Lung Retrieval from Non-Heart-Beating Donors: First Experience with an Innovative Preservation Strategy in a Pig Lung Transplantation Model

Th. Wittwer^a U.F.W. Franke^a Antonia Fehrenbach^{b,c} T. Sandhaus^a
F. Pfeifer^a N. Dreyer^b Th. Mueller^d H. Schubert^d P. Petrow^e J. Richter^b
Th. Wahlers^a

^aDepartment of Cardiothoracic and Vascular Surgery, Friedrich Schiller University, Jena; ^bDepartment of Electron Microscopy, University of Göttingen; ^cDepartment of Internal Medicine (Respiratory Medicine), Clinical Research Group 'Chronic Airway Diseases', Philipps University, Marburg, and Departments of ^dExperimental Animal Research and ^ePathology, Friedrich Schiller University, Jena, Germany

Key Words

Retrograde pulmonoplegia · Non-heart-beating donors · Perfadex lung preservation · Lung transplantation

Abstract

Objective: Lung transplantation is limited by the scarcity of donor organs. Lung retrieval from non-heart-beating donors (NHBD) might extend the donor pool and has been reported recently. However, no studies in NHBD exist using the novel approach of retrograde preservation with Perfadex solution. **Methods:** Heparinized azygotic pigs (n = 5, 30–35 kg) were ventilated for 90 min. The lungs were retrogradely preserved with Perfadex solution and stored inflated at 4°C for 3 h. Left lung transplantation in the recipient was followed by exclusion of the right lung. Results were compared to sham-operated animals. Oxygenation, hemodynamics and dynamic compliance were monitored for 4 h. Infiltration of poly-

morphonuclear cells (PMNs) and stereological quantification of alveolar edema was performed. Statistical analysis comprised Kruskal-Wallis and Mann-Whitney tests and ANOVA analysis with repeated measures. **Results:** No mortality was observed. During preservation, continuous elimination of blood clots via the pulmonary artery venting site was observed. Oxygenation and compliance were similar between groups, but sham controls showed significantly lower pulmonary vascular resistance. Stereological quantification revealed higher volume fractions of intra-alveolar edema in NHBD grafts, while PMN infiltration was comparable to sham controls. **Conclusions:** Use of NHBD lungs results in excellent outcome after 90 min of warm ischemia followed by retrograde preservation with Perfadex solution. This novel approach can optimize lung preservation by eliminating clots from the pulmonary circulation and might clinically be considered in brain-dead organ donors who become hemodynamically unstable prior to organ harvest. Further trials with longer warm and cold ischemic periods are necessary to further elucidate this promising approach to donor pool expansion.

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Thorsten Wittwer, MD, PhD
Department of Cardiothoracic and Vascular Surgery
Friedrich Schiller University
Bachstrasse 18, DE-07740 Jena (Germany)
Tel. +49 3641 934801, Fax +49 3641 934802, E-Mail Th.Wittwer-MD@t-online.de

Introduction

Although lung transplantation has been proven to be an effective therapy for patients with end-stage pulmonary disease, access to this therapy is severely limited by the increasing scarcity of suitable donor organs in the last years [1, 2]. Currently, aside from living-related organ donation, all suitable lung grafts are retrieved from brain-dead heart-beating donors. However, it is estimated that 35,000 people in the US are killed by firearms, and 47,000 deaths are related to motor vehicle accidents [3]. If, after unsuccessful resuscitation, just a small portion of these acutely injured people were candidates for non-heart-beating organ donation, this might increase the number of available organs by up to 20–30% [4, 5]. Lung retrieval from non-heart-beating donors (NHBDs) is considered to be a realistic therapeutic option as the lung is unique among the transplanted solid organs in that it is not dependent on vascular perfusion to meet its oxygen needs. Experimentally, pulmonary cells obtained from ventilated cadavers have been cultured successfully, indicating that lung parenchymal cell death does not necessarily occur at the time of clinical death [6, 7]. Previous studies in our laboratory have shown that the quality of pulmonary preservation in terms of post-ischemic lung function can be significantly improved by the innovative technique of retrograde flush perfusion (RFP) via the left atrium into the pulmonary venous system [8, 9]. However, so far there is no information concerning the feasibility of RFP with a low-potassium dextran (LPD) solution with regard to the quality of pulmonary preservation achievable when NHBD lungs at high risk of microvascular thrombi formation are used for transplantation.

Prior to extended experiments with longer ischemic intervals and detailed comparisons with standard preservation procedures, the present limited pilot study was undertaken to evaluate whether the innovative strategy of retrograde preservation of NHBD lungs is technically feasible and safe in terms of post-ischemic outcome.

Material and Methods

Experimental Groups

Female domestic pigs weighing 28–32 kg were randomized into 2 groups of 5 animals each. The first group served as a sham-operated control group, while in the second group NHBD lungs were preserved by RFP with LPD solution (Perfadex; Vitrolife, Göteborg, Sweden) after a warm ischemia time (WIT) of 90 min. In this test group, 5 additional animals were used as lung recipients.

Surgical Procedure

Donor Preparation

All animals were pre-medicated with ketamine 10% (20 mg/kg), atropine (0.04 mg/kg) and propofol (3 mg/kg). Pigs were then put in the supine position, intubated and mechanically ventilated with 50% oxygen in a pressure-controlled mode with a peak inspiratory pressure of 20 mm Hg, a rate of 18 breaths/min, an inspiratory/expiratory ratio of 1:1 and a positive end expiratory pressure (PEEP) of 8 mm Hg. Anesthesia was continued with infusion of fentanyl (0.3 µg/kg/min), midazolam (20 µg/kg/min) and pancuronium (10 µg/kg/min). All animals received 200 IU/kg of heparin intravenously. A median sternotomy was performed and the pericardium was opened longitudinally. A perfusion cannula with a sideport to measure the perfusion pressure was placed through the auricle into the left atrium. Cardiac fibrillation was induced electrically, and the cadaver was ventilated and left at room temperature for 90 min of WIT. Both pleural cavities were opened in order to evaluate the quality of the donor lungs in terms of infection or severe atelectasis. In case of such findings, animals were excluded from the study. Irrigation of the lungs was performed by instillation of 1,000 ml of 4°C cold saline solution in each pleural cavity in order to prevent the lung surface from drying. Continuous topical cooling of the lungs was not performed in order to specifically assess the impact of retrograde flush preservation. Following WIT, retrograde perfusion via the left atrium was started and the anterior aspect of the main pulmonary artery was incised for drainage of the LPD solution. Eight minutes were required to infuse 1,800 ml LPD solution at 4°C with a maximum flushing pressure of 14 mm Hg. No prostaglandins were used in either the donor animal or the preservation solution. Ventilation was continued throughout the entire perfusion period. After completion of preservation, the heart-lung bloc was excised with both lungs inflated in an end inspiratory state and stored at 4°C for 3 h.

Recipient/Sham Preparation

The anesthetic regimen was identical to the donor procedure. A Swan-Ganz catheter (7.5 french, Baxter Healthcare Corp., Irvine, Calif., USA) and a catheter to monitor the arterial pressure (Leader Cath 20G 8 cm, Vygon, France) were placed into the right carotid artery and external jugular vein, respectively. All animals were placed in a right decubitus position, and a left thoracotomy was performed in the fifth intercostal space. The pulmonary bifurcation, left main bronchus and left pulmonary veins were dissected. After clamping the left pulmonary artery and bronchus, the left pulmonary veins were ligated and pneumonectomy was performed in the NHBD test group only. The left donor lung was isolated from the heart-lung bloc and prepared for implantation with a large atrial cuff and full length of both the pulmonary artery and left main bronchus. Implantation of the donor lung started with bronchial anastomosis using a running suture with 4-0 Prolene (Ethicon Inc., Somerville, N.J., USA) followed by arterial anastomosis with a running suture of 6-0 Prolene. After clamping the left atrium, a recipient atrial cuff was designed and anastomosed to the donor atrial cuff using a running suture of 5-0 Prolene. Prior to reperfusion, the donor lung was carefully de-aired retrogradely. The pulmonary artery was then unclamped and the graft reventilated in a pressure-controlled mode with a peak inspiratory pressure of 30 mm Hg using a PEEP of 10 mm Hg with a respiratory rate of 18/min. After 15 min of reperfusion, the right pulmonary artery and bronchus were clamped both in the sham-operated control group and the NHBD test group. All lungs were reperfused for 4 h followed by termination of the experiment by intravenous injection of magnesium sulfate.

Functional Analysis

In all experiments, arterial and pulmonary artery pressures as well as central venous and left atrial pressures were recorded continuously. Dynamic lung compliance was monitored by the ventilator (Evita 2 dura, Dräger Medical Inc., Lübeck, Germany). An arterial and mixed venous blood gas analysis was performed initially and at 30-min intervals during the reperfusion period. Cardiac output was measured continuously by the Swan-Ganz catheter, and systemic and pulmonary vascular resistance was calculated.

Stereological Analysis

After completion of the reperfusion period, the entire left lung was further processed for light microscopy by fixation using vascular perfusion via the pulmonary artery with 2 liters of a 1.5% paraformaldehyde/glutaraldehyde solution in 0.1 M cacodylate buffer at 15 cm H₂O pressure. During this fixation the lung was inflated at a constant pressure of 12 cm H₂O. In order to collect lung material which is representative for the whole lung, systematic random sampling of tissue was performed according to Michel and Cruz-Orive [10]. Lungs were cut into 3-mm-thick slices. A transparent paint grid was then superimposed over the section of slices. Whenever paint hit the surface of the lung slice, a 1-cm³ tissue block was excised. The tissue was dehydrated through a graded series of ethanol and embedded in a methacrylate resin (Technovit 7100, Kulzer, Heraeus, Germany). Sections of 1 µm were cut from each block (10–12/lung) and stained with methylene blue/Azur II (1:2). The relative volume fraction of intra-alveolar edema formation was determined by established stereological techniques [11, 12] using a Cast-Grid-System 2.0 (Olympus, Denmark).

Histopathologic Studies and Polymorphonuclear Neutrophil Measurements

Lung specimens were collected prior to vascular perfusion for stereological analysis, fixed in 4% buffered formalin, and embedded in paraffin using an automated tissue processor. 4-µm tissue sections were stained with hematoxylin and eosin, and also with naphthol AS-D chloroacetate esterase according to standard methods. The polymorphonuclear neutrophils (PMNs) were identified by morphology and positive esterase staining. PMNs and alveoli were counted in 4 different fields under 400× magnification. The data are expressed as PMN per alveolus. The measurements were performed by a pathologist blind to the details of individual specimens.

Animal Care

All animals received humane care in compliance with the 'Principles 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 Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996.

Statistical Analysis

All data are expressed as mean ± SD and were analyzed with the Statistical Program of Social Sciences (SPSS for MS Windows, Version 10.0; SPSS Inc., Chicago, Ill., USA). Continuous data were analyzed using ANOVA with repeated measures. For evaluation of data without repeated measures, standard analysis of variance was used. Stereological data were analyzed using Kruskal-Wallis and Mann-Whitney tests.

Results

During retrograde flush preservation, a continuous washout of small blood and fibrin clots from the pulmonary arterial venting site was noticed in all test lungs. Following lung transplantation, all animals survived the observation period of 4 h. In the NHBD group, all reperfused lungs resumed sufficient function with a pulmonary oxygenation capacity in terms of pO₂/FiO₂ which was comparable to the outcome of sham controls (p = 0.306; fig. 1).

At early reperfusion and ligation of the contralateral lung, a 5-fold increase in pulmonary vascular resistance was calculated in the NHBD group, while the corresponding increase in the sham control group was found to be 2.5-fold. These differences were statistically significant (p = 0.032; fig. 2). In contrast, the dynamic lung compliance over 4 h of reperfusion was found to be almost unchanged in the sham group and only slightly decreased in the NHBD group, and did not reach statistical significance (p = 0.358; fig. 3). Stereological analysis revealed a higher volume fraction of intra-alveolar edema in NHBD grafts (fig. 4) compared to sham-operated (fig. 5) animals (5.92 ± 3.91 vs. 1.32 ± 0.21%; p = 0.03; fig. 6). Histologically, the amount of PMNs per alveolus was comparable between both groups (7.77 ± 1.9 [NHBD] vs. 5.18 ± 1.55 [sham]; p = 0.058; fig. 7).

Comment

Currently, lung transplantation offers a realistic therapeutic option and has become an effective method for patients with end-stage parenchymal or vascular pulmonary disease. However, the number of suitable donor lungs among the current pool of multiple organ donors is just about 20% [13] and is therefore insufficient to meet the rapidly growing demand for lung grafts. Although there has been a trend towards utilization of so-called 'marginal' donor lungs with satisfying results [14], there is still an increasing discrepancy between patients on the waiting list and the availability of suitable donor organs [15]. Organs from NHBDs, which have been used clinically in renal transplantation, might represent an important alternative organ source for a significant increase in the donor pool available [5, 16]. The lung is unique among the vital organs owing to its remarkable tolerance to warm ischemia which is premised on the fact that pulmonary parenchymal cells are able to maintain aerobic metabolism with alveolar oxygen even after cessation of vascular

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Fig. 1. Oxygenation in terms of pO₂/FiO₂ of domestic pigs before left single lung transplantation (Basis) and during the observation period of 4 h following exclusion of the native right lung (time point 0 h). Differences are not statistically significant ($p = 0.306$).

Fig. 2. Pulmonary vascular resistance of domestic pigs before left single lung transplantation (Basis) and during the observation period of 4 h following exclusion of the native right lung (time point 0 h). Differences are statistically significant ($p = 0.032$).

Fig. 3. Dynamic lung compliance of domestic pigs before left single lung transplantation (Basis) and during the observation period of 4 h following exclusion of the native right lung (time point 0 h). Differences are not statistically significant ($p = 0.358$).

circulation [6, 7, 17]. In the last years, several authors have investigated the tolerance of ventilated lung grafts to different periods of warm ischemia with promising overall results [18–21]. However, although the pulmonary vascular endothelium is considered to maintain its function over several hours of warm ischemia [22, 23], diffuse microvascular thrombosis in the pulmonary circulation is thought to represent a serious and clinically limiting problem with the use of NHBD grafts [4, 24].

Recently, the innovative approach of retrograde preservation of lung grafts has been shown to be very effective in the elimination of different kinds of thrombi from the pulmonary vasculature [6, 25–28]. Furthermore, the quality of organ preservation in terms of post-ischemic lung function was significantly improved following retrograde lung preservation [8, 9, 28, 29, 30]. This phenomenon is believed to be due to the more homogeneous distribution

in the low-pressure high-capacity pulmonary venous system [31, 32] and the concomitant preservation of the small but important bronchial circulation [33–35]. However, most of these reports used high-potassium solutions for lung preservation. Since there was clear evidence in the past that use of the modern LPD-containing Perfadex solution ameliorates reperfusion injury and improves primary graft function in lung transplantation [36, 37], we evaluated whether retrograde preservation of NHBD lungs with Perfadex solution is technically feasible and results in sufficient preservation quality in terms of pulmonary function.

In terms of oxygenation and lung compliance, in our series post-ischemic lung function was not different from the results obtained with sham-operated controls. Stereological evaluation revealed a moderate degree of intra-alveolar edema formation in NHBD grafts which was sig-

Fig. 4. Moderate formation of intra-alveolar edema (Ed) after 4 h of reperfusion in a non-heart-beating donor lung. Du = Ductus alveolaris.

Fig. 5. Parenchyma of a sham control animal 4 h after reperfusion without any intrapulmonary edema formation. Du = Ductus alveolaris.

Fig. 6. Comparison of volume fractions of intra-alveolar edema in domestic pig lungs after 4 h of observation. In non-heart-beating donor lungs, edema formation was significantly increased compared to sham-operated lungs ($p = 0.03$).

Fig. 7. Comparison of amount of polymorphonuclear cells (PMN) per alveolus in domestic pig lungs after 4 h of observation. There was no significant difference between both groups ($p = 0.058$).

nificantly higher compared to the sham group. This edema formation correlates with the significantly increased pulmonary vascular resistance in NHBD lungs and represents a mild form of ischemia/reperfusion injury. Of special clinical importance and one of the main results of the presented work is the finding of continuous washout of small blood and fibrin clots from the pulmonary arterial

venting site during retrograde preservation, although all donor animals in our series received intravenous heparin prior to cardiac arrest as recommended by several groups [18, 26, 38]. This phenomenon underlines that even with heparinization the pulmonary circulation is still prone to some degree of microvascular thrombosis which can alter the postoperative outcome following lung transplanta-

tion. However, some authors indicate that heparin is not mandatory in NHBD [23, 39]. Especially in those non-heparinized NHBD lungs, the innovative retrograde preservation strategy might display special benefit in the elimination of potential microvascular thrombi and debris in addition to the excellent preservation quality in terms of oxygenation and dynamic compliance.

A limitation of this pilot study is the short duration of warm ischemia, which was chosen to be 90 min. However, it is generally agreed that for the clinical use of NHBD lungs, the period of warm ischemia should be kept as short as possible [39, 40]. Furthermore, in accordance with the reported outcome of short-term warm ischemia, this novel technique might also be considered when an accepted brain-dead and, in most cases, systemically heparinized organ donor becomes hemodynamically unstable in the intensive care unit or the operating room prior to the onset of organ retrieval. In those cases, a time period in the area of 90 min should be enough to harvest at least the continuously ventilated lungs.

These promising results strongly encourage the initiation of more detailed studies in the field of retrograde preservation using NHBD lungs with extended periods of warm and cold ischemia, including comparisons with both antegrade preservation procedures in NHBD and retrograde preservation using standard heart-beating donors. The potential future use of this innovative preservation strategy might represent a highly relevant tool in the clinical arena to significantly expand the progressively limited pool of donor lungs [41].

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