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ORIGINAL ARTICLE

Surfactant alterations following donation after cardiac death donor lungs

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Summary

The use of lungs from donation after cardiac death (DCD) donors is one of the strategies to increase the donor pool. The aim of this study was to assess the surfactant alterations in DCD donor lungs. Pigs were sacrificed and left untouched for 1 (DCD1), 2 (DCD2) and 3 (DCD3) h. Lungs were then topically cooled with saline for 1, 2 or 3 h to reach a total ischemic time of 4 h. Heart-beating donors (HBD) served as control group. Bronchoalveolar lavage (BAL) samples were assessed for protein levels and surfactant function. Left lungs were prepared for *ex-vivo* evaluation. Pulmonary vascular resistance (PVR), oxygenation, airway pressure (AWP) and wet-to-dry weight ratio were significantly different between HBD and DCD3 groups ($P < 0.05$). BAL protein levels were statistically higher in DCD3 compared with HBD group ($P < 0.05$). Surface tension and surface tension measured at minimal bubble diameter (adsorption) were lower in HBD compared with DCD groups ($P < 0.05$). Adsorption was also lower in DCD1 compared with DCD2 ($P < 0.05$). Adsorption and surface tension were correlated with oxygenation and AWP ($P < 0.05$). This study has shown that lung function deteriorates with increasing warm ischemic time intervals. BAL protein, surface tension, adsorption, peak AWP and PVR increase significantly after 2 h of warm ischemia together with a significant reduction of the ratio PaO_2/FiO_2.

Introduction

One of the major obstacles to clinical lung transplantation is the scarcity of suitable graft. The use of lungs from donation after cardiac death (DCD) donors is one of the strategies to increase the donor pool. However, only a small number of lung transplantations have been performed from DCD donors. Recently several centers have reported their experience with category I, II, and III DCD [1–7].

Pulmonary surfactant, found at the air–liquid interface of the lung is a composition of phospholipids (90%) and four specific surfactant proteins. Surfactant is synthesized within type II cells of the alveoli where it is stored as lamellar bodies and secreted into the airspace via exocytosis. Surfactant lies as a monolayer at the air–liquid interface and reduces the surface tension in the lung. As a result of this effect, breathing in physiological transpulmonary pressures is possible and alveolar collapse is prevented [8,9].

The aim of this study was to demonstrate the surfactant alterations in uncontrolled DCD I and II lung donors that were subjected to different warm ischemic times. We used *ex vivo* lung perfusion to evaluate the functional status of lung grafts [10].

Methods

Experimental design

Domestic pigs (25–30 kg) were sacrificed and randomly assigned into one of the four study groups ($n = 5$/group). In the control heart-beating donor (HBD) group, the lungs were flushed, explanted, and stored in Perfadex® (Vitrolife, Göteburg, Sweden) for 4 h at 4 °C. In the other three study groups, lungs were topically cooled in the closed chest. Warm ischemic time was 1 (DCD1), 2 (DCD2) and 3 (DCD3) h followed by 3, 2, and 1 h of topical cooling (4 °C), respectively. Bronchoalveolar lavage (BAL) samples were taken from right lungs. Left lungs were prepared for *ex-vivo* evaluation. Warm ischemia is defined as the time interval between cardiac arrest and topical cooling.

Animal preparation

All animals received humane care in accordance with the 'Guide for the Care and Use of Laboratory Animals' (National Institutes of Health publication 85–23, revised 1985). The study protocol was approved by the local animal study committee.

Animals were premedicated with an intramuscular injection of 20 mg/kg Ketamin (Ketasol, 1 ml = 100 mg), 1 mg/kg Azaperon (Stresnil 1 ml = 40 mg) and Atropin-sulfate 0.1% (1 ml = 1 mg) 0.25 ml/10 kg. The animals were intubated and ventilated with an inspired oxygen fraction (FiO_2) of 0.5, tidal volume of 10 ml/kg, and a frequency of 20 breaths/min, a positive end-expiratory pressure (PEEP) of 5 cmH_2O. Anesthesia was maintained with 0.6–1% isoflurane.

Preservation of HBD

After sternotomy the pericardium was opened. Following heparinization (300 IU/kg) main pulmonary artery was cannulated. The superior and inferior caval veins were ligated, the left atrial appendage was incised and the lungs were flushed with 50 ml/kg cold (4 °C) Perfadex. Immediately prior to arrest, blood withdrawal is commenced via the inferior vena cava.

Preservation of the DCD lungs

The pigs were sacrificed by intravenous injection of 20 ml potassium chloride without heparinization and left untouched 1, 2 or 3 h at room temperature (21 °C). Via two chest drains, cold (4 °C) saline solution was infused and continuously circulated with a roller pump from a reservoir. The topical cooling is continued for 1, 2 or 3 h to obtain a total *in situ* ischemic time of 4 h.

Preparation of the heart–lung block

The right lung was resected and left lung was prepared for evaluation. The pulmonary artery trunk was cannulated through the right ventricular outflow tract after removal of all clots. Following removal of all clots the left atrium was cannulated through the apex of the left ventricle. Then 250–300 ml of Perfadex was perfused retrograde through the left atrium.

Preparation of the perfusate

Autologous blood was drawn from the donor animal after confirmation of death via the catheter in the left external jugular vein and collected in an empty sterile bag containing 2 ml of heparin (5000 IU/ml). Leukocytes were removed using a leukocyte filter and the blood was centrifuged using a Cell Saver (Electa, Dideco, Italy). The red blood cell concentrate was then diluted with Perfadex to a hemotocrit of approximately 25%. The perfusate also included glucose (2 ml, 50%), heparin (2 ml, 5000 IU/ml), sodium bicarbonate (0.8 mol/l) and albumin (4–5 g/dl).

Isolated perfusion circuit

The perfusion circuit comprised a bypass roller-pump, a cardiopulmonary bypass membrane oxygenator supplied with 91% nitrogen and 9% carbon dioxide gas serving as a deoxygenator, a venous reservoir, a heat exchanger and polyethylene tubing.

Pulmonary artery flow, pulmonary artery pressure (PAP) and temperature of the perfusate were recorded online on the inflow cannula. Left atrial pressure (LAP), flow and perfusate temperature were also recorded online from the outflow cannula.

Evaluation of the graft

Pulmonary artery pressure and temperature of inflow perfusate were gradually increased. During the first 15 min of the reperfusion, PAP was kept between 5 and 10 mmHg. Ventilation was started when the outflow temperature reached 32 °C. The oxygen flow through the gas exchanger was switched at that moment to a mixture of carbon dioxide (9%) and nitrogen (91%) to deoxygenate the perfusate. Total ventilation volume and PEEP were slowly increased up to approximately 40% of the *in-vivo* minute volume and 5 cmH_2O, respectively, with a frequency of 20 breaths/min ($FiO_2 = 0.5$). When PAP and the temperature of the lung reached 20 mmHg and 37.5 °C, respectively, functional assessment was performed during a 20-min period. Three consecutive measurements of pulmonary artery (PA) flow (L/min), PAP

(mmHg), LAP (mmHg), and airway pressure (AWP) (mmHg) were recorded and three blood gas samples were taken from the outflow perfusate to analyze oxygenation. Oxygenation was calculated as the ratio of partial arterial oxygen pressure to fraction of inspired oxygen (PaO_2/FiO_2). Pulmonary vascular resistance (PVR) and wet-to-dry (W/D) weight ratio were calculated.

Histological examination

The following four items were scored: alveolar edema, hemorrhage, atelectasis, and thrombus formation. Each item was graded according to a four-point scale: 0 = minimal (little) damage, 1 = mild damage, 2 = moderate damage, 3 = severe damage.

Measurement of protein and surfactant function in BAL

Bronchoalveolar lavage samples were taken from each right lung immediately after explantation by instillation of 20 ml of saline solution. Samples were centrifuged at 3500 rpm at 4 °C for 10 min. The supernatant was stored at −80 °C until assayed.

Protein concentration in 10 μl of BAL was determined with the Bio-Rad protein assay (Bio-Rad Laboratories, Reinach, Switzerland) using BSA as a standard.

Phospholipids in the small aggregate containing supernatant and in the large aggregate containing pellet were determined by phosphorus analysis. Surfactant pellet was re-suspended in saline solution supplemented with calcium chloride (1.5 mmol/l). Surfactant function was analyzed with 40 μl of large aggregate suspension by a pulsating bubble surfactometer, adjusted to a phospholipid concentration of 3 mg/ml.

The surface tension at minimal bubble size was obtained after 5 min of bubble pulsation at a rate of 20 cycles/min and a temperature of 37 °C. Before bubble pulsation was started, adsorption was determined as surface tension 10 s after formation of a bubble. Adsorption is a process that occurs when a gas or liquid solute accumulates on the surface of a solid or, more rarely, a liquid (adsorbent), forming a molecular or atomic film (the adsorbate). It is different from absorption, in which a substance diffuses into a liquid or solid to form a solution. All analog data were digitalized and recorded on a personal computer.

Statistical evaluation

Data analysis was performed with the SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). All data are expressed as mean values ± SE of mean. As it was not possible to check for the normality of the variables with only five observations per group, we used nonparametric procedures. Kruskal–Wallis test is used to test the differences among the groups. In case of significant differences, the Mann–Whitney test is performed for comparing between two groups. The Spearman rank test was used to check for correlations. A P value equal or less than 0.05 was considered significant.

Results

Surfactant function

Adsorption significantly differed between HBD and DCD1 ($P = 0.004$), HBD and DCD2 ($P = 0.008$), HBD and DCD3 groups ($P = 0.008$). DCD1 and DCD2 groups also showed significant difference regarding adsorption ($P = 0.04$) (Fig. 1, Table 1). Surface tension was also significantly higher in DCD1, DCD2, and DCD3 groups compared with HBD group ($P = 0.016$) (Fig. 2, Table 1). As observed in adsorption, surface tension was significantly higher in DCD2 group compared with DCD1 group.

BAL protein levels

We observed higher BAL protein levels in DCD3 compared with HBD ($P = 0.01$), DCD2 ($P = 0.03$) and DCD1 groups ($P = 0.03$) (Table 1).

Pulmonary vascular resistance

PVR increased with longer warm ischemic periods. PVR was also higher in DCD3 group compared with HBD and DCD1 groups ($P = 0.016$) (Table 1).

Oxygenation (PaO_2/FiO_2)

Oxygenation was higher in HBD group compared to DCD1 ($P = 0.008$), DCD2 ($P = 0.04$), and DCD3

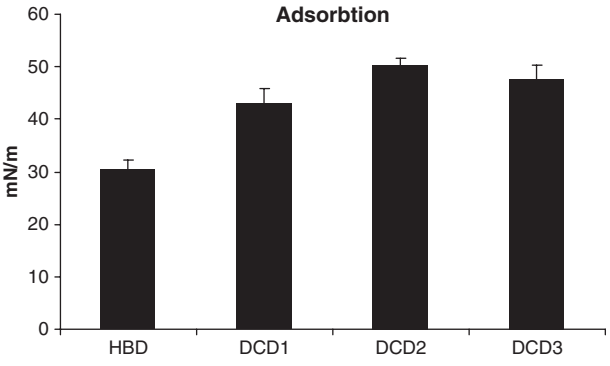

Figure 1 Adsorption significantly differed between heart-beating donor (HBD) and DCD1 ($P = 0.004$), HBD and DCD2 ($P = 0.008$), HBD and DCD3 groups ($P = 0.008$). DCD1 and DCD2 groups also showed significant difference regarding adsorption ($P = 0.04$).

Table 1. Graft and surfactant function in the study groups.

Groups	PVR	PaO_2/FiO_2	AWP	W/D ratio	BAL protein (μg/ml)	Adsorption (mN/m)	Surface tension (mN/m)
HBD	23.4 ± 2.0	540.6 ± 27.7	11.2 ± 1.02	5.42 ± 0.24	380.1 ± 81.6	30.5 ± 1.8	1.8 ± 0.3
DCD1	23.2 ± 2.5	458.1 ± 9.9	12.2 ± 0.6	5.2 ± 0.16	514.1 ± 54.6	43.08 ± 2.9	20.6 ± 1.2
DCD2	36.2 ± 5.7	286.7 ± 80.4	15.0 ± 1.4	5.8 ± 0.33	517.6 ± 25.3	50.4 ± 1.2	26.2 ± 2.5
DCD3	45.02 ± 7.8	205.8 ± 58.04	21.0 ± 3.4	7.38 ± 0.44	663.4 ± 43.3	47.6 ± 2.8	23.3 ± 2.8

All values mean ± SE of mean.

HBD, heart-beating donor; DCD1, 1 h warm ischemia followed by 3 h of topical cooling with saline; DCD2, 2 h warm ischemia followed by 2 h of topical cooling with saline; DCD3, 3 h warm ischemia followed by 1 h of topical cooling with saline; AWP, peak airway pressure (mmHg); PVR, pulmonary vascular resistance (Wood Units); PaO_2/FiO_2 (mmHg), the ratio between the partial arterial oxygen pressure and the fraction of inspired oxygen; W/D ratio, wet to dry weight ratio; mN/m, miliNewtons per meter.

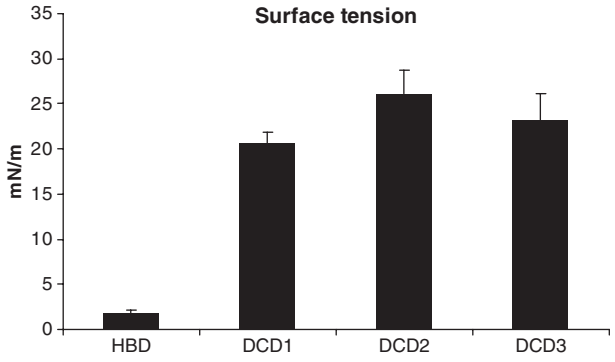

Figure 2 Surface tension was also significantly higher in DCD1, DCD2, and DCD3 groups compared with heart-beating donor (HBD) group ($P = 0.016$).

($P = 0.008$) Oxygenation was also higher in DCD1 compared to DCD3 group ($P = 0.008$). Oxygenation was also higher in DCD1 group compared with DCD3 group ($P = 0.008$) (Table 1).

Airway pressure

Airway pressure was lower in HBD group compared with DCD3 group ($P = 0.008$). AWP was also higher in DCD3 group compared with DCD1 group ($P = 0.008$) (Table 1).

Wet to dry (W/D) weight ratio

We found lower W/D weight ratio in HBD group compared with DCD3 group ($P = 0.03$). W/D weight ratio was also lower in DCD1 and DCD2 groups compared with DCD3 group ($P = 0.008$, $P = 0.04$, respectively) (Table 1).

Correlations

Oxygenation negatively correlated with adsorption ($r = -0.649$, $P = 0.01$) and surface tension ($r = -0.594$, $P = 0.01$) (Figs 3 and 4). AWP positively correlated with adsorption ($r = 0.486$, $P = 0.05$). BAL protein levels showed positive correlation with adsorption ($r = 0.577$, $P = 0.01$) and surface tension ($r = 0.489$, $P = 0.05$).

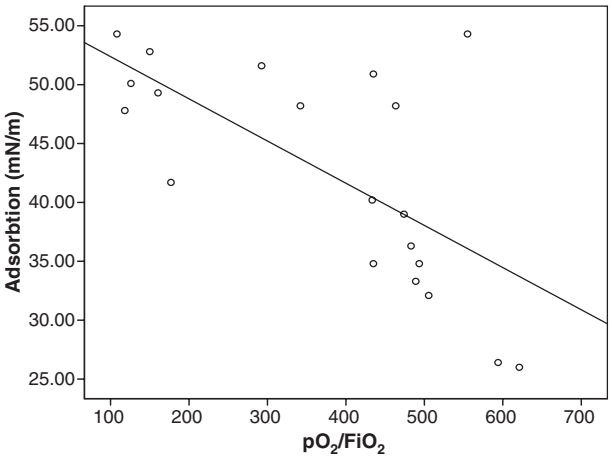

Figure 3 Adsorption negatively correlated with PaO_2/FiO_2 ($r = -0.649$, $P = 0.01$).

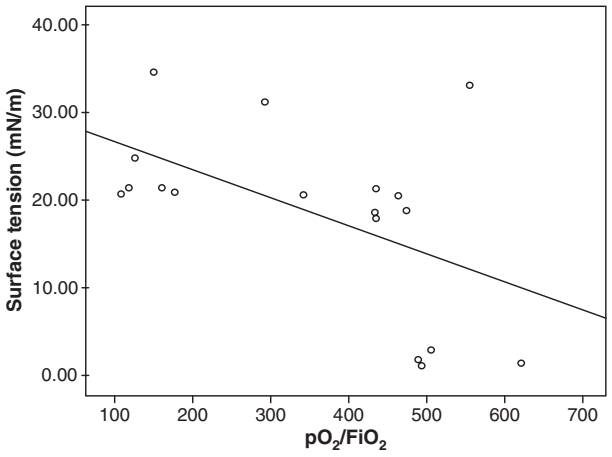

Figure 4 PaO_2/FiO_2 negatively correlated with surface tension ($r = -0.594$, $P = 0.01$).

Histology

Histology was comparable between HBD and DCD1 groups($P > 0.05$). However, significant histological differences were observed when we compared HBD group with DCD2 and DCD3 groups ($P < 0.05$). Histological abnormalities were also more severe in DCD2 and DCD3 groups compared with DCD1 group ($P < 0.05$). We observed comparable histological changes between DCD2 and DCD3 groups ($P > 0.05$).

Discussion

The main purpose of this study was to investigate the surfactant alterations in DCD lungs with increasing warm ischemic time intervals. We clearly showed that surfactant function decreases with increased warm ischemic times. This was proved by significantly different adsorption and surface tension in DCD groups compared with HBD group. We also observed statistically higher BAL protein levels in DCD3 groups compared with other groups. Our data also demonstrated significant correlation between oxygenation, BAL protein levels and surfactant function.

The endothelium and alveoli are damaged more during warm ischemia than cold ischemia, and reperfusion after warm ischemia is more damaging to grafts than that after cold ischemia [11]. Dilution from alveolar edema, inhibition by serum proteins, and type II cell injury are some of the mechanisms that lead to surfactant dysfunction [12]. Both experimental and clinical studies have shown the beneficial effect of surfactant treatment to lung transplant recipients [13–17].

Data from experimental lung transplantation models have provided evidence for surfactant alterations after lung transplantation. As the endogenous surfactant pool is damaged by reperfusion injury, the effect of surfactant replacement has been investigated in different models. Some of the studies focused mainly on the recipient treatment. Surfactant given just before or after reperfusion resulted in good graft function after reperfusion. Pretreatment with surfactant before the onset of ischemia has also been reported beneficial for graft function.

Andrade *et al.* [12] showed that the surfactant system is significantly affected in lungs preserved by the conventional pulmonary artery flushing and cold storage. Contrary to this observation, Gunther *et al.* [16] reported no alteration in surfactant function during 24 h of cold ischemic storage.

In a model of rat lung transplantation Boglione *et al.* [18] showed that after 240 min of warm ischemia intratracheal surfactant instillation resulted in better oxygenation, compliance and lower lipid peroxidation compared with the group without treatment. However, surfactant function was not measured. The animals received heparin before cardiac arrest and ventilated throughout the warm ischemic period.

In an isolated nonblood perfusion model in rats Nonaka *et al.* [19] reported that after 30 min of warm ischemia followed by 1 h of perfusion, surfactant administration before reperfusion was effective in preventing pulmonary edema. They also stated the effective value of initial low flow reperfusion which had decreased endothelial damage and graft damage. Their histological data demonstrated decreased cell death. Unfortunately, this group also did not measure the surfactant function.

In a pig left lung transplant model, surfactant function after transplantation was comparable between HBD and DCD groups [20]. Trend toward lower protein content in BAL was observed in NHBD group. In this experimental setting, the warm ischemic period was 30 min followed by *in situ* topical cooling with saline. All donors were heparinized and DCD lungs were not flushed via pulmonary artery, and the grafts were stored for 24 h before transplant [20]. Topical cooling was emphasized to be effective in terms of endothelial cell and type II pneumocyte protection. Reduced protein exudation and acceptable surfactant function in BAL confirmed the authors' hypothesis [20].

In the study presented, we utilized *ex vivo* lung perfusion system to evaluate the graft function. The *ex vivo* lung perfusion method has been proposed originally to assess the function of the graft from a non-HBD as an interim evaluation prior to transplantation [21,22]. Recently Steen *et al.* [23] successfully reconditioned and transplanted a rejected donor lung as a result of bilateral pulmonary contusion using *ex vivo* perfusion system. Toronto Group also uses *ex vivo* lung perfusion system for evaluation of a graft from category III NHBD when cardiac arrest does not result in 30 min after withdrawal of life support [5].

The length of tolerable warm ischemic time for a DCD donor lung is controversial; however, most of the experimental data suggest that lungs remain viable for at least 60–90 min after cardiac arrest [3–6,24–26]. The definition of warm ischemic time is also controversial. Most publications define it between cardiac arrest and start of cold flush preservation [24–26]. In this experimental setting, we defined the warm ischemic time as the time from cardiac arrest to the time of topical cooling. We did not perform cold flush after topical cooling. However, we performed retrograde perfusion at the back-table before *ex vivo* evaluation. In this study, different from above cited studies, we simulated three different warm ischemic time period without heparinization. Although 1 h warm ischemic time is accepted as tolerable, data for longer ischemic times regarding surfactant function is lacking.

Our data clearly show that the surfactant alteration begins at 1 h of warm ischemia (DCD1), although at this time point oxygenation, PVR and AWP were comparable with HBD group. This is in accordance with the international data accepting tolerable warm ischemic time of 1 h [3–6,25,26]. However, when we increase the warm ischemic time to 2 and 3 h, the deterioration in physiological parameters is more prominent compared with HBD and correlate with the surfactant dysfunction. Although the limited clinical DCD category III case series report comparable early and midterm outcomes compared with brain death donors [3–6,25,26], the rate of severe primary graft dysfunction (PGD) in category I DCD was 29% [1]. As our study simulates category I or II DCD donors, surfactant replacement might be an option to alleviate PGD after lung transplantation.

In conclusion, this study clears shows that surfactant function deteriorates with increasing warm ischemic time intervals. More studies with therapeutic surfactant administration are needed in DCD donors to assess its potential role in this population.

Authorship

II: designed research/study, performed the study, analyzed the data, wrote the paper. SA: performed the study, performed biochemical assessment, evaluated the data. CA: performed surfactant function analysis. WJ: analyzed the data. DI: analyzed the data, wrote the paper. PV: analyzed the histological data. WW: designed research/study, analyzed the data.

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