Alveolar expansion itself but not continuous oxygen supply enhances postmortem preservation of pulmonary grafts

Dirk E.M. Van Raemdonck a,b,*, Nicole C.P. Jannis a, Paul R.J. De Leyn b, Willem J. Flameng a, Toni E. Lerut b

a Center for Experimental Surgery and Anesthesiology, Katholieke Universiteit Leuven, Herestraat 49, B-3000 Leuven, Belgium
b Department of Thoracic Surgery, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium

Received 28 September 1997; received in revised form 2 January 1998; accepted 10 February 1998

Abstract

Objective: If lungs could be retrieved for transplant after circulatory arrest, the shortage of donors might be significantly alleviated. Great controversy still exists concerning the optimal mode of preservation of pulmonary grafts in these non-heart-beating donors. Methods: Graft function was measured in an isolated room air-ventilated rabbit lung model during reperfusion with homologous, diluted (Hb 9.8 g/dl) and deoxygenated (PaO 2 40 mmHg) blood up to 4 h. Five groups of cadavers (n = 4 in each group) were studied: In the control group, lungs were immediately reperfused. In the other groups, cadavers were left at room temperature for 4 h after death with lungs either deflated (group 1), inflated with room air (group 2), or ventilated with room air (group 3) or 100% nitrogen (group 4). Results: After 1 h of reperfusion, significant differences were noted between group 1 and groups 2, 3, and 4 in peak airway pressure (27 9 5 cmH 20 vs. 15 9 1 cmH 20, 17 9 2 cmH 20, and 16 9 1 cmH 20, respectively; P < 0.05), in weight gain (137 9 24 vs. 31 9 7, 30 9 3, and 30 9 2%, respectively; P < 0.05), and in veno-arterial oxygen pressure gradient (9 9 5 vs. 95 9 13, 96 9 7 and 96 9 4 mmHg, respectively; P < 0.05). Also, wet-to-dry weight ratio at end of reperfusion was significantly different (10.2 9 1.0 vs. 6.0 9 0.3, 5.2 9 0.3 and 5.4 9 0.5, respectively; P < 0.05). No significant differences in any of these parameters were observed between groups 2, 3, and 4. Conclusions: These data suggest that: (1) pulmonary edema will develop in atelectatic lungs if reperfusion is delayed for 4 h after death; (2) postmortem room air-inflation is as good as ventilation in prolonging warm ischemic tolerance; (3) ventilation with room air is no different from that with nitrogen; (4) therefore, prevention of alveolar collapse appears to be the critical factor in protecting the warm ischemic lung from reperfusion injury independent of continuous oxygen supply. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Lung ischemia; Lung transplantation; Non-heart-beating donor; Organ preservation; Reperfusion; Pulmonary vascular resistance

1. Introduction

Lung transplantation, as other forms of solid organ transplantation, is limited by a scarcity of good donor organs. It is estimated that < 10% of all available multi-organ donors have lungs suitable for lung transplantation [1].

In order to alleviate this critical organ shortage, there is a growing interest in increasing the potential donor pool by turning to alternative sources such as the use of lobar or split transplants [2,3], living-related donors [4], or the use of organs from circulation-arrested cadavers, so called non-heart-beating donors (NHBDs).

In the NHBD, however, there will always be a certain delay between (unexpected) circulatory arrest and the start of cold in situ flush of the organs. This period
of inevitable warm ischemia in the NHBD should be kept as short as possible. However, organizing organ retrieval and obtaining family consent for organ donation consumes precious time.

The clinical use of lungs from NHBD is still anecdotal [5,6]. Nevertheless, transplantation of lungs retrieved from cadavers after cardiac arrest is being investigated in an increasing number of animal transplant experiments during recent years [7–13]. Some of these studies [8,11–13] have suggested that modification of the preharvest condition may have a major impact on the preservation of lungs from NHDB.

In previous rabbit animal studies from our laboratory, we have investigated the effect of postmortem cadaver lung inflation, ventilation and cooling on catabolism of adenine nucleotides [14] and on pulmonary cell viability [15,16] and we also looked at the effect of external cadaver cooling on pulmonary temperatures [17] at intervals after death.

Recently, we investigated pulmonary hemodynamic and aerodynamic changes during hypothermic crystalloid flush at intervals after cardiac arrest [18]. Assessment of gas exchange at reperfusion with desoxygenated blood is probably the most reliable variable to evaluate the quality of lung preservation and permits clear differentiation between well preserved and poorly preserved lungs.

In the present study, using an isolated rabbit reperfusion model, we therefore wanted to reinvestigate the effect of postmortem lung expansion on graft function including oxygenation capacity.

2. Material and methods

2.1. Experimental groups

A total of 20 New Zealand white rabbits (mean weight 2522 ± 39 g) were sacrificed and assigned to five groups of animals (n = 4 in each group). In the control group (Contr), both lungs were immediately excised and prepared for isolated reperfusion. In the other groups, cadavers were left at room temperature (± 24°C) for 4 h with sternal edges reapprroximated. Postmortem condition of the lungs inside the cadaver differed between groups (Table 1). In group 1 (control non-heart-beating donor), cadavers were left with lungs deflated (Defl) by disconnecting the endotracheal cannula from the ventilator resulting in progressive atelectasis. In group 2, lungs were fully inflated with room air (Infl-RA) immediately after cardiac arrest by clamping the endotracheal cannula at end-tidal volume with end-expiratory pressure (Vent-RA) at a respiratory rate of 30 breaths/min, a tidal vol. of 10 ml/kg body weight and a positive end-expiratory pressure of 2 cm H2O. In group 3, the lungs were continuously ventilated with room air (Vent-N2) at a respiratory rate of 30 breaths/min, a tidal vol. of 10 ml/kg body weight and a positive end-expiratory pressure of 2 cm H2O. Finally, in group 4, lungs were ventilated identically as in group 3 but with 100% nitrogen (Vent-N2).

Weight of the animals did not differ between all study groups (Table 1).

2.2. Animal preparation

All animals received humane care in compliance with the European Convention on Animal Care. The study was approved by the institutional ethics committee.

In every experiment, three rabbits were used, one as lung donor and two as additional blood donors. Animal preparation in our laboratory has been previously described in detail [18].

In the lung donor, the main pulmonary artery and the inferior caval vein were cannulated with a 10-gauge catheter (Angiocath, Becton Dickinson Vascular Access, Sandy, UT) and secured by a purse-string in the right ventricular outflow tract and the right atrial appendage, respectively. The ascending aorta was ligated and the pulmonary artery was isolated from the right ventricle by ligature around the tip of the catheter just distal to the pulmonary valve creating pulmonary ischemia. The animal was rapidly exsanguinated through the catheter in the inferior caval vein and autologous

Table 1

Animal characteristics in different study groups a

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal weightb (g)</th>
<th>Heart–lung block weightb (g)</th>
<th>Ischemic time (min)</th>
<th>Preparation timeb (min)</th>
<th>Blood volumeb (ml)</th>
<th>Perfusate volumeb (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: Contr</td>
<td>2353 ± 44</td>
<td>40 ± 1</td>
<td>0 ± 0</td>
<td>29 ± 1</td>
<td>338 ± 11</td>
<td>451 ± 15</td>
</tr>
<tr>
<td>1: Defl</td>
<td>2466 ± 64</td>
<td>44 ± 2</td>
<td>240 ± 0</td>
<td>28 ± 1</td>
<td>335 ± 4</td>
<td>447 ± 5</td>
</tr>
<tr>
<td>2: Infl-RA</td>
<td>2618 ± 111</td>
<td>42 ± 1</td>
<td>240 ± 0</td>
<td>29 ± 1</td>
<td>387 ± 16</td>
<td>516 ± 21</td>
</tr>
<tr>
<td>3: Vent-RA</td>
<td>2576 ± 84</td>
<td>42 ± 1</td>
<td>240 ± 0</td>
<td>29 ± 0</td>
<td>363 ± 13</td>
<td>483 ± 17</td>
</tr>
<tr>
<td>4: Vent-N2</td>
<td>2596 ± 81</td>
<td>42 ± 1</td>
<td>240 ± 0</td>
<td>29 ± 0</td>
<td>366 ± 11</td>
<td>488 ± 13</td>
</tr>
</tbody>
</table>

Contr, control; Defl, deflated; Infl-RA, inflated room air; Vent-RA, ventilated room air; Vent-N2, ventilated 100% nitrogen.

a Values are means ± S.E.M. from four experiments.

b Not significant between all groups (analysis of variance with factorial analysis).

c P<0.05 control group vs. other groups (analysis of variance with factorial analysis).
blood was collected. All remaining ligatures were then tied. Both the endotracheal cannula and the pulmonary artery catheter remained in place until reperfusion.

Homologous blood was collected from two additional donors. The operative procedure was identical to that described above, except that a single catheter was placed into the inferior caval vein through the right atrial appendage.

2.3. Preparation and monitoring of the perfusate

Fresh venous blood (volume ± 35 ml/kg; hemoglobin ± 11 g/dl) from three donor animals was collected in an empty sterile bag (500 ml NaCl 0.9%, Baxter, Lessines, Belgium) and stored at 4°C. A total of 30 min prior to reperfusion, the blood was diluted 1:4 [19] with modified Krebs-Henseleit Bicarbonate Buffer solution (composition in mmol/l: NaCl, 118; NaHCO3, 25; KCl, 5.6; CaCl2, 2.9; MgCl2, 0.6; NaH2PO4, 1.2; and D-glucose, 11; pH, 7.4; osmolarity, 321 mOsml). Gelatine 3 g% (30 g/l) (Gelatin Type A ± 60 bloom, Sigma, St Louis, MO) was added to this crystalloid solution as plasma expander. The reperfusion blood reservoir was then filled with the diluted blood and the circuit was primed (± 200 ml) and deaired. No significant differences in blood and perfusate volumes were noted between all study groups (Table 1).

During the whole experiment, the temperature of the inflowing blood was recorded and samples were taken to monitor pH, arterial oxygen pressure, and the concentration of potassium, blood and free plasma hemoglobin. No significant differences in these parameters were seen at any time interval between all groups. However, with longer reperfusion time, there was a significant decrease in pH and arterial oxygen pressure and an increase in blood hemoglobin, reflecting hemoconcentration and in potassium and free plasma hemoglobin resulting from hemolysis in the extracorporeal circuit (Table 2).

2.4. Isolated reperfusion circuit

The closed reperfusion circuit is shown schematically in Fig. 1. The heart–lung block was suspended in a humidified and temperature-controlled (37–38°C) plexiglas chamber from a force displacement transducer (type TB-611T, Nihon Kohden, Tokyo, Japan) by a thin rigid tube connected at both ends to the tracheal cannula and the pulmonary arterial catheter, respectively. This tube bridges the heart–lung block, thereby preventing edematous lungs to sag during the experiment.

Pulmonary venous effluent was drained from the left atrium by gravity (60 cm H2O) into the blood reservoir (Minimax filtered hardshell reservoir 1316, Medtronic, Anaheim, CA) submerged in a temperature-controlled (39°C) water bath. The blood was recirculated using silicone tubing and a roller pump (model 503 s, Watson Marlow, Falmouth, Cornwall, UK). The perfusate passed a 40 µm blood filter (PALL blood transfusion
filter, East Hills, NY) and was then deoxygenated in a membrane gas exchanger (Minimax Hollow Fiber Oxygenator type 1381, Medtronic, Anaheim, CA) using a gas mixture of 90% N₂ and 10% CO₂ obtaining a partial oxygen and carbon dioxide pressure of 7.35 and 7.55 by adjusting the CO₂. Finally, the perfusate was monitored from the force transducer (Fig. 1). During the whole experiment, AwP, mPAP, PAF, and \( \Delta W \) were continuously recorded via an amplifier (Carrier amplifier AP-601G, Nihon Kohden, Tokyo, Japan) on a four channel recorder (Heat writing recorder model WT-045G, Nihon Kohden, Tokyo, Japan). Total pulmonary vascular resistance (TPVR) was calculated using the formula: $TPVR = (mPAP)/(PAF) \times 1000$.

Two on-line oxygen saturation probes (Bentley SMP-0110 attached to a Bentley oxy sat optical transmission cell, Baxter, Irvine, CA) were connected to an oxygen saturation meter (Bentley Oxy sat-meter SM-0100, Baxter, Irvine, CA) for continuous monitoring of arterial and venous oxygen saturation. Blood samples of deoxygenated inflowing (\( \text{PaO}_2 \)) and oxygenated outflowing (\( \text{PaO}_2 \)) were obtained from the pulmonary artery and the perfusion pressure was kept constant at 30 mmHg, respectively. The increase in weight of both lungs (\( \Delta W \)) was monitored from the force transducer (Fig. 1). During the whole experiment, AwP, mPAP, PAF, and \( \Delta W \) were continuously recorded via an amplifier (Carrier amplifier AP-601G, Nihon Kohden, Tokyo, Japan) on a four channel recorder (Heat writing recorder model WT-045G, Nihon Kohden, Tokyo, Japan). Total pulmonary vascular resistance (TPVR) was calculated using the formula: $TPVR = (mPAP)/(PAF) \times 1000$.
Statistics

Values are expressed in cm H₂O for AwP, in mmHg for mPAP, in ml/min for PAF, in Wood Units (WU) for TPVR, in percent for \( \Delta W \) and lung survival, and in mmHg for \( \Delta v-aPO₂ \). Data are presented as means ± S.E.M.

Differences within one group between values at successive time intervals of reperfusion were calculated using one-way analysis of variance with repeated measurements followed by Scheffé’s multiple comparison test [21]. Differences between study groups at the same reperfusion interval were compared using analysis of variance with factorial analysis (StatView SE + Graphics, Abacus Concepts, Berkeley, CA) on a Macintosh Performa 630 computer. Values of \( P < 0.05 \) were accepted as significant.

3. Results

Values for all study groups are presented in Table 3. No significant differences in any of the graft parameters were observed at any time interval during reperfusion between Infl-RA, Vent-RA, and Vent-N₂. Therefore, the results of these groups are presented together (\( n = 12 \)) as ‘non-deflated’ groups (Non-defl).

3.1. Hemodynamics

Hemodynamic parameters in all study groups are presented in Fig. 2 A–C. Following the onset of reperfusion, there was a gradual decrease in both TPVR (Fig. 2A) and mPAP (Fig. 2B) in all groups, reaching a minimum at 1 h. Concomitantly, PAF increased in all groups to reach a maximum at 1 h (Fig. 2C). Thereafter, mPAP and TPVR reincreased up to 4 h resulting in an important reduction in PAF.

Vascular resistance in postmortem expanded lungs, however, was significantly higher at the start of reperfusion when compared to lungs that were left deflated during the ischemic interval (4775 ± 1048 WU vs. 266 ± 50 WU at 5 min, respectively; \( P < 0.05 \)) (Fig. 2A). This was reflected by a significantly higher mPAP (21 ± 1 vs. 14 ± 1 mmHg at 5 min, respectively; \( P < 0.0001 \)) (Fig. 2B) and resulted in a significantly lower PAF (9 ± 2 vs. 54 ± 5 ml/min at 5 min, respectively; \( P < 0.0001 \)) (Fig. 2C). These differences between Defl and Non-defl disappeared at 1 h. PAF in Contr was superior during the first hour when compared to Defl (99 ± 3 vs. 66 ± 2 ml/min at 60 min, respectively; \( P < 0.05 \)) (Fig. 2C).

Lung survival in all study groups is listed in Table 4.

3.2. Aerodynamics

There was a slight increase in AwP in Contr and Non-defl with longer reperfusion (not significant Contr vs. Non-defl at all intervals) (Fig. 2D). In contrast, AwP in Defl rapidly increased as a result of alveolar edema formation to reach a maximum at 15 min (35 ± 8 cm H₂O). AwP in this group was significantly higher when compared to Non-defl and Contr (16 ± 1 and 15 ± 1 cm H₂O at 15 min, respectively; \( P < 0.01 \)).

3.3. Oxygenation capacity

The difference in partial oxygen pressure between deoxygenated inflowing and oxygenated outflowing blood is depicted in Fig. 2E. Oxygenation capacity in Defl was significantly impaired when compared to Non-defl and Contr (9 ± 5 vs. 96 ± 5 and 116 ± 12 mmHg at 1 h, respectively; \( P < 0.0001 \)). The decline in \( \Delta v-aPO₂ \) during 4 h of reperfusion was somewhat earlier in Non-defl when compared to Contr, but the difference between both groups only reached statistical significance at 150 min (\( P < 0.05 \)) and 180 min (\( P < 0.01 \)).

3.4. Edema formation

In Contr and in Non-defl, lung weight increased by 18 ± 1 and 16 ± 1%, respectively, 5 min after the onset of reperfusion (Fig. 2F). This \( \Delta W \) was the result of an increased blood volume by vascular distension and not from filtration of fluid out of the microvasculature into the lung interstitium. Thereafter, the weight slowly increased until 3 h. No significant differences were observed between these two groups. In contrast, edema formation was much more pronounced in Defl, reflected by an increase in weight to 81 ± 19% at 5 min (\( P < 0.001 \) vs. Contr and Non-defl) and further to 137 ± 24% at 1 h.

No differences in \( W/D \) were seen between Infl-RA, Vent-RA, and Vent-N₂ (Fig. 3). The difference between
9: Defl 42

4. Discussion

This study has shown that reperfusion of rabbit lungs that were left deflated for 4 h inside the cadaver yields poor function with reduced oxygenation, elevated peak airway pressure suggesting impaired lung compliance, and significant weight gain. All of these changes were prevented by postmortem alveolar expansion. The mechanism of this protective effect appears to be independent of the method of lung stretching (static or intermittent) and is not influenced by the gas mixture (room air or nitrogen) used for alveolar ventilation.

PAF was significantly reduced in ischemic lungs during the first hour of reperfusion when compared to control lungs. It is well known that the vasculature of an ischemic organ becomes more difficult to perfuse and other groups was highly significant ($P < 0.0001$).
Fig. 2. Parameters of pulmonary graft function during 4 h of isolated blood reperfusion comparing cadaver lungs (n = 4) that were immediately reperfused (Control group, open circles) versus lungs (n = 4) left deflated for 4 h postmortem (Deflated group, open squares) versus lungs (n = 12) that were kept expanded for 4 h postmortem (Non-deflated groups, closed squares compiling lungs (n = 4) that were inflated with room air (Infl-RA), lungs (n = 4) that were ventilated with room air (Vent-RA), and lungs (n = 4) that were ventilated with nitrogen (Vent-N₂)). Values are presented as means ± S.E.M. A, Total pulmonary vascular resistance; B, Mean pulmonary artery pressure; C: Pulmonary artery flow; D, Peak airway pressure; E, Veno-arterial oxygen pressure gradient; F, Weight Gain. No significant differences were observed in any of these parameters at any time interval between the groups Infl-RA, Vent-RA, and Vent-N₂. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001 for deflated group vs. non-deflated group; + P < 0.05, ++ P < 0.01, +++ P < 0.001, ++++ P < 0.0001 for control group vs. deflated group. ^ P < 0.05, ^^ P < 0.01, ^^^ P < 0.001, ^^^^^ P < 0.0001, and N.S., not significant for control group vs. non-deflated group by analysis of variance with factorial analysis.
Table 4
Lung survival during 4 h of reperfusion in different study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Reperfusion time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>0: Contr</td>
<td>100</td>
</tr>
<tr>
<td>1: Defl</td>
<td>100</td>
</tr>
<tr>
<td>2: Infl-RA</td>
<td>100</td>
</tr>
<tr>
<td>3: Vent-RA</td>
<td>100</td>
</tr>
<tr>
<td>4: Vent-N₂</td>
<td>100</td>
</tr>
</tbody>
</table>

* Percentages of lungs (n = 4) with pulmonary artery flow > 5 ml/min.
Contr, control; Defl, deflated; Infl-RA, inflated room air; Vent-RA, ventilated room air; Vent-N₂, ventilated 100% nitrogen.

As the duration of ischemia is prolonged [22]. The exact mechanism remains unclear. Hypoxic vasoconstriction, mediator-induced vasospasm, endothelial cell swelling, and microvascular plugging of cellular blood elements have all been recognized as possible causes of increased resistance. Post-ischemic endothelial damage may then lead to permeability pulmonary edema and clinically apparent acute graft dysfunction following reperfusion [23].

We chose a period of 4 h of warm ischemia based on preliminary reperfusion experiments in which we investigated, in deflated lungs, the influence of the length of the postmortem period on gas exchange. \( \Delta v-aPO_{2} \) in lungs retrieved 1 h after death did not differ from control lungs (106 ± 11 vs. 116 ± 12 mmHg at 60 min, respectively; not significant). Gas exchange in lungs extracted 2 h after death was already significantly impaired, but still acceptable (53 ± 17 mmHg at 60 min; \( P < 0.05 \) vs. control lungs) (unpublished results).

In this model, we also elected to reperfuse the lungs with a self regulating rather than a preset flow by keeping the gravitational pressure constant at 30 cm H₂O via an overflow system. This resulted in an automatic and progressive increase in PAF in all study groups to reach a maximum at 1 h. It is well known from clinical and experimental [20] practice that the first 10 min of lung graft reperfusion following an ischemic period are of critical importance. Sudden uncontrolled reperfusion with an increased microvascular hydrostatic pressure may result in additional mechanical trauma on the already damaged vascular endothelium and may further lead to additional hydrostatic edema [24]. This is also illustrated by the severe reperfusion injury that is frequently observed following single lung transplantation for pulmonary hypertension [25]. The newly implanted allograft will receive nearly 90% of the total pulmonary blood flow.

The exact mechanism of extended warm ischemic tolerance of the lung by preventing the alveolar space to collapse is not clear. The ischemic insult in all study groups was the same and presumably produced similar endothelial damage. The observed elevated TPVR in Infl-RA, Vent-RA, and Vent-N₂ might be a self protecting mechanism against reperfusion injury. As a result, the initial low flow rates in expanded lungs may have been beneficial in itself by allowing the damaged endothelium to recover its barrier function during the initial reperfusion period, thereby limiting permeability edema. The initial higher flow rates in Defl, on the contrary, might have contributed to the important reperfusion injury manifested by poor oxygenation and edema formation.

The reason for the observed difference in TPVR between atelectatic and expanded lungs is also not clear. We can only speculate that positive pressure in the alveolar space might result in more collapse of the lung vasculature and thus elevated resistance upon reperfusion.

Accepted for oral presentation at The Fifth Annual Meeting of The European Surgical Association, Milan, Italy, April 24–25, 1998; submitted to Ann Surg.
On the other hand, release of surfactant from type II pneumocytes is known to be stimulated by inflation [26] and ventilation of lungs [27] and by mechanical stretch of isolated pneumocytes in culture [28]. Alveolar surfactant activity is reduced in the atelectatic lung [29]. Although no measurements of surfactant or surfactant activity were made in the present study, it is reasonable to speculate that repetitive or continuous alveolar expansion during warm ischemia in the groups Infl-RA, Vent-RA, and Vent-N2 may have stimulated the release of pulmonary surfactant, thereby decreasing the alveolar surface tension, preventing damage to the alveolar-capillary membrane, and protecting against permeability pulmonary edema upon reperfusion [23].

The present study validates the conclusions of our previous functional study [18]. These flush experiments were used as a rapid screening method to define the length of tolerable warm ischemia in all study groups. Pulmonary edema developed in atelectatic lungs when hypothermic flush was delayed for 2 h after death. Postmortem inflation was as good as ventilation in protecting the lung against edema formation, thereby prolonging warm ischemic tolerance up to 4 h. Postmortem inflation with oxygen or ventilation with nitrogen or oxygen was no different from that with room air. From that study, we already concluded that prevention of alveolar collapse was the critical factor to protect the lung from warm ischemic damage, independent of continued oxygen delivery.

This, however, is in contrast with the findings of previous metabolic and morphologic studies from our laboratory. Investigating postmortem adenine nucleotides catabolism, we observed that adenosine triphosphate (ATP) breakdown and hypoxanthine formation were significantly delayed in cadavers that were inflated with room air and even longer if cadavers were ventilated with room air or oxygen [14]. Nitrogen-ventilation, however, was ineffective and ATP catabolism was not different from deflated lungs (unpublished results). Looking at light microscopical pulmonary cell viability using a trypan blue vital dye exclusion test, we have found that postmortem cell death was significantly delayed in rabbit lungs that were inflated with room air (19.6 ± 3.2% non-viable cells at 6 h vs. 39.2 ± 2.9% in deflated lungs; \( P < 0.01 \)). Pulmonary cells remained viable for longer periods if they were inflated with oxygen (25.0 ± 3.3% at 12 h) or ventilated with room air or oxygen (21.4 ± 2.7% and 23.4 ± 2.7% at 24 h, respectively). Ventilation with nitrogen, however, was ineffective and cell death was comparable with atelectatic lungs (79.7 ± 2.1% at 24 h) [15]. From both these studies we concluded that the alveolar oxygen reserve was the critical factor to protect the lung from warm ischemic damage. Briefly, no correlation can be found between metabolic and morphologic studies on one hand and functional studies on the other hand.

We therefore plan to continue our study on the use of lungs from NHBDs in a canine allotransplant survival model. In a previous study conducted by Uliency and coworkers, no difference in early gas exchange was seen following canine single lung transplantation and hilar occlusion of the native lung after a 4 h period of postmortem donor ventilation with either 100% oxygen or 100% nitrogen, but results were better than after non-ventilation [8]. The authors therefore concluded, as in the present study, that the mechanics of ventilation after cessation of circulation appeared to confer a functional advantage independent of a continued supply of oxygen. In a subsequent identical transplant study, postmortem ventilation with alveolar gas was inferior to ventilation with 100% oxygen [12]. On the other hand, Koyama et al. [30] comparing the effect of postmortem ventilation with room air versus oxygen versus nitrogen in a canine isolated reperfusion model, reported superior function in lungs ventilated with nitrogen and concluded that reperfusion injury seen in oxygen-ventilated animals was mediated by oxygen free radicals [30]. The optimal oxygen concentration that should be delivered to ischemic pulmonary grafts during cadaveric storage therefore remains an open question.

In summary: (1) pulmonary vascular resistance was significantly increased in rabbit lungs that were expanded for 4 h postmortem when compared to atelectatic lungs. This resulted in a significantly reduced pulmonary artery flow and attenuated ischemia-reperfusion injury; (2) postmortem room air-inflation was as good as ventilation in prolonging warm ischemic tolerance; and (3) ventilation with room air was no different from that with nitrogen.

From this study, we therefore conclude that prevention of alveolar collapse in the NHBD appears to be the critical factor to protect the lung from warm ischemic damage independent of continuous oxygen supply. Further studies are necessary to investigate whether lungs from human NHBD will become a realistic alternative to expand the pulmonary donor pool.

Acknowledgements

This work is supported by a grant from the ‘Nationale Fonds voor Wetenschappelijk onderzoek-Levenslijn 1994’ no. 7.0036.94. We thank Peter Lemmens, Magda Mathys, Eddy Vandezande, and Kanigula Mubagwa, MD, PhD for expert technical and secretarial assistance.
References


Appendix A. Conference discussion

Dr. L. Von Segesser (Lausanne, Switzerland): I have a question with regards to the statistical methods that you have used. I have seen many P values on your slides. What tests did you use?

Dr Van Raemdonck: Because of the lack of time, I didn’t show the statistical evaluation that we have used. But we used the analysis of variance and we looked at the difference between groups using factorial analysis followed by Scheffe’s multiple comparison test. So we looked at differences between the groups at all time intervals.

Dr Von Segesser: Because normally if there are repeated measures, you should have only one P value for the whole pair of curves. You can use either surfaces under the curves or ANOVA for repeated measurements.

Dr A.T. AB (Louisville, Kentucky): I must congratulate you on your excellent study. I would like to find out the parameters used for ventilating postmortem. What were the ventilating pressures and how did you ventilate the postmortem lungs in these groups?

Dr Van Raemdonck: The tidal volume was 10 ml/kg and the frequency was ~30 times per min, and the peak end-expiratory
pressure was 2 cm of water both in the group ventilated with room air as well as in the group ventilated with nitrogen.

**Dr A. Haverich (Hannover, Germany):** I have two questions. Firstly, the high pulmonary vascular resistance combined with the low pulmonary flow in the groups with inflation, number one, how would the low flow influence the oxygen transfer when compared to the higher flow in the other groups. Secondly, would you think that if you would have a double-lung transplant after this best group in your experimental setting that the heart of the recipient would overcome this high pulmonary vascular resistance?

**Dr Van Raemdonck:** That’s a good question. Maybe I can show one of the discussion slides. It is well known that the vasculature of an ischemic organ becomes more difficult to perfuse and that, as a result, the reperfusion injury becomes more apparent as the period of ischemia is prolonged. This is the so-called no-reflow phenomenon. But the exact mechanism of this increased pulmonary vascular resistance is not well known. Hypoxic vasoconstriction, mediator-induced vasospasm, endothelial cell damage, as well as microvascular plugging of cellular blood elements have all been recognised as possible causes of this increased resistance, and as a result, permeability pulmonary edema may result from this increased resistance at the time of reperfusion. Also, we do not know the protective mechanism is of this aveolar expansion. As you mentioned Dr Haverich, on one hand this increased vascular resistance resulted in a reduced flow, and it is well possible that the damaged endothelial may recover its barrier function during this initial flow rate and therefore, result in a continued release of surfactant from type 2 pneumocytes, and this may result in a decreased alveolar surface tension and a better protected alveolar capillary membrane, resulting in less permeability edema. The system we used in our reperfusion model, was a pressure-limited system, so there always was an overflow. When the pressure was too high, flow was going back to the blood reservoir and not to the lung. And, as you mentioned, indeed when you have a double-lung transplant, the chance is high that the patient will develop severe reperfusion injury because of the increased vascular resistance and this may jeopardise right ventricular function.