The effect of verapamil on ex-vivo rat lung preservation

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The development of methods for extending ex-vivo lung preservation intervals which do not increase functional impairment1), and which minimise the functional perturbation associated with reimplantation response2,3), continues to be an important focus of research. The major impediments to clinical lung transplantation are the scarcity of suitable donor organs and the current imperfect preservation methods, which cannot cope well with the extreme vulnerability of ex-vivo lungs to ischemic damage. In order to resolve these problems, we performed a series of experiments in an ex-vivo rat lung model to evaluate the protective effects of various preservative perfusates against lung ischemia and reperfusion tissue injury4). The present study was designed to determine whether or not verapamil provided a protective effect against lung ischemia and subsequent reperfusion injury.

Sprague-Dawley male rats (400–450 gr) were used in this experiment, and were divided into three groups. In group 1 (control group), the lungs were perfused with EuroCollins solution with no preservation time. In group 2, the lungs were perfused with EuroCollins solution after 3 hour preservation, and in group 3 the lungs were perfused with EuroCollins solution containing 50 μM verapamil after 3 hour preservation (Table 1). The rats were anesthetized and the trachea was cannulated, and mechanical ventilation was maintained with a Harvard rodent ventilator at 80 breaths/min, 3 ml in tidal volume using room air. The chest was opened via a median sternotomy, and the main pulmonary artery was cannulated through an incision in the right ventricle outflow tract. The left atrial appendage was partially incised. The lungs were then gently flushed in-situ with 20 ml of hypothermic preservate (4°C) for 3 minutes. The heart and lungs were removed en-bloc from the thorax and the excess heart was then trimmed away. After these procedures were completed, the lungs were weighed. Group 1 lungs were immediately perfused with heparinized normothermic isologous whole blood at a constant blood flow (16 ml/min) for 60 minutes while continuous ventilation was maintained. The ex-vivo lung perfusion apparatus used was modified from that described by Eisman et al.5). During perfusion, the pulmonary artery pressure (PAP) was monitored continuously in each group. The lung weights and perfusate hematocrit levels were measured three times: before perfusion, after 30 minutes of perfusion, and when perfusion ceased. The pulmonary vascular resistance (PVR) was described with PVR index

| Group 1 (Control) | ECS* alone | no preservation interval |
| Group 2 | ECS alone | 3 hr preservation (4°C) |
| Group 3 | ECS + VPM** (50 μM) | 3 hr preservation (4°C) |

*: Euro Collins solution **: Verapamil HCl

Table 1 Preservation methods

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(PVRI: dynes·sec·cm⁻⁵/cm²), which was converted into the body surface area (BSA: cm²). The PVR was calculated with the following formula: PVR=PAP/CO × 79.9. The BSA was calculated by the following equation: BSA=C × √(height × body weight), C=constant. All data were reported as the mean ± the standard error of the mean.

The results of this experiment are summarized in Table 2. Control data were obtained from group 1 in which there was no preservation time. The wet lung weight in group 1 showed little change before, during, and after reperfusion. Group 2 showed an increase of 4.6 ± 0.9 gr, while group 3 showed only a slight increase of lung weight. The hematocrit levels showed no marked changes in group 1 and 3, whereas in group 2 the hematocrit level increased about 8% on average. Group 1 indicated a slight increase of PVR immediately at the beginning of reperfusion and this returned to normal within 30 minutes of starting reperfusion. Group 2 and 3 showed higher PVR values at the beginning of reperfusion. Group 2 finished with the highest PVR value, while group 3 returned to the control level within 30 minutes of starting reperfusion. Histological examination showed a normal alveolar architecture with slight thickening of the alveolar septae in group 3. In contrast, group 2 showed prominent alveolar damage associated with marked edema, congestion and hemorrhage (Fig. 1, 2).

We previously developed the ex-vivo rat lung model for evaluating the function of preserved lungs as a preliminary study. We subsequently attempted to reduce ischemic tissue damage due to long preservation intervals by adding a variety of substances to the preservative used in this ex-vivo rat lung model. It is well known that elevation of the intracellular free Ca²⁺ concentration causes contraction of the myocardium and smooth muscle, increases glandular and mast cell secretion, releases neurotransmitters and promotes the accumulation of inflammatory cells like neutro-

### Table 2

<table>
<thead>
<tr>
<th>Preservative</th>
<th>N</th>
<th>Time*</th>
<th>Post preservation weight gain(%)</th>
<th>Post perfusion weight gain(%)</th>
<th>Initial PVRI**</th>
<th>% Final PVRI***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>9</td>
<td>0</td>
<td>—</td>
<td>1.0</td>
<td>296.0±121.5</td>
<td>71.2±14.4</td>
</tr>
<tr>
<td>Group 2</td>
<td>10</td>
<td>3</td>
<td>9.2</td>
<td>43.0</td>
<td>499.0±40.3</td>
<td>85.2±10.4</td>
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<tr>
<td>Group 3</td>
<td>10</td>
<td>3</td>
<td>0.2</td>
<td>5.0</td>
<td>422.3±195.1</td>
<td>53.7±12.6</td>
</tr>
</tbody>
</table>

*: Hours of preservation at 4°C. +: p<0.01 **: Pulmonary vascular resistance index (dynes·sec·cm⁻⁵/cm²). ***: Percentage of initial PVR at termination of 60 min perfusion period.

Fig. 1 Hematoxylin and Eosin (×25)  
Fig. 2 Hematoxylin and Eosin (×10)
phils. Ischemia and postischemic reperfusion induce an increase of intracellular Ca++ levels and initiate transmembrane Ca++ influx into vascular smooth muscle cells. McMurty et al.\textsuperscript{6} found that verapamil significantly inhibited pulmonary vasoconstriction in hypoxic lungs in comparison to prostaglandin F\textsubscript{2a} and angiotensin 2 which induce pulmonary vasoconstriction. Hachida et al.\textsuperscript{7} showed that in the lungs treated with verapamil the release of enzymes from ischemic lung tissue after reperfusion was significantly reduced, suggesting that ischemic tissue damage had been prevented. The protective mechanisms are suggested as follows: ischemia and postischemic reperfusion damages the alveolar capillary membrane so it results in disruption of the permeability barrier to calcium ions. Verapamil inhibits edema formation and an increase of microvascular resistance caused by an increase of vascular capillary leak. In addition, the tissue stores of ATP are preserved for maintaining intracellular homeostasis by preventing an increase in the Ca++ content in mitochondria during ischemia and subsequent reperfusion. The optimal concentration of verapamil for use in simple hypothermic preservation has not been investigated sufficiently to date. We determined the 10\textsuperscript{-5} M concentration of verapamil after studying the following reports. Angero et al.\textsuperscript{8} showed that pulmonary vasoconstrictive response was significantly attenuated by 10\textsuperscript{-5} M concentration of verapamil. In our study, we also were able to demonstrate an effect of verapamil at a similar concentration. Schwarz and Hamann et al.\textsuperscript{9,10} found that the concentration and distribution of plasma and tissue verapamil were highest for the lung, followed by the kidney, liver and heart. This suggested that verapamil is of specific value for lung preservation. Our results indicated that verapamil provided a protective effect against ischemic lung damage during preservation and also against postischemic reperfusion injury. It appears that the ex-vivo rat lung model is a reliable, reproducible and inexpensive methods for the screening of lung preservation regimens, and that verapamil prevents lung injury during ischemia and reperfusion. Further advances in preservation methods are urgently needed to increase the availability of organ transplantation in a number of fields of surgery.

References


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