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Comparison of Euro-Collins Solution, Low-Potassium Dextran Solution Containing Glucose, and ET-Kyoto Solution for Lung Preservation in an Extracorporeal Rat Lung Perfusion Model

Abstract

We have recently developed a modified ET-Kyoto solution by adding N-acetylcysteine, nitroglycerin, and dibutyryl cyclic adenosine monophosphate to the previously reported ET-Kyoto solution. The purpose of this study was to compare the new ET-Kyoto solution with the regular Euro-Collins solution and a low-potassium dextran solution containing glucose (Perfadex\textsuperscript{®}) in an isolated rat lung perfusion model. Rat lung blocks were preserved with ET-Kyoto solution, with Euro-Collins solution, or with Perfadex for 4 h. Arterial oxygen tension, shunt fraction, peak inspiratory pressure, mean pulmonary arterial pressure, and pulmonary vascular resistance were assessed up to 50 min after reperfusion. ET-Kyoto solution provided a significantly better lung function after reperfusion than Euro-Collins solution and Perfadex, while Perfadex was also superior to the Euro-Collins solution. We conclude that lung preservation with ET-Kyoto solution is significantly superior to Euro-Collins solution and to Perfadex in an isolated rat lung perfusion model.

Introduction

Current clinical lung preservation with Euro-Collins (EC) or University of Wisconsin solution provides satisfactory graft function. The tolerable ischemic time, however, is still limited to approximately 7 h [1]. Strategies to increase the ischemic tolerance and as a consequence the supply of transplantable lungs are, therefore, needed. We already reported...
that disaccharide trehalose was more effective than monosaccharide glucose in canine lung transplantation after 12-hour ischemia. Subsequently, we developed an extracellular preservation solution, ET-Kyoto (ET-K) solution, which contains trehalose, gluconate, and hydroxyethyl starch (HES), and demonstrated its efficacy in 20-hour canine lung preservation [3]. However, in a further study for 48-hour preservation with ET-K solution [4], the results were inconsistent. A number of recent studies have demonstrated that ischemia-reperfusion causes pulmonary endothelial damage and dysfunction of vasorelaxation [5–7]. Our previous study with scanning and transmission electron microscopy [8] demonstrated a significant correlation between the post-ischemic graft function and the structural changes of endothelial cells. The intracellular mechanisms of pulmonary vascular smooth muscle relaxation are ultimately mediated through either cyclic guanosine monophosphate (cGMP) or cyclic adenosine monophosphate (cAMP) [9]. Ischemia and reperfusion reduce intracellular cGMP and cAMP levels [10, 11]. Retaining the endothelial intracellular cGMP and cAMP levels, therefore, may lead to improvement of lung preservation. In order to enhance the efficacy of the ET-K solution, we have developed a modified ET-K solution by adding dibutyryl cAMP (db-cAMP), which is a membrane-permeable cAMP analogue and elevates the intracellular cAMP concentration; nitroglycerin, which is a potential nitric oxide (NO) donor and elevates intracellular NO/cGMP levels [12], and N-acetylcysteine (NAC), which protects endothelial cells from oxygen free radicals [13]. NAC has been proven in our previous study [14] to be effective in attenuating reperfusion injury after lung transplantation. We have already demonstrated that the modified ET-K solution provided excellent 30-hour canine lung preservation and was significantly superior to the previous ET-K solution [15, 16]. In the present study, we used an isolated rat lung perfusion model for evaluation of the effect of ET-K solution on pulmonary preservation compared to regular EC solution and, as a second control, to an established low-potassium dextran solution containing glucose (Perfadex®).

Materials and Methods

Twenty-one male inbred Sprague-Dawley rats (390–510 g) were assigned randomly to three groups according to the type of the preservation solution. Group 1 (n = 7): ET-K solution, group 2 (n = 7): EC solution, and group 3 (n = 7): Perfadex (Kabi Pharmacia, Uppsala, Sweden; table 1). The lungs in each group were stored for 4 h in the corresponding solution at 4°C prior to reperfusion. All animals received humane care in compliance with the ‘Principal of Laboratory Animal Care’ formulated by the National Society for Medical Research and the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Sciences and published by the National Institutes of Health [NIH Publication No. 86-23, revised 1985].

Bovine blood was drawn directly from the jugular vein of living cows. The red cells were processed within 1 day, using sterile techniques to remove plasma, white cells, and platelets. The blood was spun at 3,500 g for 10 min. The supernatant plasma was removed. The cells were diluted with 0.9% saline and spun in the same way as in the first process. The red blood cells were then diluted with Krebs-Henseleit solution to a hematocrit of 37–38%. Thereafter, leukocytes were removed through a leukocyte filter (leukocyte removal filter RC100E; PALL Europe, Portsmouth, UK).

The animals were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg) and intubated by tracheostomy. During the operation, the animals were ventilated with room air at a tidal volume of 5 ml and a respiration rate of 40 breaths/min with a positive end-expiratory pressure of 3 cm H₂O. Through a laparotomy, 100 U heparin was given into the inferior vena cava. After sternotomy, the right and left superior vena cavae and the left azygos vein were clipped. A No. 14 cannula was inserted into the pulmonary artery through the right ventricle, and the inferior vena cava was clipped. The left atrial appendage was then cut, and the lungs were flushed with 20 ml of cold...
perfusates under 20 cm of gravity pressure. Ventilation of the lungs was continued during the flush, and the duration of flushing was recorded. The heart-lung block was carefully excised and immediately immersed in cold (4°C) Ringer solution. Pulmonary artery, trachea, and left atrium were cannulated (inner diameter 1.8 mm). The lungs were allowed to deflate during the preparation. Thereafter, the lungs were reinflated with 10 ml of room air, the heart-lung block was then immersed in the respective cold solution and stored at 4°C for 4 h.

An extracorporeal circuit, described by Fukuse et al. [17], was modified and used. The perfusion circuit was primed with 600 ml of Krebs-Henseleit solution containing washed bovine red blood cells. The perfusate was continuously deoxygenated by a deoxygenator (Monolyth integrated membrane lung; Sorin Biomedica, Saluggia, Italy) and gassed with 95% N2 and 5% CO2. The heart-lung block was suspended at a 45-degree angle in a humidification chamber. Mechanical ventilation was performed with a small animal respirator (animal respirator 4601; Rhema Labortechnik, Germany). All vessels were water jacketed and the temperature controlled by a warming pump (Water thermostat type VTS 13c; Radiometer, Copenhagen, Denmark) at 37°C.

After storage for 4 h, the heart-lung block was connected to the extracorporeal circuit. The lungs were ventilated via the tracheal cannula with room air at a tidal volume of 5 ml and a respiration rate of 40 breaths/min with a positive end-expiratory pressure of 3 cm H2O and were reperfused via the pulmonary arterial cannula with the deoxygenated perfusate by a roller pump (PA21-A; Cole Parmer, Chicago, Ill., USA). The perfusion rate was gradually increased from 1.0 to 8.0 ml/min during the first 9 min, and thereafter, the lung was perfused at a constant rate of 8.0 ml/min up to 50 min after reperfusion. If the tracheal cannula was entirely filled with fluid due to pulmonary edema, reperfusion was interrupted. The pulmonary arterial pressure was assessed continuously with a transducer and a pressure monitor (Servomed; Hellige, Hamburg, Germany). The pulmonary flow rate was measured by collecting flow from the left atrial cannula. The peak inspiratory pressure was monitored continuously. Blood gas analysis, mean pulmonary arterial pressure, peak inspiratory pressure, and pulmonary flow rate were recorded every 10 min after reperfusion. The oxygen tension (PO2) of the perfusate collected from the left atrium was defined as arterial PO2 and PO2 of the perfusate after deoxygenation as venous PO2. The pulmonary vascular resistance (PVR) was calculated as follows: PVR = 80 x (mean pulmonary arterial pressure – left atrial pressure)/pulmonary flow rate (dyn-s-cm⁻⁻). In addition, the shunt fraction, which shows the fraction of arteriovenous shunts in the lungs, indicating the invalid respiration or gas exchanges, was calculated as follows: shunt fraction (%) = [(Cc – Ca)/Cc] x 100; C = (PO2 x 0.003) + (1.34 x hemoglobin concentration x O2 saturation) where Cc, Ca, and Cv are the oxygen contents of the pulmonary capillary, arterial, and venous blood, respectively.

Statistical analysis of the data was performed by analysis of variance with post hoc comparison and paired, two-tailed t test. p < 0.05 was considered significant. All results are expressed as mean values ± SD.

**Results**

The pulmonary flush time was similar in the three groups (group 1: 44.4 ± 4.6 s, group 2: 41.0 ± 6.0, and group 3: 47.6 ± 6.2 s). All the lungs in group 1 were reperfused for 50 min, while in 4 animals of group 2 and in 1 animal of group 3, the reperfusion was interrupted due to severe lung edema after 11, 14, 14, 44, and 42 min of reperfusion, respectively. The mean reperfusion times of groups 1, 2,
and 3 were 50 min, 33.3 ± 19.1, and 48.9 ± 3.0 min, respectively (p < 0.05, groups 1 and 3 vs. 2).

The arterial PO2 of group 1 (42.7 ± 18.6 mm Hg at 50 min of reperfusion) was significantly higher than those of groups 2 (12.9 ± 2.5 mm Hg at 50 min of reperfusion) and 3 (22.8 ± 6.3 mm Hg at 50 min of reperfusion) throughout the reperfusion: p < 0.01, group 1 vs. 2; p < 0.01, group 1 vs. 3 (10 and 20 min after reperfusion); p < 0.05, group 1 vs. 3 (30, 40, and 50 min after reperfusion; fig. 1). No significant differences were detected between groups 2 and 3 in PO2. The shunt fraction remained low in group 1 (23.3 ± 11.6% at 50 min of reperfusion) and was significantly lower than in groups 2 and 3 throughout the reperfusion (p < 0.01; fig. 2).

The shunt fraction of group 3 (61.1 ± 17.9% at 50 min of reperfusion) was also significantly lower than that of group 2 (85.9 ± 6.8% at 50 min of reperfusion) throughout the reperfusion (p < 0.01 at 10, 20, and 30 min of reperfusion; p < 0.05 at 40 and 50 min of reperfusion).

The mean pulmonary arterial pressure increased significantly after reperfusion in each group (p < 0.01, 10 vs. 50 min after reperfusion; table 2). The mean pulmonary arterial pressure of group 1 was significantly lower than that of group 2 throughout the reperfusion (p < 0.01) and that of group 3 after reperfusion (p < 0.01 at 20, 40, and 50 min after reperfusion; p < 0.05 at 30 min after reperfusion). During the first 20 min of reperfusion, the mean pulmonary arterial pressure in
Comparison of EC, LPDG, and ET-Kyoto Solutions for Lung Preservation


Fig. 3. PVR after reperfusion (mean ± SD). * p < 0.01 (groups 1 and 3 vs. 2); ** p < 0.01 (group 1 vs. 2), p < 0.05 (group 1 vs. 3), p < 0.05 (group 2 vs. 3); *** p < 0.01 (group 1 vs. 2), p < 0.05 (group 1 vs. 3).

Table 2. Mean pulmonary arterial pressure (mm Hg) after reperfusion (mean ± SD)

<table>
<thead>
<tr>
<th>Time of reperfusion min</th>
<th>Group 1 (ET-K)</th>
<th>2 (EC)</th>
<th>3 (Perfadex)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>11.7±1.1 (7)b</td>
<td>22.9±6.4 (7)</td>
<td>15.7±5.5 (7)e</td>
</tr>
<tr>
<td>20</td>
<td>11.6±1.0 (7)b,c</td>
<td>22.5±4.2 (4)</td>
<td>17.4±4.9 (7)e</td>
</tr>
<tr>
<td>30</td>
<td>12.1±1.2 (7)b,d</td>
<td>26.5±2.9 (4)</td>
<td>20.3±7.9 (7)</td>
</tr>
<tr>
<td>40</td>
<td>13.4±1.6 (7)b,c</td>
<td>30.5±1.7 (4)</td>
<td>25.1±10.6 (7)</td>
</tr>
<tr>
<td>50</td>
<td>14.3±1.6 (7)b,c</td>
<td>34.3±4.5 (3)b</td>
<td>25.7±9.3 (6)a</td>
</tr>
</tbody>
</table>

The number of animals at each time point is shown in parentheses.

* a p < 0.01 (10 vs. 50 min of reperfusion).
 b p < 0.01 (group 1 vs. 2).
 c p < 0.01 (group 1 vs. 3).
 d p < 0.05 (group 1 vs. 3).
 e p < 0.05 (group 2 vs. 3).

group 3 was significantly lower than in group 2 (p < 0.05). The difference, however, did not reach significance after 30 min of reperfusion.

In group 1, the PVR maintained a significantly lower level (144.8 ± 15.3 dyn·s·cm⁻² at 50 min of reperfusion) than in group 2 (359.6 ± 58.1 dyn·s·cm⁻² at 50 min of reperfusion) throughout the reperfusion (p < 0.01) and in group 3 (260.9 ± 100.1 dyn·s·cm⁻² at 50 min of reperfusion) after 20 min of reperfusion (p < 0.05; fig. 3). Although the PVR of group 3 was significantly lower than that of group 2 until 30 min after reperfusion (p < 0.01 at 10 min and p < 0.05 at 20 and 30 min of reperfusion), no significant difference was detected during the last 20 min of reperfusion.

The peak inspiratory pressure of group 1 (16.0 ± 4.4 cm H₂O at 50 min of reperfusion) was significantly lower than that of group 2 (33.9 ± 7.2 cm H₂O at 50 min of reperfusion) throughout the reperfusion (p < 0.01) and that
Fig. 4. Peak inspiratory pressure after reperfusion (mean ± SD). * p < 0.01 (groups 1 and 3 vs. 2), p < 0.05 (group 1 vs. 3); ** p < 0.01 (group 1 vs. 2 and 3), p < 0.05 (group 2 vs. 3); *** p < 0.01 (group 1 vs. 2 and 3).

Discussion

The EC solution continues to be the current clinical standard solution for lung preservation. A recent clinical report by Kshettry et al. [18] has shown that patients who received lung grafts with ischemic time >6 h achieved acceptable survival rates after transplantation. However, Snell et al. [19] reported that the survival of the patients was significantly reduced receiving a graft with an ischemic time beyond 5 h. Several animal studies have demonstrated that low-potassium dextran solutions were significantly superior to the EC solution [20, 21] and provided extended pulmonary preservation with satisfactory results [22]. In the current study, we intended to compare the recently improved ET-K solution with the clinical standard EC solution and a low-potassium solution, since ET-K is also a potassium-reduced solution. As a representative, we chose Perfadex because of its proven beneficial effects in recent experimental trials [21, 22].

The present study revealed that potassium reduction significantly improves posts ischemic lung function, since ET-K as well as Perfadex were superior to EC. The decreased shunt fractions, the lower PIPs, and the lower PVRs with the potassium-reduced solutions mean more homogenous perfusion, better pulmonary compliance, and less damage to the microvasculature than with the high-potassium solution. These findings are consistent with those of our previous study on the comparison of ET-K and EC [3] and correspond with those obtained in a previous study performed by Keshavjee et al. [20]. However, our results additionally showed a superiority of modified ET-K over Perfadex. A similar observation was reported previously by Liu et al. [16]. The ET-K solution contains a disaccharide (trehalose), HES, and gluconate and has a potassium concentration of 42 mmol/l. We have demonstrated earlier [2] that trehalose was more effective than glucose as a component of EC and Perfadex solutions in canine lung preservation. Colloids, such as HES, and dextran are thought to prevent interstitial edema and to improve posts ischemic organ...
function. The ET-K solution contains HES with a mean molecular weight of 400,000 daltons, whereas in Perfadex dextran with a mean molecular weight of 40,000 daltons is added. We recently compared EC solutions with three different additives, dextran 40, dextran 70, and dextran 160, and have found that dextran 160 was the most beneficial additive [23]. This was obviously due to the limited leakage of the larger dextran molecules into the interstitial space during pulmonary flush and storage in contrast to the dextrans of lower molecular weight. According to these results, HES, with its high molecular weight, can, therefore, be more effective for prevention of edema than dextrans with a molecular weight of only 40,000 daltons.

With regard to the potassium concentration, it is still unclear which concentration of potassium in preservation solution is optimal for lung preservation. Perfadex contains only 6 mmol/l of potassium ion, while ET-K contains 42 mmol/l. High-potassium solutions have been considered to induce pulmonary vasoconstriction during flushing, causing interruption of homogenous flushout. Although Sasaki et al. [24] proposed that a potassium concentration in flushing solution of 20 mmol/l or less assured appropriate flushing and adequate distribution of the solution, Wada et al. [4] reported recently that a medium-potassium (44 mmol/l) ET-K solution was superior to a low-potassium (20 mmol/l) ET-K solution in canine lung preservation. The use of trehalose and HES as well as the medium-potassium concentration may, therefore, serve as one of explanations for the better preservation achieved with ET-K when compared to Perfadex.

The ET-K solution contains nitroglycerin and db-cAMP which elevate intracellular NO/cGMP and cAMP levels. cGMP and cAMP are considered to relax pulmonary vascular smooth muscles and to inhibit platelet aggregation and adhesion [9]. The intracellular concentrations of both monophosphates were reported to be reduced during ischemia and reperfusion [10, 11]. Naka et al. [25] showed that nitroglycerin improved lung preservation; Oz et al. [26] indicated that sustaining higher levels of cAMP and NO/cGMP provided a better cardiac preservation. NAC, which protects endothelial cells from oxygen free radical injury [12], is also one of the components in the ET-K solution. In the present ex vivo study, however, reperfusion injury caused by oxygen free radicals may not play the same important role as under in vivo conditions because leukocytes were removed from the perfusate in our model.

In conclusion, the modified ET-K solution provided better lung preservation than EC or Perfadex solution in an isolated rat lung perfusion model and may, therefore, be useful for extended clinical lung preservation. Further studies are needed to examine the importance of various components in ET-K solution, especially the significance of db-cAMP, nitroglycerin, and NAC, in lung preservation.

**Acknowledgement**

Dr. Bando was supported by a fellowship from the Alexander von Humboldt Foundation.
References


