Interruption of bronchial circulation leads to a severe decrease in peribronchial oxygen tension in standard lung transplantation technique1

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Abstract

Objective: In clinical practice lung transplantation is the only procedure where the transplanted organ is left without its own arterial perfusion. With the interruption of the bronchial arteries the nutritive support is dependent on collateral flow by the pulmonary artery and the oxygen tension of desaturated central venous blood, representing an abnormal physiology. Methods: To analyze this problem systematically, we used a standard single left lung transplantation model in the pig (n = 12). In accordance with the clinical standard, lung preservation was performed with modified Euro-Collins solution with addition of prostacycline. The duration of ischemia was set to 4 h. Before and after single left lung transplantation tissue oxygen tension in the peribronchial tissue was measured with Licox® tissue pO2 microprobes. For validation, the myocardial tissue oxygen tension was recorded simultaneously. The hemodynamic assessment included continuous flow measurement of the left and right pulmonary artery using Transsonic ultrasound flow probes. After transplantation the animals were observed for 4 h. For hypothetic augmentation of collateral blood flow to the peribronchial tissue we administered Nitric oxide (10 ppm) to the ventilation in six pigs (group B). Six pigs (group A) served as a control without the addition of nitric oxide (NO). All pigs were ventilated with a FiO2 of 0.5 resulting in pao2 values between 160 and 200 mmHg. Results: In both groups single lung transplantation led to a significant decrease in peribronchial tissue oxygen tension throughout the observation period. Pre-Tx values of peribronchial tissue oxygen tension (38.31 ± 6.56 mmHg) decreased to 9.72 ± 2.55 mmHg in group A and 10.3 ± 3.61 mmHg in group B after 4 h, which could not be altered by a FiO2 of 1.0 (P < 0.0001). The addition of NO in group B led to a significantly augmented flow in the left pulmonary artery (0.63 ± 0.31 l/min in group B vs. 0.46 ± 0.26 l/min group A, P < 0.001) representing 67 vs. 49% of the pre-Tx flow in groups B and A, respectively, but the peribronchial tissue oxygen tension was not influenced (P > 0.05). In both groups A and B, the central venous pO2 did not differ in the postoperative period (41.83 ± 3.27 mmHg group A vs. 43.26 ± 2.98 mmHg group B) and was kept in a comparable range to the pretransplantation values (45.23 ± 3.41 mmHg pre-Tx). Conclusions: The persistence of a very low peribronchial tissue oxygen tension in the early phase after lung transplantation cannot be influenced by improved pulmonary artery flow and solely relates to the central venous pO2, which cannot be augmented by the addition of NO. This mechanism might be a trigger for anastomotic healing problems, infectious complications and later development of obliterative bronchiolitis (OB). © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Lung transplantation has become the treatment of choice for endstage pulmonary failure. Despite the fact that systemic blood supply to the bronchial structures is interrupted in conventional lung transplantation technique, bronchial healing problems could be decreased in recent years to 3–10% in single as well as sequential double lung transplantation [1–3]. Thus, the bronchial wrapping technique, based on the idea of early capillarization of the donor bronchus using omentum majus, as originally described by the Toronto group, was abandoned [4,5]. Overall, 1- and 5-years survival rates were improved to 70% single and 40–47% for bilateral lung transplantation [6].

Few groups in the world practice bronchial arterial revascularization with the idea to immediately reestablish physiologic conditions in the transplanted lungs [7–10], a procedure originally attempted by Haglin in 1960 [11]. Whether or not bronchial healing is improved using this more demanding technique has to be critically evaluated. Preliminary data from the Bordeaux group indicates that besides improved tracheal healing a decreased incidence of obliterative bronchiolitis (OB) in a small cohort of en bloc double lung transplanted patients with patent bronchial artery revascularization could be observed [12].

The aim of our study is to systematically examine the effects of bronchial arterial devascularization in an animal model. A first step was to evaluate the acute effects of loss of systemic blood supply to the peribronchial tissue and its potential reversibility by pharmacologic manipulation of the collateral blood flow.

2. Material and methods

We used a standard single left lung transplantation model in the pig. Male pigs with a body weight of 22.5 ± 2.2 kg were used as organ donors and recipient animals. The animals were preanaesthetized with azaperone (0.2 ml/kg per BW, Stresnil®, Roche, Grenzach-Whylen, FRG) and midathydrochloride (0.2 ml/kg per BW, Hypnodil®, Roche, Grenzach-Whylen, FRG) by intramuscular application. Anaesthesia was established and maintained by continuous infusion of piritramid (0.03 mg/kg BW per min, Dipidolor®, Janssen, Neuss, FRG) and midazolam (0.005 mg/kg BW per min Dormicium®, Roche, Grenzach-Whylen, FRG), followed by intravenous application of pancuronium (4 mg Pancuronium Organon®, Organon, Eppelheim, FRG). The animals were intubated with a cuffed endotracheal tube and placed on a respirator at a tidal volume of 25 ml/kg of body weight. The inspired oxygen fraction (FiO₂) was set to 0.5, the positive end-expiratory pressure was limited to 4 cm H₂O.

2.1. Donor operation

A median sternotomy and anterior pericardectomy was performed in the supine placed animals. The superior and inferior venae cavae, the ascending aorta and the pulmonary artery were mobilized and encircled with heavy silk ties. A cathether was placed into the right internal thoracic artery and connected with a pressure transducer (CR 4050, Corotec, Klein-Winterheim, FRG) for continuous arterial pressure recording. A pulmonary artery catheter was placed for pressure measurement and cardiac output as well as the administration of intravenous drugs and volume. After administration of 300 U/kg per BW of heparin, a flush perfusion cannula (9G, DLP, Medtronic, Düsseldorf, FRG) was inserted in the proximal pulmonary artery and a prostacycline (Flolan®, Welcome England) infusion (25 μg/kg per body weight) was commenced. In inflow occlusion and after aortic cross-clamping, the tip of the left atrial appendage was cut off and the pulmonary artery was flushed with modified 4°C Euro-Collins solution (60 ml/kg). The pulmonary flush solution was given by gravity drainage and adjusted in altitude to achieve a perfusion pressure of less than 25 mmHg. After the flush perfusion, the trachea was cross-clamped 4 cm above the bifurcation at endinspiration and at an inspired oxygen fraction (FiO₂) of 1.0. The total heart-lung block was excised and placed in a basin containing 4°C Euro-Collins solution. Great care was taken to minimize mechanical trauma of the lung. The heart and the right lung were removed and the left lung was placed in a plastic bag containing 4°C Euro-Collins solution and stored in a refrigerator at temperatures between 4 and 8°C, surrounded with crushed ice. According to clinical practice the total ischemic time of the donor lung was set to 4 h.

2.2. Recipient operation

The recipient animals were placed in the right lateral position, anaesthetized and ventilated as described in the donor section. A posterolateral thoracotomy was performed through the third intercostal space and the third and fourth rib were removed. The pericardium was opened posteriorly and anteriorly to the phrenic nerve. The aorta, the right and left pulmonary artery were mobilized, freed of tissue and encircled with silk ties. For subsequent flow measurement ultrasound flow probes (Transsonic®, Schubarth, Frankfurt, FRG) were placed around the left and right pulmonary artery. For hemodynamic measurements, the animals were instrumented with catheters as described in the donor section. The hilus of the left lung was mobilized, the hemi-azygos vein was ligated and transected. A clamp was applied intrapericardially on the left atrium with care taken to preserve right lower lobe venous drainage.
unhampered to the left atrium. Left pneumonectomy was performed preserving a 1 cm long stump of the recipient bronchus and the left pulmonary artery as well as a generous cuff of the left atrium. Left lung transplantation was accomplished using a continuous 5-0 polypropylene suture technique for the left atrial anastomosis, the left main bronchial anastomosis, the pulmonary artery anastomosis. Before tightening the PA and LA suture, the bronchial clamp was released and the lung gently ventilated for deairing of the pulmonary circulation after vascular clamp release. An additional catheter was placed in the left atrium for continuous measurement of left atrial pressures. The pressure transducers, the ECG and the ultrasound flow meters were coupled to a chart recorder (Gould 2000, Gould, Cleveland, OH). During the transplantation procedure the animals were ventilated with a FiO2 of 1.0, which was lowered to 0.5 after Tx, to maintain the arterial oxygen tension in a physiological range of 160–200 mmHg. Throughout the observation period the positive endexpiratory pressure was kept at 5 cm H2O.

2.3. Experimental protocol

To measure the peribronchial tissue oxygen tension, Licox TM pO2 microcatheter probes of polarographic Clark type (C1.2, GMS, Mielkendorf, Germany) were used, an established and validated method originated by Clark. Arterial blood pressure, pulmonary artery pressure, left and right pulmonary artery flow, CVP and LAP was measured continuously. Arterial and venous blood gas analysis was performed at intervals of 20 min. The Licox pO2 microprobes were connected with a two channel Licox pO2 measurement computer device (SNE 340, GMS, Mielkendorf, FRG). For online data validation myocardial tissue oxygen measurement was performed simultaneously. Tissue temperature was online recorded with an integrated thermo couple placed in the tissue in addition to the pO2 sensor. Measurements were permanently corrected and compensated for temperature changes. The diffusion error of the current pO2 probe is less 1.5%, the zero error is smaller than 0.2 mmHg.

Several pilot experiments were performed to determine the ideal location for reliable and reproducible measurements of the peribronchial tissue oxygen tension. Only the peribronchial lymph nodes located more than 1 cm distal to the bronchial anastomosis proved to provide reproducible results in tissue oxygen measurements. The very thin bronchial wall (< 1 mm) as well as the friable peribronchial fatty tissue did not allow stable positioning of the tissue pO2 probes (0.6 mm in diameter) without creating artefacts.

Our experimental protocol started after a steady state period prior to explantation (T0). After transplantation tissue oxygen measurement was started and repeated every 60 min (T1–T4). Tissue oxygen measurements were performed three times at any point of measurement.

The obtained values were only considered to be reliable and valid, if the obtained tissue pO2 values reached a steady state for more than 5 min.

To assure the steady state conditions of the preparation throughout the experiment we only included the obtained data during a postoperative period of 4 h.

A pilot study (n = 3) was performed to assess the dependence of the peribronchial tissue oxygen tension in correlation to the arterial and venous pO2 values prior and after transplantation.

Tissue oxygen tension was measured under increasing FiO2 from 0.2 to 1.0 in increments of 0.2 according to the protocol described before. Based on the results of this study the FiO2 was set to 0.5 to obtain optimal arterial oxygenation with a arterial pO2 of 160–200 mmHg and a central venous pO2 of 40–45 mmHg.

2.4. Animal groups

To prove the hypothesis whether or not the collateral blood flow to the bronchial circulation in the transplanted lung could be increased acutely, the animals were divided into two groups of six recipients each.

Group A (n = 6) was ventilated with a FiO2 of 0.5. All measurements were performed as described above.

Group B (n = 6) was ventilated with addition of nitric oxide (NO) to the ventilation in a concentration of 10 ppm. NO administration was started 30 min after beginning of reventilation and reperfusion. The chosen concentration of NO is based on a previous study, where optimal dose/response relationship for inhalative NO administration in Yucatan micropig was found to be at an inspired dose of 10 ppm [13]. The inspired NO concentration was controlled independently of the concentration of the inspired oxygen (NoxBox, Dräger, Lübeck, FRG). All measurements were performed according to the presented protocol, as described previously.

2.5. Statistical analysis

Results were expressed as mean ± S.D. of the mean. For calculation of the significance the Student’s t-test was used. Statistical significance was considered to be present if a probability value less than 0.05 was obtained.

All animals received human care in compliance with the ‘European Convention on Animal Care’ and the study was approved by the institutional ethics committee.
3. Results

According to a pilot study \((n = 3)\) the arterial \(\text{pO}_2\) (artpO\(_2\)), central venous \(\text{pO}_2\) (CvpO\(_2\)) and the peribronchial tissue \(\text{pO}_2\) (PBpO\(_2\)) could be augmented to 394, 67 and 43 mmHg, respectively, at a FiO\(_2\) of 1.0 prior to transplantation, whereas, after transplantation the PBpO\(_2\) remained unchanged at 10 mmHg despite an artpO\(_2\) of 368 and a CvpO\(_2\) of 66 mmHg (Figs. 1 and 2).

There were no significant differences between groups A and B among the weights of the donor and the recipients or the total ischemic time of the allografts (22.5 ± 1.5 kg BW group A vs. 22.33 ± 1.1 kg BW group B). The total ischemic time of the donor lung was 3.89 ± 0.39 h in group A vs. 4.06 ± 0.39 h in group B.

After single lung transplantation the peribronchial tissue oxygen tension of the transplanted lung decreased significantly from the pre-transplantation baseline \(\text{pO}_2\) values of 38.31 ± 6.56 mmHg to 9.72 ± 2.55 mmHg in group A and to 10.3 ± 3.61 mmHg in group B 4 h after transplantation (mean of T1–T4; \(\pm\) S.D.) \(P < 0.0001\) (Figs. 3 and 4a,b).

The simultaneously recorded myocardial tissue oxygen tensions remained unchanged after transplantation (33.83 ± 5.32 mmHg pre-Tx vs. 32.72 ± 6.88 mmHg in group A and 34.74 ± 7.64 mmHg in group B post-Tx, mean of T1–T4; \(\pm\) S.D.) \(P > 0.05\).

The central venous \(\text{pO}_2\) as the only source of oxygen supply for the collateral flow dependent peribronchial tissue showed no significant differences between pre- and posttransplantation values and was kept in its physiological range (45.23 ± 3.41 mmHg pre-Tx vs. 41.83 ± 3.27 mmHg in group A and 43.26 ± 2.98 mmHg in group B post-Tx, mean of T1–T4; \(\pm\) S.D.).

Postoperatively, the flow in the pulmonary artery of the transplanted lung decreased significantly in both groups from the pre-Tx value of 0.94 ± 0.36 l/min to T1 after transplantation to: group A: 0.32 ± 0.1 l/min at T1, \(P < 0.001\); to group B: 0.30 ± 0.08 l/min at T1, \(P < 0.001\); (mean \(\pm\) S.D.).

The addition of NO to the ventilation in a concentration of 10 ppm in group B led to a significant increase of flow in the pulmonary artery of the transplanted lung from T1 to T4 compared to group A without NO administration. (Group B: 0.3 ± 0.08 l/min at T1 to 0.63 ± 0.9 l/min at T4 \(P < 0.001\); group A: 0.32 ± 0.1 l/min at T1 to 0.49 ± 1.1 l/min \(P = 0.001\) (Figs. 4 and 5).

No change in the flow of the right pulmonary artery was observed, no matter of NO application or not (2.15 ± 0.35 pre-Tx vs. 1.95 ± 0.41 in group A or 2.04 ± 0.46 l/min in group B; mean of T1–T4 \(\pm\) S.D.).
4. Discussion

The improved results in clinical single and double lung transplantation over the last 10 years, including the decreased incidence of airway healing problems, have justified the current practice of standard lung transplantation technique, which does not provide the routine revascularization of the bronchial arteries [14]. However, concern still persists, that the interruption of the bronchial artery circulation leads to a nonphysiologic milieu in the airways and the peribronchial tissue. With cessation of the physiological arterial blood circulation, the nutritive perfusion of the transplanted lung and, therefore, the airway supply depends solely on retrograde perfusion with desaturated central venous blood driven by the pulmonary artery [15]. Airway ischemia was addressed to be the most important pathogenetic factor in development of early and late bronchial complications and related sequelae, represented by anastomotic dehiscence, bronchus necrosis and airway stricture [8].

The initial poor results in en bloc double lung transplantation with a mortality rate between 25 and 50% [16] due to airway necrosis led to different strategies and technical modifications to improve airway healing and to prevent ischemic damage of the bronchial system.

Corticosteroids have been accused to provoke bronchial complications and to be responsible for disturbance in bronchial anastomotic healing. But experimental studies showed that this is dependent to a dose related application [5]. Additionally, it was shown that early corticosteroid administration has an protective effect, because of the attenuation of the reperfusion injury leading to early improved collateral bronchial blood flow [17].

Technical modifications as bilateral sequential lung transplantation and consequent donor bronchus shortening and bronchial omentopexy were applied to further reduce bronchial complications [5,16,18,19]. Though the mortality could be reduced by technical improvements, the incidence of bronchial related complications persisted [20,21].

In contrast to that, groups performing direct revascularization of the bronchial arteries report about excellent airway healing and a lower frequency of bronchial
complications in their series [8,9,12,22]. The follow up studies revealed, that in patients with angiographic proven restoration of the bronchial circulation, an excellent bronchial healing was achieved. Bronchoscopic examination showed healthy and viable distal mucosa.

Despite these promising results, the discussion remains, whether bronchial artery revascularization should be performed or not [23]. A number of studies appeared, trying to assess the postoperative conditions of the bronchial circulation. It was demonstrated that without bronchial revascularization a sufficient bronchial collateral circulation is reestablished after a delay of 2–4 weeks after transplantation [24]. In the immediate postoperative course the mucosal blood flow is impaired. In opposite to that, direct revascularization led to a significant improvement in graft bronchial blood flow at once [25,26]. The measurements performed in these experimental protocols relied on laser doppler velocimetry (LDV), allowing only a semiquantitative analysis of bronchial mucosal blood flow. An analysis whether the oxygen content of the delivered blood meets the tissue demands is not provided by this method. Furthermore, the LDV method is not a well accepted and valid method for blood flow measurement in the distal parts of the bronchial system [27,28].

Our intention was to determine the quality of the collateral blood flow to the bronchial circulation by assessing the resulting peribronchial tissue oxygen tensions in a transplantation model according to the current clinical standard.

To assess the tissue oxygen tension of the bronchial wall itself was impossible, because the airway wall is too thin and friable for reliable probe placement and, therefore, valid data acquisition. However, the peribronchial tissue and the related lymph nodes distal the tracheal bifurcation rely on arterial perfusion provided by the belonging bronchial artery [29]. This was confirmed by our own findings. There was only a slight decrease in tissue oxygen tension observed, after proximal dissection of the donor bronchus, assuring the distal bronchial artery depending perfusion of peribronchial lymph nodes.

For this reason and to avoid artefacts, peribronchial lymph nodes located more than 1 cm distal the tracheal bifurcation were chosen for tissue oxygen measurement. To objectify the physiological range and the predictive value of the measured peribronchial tissue oxygen tensions, in the pilot study \((n = 3)\) group the relationship between arterial oxygen tension, central venous oxygen tension and peribronchial tissue oxygen tension at constant flows was measured to obtain reference values. The peribronchial tissue oxygen tensions varied in a physiological range at different inspired oxygen fractions \((0.2–1.0)\) demonstrating the dependence to the arterial pO\(_2\) values (Figs. 1 and 2).

After standard single lung transplantation the peribronchial tissue oxygen tension decreased unanimously in all animals of both groups to a level below 10 mmHg, despite the fact that the central venous pO\(_2\), as the responsible source of oxygen delivery, remained unchanged. With the addition of inhalative NO a significant increase of pulmonary artery flow in the transplanted lung was achieved, but this had no effect on the resulting peribronchial tissue oxygen tensions. It is well known from traumatology, that constant tissue oxygen tensions below 10 mmHg in skeletal muscle are critical in regard to the viability of the tissue and its capability to recover from trauma and ischemic injury [30,31]. Whether or not this finding can be extrapolated to the situation immediately after lung transplantation remains to be seen.

The obtained tissue oxygen tension data demonstrate that at least for the first 4 h postoperatively, the peribronchial tissue remains under hypoxic conditions. The finding that in the early phase after transplantation an increased pulmonary artery flow does not result in an increase in peribronchial tissue oxygen tension probably is related to persisting disturbances of microcirculation after ischemia and reperfusion injury.

The physiological role of the bronchial circulation is not only restricted to airway healing. The capability of the lung to respond to pathologic conditions was demonstrated to depend on intact bronchial artery supply. In pulmonary infections the bronchial arteries were shown to provide the involved tissue reactions [22]. Other studies revealed, that intact bronchial artery circulation relates to a reduced tissue edema after ischemia and subsequent reperfusion [32]. These findings contribute to observations made by centres performing routine revascularization of the bronchial arteries, reporting that infectious complications are rare.

Fig. 5. Comparison of the pulmonary artery flow in the transplanted lung prior to transplantation (pre-Tx) and 1–4 h (T1–T4) after transplantation between groups A and B (10 ppm NO). (x) The significant decrease of flow in the pulmonary artery in group A from pre-Tx to T1 \((P < 0.001)\). (o) The significant decrease of flow in the pulmonary artery in group B from pre-Tx to T1 \((P < 0.001)\). (+) The significant increased flow in group B by NO administration at T4 compared to group A \((P < 0.001)\).
OB as the most important limiting factor of long term survival in lung transplantation remains a matter of controversial discussion. In the beginning of lung transplantation chronic rejection was thought to be synonymous with OB [33,34]. In recent years evidence has emerged that chronic airway ischemia or hypoxia may contribute to the development of OB [35]. Mucosal blood flow has been shown to be reduced in pulmonary rejection periods, intensifying a preexisting hypoxia or chronic ischemia [36]. Additionally the risk of developing OB is increased with repeated rejection episodes [35,37]. Whether bronchial revascularization can reduce the current incidence of OB remains unclear [22], but as reported by the Bordeaux group, in five out of 15 double lung transplanted patients, OB occurred 1 year after transplantation. In all 5 patients revascularization had failed [7]. Persisting hypoxia may be a predisposing factor in the development of infectious complications, repeated rejection episodes or chronic rejection and a potential risk factor for the development of OB [35,37].

The limitation of our study has to be seen in the indirect assessment of the peribronchial tissue oxygen tension by measuring tissue oxygen tension in lymph nodes as a substitute for bronchial or mucosal tissue. But as shown in our pilot studies, these lymph nodes depend on the bronchial circulation of the transplanted lung. A second limitation has to be seen in the shortness of the observation period. Compared to the clinical situation, it is not possible to detect, whether there are time dependent changes in the resulting peribronchial pO2 values or not. Furthermore, the current technique of lung preservation does not include the antegrade perfusion of the bronchial artery system. Therefore, the bronchial arteries and the lymphatic system of the donor lung is filled with donor blood and immunocompetent cells, probably intensifying the early disturbance in microcirculation, which cannot be ruled out by our study set-up [38].

However, the obtained tissue oxygen tension data reliably indicates, that lung transplantation performed according to the current standard technique causes a severe decrease of oxygen supply to the tissues depending on the retrograde bronchial artery flow, which can not be compensated by the augmentation of collateral blood flow, driven by the pulmonary artery for at least 4 h.

Thus, in a chronic model of single lung transplantation tissue oxygen measurements have to be repeated to answer the question whether or not severe peribronchial hypoxia persists with time.

References