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Antegrade versus retrograde perfusion of the donor lung: impact on the early reperfusion phase

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Abstract Transpulmonary thermodilution was used to evaluate the effect of flush route during harvest on hemodynamic and respiratory function of the pulmonary graft in the early post-transplant phase. Single lung transplantation was performed in two piglet groups after 24 h of cold storage. Donor organs for group A underwent antegrade perfusion, and those for group R retrograde perfusion. PaO_2, compliance (C), airway resistance (R), extravascular lung water index (EVLWI), pulmonary blood volume index (PBVI), intrathoracic blood volume index (ITBVI), capillary leak (CL), and cardiac function index (CFI) were assessed by transpulmonary thermodilution at baseline, 1, 3, and 6 h after reperfusion. EVLWI was significantly lower in group R. Compliance and PaO_2 were higher in the same group. The two groups did not differ significantly with regard to CFI, PBVI, ITBVI, and airway resistance. Retrograde perfusion of the donor lung had a positive impact on graft function during early reperfusion. Transpulmonary hemodynamic monitoring can be a powerful tool for intra- and postoperative management of transplant patients.

Keywords Organ harvest · Retrograde flush perfusion · Lung transplantation · Transpulmonary thermodilution

Introduction

Lung transplantation is an established therapeutic option for selected patients with end-stage pulmonary disease. A series of significant advances, including improved surgical technique, better procurement procedures, and state-of-the-art immunosuppression protocols, has led to better postoperative outcomes. Yet, complications can still occur, leading to significant morbidity and mortality. Among these, severe reperfusion injury can result in lung edema, pulmonary hypertension, and loss of respiratory function. Clearly, the delicate alveolar–capillary membrane network of the lung is markedly sensitive to ischemia. Our understanding of the underlying mechanisms of pulmonary tissue response to ischemia and reperfusion is currently incomplete. One critical factor mediating reperfusion injury is the interaction between white blood cells and the endothelium at the onset of reperfusion. Leukocytes migrate out of pulmonary capillaries and induce an inflammatory response through the release of an array of mediators and free radicals [1]. These events manifest themselves as increased pulmonary capillary permeability, altered vascular tone, pulmonary edema, and clinically apparent acute graft dysfunction [2]. In order to minimize the development of reperfusion injury, it is essential that procurement, storage, and reperfusion protocols be optimized.

Concern over bronchial healing remains one of the dominant issues in lung transplantation. Currently, the most common perfusion method with respect to donor lung procurement is antegrade flush perfusion into the pulmonary trunk [3]. However, there is evidence that retrograde perfusion may result in an improved distribution of preservation solution in the parenchyma and airways, both in the experimental and the clinical setting [4, 5, 6]. This was also demonstrated in experimental models using dye-labeled microspheres [7]. Nevertheless, retrograde flush perfusion has not yet been used widely, and its impact on early hemodynamic and respiratory function after the completion of graft anastomoses has so far not been elucidated [8].

Extensive hemodynamic and respiratory monitoring is mandatory in the intra- as well as early postoperative course after single or double lung transplantation. Until now, much of the information on patient hemodynamic and volume status has been inferred from filling volumes. The pulmonary artery thermodilution catheter is the clinical standard for the measurement of cardiac output and cardiac preload. However, its use carries several disadvantages and risks, including relatively time-consuming placement, the risk of endocarditis, endocardial lesions, and arrhythmia, a significantly higher rate of catheter-related infection compared to central venous and arterial lines, and the misintepretation of wedge pressure-related data [9]. Several years ago, the transpulmonary hemodynamic monitoring method based on the placement of an intraarterial, transfemoral catheter was established [10]. This method is less invasive since it does not require insertion of a Swan–Ganz pulmonary catheter. The transpulmonary thermodilution method allows continous monitoring of several parameters, including total blood volume (TBV), intrathoracic blood volume (ITBV), global end-diastolic blood volume (GEDV), and extravascular lung water (EVLW), which in itself is an indicator of capillary leak.

In this study, we compare hemodynamic and respiratory findings of the early reperfusion phase in animals that received donor lungs in connection with either antegrade or retrograde perfusion. We also demonstrate the value of transpulmonary thermodilution monitoring, a powerful tool that can guide early postoperative management in the pulmonary transplant recipient.

Materials and methods

Animals

Single lung transplantation was performed in two groups of pigs, each consisting of six animals. Donors and recipients all weighed between 26 and 33 kg, with an average weight of 28.7 ± 4.2 kg. The left lung was selected for transplantation because of its simpler anatomy. All animals received humane care in compliance with the *Principles of Laboratory Animal Care*, as formulated by the German National Society for the Advancement of Medical Research, and the *Guide for the Care and Use of Laboratory Animals*.

Anesthesia

The animals were premedicated with propofol at 0.7 mg/kg. The piglets were intubated and placed in a recumbent position for organ harvesting and a right-side recumbent position for transplantation. Ventilation was carried out by means of a SERVO 900C ventilator with a FiO_2 of 0.5, respiratory rate of 12–15 breaths per minute, maximal inspirational support pressure of 30 mmHg, and positive end-expiratory pressure (PEEP) of 5 mmHg. We maintained general anesthesia with inhalational NO_2 and isoflurane. Once deep anesthesia was reached, ventilation was performed in a controlled mandatory mode. A continous infusion of fentanyl at a rate of 12 μg/kg per hour was used for analgesia, and pancuronium was given intermittently at doses of 1 mg for muscle relaxation.

Donor operation

After introduction of a central venous line into the right jugular vein, sternotomy, thymectomy, and anterior pericardectomy were performed. The animals received heparin (300 U/kg b.w.) and methylprednisolone (250 mg).

For antegrade perfusion (group A), the pulmonary artery was doubly cannulated; one line was placed for perfusion and a second line to serve as a pressure transducer during flush perfusion. The superior and inferior vena cava and the proximal right ventricular outflow tract were ligated and the aorta cross-clamped. Immediately after that, we started perfusion and incised the inferior vena cava. PEEP was increased to 8 mmHg and occlusion of inflow was effected. We ligated the pulmonary artery proximally and initiated flush perfusion. The inferior vena cava was transected above the ligation to prevent distension of the right ventricle.

For retrograde perfusion (group R), the left atrium was opened and blood was allowed to empty under suction. We introduced a double-head balloon catheter into the left pulmonary vein and initiated flush perfusion. Discharge of the perfusate was facilitated through the proximal part of the pulmonary trunk after ligation of the proximal right ventricular outflow tract. In all cases, we used 2 l of cold Perfadex solution. Perfusion pressure was maintained at 12–18 mmHg, and during perfusion the lungs were ventilated with approximately half the normal tidal volume (approximately 300 ml). After 8 min of perfusion, the lungs were ventilated vigorously to reinflate atelectatic areas, and the trachea was clamped after the lungs were moderately inflated. We then removed the heart-lung block and dissected the left lung away from the heart, leaving sufficient arterial and bronchial cuffs as well as an atrial margin for later anastomoses. The left lung was stored in cold Perfadex at 4°C for 24 h.

Recipient operation

While in the recumbent position, the right external jugular vein and carotid artery were dissected free, and an 8-French venous line and arterial pressure line were placed. An additional three-lumen central venous line was placed in the left internal jugular vein. We performed inguinal skin incision, isolated the right femoral artery, and placed an intra-arterial catheter (PULSION, Munich, Germany); its tip was positioned 25 cm within the descending aorta. The piglet was then placed in the right lateral position and left thoracotomy through the 5th intercostal space was performed. After pericardiotomy, we placed a left atrial line for pressure and blood gas monitoring. All lines were connected to a standard monitoring unit, which in turn was connected to a computer for data collection. The PULSION transfemoral thermodilution catheter was connected to a PiCCO-monitor (PULSION, Munich, Germany). We obtained a baseline blood test from the arterial and venous lines and performed bronchoscopy with lavage. The hemiazygos vein overlying the left pulmonary veins was isolated. We isolated the left main pulmonary artery and divided the ligamentum arteriosum. A clamp was placed medially on the pulmonary trunk, encompassing the origin of the left pulmonary artery. This was ligated distally and divided. The left pulmonary veins and left atrium were dissected away from the pericardium, and a clamp was placed as far medial as possible on the left atrium. We also dissected the right main and accessory pulmonary arteries and the right mainstem bronchus free from surrounding tissue. Before explanting the left host lung, we confirmed the feasibility of clamping all structures on the right side and released the contralateral clamps. The left lung was then removed as follows: the left atrium was divided from the superior to the inferior pulmonary veins, and the left mainstem bronchus was clamped and incised just proximal to its bifurcation into lobar bronchi. We anastomosed the atrium, bronchus, and artery in that order using running monofilament sutures (5–0 prolene for artery and atrium, 4–0 prolene for the bronchus). The arterial anastomosis was done end-to-side to the pulmonary artery. Ventilation of the transplanted left lung was begun and the graft was further inflated by increasing PEEP for a few minutes. The clamps were released after meticulous de-airing. After 15 min of reperfusion, we clamped the right pulmonary arteries and the right main bronchus while performing hemodynamic monitoring. We administered an epinephrine infusion (up to 1.5 mg/h) titrated to maintain a mean arterial pressure above 50 mmHg. After a hemodynamic steady state was achieved, we obtained blood gases and registered the hemodynamic parameters. Monitoring was continued for a minimum of 6 h.

Hemodynamic and respiratory monitoring: transpulmonary thermodilution measurements

For arterial thermal dilution, a single central venous line was needed for the injection of a cold bolus. Cardiac output, mean transit time, and exponential downslope time were calculated automatically from the thermodilution curve by the PULSION device. After injection, the thermal bolus spreads in the intrathoracic intravascular blood volume (ITBV) and extravascular lung water (EVLW) space. The ITBV is composed of the end-diastolic volumes of the right atrium and right ventricle, pulmonary blood volume (PBV), and the end-diastolic volumes of the left atrium and left ventricle. The global end-diastolic volume corresponds to the global cardiac preload. It is the theoretical sum of all end-diastolic partial volumes of the heart, which do not occur simultaneously. The capillary leak (CL) is defined as EVLW / PBV and normally does not exceed 3%. By the same computed algorithms, we obtained the cardiac function index (CFI), defined as cardiac output / global end-diastolic volume. This represents a preload-independent index of cardiac function. The suffix "I", as in EVLWI, stands for the index of the arithmetic value divided by body weight in kilograms. The following parameters were recorded for this study at baseline, and at 1, 3, and 6 h after the start of reperfusion: EVLW(I), CFI, PBVI, CL, PaO_2, compliance (C), and airway resistance (R). The experiments were terminated after 6 h of reperfusion and the animals killed.

Statistics

Descriptive analysis with mean and standard deviation was performed for the above parameters. The Students' t-test for unpaired samples was used for comparison of the antegrade and retrograde perfusion groups. For bivariate analysis, a linear correlation was calculated. All data were expressed in units of the International System (SI), and significance was assumed at P values of less than 0.05. All statistical tests were performed using the Statistical Package for the Social Sciences (SPSS), version 8.01.

Results

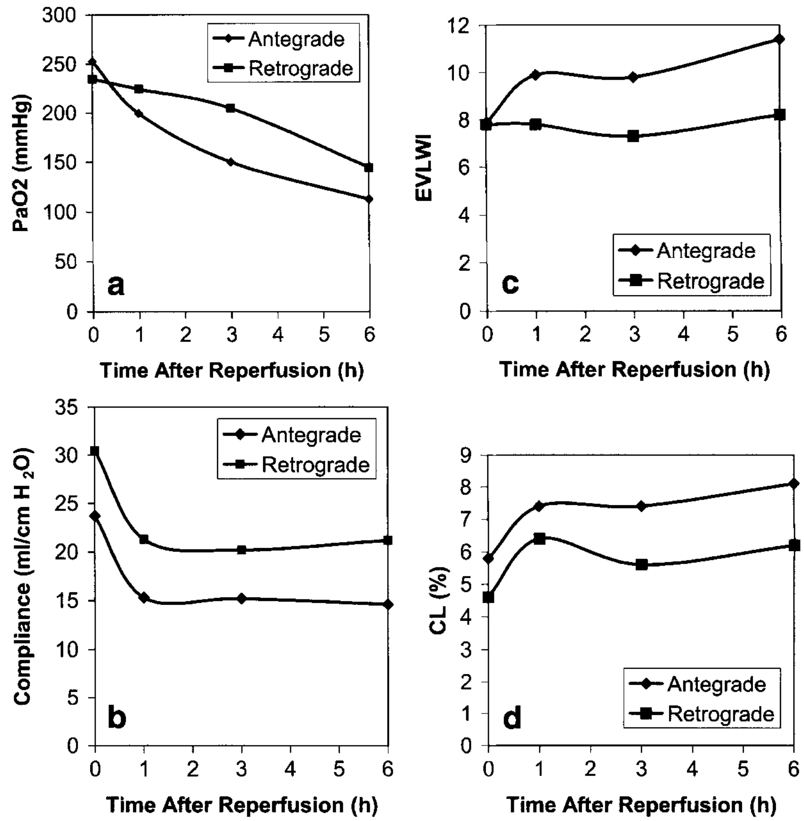
Single lung transplantation after 24 h of cold storage was successfully performed in all animals. The animals tolerated clamping of the contralateral native pulmonary arteries and bronchus. The left lung graft was observed throughout the experiment and was noted to have become progressively more edematous. Toward the end of the experimental session, edematous fluid had to be removed periodically from the endotracheal tube.

Over time PaO_2 deteriorated in both groups (Table 1, Fig. 1a). This process was more gradual in the group with retrograde perfusion ($P = 0.038$). There was a significant fall in pulmonary compliance between the baseline and the first hour of reperfusion, but steady-state compliance was reached in both groups by 3 h of reperfusion (Fig. 1b). Compliance in the group with retrograde perfusion was significantly higher at all time points ($P = 0.031$), including during the initiation of reperfusion. By contrast, airway resistance of the transplanted lung did not differ among the two groups. Again, a marked increase was observed within the first hour of reperfusion, after which a plateau was reached (Table 1). The cardiac function index (CFI) was not significantly different between the two groups. It remained stable during the first half of the experiment

Table 1 Hemodynamic and respiratory parameters recorded at the baseline and at 1, 3, and 6 h after clamping the opposite, non-transplanted hilus (*A* group with antegrade perfusion ($n = 6$), *R* group with retrograde perfusion ($n = 6$), *EVLWI* extravascular lung water index, *PBVI* pulmonary blood volume index, *ITBVI* intrathoracic blood volume index, *CL* capillary leak, *CFI* cardiac function index)

Parameters	Baseline (A)	Baseline (R)	1 h (A)	1 h (R)	3 h (A)	3 h (R)	6 h (A)	6 h (R)
PaO_2 (mmHg)	252.7 ± 35.4	234.8 ± 38.5	199.7 ± 50.7	224.3 ± 42.8	150.0 ± 93.6	204.8 ± 59.0	112.3 ± 45.8	144.2 ± 44.6
Compliance (ml/cm H_2O)	23.7 ± 6.5	30.5 ± 5.7	15.3 ± 4.8	21.3 ± 3.0	15.2 ± 4.7	20.2 ± 3.1	14.6 ± 5.4	21.2 ± 4.1
Airway resistance (cm H_2O×s/l)	16.2 ± 4.5	16.2 ± 1.9	25.7 ± 4.5	24.5 ± 5.0	24.2 ± 5.7	20.3 ± 1.7	28.1 ± 6.3	26.8 ± 3.4
EVLWI	7.9 ± 1.6	7.8 ± 1.1	9.9 ± 3.8	7.8 ± 1.7	9.8 ± 1.9	7.3 ± 2.1	11.4 ± 2.7	8.2 ± 2.4
PBVI	139.5 ± 35.6	169.8 ± 20.2	132.3 ± 26.6	131.2 ± 41.8	133.0 ± 11.6	134.5 ± 39.8	144.2 ± 44.6	130.7 ± 34.4
ITBVI	586.8 ± 101.3	702.0 ± 41.5	529.0 ± 101.1	582.2 ± 127.6	653.8 ± 107.8	602.5 ± 110.6	688.8 ● 134.1	696.3 ± 108.5
CL (%)	5.8 ± 1.0	4.6 ± 1.0	7.4 ± 2.4	6.4 ± 2.3	7.4 ± 3.0	5.6 ± 1.8	8.1 ± 3.4	6.2 ± 1.9
CFI	7.4 ± 1.1	7.3 ± 0.8	7.7 ± 1.5	7.6 ± 1.9	6.3 ± 1.4	6.8 ± 1.7	6.0 ± 2.1	6.4 ± 2.2

Fig. 1 Significant differences between groups with antegrade vs retrograde flush perfusion. (**a**) The decrease in PaO_2 was greater in the group with antegrade perfusion ($P=0.038$), (**b**) compliance was higher in the group with retrograde perfusion ($P=0.031$), (**c**) the extravascular lung water index (*EVLWI*) was significantly lower in the group with retrograde perfusion after 1 h of reperfusion ($P=0.028$), (**d**) the capillary leak (*CL*) was significantly lower throughout the entire experiment in the group with retrograde perfusion ($P=0.047$)



(>7) and decreased progressively thereafter. The EVLWI was initially similar in the two groups, but showed a trend toward higher values after 1 h of reperfusion in the group with antegrade perfusion ($P=0.028$, Fig. 1c). There was no difference in ITBVI or PBVI between the two groups. The capillary leak (CL) was significantly lower throughout the entire experiment in the group with retrograde perfusion ($P=0.047$, Table 1, Fig. 1d).

To validate the accuracy of the transpulmonary thermodilution method, we compared the measurements obtained by this method with conventionally obtained values, i.e., by Swan–Ganz monitoring. The capillary leak correlated reciprocally with PaO_2 in the same animals. This suggests that PaO_2 decreases with increasing CL ($P=0.01$). Similarly, pulmonary airway resistance increased with increasing EVLWI ($P=0.037$). Finally, we compared the calculated CFI with the cardiac index derived intrapulmonarily (measured with a Swan-Ganz catheter) and found that both measures responded equally to various hemodynamic fluctuations.

Discussion

Our study demonstrates that retrograde flush perfusion of the donor lung leads to better respiratory function in the early postoperative phase. The hypothesis that retrograde perfusion might have a positive effect on respiratory function immediately after transplantation is supported by a series of organ procurement studies in piglets using modified dye-labeled microspheres [7]. In these studies, the distribution pattern of hyperosmolar lung preservation solutions could be assessed; importantly, one could determine whether the solution reaches areas in the peripheral bronchial tree that are normally perfused by the vasa privata, i.e., the bronchial arteries of the lung. It has been suggested that with antegrade perfusion, this microvascular target volume might be "neglected". As a consequence, the quality of bronchial protection during lung procurement may be inadequate and ultimately result in impaired bronchial healing [11]. Another explanation for the superiority of the retrograde flush route may be related to underlying pressure differences in the flush routes [12, 13]. For example, with antegrade delivery the solution moves toward the arterial microvascular bed, which is characterized by high overall vascular resistance [14]. By contrast, with retrograde delivery the solution enters the more distensible venous compartment, and greater vascular recruitment may result.

Our animal model is sensitive enough to detect the physiological sequelae of the differences in flush direction, and it may serve as a powerful tool for documenting the time course of the resulting respiratory impairment. Of significance to this model, we have chosen prolonged storage times of 24 h and have clamped the contralateral native lung, allowing only the transplanted organ to function. Evidence of less EVLW, less CL, and better compliance in the organs with retrograde perfusion confirms the superiority of the retrograde route. A higher PaO_2 in the same group also validates our hypothesis. The initial difference of compliance between the two groups may have been due to the combination of adequate perfusion and airway elasticity (tone) in the group with retrograde perfusion. It seems unlikely that variations in cardiac function between the groups can explain this finding since the arterial, transpulmonary measurements (CFI) did not differ among the two groups. At the same time, both groups did experience progressive deterioration in cardiac function over time, particularly at the onset of reperfusion. This is likely a consequence of impairment of right ventricular function due to emerging pulmonary hypertension.

The transpulmonary thermal dilution method for hemodynamic monitoring greatly facilitates intraoperative and postoperative management. From the viewpoint of both safety and efficacy, there are significant benefits [15]. It is less invasive compared to the traditional Swan-Ganz method. It also avoids the use of intrapulmonary catheters, which is particularly advantageous in children. The collected data may facilitate early diagnosis of emerging respiratory dysfunction. For example, an increase in capillary leak measurements will instantly direct therapeutical measures. A rapid increase in EVLW, which can almost be demonstrated beat-to-beat, will call for immediate volume management [9, 15]. The physiological effect of medication commonly used during and after transplantation, including corticosteroids and other vasoactive substances or catecholamines, can be determined and assessed. Chest X-ray can be used less frequently for diagnosis and for the evaluation of pulmonary edema or pleural effusions. The intra-arterial catheter does not obscure the operative field during transplantation and can be left in place for longer than 10 days. It functions as a dual-purpose line, allowing pressure monitoring and withdrawal of blood gas samples. Clearly, the major advantage of this method lies in the ability to collect data on many parameters, to monitor continuously, and to easily store the data. The use of this monitoring device may lead to the development of standard algorithms for patient management during and after transplantation.

In conclusion, we have developed an experimental model that may be useful in further studies of procurement, storage, and operative aspects of transplantation research. We have demonstrated that the hypothesis that retrograde flush perfusion of donor organs will yield benefits in the early postoperative phase is sound. Moreover, the employment of transpulmonary monitoring provides more detailed data on hemodynamic and respiratory function and may help direct management during and after pulmonary transplantation.

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