tissue\(^{-1}\); †P = 0.05 compared with control; ‡P = 0.05 compared with 60-min \(O_2\)-ventilated group.

Standard curves were made by performing serial dilutions for ATP, ADP, AMP, xanthine, and hypoxanthine (Sigma Chemical).

Specific protocol. Forty-eight pairs of lungs were divided into eight groups (n = 6 lungs/group): group I, controls, retrieval immediately after death; group II, retrieval 30 min postmortem and \(O_2\) ventilation; group III, retrieval 30 min postmortem and no ventilation; group IV, retrieval 60 min postmortem and \(O_2\) ventilation; group V, retrieval 60 min postmortem and no ventilation; group VI, retrieval 120 min postmortem and \(O_2\) ventilation; group VII, retrieval 120 min postmortem and no ventilation; and group VIII, retrieval 240 min postmortem and \(O_2\) ventilation. Control lungs were extirpated immediately and reperfused within 5 min of death. All lungs were allowed to equilibrate for 15–20 min to achieve an isogravimetric state. Lungs that could not reach an isogravimetric state were discarded, and the experiment was repeated. During equilibration, the Ppa, Ppv, and Paw were measured and recorded every minute. After equilibration, the Ppc was obtained, and the \(K_{fc}\) was measured. The left lung was then perfused with trypan blue to determine cell viability, and portions of the right lungs were frozen or weighed to calculate the W/D ratio as described above.

Statistics. All results are expressed as means ± SE. Comparisons among groups were made using analysis of variance with Fisher’s post hoc test for multiple comparisons. Significance was determined to be present when \(P < 0.05\). Linear correlations were obtained using Pearson’s correlation coefficient.

RESULTS

The number of lungs unable to achieve an isogravimetric state for each group was as follows: group I, 1; group II, 2; group III, 0; group IV, 0; group V, 2; group VI, 3; group VII, 3; and group VIII, 4.

\(K_{fc}\). Changes in microvascular permeability as measured by \(K_{fc}\) are shown in Table 1. In nonventilated lungs, a marked increase in pulmonary microvascular permeability occurred as the postmortem ischemic time increased (\(r = 0.96\)) (Fig. 1). In lungs ventilated with \(O_2\), \(K_{fc}\) did not increase significantly compared with the control group for up to 60 min postmortem. In contrast, in nonventilated rats, the \(K_{fc}\) increased threefold after 30 min (\(P = 0.05\)) and sevenfold after 60 min (\(P = 0.0001\)) postmortem. After 120 min of postmortem time, the \(K_{fc}\) increased despite \(O_2\) ventilation. Attempts to evaluate \(K_{fc}\) after 240 min postmortem in nonventilated lungs were unsuccessful because the lungs failed to reach an isogravimetric state and had near-instantaneous pulmonary edema on reperfusion. However, \(K_{fc}\) could be assessed in \(O_2\)-ventilated lungs 240 min postmortem (Fig. 1).

W/D ratios. As the pulmonary microvascular permeability increased, lung weight gain increased, as measured by the W/D weight ratio (Table 1). The W/D ratio increased after 30 min postmortem ischemia for both the \(O_2\)-ventilated and nonventilated groups. The W/D ratio was significantly less with \(O_2\) ventilation after 60 min compared with the 60- and 120-min nonventilated groups (\(P < 0.02\)). \(K_{fc}\) correlated with W/D ratio (\(r = 0.91\)) for all groups (Fig. 2).

Viability. Pulmonary parenchymal cell viability was determined after IR by using the trypan blue exclusion method. \(K_{fc}\) correlated with W/D ratio (\(r = 0.91\)) for all groups (Fig. 2).
technique. The percentage of viable cells for each group is shown in Table 1. All groups had significantly decreased viability compared with control lungs except the 30-min postmortem ischemia group ventilated with O2. Viability correlated with Kfc and postmortem time in the O2-ventilated and nonventilated groups (Fig. 3).

Hemodynamics. Hemodynamic data are shown in Table 2. There were no significant differences among groups with respect to the Ppa or Ppv. Peak Paw was significantly increased in the 240-min postmortem ischemic group. All groups except the 30-min O2-ventilated lungs showed significant reductions of ATP and ADP compared with control values. No significant differences were found in adenine nucleotide levels between rats with or without O2 ventilation at any postmortem time interval. TAN levels were significantly decreased from control values in all groups. In addition, TAN levels decreased with increasing postmortem ischemic time (r = 0.76).

Xanthine and hypoxanthine levels increased with decreasing TAN levels for all groups. The 30- and 60-min O2-ventilated groups had decreased xanthine levels compared with other groups relative to control rats.

Figure 4 shows the relationship between mean TAN levels and Kfc. As lung parenchymal adenine nucleotide levels decreased, the Kfc increased. This suggests that the vascular permeability and resulting pulmonary edema may be related to lung total high-energy phos-

Fig. 3. Relationship between parenchymal cell viability and Kfc over postmortem ischemic time in O2-ventilated lungs is shown. As viability of cells decreases, Kfc increases (r = 0.84).

Table 2. Hemodynamic data

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>Ppa, cmH2O</th>
<th>Ppv, cmH2O</th>
<th>Paw, cmH2O</th>
<th>Ra, cmH2O·min·ml⁻¹</th>
<th>Rv, cmH2O·min·ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>20.26 ± 0.84</td>
<td>5.46 ± 0.03</td>
<td>8.93 ± 0.64*</td>
<td>0.65 ± 0.08*</td>
<td>0.43 ± 0.05*</td>
</tr>
<tr>
<td>II</td>
<td>30/O2</td>
<td>22.35 ± 1.15</td>
<td>5.61 ± 0.10</td>
<td>8.73 ± 0.16</td>
<td>0.99 ± 0.13</td>
<td>0.49 ± 0.02*</td>
</tr>
<tr>
<td>III</td>
<td>30/NV</td>
<td>21.88 ± 0.89</td>
<td>5.46 ± 0.13</td>
<td>8.64 ± 0.22*</td>
<td>0.87 ± 0.08</td>
<td>0.71 ± 0.10</td>
</tr>
<tr>
<td>IV</td>
<td>60/O2</td>
<td>21.63 ± 1.10</td>
<td>5.54 ± 0.08</td>
<td>8.76 ± 0.41*</td>
<td>0.69 ± 0.08</td>
<td>0.45 ± 0.04*</td>
</tr>
<tr>
<td>V</td>
<td>60/NV</td>
<td>23.27 ± 1.71</td>
<td>5.53 ± 0.11</td>
<td>9.12 ± 0.31*</td>
<td>0.82 ± 0.09</td>
<td>0.59 ± 0.09</td>
</tr>
<tr>
<td>VI</td>
<td>120/O2</td>
<td>21.85 ± 1.45</td>
<td>5.35 ± 0.08</td>
<td>9.98 ± 0.84</td>
<td>0.84 ± 0.07</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>VII</td>
<td>120/NV</td>
<td>21.19 ± 1.32</td>
<td>5.50 ± 0.09</td>
<td>9.36 ± 0.28</td>
<td>0.76 ± 0.08</td>
<td>0.59 ± 0.04</td>
</tr>
<tr>
<td>VIII</td>
<td>240/O2</td>
<td>23.08 ± 0.56</td>
<td>5.39 ± 0.05</td>
<td>10.55 ± 0.71</td>
<td>0.90 ± 0.03</td>
<td>0.66 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ppa, pulmonary artery pressure; Ppv, pulmonary venous pressure; Paw, pulmonary airway pressure; Ra, pulmonary artery resistance; Rv, pulmonary venous resistance. Group descriptions show postmortem time (min)/O2 or no ventilation (NV). *P = 0.05 compared with 240-min O2-ventilated lungs.

Table 3. Adenine nucleotides

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>TAN*</th>
<th>Xanthine</th>
<th>Hypoxanthine</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>3.85 ± 0.25</td>
<td>2.13 ± 0.14</td>
<td>1.88 ± 0.47</td>
<td>7.86 ± 0.47</td>
<td>0.72 ± 0.14</td>
<td>0.91 ± 0.17</td>
</tr>
<tr>
<td>II</td>
<td>30/O2</td>
<td>3.44 ± 0.40</td>
<td>1.13 ± 0.11†</td>
<td>1.37 ± 0.27</td>
<td>5.94 ± 0.12†</td>
<td>0.93 ± 0.17</td>
<td>0.64 ± 0.15</td>
</tr>
<tr>
<td>III</td>
<td>30/NV</td>
<td>2.88 ± 0.19†</td>
<td>1.62 ± 0.10†</td>
<td>1.41 ± 0.17</td>
<td>5.91 ± 0.21†</td>
<td>1.68 ± 0.17†</td>
<td>1.15 ± 0.15</td>
</tr>
<tr>
<td>IV</td>
<td>60/O2</td>
<td>2.29 ± 0.37†</td>
<td>1.46 ± 0.12†</td>
<td>0.90 ± 0.10†</td>
<td>4.65 ± 0.53†</td>
<td>1.34 ± 0.23</td>
<td>0.99 ± 0.26</td>
</tr>
<tr>
<td>V</td>
<td>60/NV</td>
<td>2.24 ± 0.36†</td>
<td>1.25 ± 0.17†</td>
<td>1.14 ± 0.19†</td>
<td>4.63 ± 0.60†</td>
<td>2.05 ± 0.21†</td>
<td>0.92 ± 0.19</td>
</tr>
<tr>
<td>VI</td>
<td>120/O2</td>
<td>1.68 ± 0.42†</td>
<td>1.24 ± 0.15†</td>
<td>0.95 ± 0.12†</td>
<td>3.87 ± 0.56†</td>
<td>2.05 ± 0.20†</td>
<td>0.92 ± 0.19</td>
</tr>
<tr>
<td>VII</td>
<td>120/NV</td>
<td>1.52 ± 0.28†</td>
<td>1.19 ± 0.19†</td>
<td>1.24 ± 0.25</td>
<td>3.89 ± 0.64†</td>
<td>2.02 ± 0.39†</td>
<td>1.22 ± 0.21</td>
</tr>
<tr>
<td>VIII</td>
<td>240/O2</td>
<td>1.09 ± 0.22†</td>
<td>1.03 ± 0.13†</td>
<td>0.90 ± 0.21†</td>
<td>3.02 ± 0.53†</td>
<td>2.33 ± 0.20†</td>
<td>1.14 ± 0.17</td>
</tr>
</tbody>
</table>

Values are means ± SE. TAN, total adenine nucleotide. Group descriptions as in Table 2. *TAN = ATP + ADP + AMP; †P = 0.05 compared with control; ‡P = 0.05 compared with 60-min O2-ventilated lungs.
CIRCULATION-ARRESTED DONORS, $K_{fc}$, AND ADENINE NUCLEOTIDES

DISCUSSION

The duration of postmortem ischemic time whereby lung function remains acceptable for transplantation is unknown. Identification of this time range and modifications in preservation techniques that could potentially increase the time are mandatory if transplantation of lungs from circulation-arrested donors is to become a reality. Previous studies from this laboratory have shown preserved parenchymal cell viability in adenine nucleotide levels in cadaveric rat lungs retrieved up to 12 h postmortem (3). This study was designed to evaluate viability and adenine nucleotide levels, as well as lung function, as measured by the capillary permeability coefficient after reperfusion.

The model used in this study is different from other isolated perfused rat lung models. Lungs were left in situ after the animal was euthanized, instead of the usual protocol of harvesting, perfusing, and then making the lungs ischemic. This model most closely resembles the clinical scenario of the circulation-arrested donor, which is of interest to us.

The degree of capillary permeability, as measured by $K_{fc}$, and the W/D ratio increased in all groups compared with controls. Interestingly, ischemic lungs ventilated with $O_2$ had similar $K_{fc}$ values and W/D ratios compared with the control group up to 60 min postmortem. Lungs reperfused after 120 min of ischemia had similar $K_{fc}$ and W/D, regardless of preharvest ventilation. $O_2$ ventilation did allow measurement of $K_{fc}$ in lungs with postmortem ischemic time interval of 4 h, although the $K_{fc}$ and W/D ratio were significantly increased compared with controls. This suggests a beneficial effect of $O_2$ ventilation on the capillary permeability of the reperfused lungs. We have shown a similar benefit in $O_2$-ventilated cadaveric lungs transplanted in dogs, as measured by improved gas exchange and recipient survival (19).

Although there is considerable evidence that reduced $O_2$-derived species are responsible for oxidative injury to tissues, the degree of damage necessary to cause irreversible lung injury is unknown. Ayene et al. (2) have demonstrated increased oxidative stress as measured by lipid peroxidation in IR rat lungs ventilated with 100% $O_2$. Unfortunately, no correlation was made between the increased lung oxidized protein levels and actual lung function. Haniuda et al. (9) have shown that lung function, as measured by $K_{fc}$, is not influenced by differences in the inspired $O_2$ fraction during ischemic intervals up to 8 h. We have shown that $O_2$ ventilation of cadaver dog lungs for 4 h before retrieval leads to improved lung function in recipients compared with lung function in recipients of nonventilated lungs (19). In addition, $O_2$ ventilation of cadaveric rat lung resulted in improved viability compared with $N_2$ ventilation or no ventilation (4).

In general, lung viability was decreased in lungs subjected to an ischemic period before perfusion (except 30-min $O_2$-ventilated lungs, which did not differ from controls). Preharvest $O_2$ ventilation did not increase the percentage of viable parenchymal cells compared with nonventilated lungs. This finding is contrasted by lung viability studies before reperfusion in the cadaveric rat lung. Prior studies have found that $O_2$ delivery to the ischemic airway, rather than mechanical ventilation per se, was the critical factor in delaying cell death in nonperfused lungs (3). Approximately 20% more lung cells were viable before reperfusion than what we found after reperfusion for each postmortem ischemic time interval. Therefore, IR appears to decrease cell viability in this model, compared with viability in ischemic but nonreperfused lungs. Additionally, the beneficial effect of preharvest $O_2$ ventilation on cell viability could not be demonstrated after lungs were reperfused.

This study shows a strong correlation between cell viability and microvascular capillary permeability ($K_{fc}$). This relationship was more apparent in $O_2$-ventilated lungs. Realizing that lungs with high $K_{fc}$ values will have a poorer gas exchange, it was particularly interesting to find a strong relationship between $K_{fc}$ and cell viability. This implies that if lung parenchymal cell viability could be ascertained noninvasively preharvest, one would have an idea of the suitability of the lungs for transplant and what their immediate posttransplant lung function would be.

Tissue TAN levels decreased at different rates, depending on the organ. For example, TAN levels in rat liver decreased > 75% after 2 h of ischemia (11) and > 65% in mouse kidney after 2 h of ischemia (20). We have shown that TAN levels in nonventilated rat lung decreased 66% from baseline at 2 h postmortem. By comparison, $O_2$ ventilation of rat lungs after 2 h of ischemia decreased TAN levels by 32% (4). In the present study, we found no difference in TAN levels in ventilated and nonventilated lungs. This suggests that TAN levels after reperfusion correlate better with length of postmortem ischemic time than with preharvest ventilation status. Alternatively, TAN levels may be somehow restored by reperfusion with Earle’s solution.

The present study shows a clear relationship between $K_{fc}$, cell viability, and adenine nucleotide levels in the IR rat lung. Whereas previous studies from our laboratory suggested a benefit from preharvest $O_2$ ventilation in circulation-arrested cadavers with respect to cell viability and adenine nucleotide levels, this benefit is not as apparent after the lungs are reperfused. $O_2$ ventilation resulted in a significantly decreased $K_{fc}$ in lungs ventilated up to 1 h postmortem and allowed for $K_{fc}$ assessment in lungs retrieved 4 h postmortem. Failure of the $K_{fc}$ to remain decreased after 60 min postmortem ischemia may be related to decreased cell viability and/or increased adenine nucleotide breakdown. Other possible explanations for this finding that were not explored in this study include the sodium, potassium, and calcium fluxes between the cell and the interstitium. Finally, alterations in the endothelial cell cytoskeleton may affect capillary permeability; however, it is unclear what length of ischemic time...
interval is necessary to result in contraction of the actomyosin fibril in the cell, which results in this increased permeability. Seibert et al. (17) have suggested that compounds that increase adenosine 3',5'-cyclic monophosphate can reverse increased capillary permeability, presumably through an adenosine 3',5'-cyclic monophosphate-dependent endothelial cell relaxation.

In conclusion, this modification of traditional IR rat lung models has allowed us to further evaluate our hypothesis that lungs retrieved from circulation-arrested donors may be suitable for transplantation. This experimental design evaluates the acute effects of the postmortem ischemic time and ventilation on $K_t$, cell viability, and adenine nucleotide levels. It does not address whether these observations are irreversible or constant over time, because our reperfusion time period was only – 45 min. A recent study suggests that the increase in permeability may be transient, even in lungs retrieved 4 h after death. Using a canine double-lung transplant model, we showed improvement in alveolar-to-arterial gradient and reduction in extravascular lung water over an 8-h period following lung transplant from O2-ventilated cadaver dogs (16). To investigate the time course of changes in permeability and gas-exchange characteristics, we plan to perform rat lung allograft transplantation with lungs retrieved from cadavers at intervals after death. These studies will further elucidate our understanding of lungs retrieved after death, perhaps culminating in the use of circulation-arrested donor lungs for lung transplantation.

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