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ORIGINAL ARTICLE

Normothermic *ex vivo* lung perfusion of non-heart-beating donor lungs in pigs: from pretransplant function analysis towards a 6-h machine preservation

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Summary

Donor shortage urges optimal use of all lungs available. *Ex vivo* lung perfusion (EVLP) is a method to evaluate lung function before implantation. EVLP was performed in pigs to evaluate lung function, using two different clinical non-heart-beating (NHS) donor protocols: flush perfusion and topical cooling after 1-h warm ischaemia ($n = 5$ each). Secondly, we investigated whether EVLP can be used for 6 h *ex vivo* machine preservation ($n = 4$). In comparison with topical cooling, flush perfusion preserved lung function better during EVLP. During 6 h normothermic EVLP, gas exchange remained stable; however, the pulmonary artery pressure and ventilation pressure showed a significant increase. EVLP is a reliable method for evaluation of lung graft function. Flush perfusion with Perfadex is preferred above topical cooling in NHB lung donation. Six-hour normothermic EVLP is feasible but should be further improved to make *ex vivo* machine preservation or treatment of lung grafts successful.

Introduction

The continuing donor shortage makes it necessary to expand the donor pool. Nowadays, many lung transplant centres use already so-called marginal donors, with extended criteria for age, smoking, contusion and infiltrate. Also, the concept of non-heart-beating lung donation (NHBLD) has been clinically re-introduced by Steen *et al.* [1]. Since their successful transplantation of a lung from a non-heart-beating (NHB) donor after failed cardiac resuscitation, several groups have reported successfully transplanted lungs from NHB donors [2–4]. Steen used a method of *in situ* topical cooling, after an *in situ* warm ischaemia period, in the setting of failed resuscitation NHBLD. In this protocol, he performed preimplant function testing of the NHB lungs on an *ex vivo* perfusion circuit and showed excellent results [5]. The others used immediate flush perfusion preservation in the setting of controlled NHBLD. With the use of normothermic *ex vivo* lung perfusion (EVLP), preimplant identification of poorly preserved lungs can be made. Furthermore, when the EVLP can be extended, *ex vivo* machine preservation or treatment of poorly functioning lungs might be possible. In this study, we investigated in pigs, the reliability and sensitivity of EVLP to evaluate lung function after preservation. Firstly, the two successfully used clinical NHB-donor protocols were evaluated in the setting of NHBLD after ventilator switch-off: antegrade and retrograde Perfadex flush followed by EVLP and *in situ* topical cooling preceded by a 1-h *in situ* warm ischaemia period. Secondly, we investigated whether prolonged normothermic *ex vivo* perfusion of lung grafts is feasible to make new preservation and treatment strategies possible.

Materials and methods

Female pigs in the range of 48–72 kg were used. All animals received human care in compliance with the Dutch regulations and law. After anaesthesia, the pigs were intubated in prone position and paralysed by pancuronium bromide. Ventilation was set at 150–160 ml/kgBW/min, 12 breaths/min, PEEP of 5 cmH_2O, using 100% oxygen. About 1.5 l of blood was taken by bleeding the carotid artery before the NHB procedure for later usage in the *ex vivo* perfusion circuit. One thousand international units (IU) of heparin was added the blood. To stabilize blood pressure of the pigs, a half litre of Haes 6% (Kabi Frensenius, Emmercompascum, the Netherlands) was given intravenously with concomitant placement in Trendelenburg position.

The NHB donation procedure consisted out of disconnecting the tracheal tube, causing apnoea and, subsequently, cardiac arrest. Cardiac arrest was defined as an equalled low systolic and diastolic blood pressure. The collected donor blood was run through a Cell Saver (Cat II®; Frensenius Hemocare, Emmercompascum, the Netherlands) to eliminate leucocytes and platelets and mixed with the experimental Steen solution® to a Hct of 15%. This mixture was used as *ex vivo* perfusion fluid to test the lung function. The *ex vivo* perfusion circuit was composed of a heparin-coated tubing (Carmeda®; Medtronic, Eindhoven, the Netherlands), a centrifugal pump (Biomedicus®; Medtronic, Eindhoven, the Netherlands), an oxygenator (Carmeda Affinity®; Medtronic, Eindhoven, the Netherlands), a heat exchanger and a leucocyte filter (LG6®; Pall, Zaventern, Belgium), all designed to minimize blood cell activation. No heparin was used. The heart–lung block was placed in a transparent box (Vitrolife; Göteborg, Sweden).

Experimental groups

Procurement with topical cooling (Topical group; n = 5)
After a 10-min no-touch period, the lungs were re-inflated a few times with 50% oxygen. After 1-h warm ischaemia, the pleural cavity was opened and filled with cold saline and Haes 6% to cool the collapsed lungs. Also, the abdomen was opened and cooled with ice. Every 15 min, the cooling fluid was renewed. At a lobar bronchial temperature of 10 °C, the lungs were inflated with 50% oxygen, the trachea clamped and the heart and lungs en-block removed. The left and right ventricle were incised to check the pulmonary artery and left atrium for clots. The heart–lung block was weighed and further cooled on melting ice.

Procurement with flush perfusion (Flush group; n = 5)
After a 10-min no-touch period sternotomy was performed, the pleurae were opened and the chest filled with cold saline and Haes 6%. The pulmonary artery (PA) was canulated, the left atrium was opened and both cavas clamped. Fifty millilitre per kilogram Perfadex® (2.8 l bag with 0.0017 mmol $CaCl_2$, 56 μg prostacycline and 0.0028 mmol Tham added) was flushed antegrade via the PA and a few hundred millilitre retrograde via the left auricle until the fluid running out of the PA was clear. The lungs remained inflated with 50% oxygen by clamping the tube. After flushing, the heart–lung block was treated in an identical way as in the Topical group. Both the Topical and the Flush group were reperfused after 5 h of cold storage with the lungs in an inflated state.

Ex vivo long-term machine perfusion (long-term group; n = 4)
The same harvesting technique of the heart–lung block was used as in the flush procurement group. After dissection, the heart–lung block was weighed and stored on melting ice. After a short 1 h cold storage period, the lungs were prepared for 6-h EVLP.

Ex vivo lung perfusion (EVLP)

Ex vivo lung perfusion for lung function analysis of the first two groups was performed as described by Steen *et al.* [5]. In short, the PA and the left atrium (LA) were canulated via the right and left ventricle, respectively. Pressure catheters were placed in the PA and LA. The trachea was intubated. The perfusion started at 20 °C after the first 150 ml was flushed out of the LA. The perfusion was run pressure controlled with a maximum mean PA pressure (mean PAP) of 20 mmHg. A flow of 4 l/min was considered full flow and was tried to reach within the mean PAP limit. Ventilation was started with small tidal volumes at 35 °C. In about half an hour, the perfusion fluid temperature was warmed to 37 °C. The gas mixture over the oxygenator was changed from O_2 into a N_2/CO_2 mixture to de-saturate the perfusion fluid aiming at an end-tidal CO_2 between 3.5 and 4.5 kPa during ventilation. The gas flow: perfusion flow ratio was 1:1. Ventilation settings were fixed after about 1.5 h of EVLP: 5 cmH_2O PEEP, 12 breaths/min, 140 ml/kgBW/min at 37 °C. The perfusion flow was set at full flow of 4 l/min. Lung function was measured at an FiO_2 of 0.5 and 1 and at a perfusion flow of 4 l/min (or lower when not reached within the mean PAP limit) after about 2 h of EVLP. Lung function measurements were: blood gasses from PA and LA, mean left atrial pressure (mean LAP), and mean PAP, maximum ventilation pressure (MPV) and end-tidal CO_2. With these measurements, we calculated the A-a DO_2 gradient [6] and the pulmonary vascular resistance (PVR) in Woods units and PVR × body surface area (BSA) in Woods units × m^2. BSA was estimated: BSA (m^2) = 0.112 × body weight (in kg)$^{2/3}$. Lung oedema in the

trachea was scored after removal of the ventilation tube using four categories (0–3), absent, some, evident or massive. Also, the heart–lung block was weighed before and after EVLP.

In the long-term EVLP group, some additional medications were given and measures taken to optimize the system. To keep perfusion pressures as low as possible, continuous prostacycline infusion was added at a rate 20 μg/h. To correct the pH and prevent high sodium levels, continuous infusion of trometamol buffer was given, based on the measured base excess. Between the lung function measurements, MVP was kept below 15 cmH_2O at a PEEP level of 3 cmH_2O to prevent baro trauma and the perfusion flow was reduced until a mean PAP of 15 mmHg was reached to prevent development of lung oedema. Every 10 min, a few sighs with 8 cmH_2O was applied to prevent atelectasis. Finally, to prevent desiccation and heath loss, the lungs were wrapped in a double layered plastic shield within the evaluation box. All measurements were performed in the same way as in the other two groups. Lung function was measured after 2, 3, 4, 5 and 6 h of EVLP.

Statistical analysis

For comparison of oedema scores, a Mann–Whitney *U*-test for unpaired nonparametric values was performed. For comparison of the other data, a student's *t*-test was performed. A difference with $P < 0.05$ was considered statistically significant.

Results

In all pigs, the heart stopped beating between 7 and 23 min after the ventilator was switched off (Table 1a).

In the Topical group, a tracheal temperature of 10 °C was reached after 1-h of *in situ* cooling. In both groups, no clots had to be removed or were flushed from the pulmonary artery. During EVLP, two lungs of the Topical group did not reach the 4 l/min maximum flow (Table 1b). Compared with the control donor value (baseline values), a large increase in A-a DO_2 gradient was observed in three animals of the Topical group. Substantial lung oedema was seen in all topically cooled lungs in parallel with an increased weight of the heart–lung blocks after EVLP (101 ± 79.2 g) (Table 1b).

In the Flush group, the mean warm ischaemia time was 23 ± 2.7 min (Table 1a). In the Flush group, in contrast to the Topical group, all lungs reached 4 l/min flow during EVLP (Table 1b). However, the calculated PVR in the Flush group was not significant lower than the resistance in the Topical group. The A-a DO_2 gradient in donor controls minus the A-a DO_2 gradient during EVLP, as a measure for impairment of lung function, was equal in the Topical and Flush group when all the lungs were compared (Fig. 1). Not reaching a significant difference was caused by one lung out of the five in the Flush group who showed a substantial increase instead of decrease in A-a DO_2 gradient during EVLP. The measured A-a DO_2 gradient during EVLP was significantly lower in the Flush group compared with the Topical

Table 1. (a) Donor control data (b) lung function after 2-h *ex vivo* lung perfusion (EVLP).

	Topical group ($n = 5$)	Flush group ($n = 5$)
(a)		
Body weight (kg)	63.4 ± 7.7	60.4 ± 10.5
Time switch-off-cardiac arrest (min)	12.2 ± 5.6	10.3 ± 2.6
A-aDO_2, 100% O_2 (kPa)	24.7 ± 6.1	24.7 ± 6.4
MVP (cmH_2O)	18.4 ± 1.3	18.2 ± 1.1
In situ warm ischaemia (min)	60.0 ± 0.4	23.0 ± 2.7
(b)		
A-aDO_2, 100% O_2(kPa)	33.0 ± 4.7	26.1 ± 3.4**
A-aDO_2, 50% O_2 (kPa)	12.3 ± 5.6	3.1 ± 1.2*
PVR (Woods units)†	3.3 ± 1.3	2.8 ± 0.7
PVR × BSA (Woods units × m^2)†	6.0 ± 2.5	4.8 ± 1.3
MVP (cmH_2O)	24.4 ± 2.9	17.8 ± 1.8**
Lung oedema score (range: 0–3)	2.4 (2–3)	0.6 (0–1)*
Weight change (g)	101 ± 79.2	−3.5 ± 64.5

Values are mean ± SD. A-aDO_2, alveolar-arterial oxygen gradient; MVP, maximum ventilation pressure.
In the Topical group, lungs were topically cooled *in situ* after1-h *in situ* warm ischaemia. In the Flush group, the lungs were flushed after preparation and canulation. Values are mean ± SD except for lung oedema score. PVR, pulmonary vascular resistance; BSA, body surface area; *$P < 0.002$, Flush versus Topical; **$P < 0.05$, Flush versus Topical. †Two lungs in the Topical group did not reach maximal perfusion flow of 4 l/min.

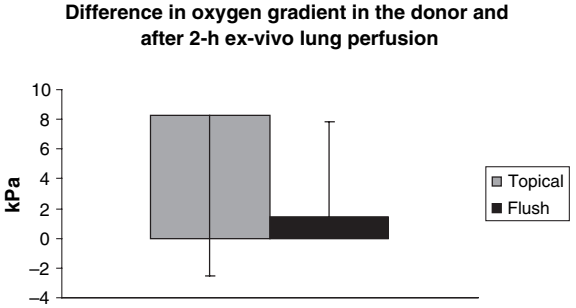

Figure 1 The A-a DO_2 gradient difference (donor A-a DO_2 gradient minus 2-h EVLP A-a DO_2 gradient) was not significantly greater in the Topical cooling group ($n = 5$) compared to the Flush perfusion group ($n = 5$) ($P = 0.08$). However, only one lung in the Flush perfusion group showed a large increase in oxygen gradient during EVLP as compared to its exceptionally low control gradient in the donor. Excluding this donor in the Flush perfusion group, a significant difference in oxygen gradient difference exists ($P < 0.05$) between the groups.

Table 2. Lung function during extended 6-h EVLP ($n = 4$).

	Donor	2-h	3-h	4-h	5-h	6-h
A-aDO_2, 50% O_2 (kPa)	6.6 ± 2.9	8.4 ± 3.3	7.1 ± 2.3	7.2 ± 1.6	7.9 ± 2.1	7.6 ± 3.1
PVR (Woods units)	–	3.8 ± 0.4	5.2 ± 2.3	4.3 ± 1.8	4.4 ± 1	4.6 ± 0.3*
PVR × BSA (Woods units × m^2)	–	6.2 ± 0.6	8.3 ± 3.8	6.9 ± 2.8	7.0 ± 1.5	7.4 ± 0.6*
MVP (cmH_2O)	18.1 ± 1.1	18 ± 3.7	18 ± 3.7	18.5 ± 5.1	18.3 ± 5.4	20.6 ± 5.0*†
Lung oedema score (0–3)	–	–	–	–	–	0.5 (0-1)
Weight change (g)	–	–	–	–	–	1.7 ± 66.8

Values are mean ± SD except for lung oedema score. A-aDO_2, alveolar-arterial oxygen gradient; PVR, pulmonary vascular resistance; MVP, maximum ventilation pressure.
*$P < 0.05$, 6-h versus 2-h. †$P < 0.05$, 6-h versus donor.

group (Table 1b). Also, the MVP and lung oedema scores were lower in the Flush group than in the Topical group (Table 1b). Finally, three heart–lung blocks in the Flush group lost weight during *ex vivo* perfusion, whereas in the Topical group all heart–lung blocks gained weight (Table 1b).

In the long-term EVLP group, all lungs were perfused for 6 h. The A-a DO_2 gradient during perfusion remained stable and low but was significantly higher at all time points when compared with the donor baseline value. Both the PVR and the MPV increased gradually to significant higher values at 6-h when compared with 1–2-h EVLP. Lung oedema scores remained low whereas two lungs increased in weight and two lost weight after 6-h EVLP (Table 2).

Discussion

Ex vivo lung perfusion using the 'Steen solution' mixed with leucocyte depleted blood in a heparin-coated circuit is well tolerated and proved to be a sensitive tool for evaluation of pretransplant lung function assessment. The method discriminated well between two clinically used procurement protocols in NHB-lung donation. In individual cases, especially A-a DO_2 gradient at 50% oxygen, perfusion flow (e.g. vascular resistance), MVP and degree of lung oedema indicated optimal and sub-optimal procurement. In addition, 6 h EVLP, using a preservative perfusion and ventilation strategy, is feasible although impairment of lung function does occur after 6-h with the used set-up.

Lung function after ventilator switch off was somewhat impaired in the 1-h warm ischaemia and subsequent topical cooling group. This is in sharp contrast with the excellent function described by others who used 1-h *in situ* warm ischaemia and topical cooling in NHB donor pigs [5,7]. The main difference between their studies and ours is that they use ventricular fibrillation as induction of cardiac death instead of ventilator switch-off. After the ventilator was disconnected, we always observed a period of central hypertension preceding cardiac arrest. This hypertension might have caused endothelial damage by mechanical stretching with release of pro-inflammatory cytokines as seen after brain death [8,9]. We hypothesize that lung grafts obtained after induction of ventricular fibrillation are less injured when compared with lung grafts obtained after ventilator switch-off donation. In the present study, the 1-h warm ischaemia in a situation with endothelial damage and release of pro-inflammatory cytokines in the topical cooled group may be the cause of the lesser lung function during EVLP in comparison to flush perfusion.

The lungs with ante- and retrograde flush Perfadex perfusion and a short warm ischaemia time showed almost no lung function impairment during EVLP for 2 h. In these lungs, the A-a-DO_2 remained at the baseline level in the donor, the perfusion flow of 4 l/min was reached easily, the MVP did not increase, and no or a minimal physical amount of pulmonary oedema developed after 2-h perfusion. When compared with the results of flushed lungs from heart beating pigs without warm ischaemia as tested by Rega using a similar *ex vivo* perfusion circuit, the present results are almost identical indicating minimal damage [7].

Of course, lung function during EVLP will differ from the function during reperfusion after reimplantation. In our EVLP set-up, leucocytes were filtered out, a preservative perfusion fluid was used, the haematocrit lowered, perfusion flow was limited to 4 l/min and the re-warming of the lung was controlled. All these measures are thought to minimize reperfusion damage. However, the strength of our set-up is that differences in lung function solely depend on the difference in preservation method. Therefore, we believe that measured differences in lung function are significant and have clinical value. The clinically well functioning lung grafts after ventilator switch-off lung donation by Love [2] and by J. Dark (pers. comm.; Freeman Hospital, Newcastle-upon-Tyne) support our finding that flush perfusion preservation is reliable and safe. Recently our Lung Transplant Group in Groningen

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performed also six successful clinical NHB lung procedures with the flush perfusion method as described in this article [10].

In the clinical situation, allowing warm ischaemia and applying *in situ* topical cooling are of a great logistic advantage. The thoracic surgeon can wait till cardiac arrest occurs (which may last for 2 h) and can decide after the switch-off whether the lungs will be procured. The transplant co-ordinator can organize the topical cooling. Therefore, strategies of combined *in situ* topical cooling with subsequent flush perfusion are worthwhile to be investigated. Based on the lung function before switch-off or because of haemodynamic instability after swich-off, EVLP might be necessary. In our clinical series, we transplanted lungs after a warm ischaemia time of 43 min without EVLP [10]. EVLP facilitates the use of NHB-lungs after failed resuscitation [1] or lungs with a marginal function [11].

Long-term EVLP showed stable A-a DO_2 gradients. However, the increase in vascular resistance and MVP after 6-h of EVLP indicates injury; therefore, the set-up should be optimized further. In our set-up, we could not correct the dilution by trometamol buffer which lowered the haematocrit to an average of 10.5 (at 6 h of EVLP) with additional blood. Furthermore, we did not add any additional glucose to the perfusion solution. Experience of others is that glucose is consumed fast during normotherm lung perfusion and should be added continuously to prevent oedema.

This study shows that EVLP is a reliable and sensitive method for evaluation of lung graft function before implantation. Based on the preserved lung function after combined antegrade and retrograde flush perfusion during EVLP in this study, we use this method clinically after switch-off ventilator NHB lung donation. Long-term EVLP is feasible but should be further improved to make machine preservation or *ex vivo* treatment of lung grafts successful.

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