Celsior solution for improvement of currently used clinical standards of lung preservation in an ex vivo rat model

Thorsten Wittwer*, Thorsten Wahlers, Jan F. Cornelius, Sebastian Elki, Axel Haverich
Division of Cardiothoracic and Vascular Surgery, Medical School Hannover, Carl–Neuberg-Strasse 1, 30625 Hannover, Germany

Received 21 September 1998; received in revised form 18 January 1999; accepted 27 January 1999

Abstract

Objective: The introduction of Euro–Collins solution with its intracellular electrolyte composition has allowed for clinically accepted pulmonary preservation for up to 7 h of ischemic time. In recent years several alternative solutions have been developed for the improvement of pulmonary preservation. Celsior® is an extracellular solution which has significantly reduced the ischemia/reperfusion (IR)-induced pulmonary damage in animal studies. So far, no larger experimental studies exist concerning the influence of Celsior on pulmonary gas exchange following IR.

Methods: In an extracorporeal rat lung model ten lungs, were each preserved with Celsior (CE) and Celsior/prostacycline (CEPC, 6 mg/100 ml) at 4°C and compared with preservation with low-potassium-Euro–Collins solution (LPEC, 40 mmol/l of potassium). After 2 h of ischemia the lungs were re-ventilated and reperfused using a roller-pump. Relative oxygenation capacity (ROC), pulmonary vascular resistance (PVR), peak inspiratory pressure (PIP) and wet/dry ratio were monitored for 50 min. Statistical analysis was performed using ANOVA.

Results: ROC was increased in all Celsior preserved organs compared with the EC group (P < 0.032). Though the CEPC group was found to have the lowest PIP and the least amount of lung water as assessed by wet/dry ratio, PVR was highest after 30–50 min. The significantly lowest PVR was determined in the CE group (P < 0.02).

Conclusions: Celsior provides better lung preservation than LPEC solution, as demonstrated by a significantly increased oxygenation ability, a lower PVR and a decreased wet/dry ratio. In vivo experiments and additional histological analysis are warranted for further evaluation of Celsior in lung preservation.

Keywords: Lung transplantation; Preservation solution; Stereological analysis; Ischemia/reperfusion injury

1. Introduction

Lung transplantation has proven to be an effective therapeutic option for patients with end-stage lung disease [1–4]. The ongoing development of different techniques for organ procurement [5–9] has significantly improved the limited initial results [10,11]. Nevertheless, the poor tolerance of the lung to ischemia and reperfusion (IR) still represents one of the limitations to broad and successful clinical application of lung transplantation today. Modified Euro–Collins (EC) solution is currently the most widely used for lung preservation [12]. However, it is well known that the high potassium concentration of EC can cause severe pulmonary vasoconstriction followed by edema formation [13,14]. Therefore, in recent years several low-potassium-solutions with extracellular electrolyte composition have been developed for improvement of pulmonary preservation by minimizing vasospasm, consequently leading to a homogeneous distribution of the flush solution. Celsior® is a new extracorporeal preservation solution (Table 1) designed to prevent edematous swelling, free radical injury, calcium overload and energy depletion [15]. In animal studies, the markedly IR-induced increase in pulmonary microvascular permeability was consistently reduced [16]. So far no larger experimental studies exist concerning the influence of Celsior on pulmonary gas exchange following IR.

To assess the influence of Celsior on post-preservation lung function in terms of oxygenation ability, lung preser-
avoid development of atelectasis, thereby reducing the expiratory pressure of 3 cm H₂O was maintained in order to ml and a rate of 40 respiratory cycles/min. A positive end-

Munich, Germany) with room air at a tidal volume of 5 mechanical ventilation was maintained with a small animal respirator (animal respirator 4601, Rhema Labortechnik, London, UK).

40%. Thereafter, the remaining white cells were removed in the same way. The red cells were then diluted with Krebs–Henseleit buffer solution to a hematocrit of 38–40%. Thereafter, the remaining white cells were removed with a leucocyte filter (RC100E; PALL Europe, Portsmouth, UK).

The rats were anesthetized with pentobarbital using 1 mg/kg body weight intraperitoneally. After tracheotomy, mechanical ventilation was maintained with a small animal respirator (animal respirator 4601, Rhema Labortechnik, Munich, Germany) with room air at a tidal volume of 5 ml and a rate of 40 respiratory cycles/min. A positive end-expiratory pressure of 3 cm H₂O was maintained in order to avoid development of atelectasis, thereby reducing the extent of venoarterial shunt. A laparotomy was performed, and the animals were heparinized intravenously (100 IU). The chest was opened bilaterally and the main pulmonary artery was cannulated. Rats were assigned to three experimental groups consisting of ten animals per group: preservation with LPEC, CE and CEPG. Both lungs were flush-perfused using 20 ml of the corresponding preservation solution at 4°C and 20 cm H₂O and the flush-time was recorded. Throughout the entire perfusion period the lungs were ventilated to allow for a homogenous distribution of the perfusate. The heart–lung block was then carefully excised. Trachea and left atrium were cannulated while the heart-lung block was immersed in iced normal saline. Thereafter, lungs en bloc were inflated with 10 cm² of room air and stored in the preservation solution for 2 h at 10°C. After storage, the lungs were reventilated and reperfused with deoxygenated blood via the pulmonary cannula using an extracorporeal circuit for 50 min to specifically assess the early post-ischemic period. This circuit (Fig. 1) consisted of a reservoir and a roller pump (Multiflow bloodpump, Stoeckert Instruments, Munich, Germany) to raise the perfusate to the reservoir. Also, a 40 mm blood filter (PALL Europe, Portsmouth, UK), a membrane oxygenator (Mono-lyth integrated membrane lung, Sorin Biomedica, Saluggia, Italy) which was gassed with 95% N₂ and 5% CO₂ and a second roller pump (Reglo-Digital, Ismatec, Zurich) for continuous lung perfusion were included. The perfusion rate was gradually increased from 1.0–8.0 ml/min during the first 9 min and was maintained at a constant rate of 8.0 ml/min throughout the remaining reperfusion period. All vessels were water-jacketed and temperature controlled by a warming pump (water thermostat type Lauda M3B, Omnilab, Germany) at 37°C. Constant deoxygenation of the perfusate to a pO₂ of 10–15 mmHg was achieved by regulation of gas flow. The opening of the left atrial cannula was positioned above the level of the atrium ensuring a constant positive pressure of 2 cm H₂O (LAP). Throughout the entire experiment, the pulmonary artery pressure (PAP) was assessed with a transducer and a pressure monitor (Servomed; Hellige, Hamburg, Germany). Pulmonary vascular resistance (PVR) was calculated (mean PAP–LAP/roller pump[cardiac output] output × 80 [dyn/s per cm⁻²]). Blood gases were determined in 10-min intervals. The pO₂ measured in the effluat of the left atrium was defined as arterial pO₂ (paO₂), and pO₂ from the deoxygenated blood reservoir as venous pO₂ (pvO₂). For assessment of the oxygenation ability of the reperfused lungs the relative oxygenation capacity (ROC) was calculated [(pO₂ – pvO₂) × 100/ pvO₂]. Peak inspiratory pressure (PIP) was measured every 10 min. At the end of each experiment, the entire left lung was excised and the wet to dry (W/D) lung weight ratio was determined using the fresh weight of the lung and the weight after storage for 48 h in a cabinet dryer at 60°C.

Statistical evaluation of wet-to-dry ratio and flush perfusion time was performed using factorial analysis of variance (ANOVA) with multiple comparisons. Oxygenation and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Electrolyte composition of EC-40 and Celsior</th>
<th>EC-40</th>
<th>Celsior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ (mmol/l)</td>
<td>85</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>K⁺ (mmol/l)</td>
<td>40</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Mg²⁺ (mmol/l)</td>
<td>–</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Ca²⁺ (mmol/l)</td>
<td>–</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Cl⁻ (mmol/l)</td>
<td>15</td>
<td>41.5</td>
<td></td>
</tr>
<tr>
<td>PO₄³⁻ (mmol/l)</td>
<td>57.5</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/l)</td>
<td>10</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Histidine (mmol/l)</td>
<td>–</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Mannitol (mmol/l)</td>
<td>–</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Glucose (%)</td>
<td>3.5</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Glutamate (mmol/l)</td>
<td>–</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Lactobionate (mmol/l)</td>
<td>–</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Glutathione (mmol/l)</td>
<td>–</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Osmolarity (mosm/l)</td>
<td>370</td>
<td>360</td>
<td></td>
</tr>
</tbody>
</table>
pulmonary vascular resistance during the reperfusion period was analyzed using ANOVA with repeated measurements. All values are expressed as standard deviation of the mean. Significance was assumed when the $P$-value was $< 0.05$.

3. Results

3.1. Flush perfusion time

The perfusion time was slightly decreased when using CE solution compared to EC-40, but a significant decrease ($P = 0.01$) was noticed with additional prostacyclin (CEPG, Table 2).

3.2. Relative oxygenation capacity (ROC)

After 2 h of ischemia all reperfused lungs resumed sufficient function. However, the ROC of all Celsior preserved organs was significantly higher as compared with the EC group (Table 3). Additional application of prostacyclin did not have any significant effect on the oxygenation capacity.

3.3. Pulmonary vascular resistance (PVR)

In all study groups PVR increased towards the end of the reperfusion period but was lowest ($P < 0.02$) in CE preserved lungs (Table 4).

3.4. Peak inspiratory pressure (PIP)

In all groups PIP increased during reperfusion but was highest in the EC-40 group ($P < 0.03$, Table 5).

3.5. Wet-to-dry ratio (W/D ratio)

Preservation with CE and CEPG was associated with a lower W/D ratio compared with EC-40 (Fig. 2). However, these results did not reach statistical significance.

4. Discussion

Though lung transplantation has proven to be an effective therapy in end-stage pulmonary disease, this option is limited mainly by severe scarcity of suitable donor organs. Consequently, adequate preservation of the donor organ function is the most important factor regarding perioperative mortality of thoracic transplantsations. Currently, most

Table 2
Flush-perfusion time (mean ± SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>Flush-time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC-40, 4°C*</td>
<td>83 ± 13</td>
</tr>
<tr>
<td>CE, 4°C**</td>
<td>79 ± 18</td>
</tr>
<tr>
<td>CEPG, 4°C</td>
<td>59 ± 11</td>
</tr>
</tbody>
</table>

$*P < 0.01$ (EC-40 vs. CEPG); $**P = 0.01$ (CE vs. CEPG).

Table 3
Relative oxygenation capacity (ROC) after 2 h of ischemia (mean ± SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (min)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC-40*</td>
<td></td>
<td>491</td>
<td>494</td>
<td>446</td>
<td>446</td>
<td>446</td>
</tr>
<tr>
<td>CE**</td>
<td></td>
<td>755</td>
<td>787</td>
<td>732</td>
<td>732</td>
<td>732</td>
</tr>
<tr>
<td>CEPG</td>
<td></td>
<td>1208</td>
<td>1327</td>
<td>1134</td>
<td>1134</td>
<td>1134</td>
</tr>
</tbody>
</table>

$*P < 0.02$ (EC vs. CE and CEPG); $**P < 0.05$ (CE vs. CEPG).

Table 4
Pulmonary vascular resistance (PVR, [dyn/sec/cm$^2$]) after 2 h of ischemia (mean ± SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (min)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC-40</td>
<td></td>
<td>244</td>
<td>225</td>
<td>225</td>
<td>227</td>
<td>248</td>
</tr>
<tr>
<td>CE*</td>
<td></td>
<td>139</td>
<td>145</td>
<td>150</td>
<td>152</td>
<td>152</td>
</tr>
<tr>
<td>CEPG</td>
<td></td>
<td>208</td>
<td>224</td>
<td>238</td>
<td>242</td>
<td>253</td>
</tr>
</tbody>
</table>

$*P < 0.02$ (EC vs. EC-40 and CEPG); $**P > 0.05$ (EC-40 vs. CEPG).
institutions use single hypothermic flush perfusion of the pulmonary artery for lung preservation. Since the introduction of Euro–Collins (EC) solution for renal transplantation in 1969 [17], pulmonary preservation using EC is possible for at least 7 h of ischemia [18]. The biochemical basis of action is due to the high potassium content of EC, which resembles intracellular ion distribution and minimizes the transmembrane ion shift with limitation of the potassium ion leakage from the preserved cells, resulting in a decreased intracellular edema [17]. However, a high potassium concentration can cause severe pulmonary vasoconstriction followed by cell edema [13,14]. Since the optimal EC potassium concentration in terms of improved lung preservation was found to be in the range of 40 mmol/l [19], this has been the preferred comparative preservation solution in our study.

The isolated rat lung seems to be an ideal screening model for assessment of early post-preservation lung function and has been extensively used to study pulmonary IR-induced injury [20–22]. The main limitation of those models, however, has been the absence of gas exchange studies which require the use of deoxygenated blood. Therefore, we used an established small animal model containing washed bovine erythrocytes as described previously [19,23]. Obviously, autologous blood would have been the optimal perfusate for the experiments. However, in this rat model only 6 ml/100 g whole blood can be withdrawn from a single rat. Therefore, the amount of animals which would have to be killed for one experiment contradicts the advantage of a ‘low-cost screening model’. For this reason we used bovine red blood cells as described by Lieberthal [24] which do not restrict the experiments to the use of rodent red blood cells [23].

In recent years, numerous improvements of lung preservation have been made by changing the ion balance of the preservation solutions or by adding vasodilating agents, radical scavengers, membrane stabilizers or antiaggregants [25]. The development of Celsior solution showed promising results in heart preservation due to excellent properties in prevention of cell swelling, free radical injury, calcium overload and energy depletion [15]. In isolated rat lung models, Celsior showed a significant reduction in the ischemia/reperfusion-induced increase in pulmonary microvascular permeability [16]. However, this study lacks any gas exchange studies as the most important indicator of post-ischemic graft function.

In our study, we found a significantly increased oxygenation capacity of lungs preserved with Celsior compared with EC solution, as well as a significantly, reduced PVR and PIP. Similar results were briefly reported by Barr [26].

To improve the uniformity of distribution of single flush perfusion solutions through the lungs, prostaglandin E$_1$ or prostacyclin are often applied as an adjunct to the perfusate [12,25]. These agents have a wide variety of actions that are of theoretical advantage in lung transplantation. The potent pulmonary vasodilatory effect can counteract reflex cold vasoconstriction which in turn might be the reason for the significantly, shorter flush perfusion time measured in the CEPG group. Furthermore prostaglandins modulate white cell and platelet function by attenuating leucocyte sequestration in damaged tissues, inhibiting platelet aggregation and preventing lysosomal enzyme release and superoxide anion production by neutrophils [27]. Additionally, prostaglandins have a cytoprotective effect and mitigate the vascular permeability which is induced by vasoactive inflammatory mediators [27]. Adding prostacyclin to the Celsior solution let to an absolute improvement of the

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (min)</th>
<th>PIP (mmHg) after 2 h of ischemia (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC-40*</td>
<td>10</td>
<td>11.31 ± 1.13</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>11.56 ± 1.18</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>11.63 ± 1.38</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>11.82 ± 1.59</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>11.88 ± 1.93</td>
</tr>
<tr>
<td>CE</td>
<td>10.83 ± 0.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>11.06 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>11.32 ± 0.66</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>11.19 ± 0.72</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>11.18 ± 0.63</td>
</tr>
<tr>
<td>CEPG</td>
<td>9.4 ± 0.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>9.72 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>9.78 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>9.98 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>10.2 ± 0.47</td>
</tr>
</tbody>
</table>

*P < 0.02 (CE vs. EC-40 and CEPG)
 ROC, but these results failed to reach statistical significance. As an explanation for this slightly better outcome, the signifi-

cantly shorter flush perfusion time in the CEPG group compared with CE might provoke a rapid washout of blood

which prevents the cells of the pulmonary microvasculature from being submitted to toxic agents released from leuco-
cytes which in turn reduces the mediated membrane leakage resulting in edema. This theory might be supported by the

slightly, but not significantly, lower amount of lung water in terms of W/D ratio in those lungs preserved at shorter flush
times (CEPG < CE). Surprisingly, a marked increase of PVR was measured in the reperfused lungs preserved with

CEPG (P = 0.011) compared with CE. A similar effect of prostacyclin in the flush solution with significantly, elevated

mean pulmonary artery pressures a long time after flushing was observed by others [28] and is not well explained.

The results shown in our study clearly prove the findings of BARR [26] that Celsior provides better lung preservation

than EC solution as demonstrated by a significantly

improved oxygenation ability, a lower PVR and a decreased

W/D ratio. To further elucidate these promising results we

increased oxygenation ability, a lower PVR and a decreased

have initiated specific histological evaluations and addi-

tional experiments using an in vivo large animal model

with mimi pigs.

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