In-situ topical cooling of lung grafts: early graft function and surfactant analysis in a porcine single lung transplant model

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Abstract

Objective: Improvement of organ preservation is essential to facilitate acceptance of marginal donor lungs for transplantation. Thus, recruiting non-heart-beating donors (NHBD) may be one reasonable strategy to augment the organ-pool especially in the field of pulmonary transplantation. Topical cooling (TC) of donor lungs could provide fast organ-protection and is an available procedure even in smaller centers. In this study transplanted lung function and surfactant activity in same lungs, which were preserved by TC, were assessed following transplantation.

Methods: Twelve porcine allogeneic single lung transplants were performed. Six lungs that were flush preserved through the antegrade route served as controls. The other six lungs were preserved by TC for 30 min after induction of cardiac arrest by repeated application of cold saline (8°C) to both pleural cavities. Lungs of both groups were stored in LPD solution for 24 h at 8°C. After transplantation, the recipient’s right bronchus and right pulmonary artery were clamped. Major endpoints included early graft function over a period of 7 h. Hemodynamic measures and respiratory functions were recorded in 30-min intervals. Surfactant function was determined before transplantation and 2 h after reperfusion by broncho-alveolar lavage fluid analysis.

Results: Only four animals of the control-group survived the 7 h reperfusion period. Right heart failure occurred in two animals after 150 and 240 min of reperfusion. All six animals in the TC group survived the observation period. Pulmonary vascular resistance ($p = 0.01$), pulmonary artery pressure ($p = 0.03$), and lung tissue water content remained significantly lower in topically cooled allografts ($p = 0.01$) vs. controls. Surfactant function after transplantation was comparable in both groups with a trend towards lower protein contents ($p = 0.07$) in the broncho-alveolar fluid of grafts after TC.

Conclusions: In-situ TC seems to be a reliable strategy to preserve lungs for up to 24 h. It even surpasses the results of LPD-perfused grafts in hemodynamic function and survival time.

Keywords: Lung transplantation; Ischemia–reperfusion injury; Surfactant function

1. Introduction

Lung transplantation has evolved to an accepted treatment modality for patients suffering from various end-stage lung diseases. The currently achieved outcome is encouraging. Nevertheless, a major contributor to early mortality following lung transplantation still remains initial graft failure due to ischemia–reperfusion (IR) injury. The incidence of clinically relevant IR injury in transplanted lungs has been reported to range from 20 to 40% [1,2]. Therefore, many experimental studies have been conducted to investigate and improve novel preservation techniques for lung graft storage. We and others have recently described the preferential use of low-potassium-dextran (LPD) solution in clinical lung transplantation [6]. Several experimental studies reported improved graft function with LPD preservation even after prolonged cold ischemic periods of up to 24 h [2–4]. Based on these findings many
lung transplant centers throughout the world have changed their preservation strategies from high-potassium-intracellular-type solutions to LPD and reported superior outcome [5,6]. The standard route for lung flush preservation is the antegrade perfusion through the pulmonary trunk. There is only little evidence whether graft function can be additionally enhanced using alternative preservation strategies such as retrograde flush perfusion [2] or additional perfusion of the bronchial arteries [4]. In contrast to these novel techniques, even a ‘simple’ in-situ topical cooling (TC) approach of the atelecctic lung is known to lead to acceptable lung graft preservation [7–10]. In the beginning of lung transplantation, in-situ TC of lung grafts was the method of choice [11] and satisfying results were published from the Toronto Lung Transplant group in 1988 [12]. Apart from this early data, there is only little experience on TC of lung tissue in the clinical practice, but recently advanced experimental models have been developed [13]. Since LPD preservation solutions have led to improved graft function and prolonged cold ischemic tolerance, the TC concept using LPD solution may be reconsidered especially with regards to the retrieval of lung grafts from non-heart-beating-donors (NHBD).

The aim of this study was to determine early transplanted graft function of porcine lungs from ventilated NHBD pigs after in-situ TC and long-term storage under clinical conditions. As a control group pigs received lungs that were preserved with LPD solution by antegrade cold flush perfusion. We used a porcine left-sided single lung transplantation model followed by occlusion of the contralateral bronchus and main pulmonary artery, which is currently the gold standard in experimental lung preservation research [8].

2. Materials and methods

The following protocol was reviewed and approved by the Sub-committee on Research Animal Care, Hannover Medical School. All animals received humane care in compliance with the European Convention on Animal Care and with the ‘Principles of Laboratory and Animal Care’ and the ‘Guide for the Care and Use of Laboratory animals’ prepared by the Institute of Laboratory Animal Resources published by the National Institutes of Health (NIH publication no. 85-23, revised 1985).

2.1. Experimental groups and animals

In 12 female pigs, German landrace (20–25 kg), a left single lung transplantation was performed. The animals were subdivided and randomised into either the control-group or the study-group. Donor animals in the control-group (n = 6) received antegrade flush perfusion with LPD solution (LPD; Vitrolife, Göteborg, Sweden). Preservation in the study-group (n = 6) was performed by in-situ immersing of the atelecctic lung in cold saline solution (TC) for 30 min. In both groups the retrieved grafts were stored for 24 h in 8 °C cold LPD solution.

2.2. Donor operation of the control-group

Anaesthesia was induced with azaperon (5 mg/kg, i.m.), atropine (0.5 mg total dose, i.m.) and thiopental sodium (15 mg/kg, i.v.). After intubation analgesia was maintained with thiopental sodium (5 mg/kg/h) and fentanyl (1 µg/kg/h) continuously administered intravenously. Mechanical ventilation (Evita II, Dräger, Germany) was performed in a pressure-controlled mode with a ventilation rate of 15 breaths/min and a maximum inspiratory pressure (P(\text{max})) of 25 mmHg at an inspiratory oxygen-tension (FiO₂) of 0.5. The positive end-expiratory pressure (PEEP) was adjusted to 5 mmHg. Ventilation was not changed during the entire harvesting procedure. After median sternotomy the pericardium and both pleural cavities were opened, the inferior and superior vena cava, the aorta and the pulmonary artery were isolated. Systemic anticoagulation was performed with 3 mg/kg heparin. An arterial blood gas analysis was obtained and a broncho-alveolar lavage (BAL) of the right lung was performed. After administration of 250 mg methylprednisolon a flushing tube was inserted into the main pulmonary artery. After complete inflow occlusion, the auricle of the left atrium was incised, the pulmonary artery was clamped and flush perfusion with 1 l of 8 °C-cold LPD-solution supplemented with 0.3 ml of Tris–buffer was induced. Ventilation of lungs was maintained throughout the entire procedure. During storage the trachea was clamped, keeping the lungs mildly inflated. The heart and the lungs were excised on bloc. After the main left bronchus was clamped, the left lung was isolated from the heart-lung-block with a generous atrial cuff and full length of the pulmonary artery and the main bronchus. Finally the graft was stored in a mildly inflated status for 24 h in 8 °C cold LPD solution.

2.3. Donor operation of the TC-group

Anaesthesia and ventilation were induced and maintained as described above. After administration of heparin (3 mg/kg) and methylprednisolon (250 mg) arterial blood gas analysis was performed and broncho-alveolar lavage fluid (BALF) of the right lung was obtained. A temperature probe was placed into the left main bronchus. Median sternotomy was performed and the heart was arrested by an injection of cardioplegic solution into the left ventricle. The pericardium and both pleural cavities were opened. Mechanical ventilation was disconnected and the lungs were allowed to deflate. Immediately after cardiac arrest, TC was induced to both lungs for 30 min by infusion of 4 °C cold saline into both pleural cavities. Approximately 1.5 l of saline solution was necessary to fill the entire thoracic cavity. This solution was renewed in 3-min intervals to
optimise the cooling effect. A total amount of 12–15 l of cold saline solution was required for each TC procedure. With the described setup we reached an endobronchial temperature in the donor of 17.3 ± 1.8 °C after 20 min and 15.9 ± 2.1 °C after 30 min of cooling. During in-situ TC and storage, the lung was kept totally deflated. No graft perfusion was performed in these animals. After harvesting, the left lung was stored for 24 h in 8 °C-cold LPD solution, also in a deflated state.

2.4. Transplant procedure

Anaesthesia was induced with azaperon (5 mg/kg, i.m.), atropine (0.5 mg total dose, i.m.) and thiopental sodium (15 mg/kg, i.v.) followed by a continuous infusion of fentanyl and thiopental sodium as described above. The animals were ventilated mechanically at an FiO2 of 0.5 in a pressure-controlled mode (Evita, Dräger Germany). The ventilator settings were adjusted at a ventilation rate of 15 breaths/min, a maximal airway pressure of 25 mmHg, a PEEP of 5 mmHg and an inspiratory/expiratory ratio of 1:1. A venous catheter was placed into the right jugular vein for assessment of the central venous pressure. An arterial catheter was introduced into the left carotid artery for blood sampling and blood gas analysis. A second arterial catheter for hemodynamic monitoring was placed into the femoral artery (Picco, Pulsion Medical Systems AG, Munich, Germany). After a left-sided lateral thoracotomy in the fifth intercostal space, catheters were introduced into the left and right atrium. A Swan-Ganz catheter (7.5 F; Baxter Healthcare Corp., Irvine, CA) was placed into the pulmonary artery. After opening the pericardium, the left pulmonary artery, the tracheal bifurcation and the pulmonary veins were dissected. Umbilical tapes were applied to the right and left pulmonary arteries and the right main bronchus. Heparin (3 mg/kg) was administered intravenously. After clamping of the left main bronchus and the left pulmonary artery, the main stems of left pulmonary veins were ligated. Pneumonecctomy was performed. The left atrium was clamped. The ligated upper and lower left pulmonary veins were incised opening an atrial cuff. The donor lung was implanted starting with an end-to-end anastomosis of the bronchus using a 4.0 Prolene running suture (Ethicon, Inc., Somerville, NJ). Then the atrial cuff followed by the left pulmonary artery was anastomosed with 5.0 Prolene running sutures. Before reperfusion the graft vasculature was deaired by retrograde perfusion. The pulmonary artery was declamped and the graft was ventilated. After a reperfusion period of 15 min the contralateral pulmonary artery and bronchus were clamped, in order to clearly evaluate only the function of the transplanted lung. If necessary, hemodynamic support was applied by administration of epinephrine (maximum 0.5 μg/kg body weight/min). Each experiment was terminated after an observation period of 7 h by a thiopental sodium overdose injection.

2.5. Monitoring and measurements

2.5.1. Lung function and hemodynamic measures

The post-reperfusion observation period in each animal was 7 h. Hemodynamic data (Picco System, Pulsion Medical Systems AG, Munich, Germany and Swan-Ganz Catheter, Baxter Healthcare Corp., Irvine, CA) were recorded continuously. Arterial and venous blood gas analysis (Omni, AVL, Austria) was performed in 30 min intervals. Dynamic compliance of the lung was measured using a modified EVITA II ventilator (Dräger, Lübeck, Germany). The pulmonary vascular resistance (PVR) and the extra vascular lung water were calculated from the cardiac output by means of the transfemoral thermodilution catheter system (Picco, Pulsion Medical Systems AG, Munich, Germany). The system was calibrated by injection of cold saline solution via the jugular vein and cardiac output was calculated by pressure curve analysis [14]. Biopsies of the transplanted lung were taken at the end of the experimental period. As control specimens of the donor’s and recipient’s right lungs were taken at the beginning of the experiment, the specimens were processed for determination of lung tissue water. Wet/dry weight ratios were calculated and tissue water content was expressed as a percentage of wet weight.

2.5.2. Surfactant analysis

A BAL was obtained from the donor’s and the recipient’s right lungs as a control at the beginning of the experiment. A second BAL was taken from the left lower lobe 2 h after reperfusion. After filtration through sterile gauze, the lavage was centrifuged at 150 g and the cell-free supernatant was frozen at −80 °C. Differential cell counts were performed on the pelleted cells by standard techniques. For surfactant analysis the cell-free supernatant was centrifuged at 48,000 × g for 60 min at 4 °C to pellet large surfactant aggregates (LA). The supernatant, containing small surfactant aggregates (SA), was removed and the LA pellet was resuspended in Ringer’s solution. The phospholipid contents of the LA fraction and the SA supernatant were determined by a phosphorus analysis according to the method of Bartlett [15]. This assay is based on a phosphorus analysis carried out on the lipids extracted with chloroform/methanol [16]. The results were expressed as a small aggregate/large aggregate (SA/LA) ratio. All assays were performed in two separate samples and reported as a mean value. After adjusting the phospholipid concentration of the LA-suspension to 1 mg/ml the surfactant function was analysed with a pulsating bubble surfactometer (Electro-netics, Inc., Buffalo, NY) [17]. Forty microlitres of the LA suspension was filled into the sample chamber. The adsorption rate was evaluated by determining the surface tension 10 s after formation of a bubble (γads). The surface...
tension at minimal bubble size ($\gamma_{\text{min}}$) was obtained after 5 min of bubble pulsation at a rate of 20 cycles/min and a temperature of 37 °C. All data were digitised and stored on hard disk.

2.6. Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). Continuous data were analysed by repeated-measure analysis of variance (ANOVA) [18]. For data without repeated measurement a Mann Whitney-U test was performed. $p$-Values less than 0.05 were considered significant. All data were analysed with the statistical program of social sciences (SPSS for MS Windows, Version 10.0, SPSS, Inc., Chicago, IL).

3. Results

3.1. Survival

Early graft failure due to right heart failure developed in two out of six animals in the control-group. The animals died 150 and 240 min after initiation of reperfusion. The mean survival in the perfusion group was 5.75 ± 2 h. In the TC-group all pigs survived for the entire observation period.

3.2. Respiratory parameters

There was a rapid decrease in the arterial $p_{O_2}/FiO_2$ ratio within the first 30 min after initiation of reperfusion in the flush-perfused lungs from 495 ± 63 to 374 ± 96 mmHg. Thereafter the two animals with graft failure had a further decrease until death after 150 and 240 min after reperfusion, respectively. A third animal in this group had a continuous decrease in $p_{O_2}/FiO_2$ to 82 mmHg after 7 h of reperfusion but survived for the observation period. The remaining animals in this group showed a stable course. The $p_{O_2}/FiO_2$ ratio in the TC-group remained stable within the initial reperfusion period of 30 min (445 ± 51 vs. 438 ± 82 mmHg). Thereafter, the $p_{O_2}/FiO_2$ mean values decreased slowly from 438 ± 82 to 372 ± 157 mmHg. At the end of the experiment four animals of this group (TC) showed excellent arterial blood gas values ($p_{O_2}/FiO_2$ ratio > 400 mmHg). Although there was a trend towards improved gas exchange in the TC-group no statistical significance in the repeated-measure analysis was reached.

The dynamic compliance of flush-perfused grafts decreased from 24 ± 6 to 16 ± 5 ml/mmHg ($p = 0.08$) within the first 30 min after reperfusion. During the observation period these values remained stable. In contrast, the mean dynamic compliance in the TC-group decreased continuously from 16 ± 1 to 12 ± 2 ml/mmHg at the end of the experiment. Differences among both groups were not statistically significant.

3.3. Hemodynamic parameters

PVR was comparable in both groups prior to surgery. Clamping of the right main pulmonary artery resulted in a significant increase of PVR in all animals. An early increase of the PVR resulted in right heart failure and death in two animals in the control-group. The PVR increased from 420 ± 149 to 1605 ± 653 dyn/s/cm$^5$ after contralateral pulmonary artery clamping in the control-group. The initial rise of PVR was notably lower in the TC-group from 388 ± 167 (baseline) to 937 ± 392 dyn/s/cm$^5$ after 30 min of reperfusion. During the observation period PVR in the perfusion group was continuously elevated to more than 1200 dyn/s/cm$^5$ while the animals in the TC group presented with mean values below 900 dyn/s/cm$^5$ ($p < 0.01$, Fig. 1).

PAP values increased markedly in both groups within the first 30 min after reperfusion (16 ± 3–32 ± 4 mmHg, TC; 22 ± 3–46 ± 4 mmHg, control) and were significantly higher in the control-group (mean values: 39–46 mmHg) compared to the TC-group (mean values: 26–32 mmHg) during the observation period ($p = 0.03$). With regards to cardiac performance we noticed a trend towards elevated cardiac output ($p = 0.08$) and increased mean arterial blood pressure ($p = 0.09$) in animals, that received a TC-graft compared to control animals but it did not reach statistical significance.

3.4. Lung water content

Lung water content calculated from wet/dry ratios was increased after 7 h of reperfusion in control-grafts compared to native right lungs (84.2 ± 2.5 vs. 87.5 ± 1.0%; $p = 0.02$, Fig. 2). Grafts in the TC-group showed no increase in lung water content 7 h after reperfusion (84.2 ± 1.8 vs. 85.0 ± 1.4%; $p = 0.37$, Fig. 2). The comparison of both groups at the end of the experiment revealed significantly higher lung water content in flush-perfused grafts ($p < 0.01$; Fig. 2).

3.5. BALF-data and surfactant analysis

There was a normal cell count in the baseline BALF without significant differences between the two groups. Macrophages were the predominant cell-type (>80%) found in right lungs of donor animals. Only a few lymphocytes and neutrophils were detectable. After preservation and reperfusion of the lungs, the total cell count was very stable in both groups, but there was a macrophages-to-neutrophils shift in BALFs with a significant increase of the number of neutrophiles in both groups. No differences were seen between the two groups (Table 1). Lymphocyte counts were unchanged compared to pretreatment values.

The phospholipid content in lavage fluids remained unchanged in both groups after reperfusion compared to baseline measurements. The ratio of small aggregates to large aggregates of the phospholipids-fraction (SA/LA
ratio) was low at baseline conditions in both groups. Two hours after reperfusion a significant increase of these values \((p = 0.03)\) was seen in the control-group while the SA/LA ratio in the TC-group was only moderately elevated without a statistical difference compared to the baseline status (Fig. 3).

The protein content of the baseline BALF was low and comparable in both groups. After 2 h of reperfusion an increase in protein content (control: \(p = 0.03\); TC: \(p = 0.01\)) was detected in the BAL-fluids in both groups (Fig. 4). After reperfusion the protein levels in the TC-group were lower compared to controls \((p = 0.07)\). However, both strategies of graft preservation led to a marked increase of the protein-to-phospholipid ratio (Table 1). With regards to surface activity in BALF there was a slight but not significant increase of surface tension at minimal bubble

![PVR](image1)

Fig. 1. PVR over 7 h of reperfusion. Values are given as mean levels. PVR did not differ among groups before surgery. During the observation period PVR was significantly lower in grafts after TC compared to the control-grafts which received antegrade LPD-flush perfusion \((p < 0.01\), repeated-measures analysis of variance, ANOVA).

![Wet/dry ratio](image2)

Fig. 2. The wet/dry ratio (percentage of wet weight) of lung specimens prior to lung preservation (controls) and 7 h after reperfusion. Values are given as mean ± SEM. Compared to the controls the wet/dry ratio was significantly increased after LPD perfusion \((p = 0.02)\), while values after TC were stable. The comparison of both preservation methods revealed a significantly lower wet/dry ratio after TC \((p = 0.01\), Mann Whitney-U test).
size ($\gamma_{\text{min}}$) after reperfusion in both groups (Table 1). The adsorption rate ($\gamma_{\text{ads}}$) was unchanged in both groups compared to baseline levels (Table 1).

### 4. Discussion

Since there is a persistent lack of organ donors a widespread application of lung transplantation is limited. In a survey of 150 consecutive cardiac donors, reported to the United Network of Organ Sharing (UNOS), donor lung function was determined to be acceptable for clinical lung transplantation in only 25% of the donors [7]. Other authors describe an overall acceptance rate of lung grafts of only 10% [3,19]. Novel preservation strategies may help to improve graft quality, which could eventually lead to a liberalization of the generally accepted donor selection criteria. Additionally, a decreased rate of primary organ failure led to a lowered number of organs urgently needed for retransplantation. In order to increase organ availability the use of NHBD was mentioned in the past. This concept of retrieving cadaveric organs, which is still subject of intense medical and ethical debate, may be an effective approach to increase the number of suitable organs to up to 20% [20]. Since the first clinical transplant of a NHBD lung was performed and reported recently by Steen et al. [10], this strategy has obviously become clinical reality. Several experimental studies revealed excellent lung function after in-situ TC of cadaveric lungs [7–10]. The ‘non heart beating donor’ concept was shown to be possible from a biological point of view, but the logistic and ethical questions of this procedure are still not fully discussed and solved [10]. Thus, the number of NHBD may remain low in the near future.

Another important and still intensely discussed aspect is the limit of acceptable cold ischemic preservation time periods for lung grafts. Many clinical and experimental studies have been conducted to increase ischemic time and

Table 1

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<thead>
<tr>
<th></th>
<th>LPD Baseline</th>
<th>LPD Reperfusion</th>
<th>TC Baseline</th>
<th>TC Reperfusion</th>
<th>p-Valuea</th>
<th>p-Valueb</th>
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<tbody>
<tr>
<td>$\gamma_{\text{min}}$ (mN/m)</td>
<td>3.05 ± 1.00</td>
<td>6.64 ± 2.78</td>
<td>1.59 ± 0.25</td>
<td>5.30 ± 3.74</td>
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<td>Neutrophil count (%)</td>
<td>1.00 ± 0.45</td>
<td>26.83 ± 11.42</td>
<td>3.60 ± 2.23</td>
<td>38.60 ± 15.31</td>
<td>&lt;0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Protein (g/µl)</td>
<td>188.24 ± 32.90</td>
<td>971.28 ± 594.81</td>
<td>87.40 ± 19.65</td>
<td>640.20 ± 414.87</td>
<td>0.03</td>
<td>0.01</td>
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<tr>
<td>Protein/phospholipid ratio</td>
<td>1.08 ± 0.37</td>
<td>5.98 ± 2.04</td>
<td>2.10 ± 0.65</td>
<td>6.83 ± 3.04</td>
<td>0.02</td>
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<tr>
<td>SA/LA ratio</td>
<td>0.47 ± 0.27</td>
<td>1.76 ± 0.56</td>
<td>0.16 ± 0.05</td>
<td>0.90 ± 0.49</td>
<td>0.03</td>
<td>0.32</td>
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BAL was performed before ischemia (baseline) and after 120 min of reperfusion.

a p-Values for within treatment group comparisons (baseline vs. reperfusion).

b p-Values for between treatment group comparisons (LPD-perfusion vs. TC).

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**Fig. 3.** Protein concentration of the BALF in micrograms per millilitre. Values are given as mean ± SEM. The results display the protein concentration of the BALF of right lungs before preservation (controls) and in left lungs after TC vs. LPD-perfusion 2 h after reperfusion. Protein concentration after reperfusion was significantly increased in both groups (LPD: $p = 0.03$; TC: $p = 0.01$) with a trend to lowered protein levels after TC ($p = 0.07$, Mann Whitney-U test).
enhance primary graft function. It has been shown that the integrity of endothelial cells and type II cells plays a major role in the pathophysiologic mechanisms of IR-injury [21]. Currently, the worldwide mostly used strategy was a flush perfusion with Euro-Collins (EC) solution. It has been successfully applied for more than a decade, but the accepted cold ischemic time limit was set to 6 h only [22]. Recently, several experimental studies showed clearly that the utilization of LPD-solution ameliorates IR injury and enhances primary graft function following lung transplantation [3,4,6]. There is growing evidence that successful lung preservation in an atelectatic state of the lung with cold LPD solution (8 °C) after flush perfusion is safe for at least 24 h [2,4]. These strategies may have some impact on the quality of lung graft protection in beating as well as in NHBD. As reported by Steen et al. [8] even in-situ TC with LPD-solution without graft flush perfusion allowed a safe cold ischemic storage period of up to 24 h. If safe prolonged cold ischemic periods became indeed realistic in experimental large animal lung transplantation, it may theoretically help to increase the number of donor organs in the clinical practice by the use of NHBD.

Based on these encouraging results we compared a standard flush-preservation method with a TC model after an extended cold storage period of 24 h using LPD solution. We recorded an excellent outcome regarding early postoperative hemodynamics, and gas exchange in transplanted lungs after TC. These results are concordant with previous studies by various investigators [8,9]. The most important observation in this study was probably the significant decrease in PAP and PVR in the TC group compared to controls. This may reflect the extent of reperfusion injury followed by pulmonary vasoconstriction, which might severely affect right heart function. While all animals in the TC-group survived, two early deaths due to right heart failure occurred after transplantation of LPD perfused lungs. Since we strived for an optimised cooling effect, we had to exchange the cooling solution 10 times in each donor procedure in the TC-group. Therefore, an amount of approximately 12–15 l of cooling solution was required. In order to achieve acceptable costs using this model we had to use saline solution instead of LPD. To our opinion, this approach should not influence graft preservation quality, because there is no direct contact between saline solution and pulmonary artery endothelium or endobronchial surface during in-situ TC. The absence of a relevant reperfusion edema in topically cooled grafts may support this hypothesis. Mean wet/dry weight ratios of the TC-grafts were unchanged after the reperfusion period of 7 h and were significantly lower than those in the control-group at the end of the experiment. This shows a relevant reduction of reperfusion edema in TC-lung grafts compared to flush-perfused lungs.

The surface activity of surfactant in the BAL-fluid 2 h after reperfusion was slightly impaired in both groups probably as a result of surfactant inhibition by exuded proteins, which were well known inhibitors of surfactant function [23]. Overall, surfactant activity was maintained in both groups. Conversion of surfactant subtypes from LA into SA, which is an accepted indicator of metabolic surfactant alteration, was increased after LPD preservation but was prevented following TC. Protein exudation into the alveoli was even less after TC, which probably reflects a reduced deterioration of the capillary-endothelial barrier. Although the remaining surfactant data showed no major differences between both groups (Table 1), all data express an acceptable surfactant protection achieved by TC, which might be even superior to antegrade flush perfusion regarding protein exudation and SA/LA conversion.
The advantage of TC might be due to a smoother delivery of the coldness into the lung tissue. The decrease of tissue temperature is slower in TC compared to cold flush perfusion, but even this fact might contribute to the excellent results of this technique. Ingemansson et al. [24] demonstrated in a series of experiments that cold preservation severely impairs vascular relaxation after reperfusion and induces pulmonary vascular obstruction. Similar results were presented by Steen et al. [4] who performed a safe long-term preservation using a warm LPD-perfusion followed by moderate cold storage of the atelectatic lungs. Currently, moderate cooling temperatures of 8–10 °C are recommended by several authors [8,25]. As shown in this study, in-situ TC achieves low endobronchial temperatures of 15 °C within 30 min. The atelectatic lung tissue reaches even lower temperatures. Additionally there is no mechanical stress of endothelial cells with TC compared to flush perfusion. This might result in an optimised protection of endothelial cells and type II pneumocytes. The analysis of the BAL-fluid in these grafts confirmed this hypothesis with reduced protein exudation into the alveoli and acceptable surfactant function.

The limitations of this study were the short observation period and a rather small number of animals, which resulted in increased standard error values. Certainly, more data should be retrieved in future studies to confirm these first but promising results. However, this model is known to be a challenging evaluation method for early transplant function, and significant effects on hemodynamic and surfactant function could be demonstrated.

In conclusion, immediate TC with cold saline solution and long-term storage of lung grafts for transplantation in 8 °C LPD solution provides a safe 24 h preservation of lung grafts in our experiments. This procedure is as effective as flush perfusion with regards to early graft function and may be even more advantageous due to reduced reperfusion edema and PVR. In-situ TC is a reliable and reproducible strategy for lung preservation and it may offer a reasonable approach to increase the donor pool in clinical lung transplantation. Since recent experimental studies display excellent results of graft function using improved local preservation techniques, we should reconsider whether this concept should be transposed into clinical practice again.

Acknowledgements

We express our appreciation to Claudia Kelle and Adine Timke for their valuable assistance.

References

Appendix A. Conference discussion

Dr W. Weder (Zurich, Switzerland): Between the two study groups you change just one parameter. In one group—the topical cooling group—the lungs were atelectatic and in the other one they were ventilated. In your conclusion you address only one parameter as being relevant (topical cooling) and ignore the fact that the lungs were atelectatic in this group. Could you clarify this?

Dr Kutschka: We wanted to compare a currently used method, an antegrade perfusion with perfadex, to the topical cooling concept. It is a standard to ventilate the lungs during perfusion, but if you use topical cooling complete atelectasis is necessary to reach adequate low tissue temperatures. This is a limitation of the study, but it was unavoidable.

Dr W. Klepetko (Vienna, Austria): The results you are giving us are in concordance with reports that we can gather from the nonheartbeating donations, which give evidence that topical cooling is of importance. What I do not understand in the study is why you did not compare the group with topical cooling only with the combination of Perfadex flush perfusion and topical cooling, which reflects more or less the clinical reality.

So could you comment why you choose this model.

Dr Kutschka: You mean perfusion afterwards, after topical cooling?

Dr Klepetko: Yes, afterwards or simultaneously. It might even give you better results.

Dr Kutschka: This may be true, but the aim of this study was to compare a currently used method to a new method which is quite simple and offers the possibility to use it for an increased number of donors. Further studies are in progress and I think we should probably consider your concept.

Dr D. van Raemdonck (Leuven, Belgium): You have used, as we are doing in our laboratory, cold saline for topical cooling. Professor Steen has previously pointed out that cold saline will give an inflammatory reaction on the outside of the lungs. Have you seen this as well?

Dr Kutschka: We did not see any disadvantages. Thirty minutes cooling is a quite short period, and we focused on an efficient cooling of the lung. Therefore, we had to renew the saline solution continuously. To achieve acceptable costs we used saline for cooling and Perfadex for storage. The pulmonary vessels and the bronchus were closed during the in situ cooling and it was only the surface of the lung, which was in contact with the saline solution. I think, this was a reasonable strategy and it worked well.