The impact of alteplase on pulmonary graft function in donation after circulatory death – An experimental study

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ABSTRACT

Objective: Lung transplantation is hampered by the lack of organs resulting in deaths on the waiting list. The usage of donation after circulatory death (DCD) lungs would dramatically increase donor availability. The most optimal organ preservation method, and the need for antithrombotic and fibrinolytic treatment to prevent thrombosis in the donor lungs is currently on debate. The present study investigated, in a simulated clinical DCD situation, whether the addition of alteplase in the flush-perfusion solution at the time of pulmonary graft harvesting could prevent thrombosis in the donor lung and thereby improve pulmonary graft function.

Methods: Twelve Swedish domestic pigs were randomized into two groups. All animals underwent ventricular fibrillation and were then left untouched for 1 h after declaration of death. None of the animals received heparin. The lungs were then harvested and flush-perfused with Perfadex® solution and the organs were then stored at 8 °C for 4 h. In one group alteplase was added to the Perfadex® solution (donation after cardiac death with alteplase (DCD-A)) and in the other, it was not (DCD). Lung function was evaluated, using ex vivo lung perfusion (EVLP), with blood gases at different oxygen levels, pulmonary vascular resistance (PVR), lung weight, and macroscopic appearance.

Results: During EVLP, there were no significant differences between groups in PaO2 at any investigated FiO2 level (1.0, 0.5, or 0.21). At FiO2 1.0, the PaO2 in the DCD and DCD-A was 51.7 ± 2.05 kPa and 60.3 ± 3.67 kPa, respectively (p = 0.1320). There were no significant differences between groups PVR levels, in the DCD (372 ± 31 dyne x s/cm5) and in the DCD-A (297 ± 37 dyne x s/cm5) groups (p = 0.1720). There was no significant difference between groups in macroscopic appearance.

Conclusions: All the lungs showed excellent blood gases after EVLP, and they all meet the criteria’s for clinical lung transplantation. The use of alteplase did not seem to have any obvious benefit to the donor lungs in a DCD situation. The donor lungs treated with alteplas showed slightly better blood gases and slightly lower PVR compared to the group without alteplas, however the difference was not significant. DCD appears to be a safe and effective method to expand the donor pool.

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1. Introduction

Lung transplantation (LTx) is still hampered by the lack of organs [1,2]. This leaves a growing number of patients with end-stage pulmonary disease remaining indefinitely on the waiting list for lung transplantation. Survival in LTx recipients has also increased over the years, mainly due to careful patient selection; improved lung preservation, surgical techniques, immunosuppressive regimes, management of ischemia/reperfusion injury; and forceful regimes in antibiotic prophylaxis and treatment. However, acute and chronic rejection and dysfunction continues to be a major problems. The primary cause of death after LTx is chronic rejection or bronchiolitis obliterans syndrome (BOS), and pulmonary re-
transplantation (re-LTx) is currently the only treatment option for these patients. In recent years there has also been an increasing demand for pulmonary re-transplantation (re-LTx) which also raises ethical issues on the correct allocation of the scarce donor pool [3].

In the setting of donor lung shortage and waiting list mortality, the interest in donation after cardio-circulatory death (DCD) is increasing [4–6]. Ex vivo lung evaluation (EVLP), has shown to be an excellent method for evaluating pulmonary graft function post mortem in DCD settings. The EVLP method is today mainly used for lung evaluation in donor lungs from heart beating donors (HBD), where the lungs have initially been rejected due to poor blood gases prior to an eventual clinical lung transplantation at many cardiothoracic clinics all over the world [7–13]. The use of EVLP method is today the standard method for evaluating whether the lungs are good for transplantation or not. The most optimal organ preservation method, and the need for antithrombotic and fibrinolytic treatment to prevent thrombosis in the donor lungs is currently on debate.

The increasing interest in DCD to increase donor organs has led to extensive research in the field of EVLP and the ideal preservation method. There is now convincing evidence that a time frame of 60 min of warm ischemia does not seem to compromise the pulmonary graft [14–16]. The golden standard in clinical transplantation today is to give the donor intravenous heparin prior to lung harvesting to avoid lung thrombosis in the lung grafts. In DCD lungs, heparin would need to be recirculated. This do create problems in a DCD situation where a DCD donor do not have any circulation at the time of organ harvesting. The allowance of donor preparation before the termination of circulation is disputable and the none use of heparin would significantly make the DCD donation easier. It is currently being debated whether it is ethically permissible to give a patient heparin after death has been declared but before permission for donation has been received, particularly given the cardiac compressions required to circulate heparin. The avoidance of heparin would help overcome this ethical challenge. Recently we have shown that heparin is not necessary in DCD settings [17]. The question remains how to optimally preserve the pulmonary graft.

The risk of post circulatory arrest thrombosis in the prospective DCD graft with the possible development of ischemia-reperfusion injury has led to different approaches. Fibrinolytic treatments have been under investigation with various results [18,19]. In this study we investigate the use of alteplase infusion prior to lung harvesting in a DCD experimental model. We hypothesize that adding a plasminogen activator (alteplase) to the perfusion solution at the time of harvest would dissolve possible thrombi and improve lung quality and performance. The DCD group who did not receive alteplase was used as control.

2. Material and methods

2.1. Animal preparation

Twelve Swedish landrace pigs were fasted overnight with free access to water. The experimental protocol for this study was approved by the Ethics Committee for Animal Research, Lund University, Sweden, Dnr M 172–11. All animals received care according to the European Convention of the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, the National Society for Medical Research’s Principles of Laboratory Animal Care, and the Institute of Laboratory Animal Research’s Guide for the Care and Use of Laboratory Animals. The study were designed as a controlled none blind randomized study. The randomization was done through computerized random selection in two equally sized groups. The inclusion criteria was pigs with a mean size of 55–65 kilos. Exclusion criteria’s: signs of infections as for example fever and cough or other signs of sicknesses as for example malignancy or anatomical anomalies.

The pigs were randomly assigned into 2 groups: DCD (DCD), and DCD with alteplase (DCD-A) (Actilyys, Boehringer Ingelheim AB, Stockholm, Sweden). The DCD group was used as control. The controls have also been used in a recently published paper [17]. Premedication was performed with an intramuscular injection of Xylazine (Rompun®) vet. 20 mg/ml; Bayer AG, Leverkusen, Germany; 2 mg/kg) mixed with ketamine (Ketamin® vet. 100 mg/ml; Farmaceutici Gellini S.p.A., Aprilia, Italy; 20 mg/kg) in their stables, and a peripheral iv access was established in the earlobe. The pig was then transferred to the laboratory and placed in supine position on the operating table. Oral intubation was performed using a 7.5 size endotracheal tube after anesthesia induction with sodium thiopental (Pentothal; Abbott Laboratories, North Chicago, Illinois, USA) and pancuronium bromide (Pavulon; N.V. Organon, Oss, the Netherlands). Anesthesia was maintained with a ketamine (Ketamin® vet), midazolam (Midazolam Panpharma®, Oslo, Norway), and fentanyl (Leptanal®, Lilly, France) infusion. Fluid loss was compensated for by continuous infusion of Ringer’s Acetate. Mechanical ventilation was established with a Siemens-Elema ventilator (Servo Ventilator 300, Siemens, Solna, Sweden) with an inspired oxygen fraction (FiO2) of 0.5, a frequency of 15 breaths/min, a minute ventilation of 6 l/min, and a positive end-expiratory pressure (PEEP) of 5 cmH2O.

2.2. Experimental timeline

The experimental timeline is demonstrated in Fig. 1.

2.3. Preservation of DCD lungs

A median sternotomy was performed. Ventricular fibrillation was induced electrically. The tracheal tube was disconnected from the ventilator when circulatory arrest was confirmed and left open to air. The sternotomy and the skin were temporary closed again and the animals were left untouched for 1 h at room temperature. After one hour after the declaration of death the median sternotomy was reopened.

The pulmonary artery was cannulated via the right ventricle with a 28 F cannula secured with a purse string suture placed in the outflow tract of the pulmonary artery. A clamp was put on the superior vena cava, and another clamp was put on the inferior vena cava. A clamp was put on the ascending aorta. The left atrium and inferior vena cava was opened.

In the study group, DCD, the lungs were perfused antegradely with 2 l of cold Perfadex with added isotonic trometamol 1.0 ml (Addex-THAM 3.3 mmol/ml; Fresenius Kabi AB Uppsala, Sweden), calcium chloride 2 ml (0.45 mmol/ml) and nitroglycerine 3 ml (5 mg/ml; BMM Pharma AB, Stockholm, Sweden) distributed at low perfusion pressure (<20 mmHg).

In the study group DCD-A, the lungs were perfused antegradely with 2 l of cold Perfadex with added alteplase 15 mg (Actilyys 1 mg/ml, Boehringer Ingelheim AB, Stockholm, Sweden), isotonic trometamol 1.0 ml (Addex-THAM 3.3 mmol/ml; Fresenius Kabi AB Uppsala, Sweden), calcium chloride 2 ml (0.45 mmol/ml) and nitroglycerine 3 ml (5 mg/ml; BMM Pharma AB, Stockholm, Sweden) distributed at low perfusion pressure (<20 mmHg).

The cannula was removed from the pulmonary artery. The lungs were harvested en bloc in a standard fashion. After harvesting, the lungs were put on a scale and the lung weight was noted. During the retrieval, a segment (~8 cm) of the descending aorta was also excised. The lungs were immersed in cold Perfadex with the aortic
segment and put in cold storage at 8°C for 4 h.

2.4. Ex vivo lung perfusion

Ex vivo lung perfusion (EVLP) was performed using the extracorporeal perfusion circuit by Medtronic (Medtronic AB, Kerkrade, the Netherlands; Ex Vivo Lung Evaluation Set).

The system was primed with albumin (500 ml, 50 g/l and 200 ml 200 g/l; Albumin Baxter, Baxter Medical, Kista, Sweden), and 2 units of autologous blood, earlier withdrawn from each donor. Imipenem (0.5 g; Tienam, Merck Sharp & Dohme, Sollentuna, Sweden), insulin (20 IU; Actrapid; Novo Nordisk, Bagsvaerd, Denmark), and heparin (10,000 IU; Leo Pharma, Malmö, Sweden) were added, and isotonic trometamol (Addex-Tham, Kabi, Sweden) was used to buffer the mixed solution to a temperature adjusted pH of 7.4. Gas was supplied to the membrane oxygenator; first oxygen and CO2 during the reconditioning phase, and then 93% nitrogen and 7% CO2 during the testing phase, creating a normal venous blood gas in the perfusate to the pulmonary artery (i.e., the oxygenator is used to deoxygenate the perfusate). Before the perfusion was started, the pulmonary artery was prolonged by a segment of the descending aorta to make cannulation easier. The pulmonary artery cannula was then connected to the corresponding tube of the extracorporeal circuit, the air was removed, and the shunt of the circuit was clamped. An endotracheal tube was secured in the trachea with a cotton band and connected to the ventilator. The remnant of the left atrium was left open, prohibiting pulmonary outflow obstruction, and maintaining a constant left atrium pressure around 0 mmHg.

A low-flow perfusion at 25°C was initiated through the lungs. The lungs were gradually warmed by increasing the temperature of the perfusate. When the temperature reached 32°C, ventilation was started with a FiO2 of 0.5 and a minute volume of 1 l/minute, and no positive end-expiratory pressure (PEEP). The pump flow was gradually increased, never allowing the pulmonary arterial pressure to exceed 20 mmHg. With the temperature increase of each 1°C, ventilation was augmented with a corresponding 1 L minute volume. After 20–30 min, normothermia was reached and positive end-expiratory pressure was added to fully expand the lungs and eliminate atelectasis. Blood gases were analyzed throughout the perfusion and under full ventilation with decreasing inspired oxygen fraction levels. Pulmonary vascular resistance (PVR) was calculated at various points of ventilation using the formula PVR (dyne·sec/cm5) = \(80 \times (\text{Mean Pulmonary Artery Pressure (PAP)}) - (\text{Pulmonary Cap Wedge Press (PCW)}) / (\text{Cardiac Output (CO)})\)

e.g. Pulmonary Artery Flow).

The lungs were then disconnected from the EVLP. The lungs were put onto a scale and weighed. The pulmonary arterial branches were macroscopically studied for thrombotic material by opening the arteries as far distally as possible.

2.5. Calculations and statistics

Descriptive statistics, in the form of the number of experimental animals, mean, and the standard error on the mean (SEM) for the different parameters were analyzed. The results are presented for the different parameters divided into the different groups (DCD, and DCD-A). Statistically significant difference between the different groups was tested by a non-parametric Mann-Whitney. All statistical analysis was performed, using SPSS version 20. Significance was defined as: \(p < 0.001 (***), p < 0.01 (**), p < 0.05 (*),\) and \(p > 0.05\) (not significant, n.s.).

3. Results

3.1. Study groups

Animal weights in the two groups were as follows: 62.7 ± 1.3 kg in the DCD-A group, and 61.2 ± 1.4 kg in the DCD group \((p = 0.0463)\). Pre-operative arterial oxygen partial pressure (PaO2) at an FiO2 of 0.5 were in the DCD-A group 29.2 ± 0.4 kPa, and in the DCD group 29.1 ± 0.9 kPa \((p = 0.0954)\). Pre-operative arterial carbon dioxide partial pressure (PaCO2) at an FiO2 of 0.5 were in the DCD-A group 5.9 ± 0.5 kPa, in the DCD group 6.0 ± 0.2 kPa \((p = 0.1675)\).

The EVLP time was 56 ± 3.3 min for the DCD group, and 55 ± 4.0 min for the DCD-A group \((p = 0.1740)\). No anatomical anomalies, signs of infection, or malignancy were found in any of the animal at autopsy.

|                  | DCD  |                  |                  |                  |                  |                  |                  |                  |
|------------------|------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | Hands | Organ harvesting | Cold Perfadox     | Cold storage     | Weight | Prep time | EVLP | Weight + macroscopic assessment |
|                  | off   |                  | antegrad perf.   |                  |        |          |     |                               |
| 60 ± 0.0 min     | 29 ± 0.0 min | 240 ± 0.0 min | 22 ± 1 min | 56 ± 3.0 min | 15 ± 1 min |                  |                  |                  |

|                  | DCD-A |                  |                  |                  |                  |                  |                  |                  |
|------------------|-------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | Hands | Organ harvesting | Cold Perfadox     | Cold storage     | Weight | Prep time | EVLP | Weight + macroscopic assessment |
|                  | off   |                  | antegrad perf. With Alteplas |                  |        |          |     |                               |
| 60 ± 0.0 min     | 29 ± 0.0 min | 240 ± 0.0 min | 22 ± 2 min | 55 ± 4 min | 15 ± 2 min |                  |                  |                  |

Fig. 1. The figure shows a timeline of the experimental setup for the different groups: donation after circulatory death without alteplase (DCD) and with alteplase (DCD-A). The time for each procedural step is given as mean and SEM. In the DCD-A group the lungs were perfused with Perfadox with the addition of alteplase. Ex Vivo Lung Perfusion is mentioned as EVLP in the timeline.
3.2. Pulmonary gas function

3.2.1. Arterial and venous blood gases
Both arterial and venous blood gases after 5 min of ventilation at the different FiO2 1.0, 0.5, and 0.21 are presented in Table 1. In the DCD-A group, the PaO2 was 60.25 ± 3.67 kPa; and in the DCD group, the PaO2 was 51.67 ± 2.05 kPa after completed EVLP and FiO2 1.0 (p = 0.1320).

3.3. Hemodynamic data

3.3.1. Pulmonary artery flow and pulmonary artery pressure
Pulmonary artery flow (PAF), i.e. cardiac output (CO) in the ex vivo model and pulmonary artery pressure (PAP) was measured continuously and data was collected after 5 min of ventilation at different fractions of oxygen 1.0, 0.5, and 0.21.

PAF at FiO2 1.0 in the DCD-A group was 4.00 ± 0.02 l/min, and in the DCD group 3.87 ± 0.10 l/min. There was a statistical significant difference between the DCD group vs DCD-A group (p = 0.043). PAF at FiO2 0.5 and FiO2 0.21 were identical to PAF values at FiO2 1.0, as shown in Fig. 2.

The PAP at FiO2 1.0 in the DCD-A group was 14.83 ± 1.85 mmHg, and in the DCD group 17.83 ± 1.17 mmHg (p = 0.1720). PAP at FiO2 0.5 and FiO2 0.21 were similar to PAP values at FiO2 1.0, as shown in Fig. 3.

3.3.2. Pulmonary vascular resistance
The PVR was calculated at the different FiO2 at 1.0, 0.5, and 0.21 and is also presented in Fig. 4. The PVR at FiO2 1.0 was calculated to 297 ± 37 dyne x s/cm5 in the DCD-A group, and in the DCD group PVR was 372 ± 31 dyne x s/cm5 (p < 0.1720). PVR at FiO2 0.5 and FiO2 0.21 were similar to PVR values at FiO2 1.0, as shown in Fig. 4.

3.4. Pulmonary graft compliance
After evaluation of the lungs, they were disconnected from the ventilator and a collapse test was performed. If the lungs do not collapse this may indicate lung injury, lung edema, or pneumonia. All the lungs from both study groups collapsed as they should, and

3.5. Weight of lungs
The lungs were weighed after harvesting, before EVLP, and after

Table 1
The table demonstrates the following parameters of blood gases PaO2, PaCO2, PVO2 and PvCO2 for inspired oxygen fractions of 1.0, 0.5, and 0.21 for the three different groups: DCD-A, and DCD. Statistical analysis was performed using Mann-Whitney test to compare DCD-A, and DCD.

|        | DCD-A      | DCD       | p-value |
|--------|------------|-----------|---------|
| PaO2 (kPa) |            |           |         |
| FiO2 1.0 | 60.3 ± 3.67| 51.7 ± 2.05| 0.142   |
| FiO2 0.5 | 26.4 ± 1.37| 23.4 ± 0.80| 0.493   |
| FiO2 0.21 | 8.5 ± 0.43 | 9.0 ± 0.35 | 0.951   |
| PaCO2 (kPa) |            |           |         |
| FiO2 1.0 | 3.8 ± 0.32 | 3.5 ± 0.09 | 1.000   |
| FiO2 0.5 | 3.2 ± 0.05 | 3.3 ± 0.09 | 1.000   |
| FiO2 0.21 | 3.1 ± 0.12 | 3.6 ± 0.10 | 0.060   |
| PVO2 (kPa) |            |           |         |
| FiO2 1.0 | 6.8 ± 0.29 | 7.1 ± 0.14 | 0.966   |
| FiO2 0.5 | 7.3 ± 0.13 | 6.9 ± 0.20 | 0.237   |
| FiO2 0.21 | 4.2 ± 0.21 | 5.9 ± 0.40 | 0.001   |
| PvCO2 (kPa) |            |           |         |
| FiO2 1.0 | 3.9 ± 0.07 | 3.8 ± 0.09 | 1.000   |
| FiO2 0.5 | 3.7 ± 0.05 | 3.6 ± 0.18 | 1.000   |
| FiO2 0.21 | 3.6 ± 0.08 | 4.1 ± 0.08 | 0.001   |

FiO2 – Inspired oxygen fraction, PaO2 – arterial oxygen partial pressure, PaCO2 – arterial carbon dioxide partial pressure, PVO2 – venous oxygen partial pressure, PvCO2 – venous carbon dioxide partial pressure. DCD-A – donation after cardiac death with alteplase and non-heparin group, DCD – donation after cardiac death non-heparin group.
EVLP to assess the degree of lung edema. Before EVLP, the mean lung weight in the DCD-A group was 546.33 ± 29.06 g, and in the DCD group it was 558.33 ± 21.39 g (p = 0.688). After EVLP, the lung weight for the DCD-A group was 541.50 ± 31.25 g, and in the DCD group the lung weight was 589.17 ± 24.27 g (p = 0.1727).

3.6. Macroscopic appearance

After completing the lung evaluation, the pulmonary arterial branches were macroscopically studied for thrombotic material by opening the arteries as far distally as possible. No thrombotic material was observed in any of the groups.

4. Discussion

Lung transplantation can extend life expectancy and enhance the quality of life for select patients with end-stage lung disease. In the setting of donor lung shortage and waiting list mortality, the interest in DCD is increasing [20].

The tolerance of warm ischemia of the lungs and the remaining suitability of lung function has been investigated broadly. There is now convincing evidence that a time frame of 60 min of warm ischemia does not seem to compromise the pulmonary graft [14–17]. EVLP has become an important tool in assessing DCD lungs [5,7–9,11–13]. While different approaches to avoiding post circulatory thrombosis in DCD have been investigated, the most beneficial preservation method is still unclear. As heparin does not act on preformed fibrin or microthrombi, removing the potential appearance of microthrombi should only be possible using fibrinolytic agents. There have been reports of successful results using fibrinolytics on DCD [18–21]. The risk of thrombosis in the microvasculature of DCD organs has also been studied in heart, liver, and kidney models. Functional recovery of canine hearts by flushing the microvasculature with plasminogen activator in the perfusion solution was thought to lead to improved results [22]. When using streptokinase in the topical flush solution in a porcine model, an improvement of pre-transplant kidney preservation could be noticed [23–25]. In a DCD lung model, left lung allotransplantation was performed in dogs after 1 and 2 h of warm ischemia and 3 h of cold storage. The application of urokinase into the pulmonary artery after Perfadex flush resulted in significantly better gas exchange than the 2-h-warm-ischemia control group without urokinase and showed the same cardiopulmonary function as the 1-h warm ischemia controls [18]. In a similar model in dogs investigating different dosages of urokinase, improved results were shown with the use of recombinant tissue-type plasminogen activator (rt-PA) into the main pulmonary artery prior to reperfusion. The higher the dose, the better the gas exchange and even the cardiopulmonary performance. However, the risk of potential hemorrhages could not be ruled out [21]. Applying plasminogen activators for achieving additional fibrinolysis and dissolving microthrombi during reperfusion in DCD lungs of 30–38 kg pigs has proven to lead to a significant reduction in PVR and improve lung function after a long warm ischemic period of 3 h [19]. This fibrinolytic DCD model with larger animals simulated well a potential Maastricht category 1 in terms of warm ischemia duration. However, the small size of the animals does not reflect a normal human bodyweight in a standard clinical situation. A regular size adult human body with an estimated weight of 70 kg cools down much slower than smaller bodies do. This might implicate a shorter period of warm ischemia with regular body temperature in a smaller body.

In our study, we compared DCD lungs after 60 min of warm in situ ischemia both non-heparinized but one group with the addition of a plasminogen activator (DCD-A), the other DCD group served as a control. Our results showed no difference in blood gases when evaluating at FiO2 1.0, 0.5, or 0.21 between the two groups. All the PaO2 values of both the DCD and the DCD-A lungs fully meet the standard criteria for the acceptance for lung transplantation according to international guidelines [26]. Even the hemodynamic parameters showed excellent results for both groups. The PAF reveals statistical significantly higher flow rates in favor to the DCD-A group, however the difference in PAF between the two groups were only 0.13 l/min and probably must be seen without any clinical relevance. PVR in both groups were satisfyingly low. The results were likewise in favor for the DCD-A group, but showed no significance. One can only speculate that the results might have been significant in a larger study group.

Inci et al. earlier reported the use of additive urokinase in DCD to be superior over untreated DCD and HBD [19]. The long warm ischemic period in their model followed by topical cooling may have facilitated the formation and occurrence of thrombosis in the pulmonary vasculature leading to favorable results for the use of urokinase. In our study we investigated the effect of fibrinolytic agent alteplase after 60 min of warm ischemia from which we know from earlier studies that the pulmonary microvasculature remains rather unaffected. As our findings show equal on the hemodynamics for the investigated DCD groups with or without alteplase as an additive, they differ from previous study results where even topical cooling was applied after warm ischemia. It leaves the hypothesis that additional or prolonged cooling might alter the epithelium and maybe rather predispose it to formation of thrombosis. Furthermore, our tests for lung compliance post EVLP found no differences between the investigated groups. Neither did we find differences on the absence of macroscopic evidence for potential thrombi. None of the lungs in our study developed pulmonary edema.

5. Conclusions

As a functional clinical model for DCD could generate an
increased number of grafts for transplantation, a facilitated way of treating potential donor organs is required. Retrieving organs without applying heparin will aid in that process. A facilitated procedures where donors can be left untouched for 1 h can be a promising step in meeting the present organ shortage. The additional use of a plasminogen activator, alteplas, did not indicate any obvious increase in the outcome of DCD graft function and performance in the present study. Nevertheless all lungs, with or without alteplas, reached the clinical criteria’s for lung transplantation with a wide marginal. The results were likewise in favor for the DCD-A group, but showed no significance. Limitations in our study due to the rather short evaluation time of graft function and performance as well as relatively small study groups need to be considered. According to our results, DCD can be safely used without heparin in settings where topical cooling is eluded.

Ethical approval

The experimental protocol for this study was approved by the Ethics Committee for Animal Research, Lund University, Sweden, Dnr M 172-11.

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Author contribution

All authors contributed equally to all parts of the study as study design, data collections, data analysis and writing.

Conflicts of interest

None.

Guarantor

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