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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 PaO_2 at any investigated FiO_2 level (1.0, 0.5, or 0.21). At FiO_2 1.0, the PaO_2 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/cm^5) and in the DCD-A (297 ± 37 dyne x s/cm^5) 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.

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-

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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 since 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 alteplas 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) (Actiylys, 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 (Ketaminol® 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 (Ketaminol® 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 (FiO_2) of 0.5, a frequency of 15 breaths/min, a minute ventilation of 6 l/min, and a positive end-exspiratory pressure (PEEP) of 5 cmH_2O.

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 nitro-glycerine 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 (Actiylys 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