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Retrograde flush is more protective than heparin in the uncontrolled donation after circulatory death lung donor

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ARTICLE INFO

Article history:
Received 9 August 2013
Received in revised form
15 November 2013
Accepted 20 November 2013
Available online 25 November 2013

Keywords:
Heparin
Donation after circulatory death
Uncontrolled
Retrograde flush
Topical cooling

ABSTRACT

Background: Formation of microthrombi after circulatory arrest is a concern for the development of reperfusion injury in lung recipients from donation after circulatory death (DCD) donors. In this isolated lung reperfusion study, we compared the effect of postmortem heparinization with preharvest retrograde pulmonary flush or both.

Methods: Domestic pigs ($n = 6$/group) were sacrificed by ventricular fibrillation and left at room temperature for 1 h. This was followed by 2.5 h of topical cooling. In control group [C], no heparin and no pulmonary flush were administered. In group [R], lungs were flushed with Perfadex in a retrograde way before explantation. In group [H], heparin (300 IU/kg) was administered 10 min after cardiac arrest followed by closed chest massage for 2 min. In the combined group, animals were heparinized and the lungs were explanted after retrograde flush [HR]. The left lung was assessed for 60 min in an *ex vivo* reperfusion model.

Results: Pulmonary vascular resistance at 50 and 55 min was significantly lower in [R] and [HR] groups compared with [C] and [H] groups ($P < 0.01$ and $P < 0.001$) and at 60 min in [R], [H], and [HR] groups compared with [C] group ($P < 0.001$). Oxygenation, compliance, and plateau airway pressure were more stable in [R] and [HR] groups. Plateau airway pressure was significantly lower in [R] group compared with the [H] group at 60 min ($P < 0.05$). No significant differences in wet–dry weight ratio were observed between the groups.

Conclusions: This study suggests that preharvest retrograde flush is more protective than postmortem heparinization to prevent reperfusion injury in lungs recovered from donation after circulatory death donors.

1. Introduction

The first human lung transplantation in 1963 was performed with a left lung recovered from an uncontrolled donation after circulatory death (DCD) donor [1]. After failed resuscitation heparin was injected in the heart of the deceased patient and closed cardiac massage and ventilation were continued until explantation. The left lung was flushed with a cold heparinized glucose solution and rhythmically inflated with pure oxygen until the moment of implantation. The following

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0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2013.11.1100

milestone in uncontrolled DCD donor was the transplantation of lungs after *ex vivo* evaluation in 2001 [2]. In this case, 50,000 IU of heparin was administered through a central venous line 10 min after the declaration of death, followed by 20 chest compressions. The lungs were preserved by topical cooling, retrieved without flush, and evaluated *ex vivo*.

Intrapulmonary thrombi formation after circulatory arrest is a concern in DCD donor. In controlled DCD donor, pretreatment (i.e., heparin or phentolamine) can be given before death [3–5] or after the 5-min no-touch interval [6]. In some centers, donors are optimized but no heparin is given [7]. In uncontrolled donation, there is evidence that retrograde flush after topical cooling results in a better washout of blood and microthrombi and reduces pulmonary vascular resistance (PVR) [8]. Heparinization of the uncontrolled DCD donor followed by chest compression can potentially cause lung contusions and subsequent pulmonary hematomas. There is a concern of dispersing microthrombi through the lung.

The aim of this study was to compare the possible benefit of postmortem heparinization *versus* a preharvest retrograde flush or both in uncontrolled lung donors.

2. Materials and methods

2.1. Experimental groups

Domestic pigs were randomly divided into four groups ($n = 6$/group). In all four groups lungs were subjected to 1 h of warm ischemia and 2.5 h of topical cooling. Pulmonary grafts were recovered without heparinization or flush in the control group [C]. A retrograde flush with Perfadex (Vitrolife, Göteborg, Sweden) was performed after topical cooling in the second group [R]. In the third group [H], animals were given heparin (300 IU/kg) *via* the central venous line 10 min after cardiac arrest, and then closed chest massage was performed for 2 min. The pigs in the fourth group were heparinized in an identical manner and the lungs were explanted after a retrograde flush [HR].

2.2. Animal preparation

Animals were premedicated with an intramuscular injection of Xylazine (5 mL Xyl-M 2%; V.M.D. nv/sa, Arendonk, Belgium) and Zolazepam-Tiletamine (3 mL Zoletil 100; Virbac s.a., Carros, France), installed in a supine position, intubated with an endotracheal tube 7.5 (Portex Tracheal Tube; SIMS Portex, Ltd Hythe, Kent, UK), and ventilated with a volume-controlled ventilator (Titus; Dräger, Lübeck, Germany) with an inspiratory oxygen fraction (FiO_2) of 0.5 and a tidal volume of 10 mL/kg body weight. Respiratory rate was adjusted to achieve an end-tidal CO_2 of 40 mm Hg. Positive end-expiratory pressure was set to 5 cmH_2O. Anesthesia was maintained with isoflurane 0.8%–1% (Isoba Vet; Schering-Plough Animal Health, Harefield, Uxbridge, UK) and muscle relaxation with intermittent boluses of pancuronium bromide (Pavulon 2 mg/mL; Organon Teknika, Boxtel, The Netherlands). A 14 G catheter (Secalon T; Becton Dickinson Ltd, Singapore, Singapore) was placed in the right common carotid artery for the measurement of the systemic arterial pressure and sampling of arterial blood. A Swan-Ganz catheter was inserted through the internal jugular vein into the main pulmonary artery to measure pulmonary artery pressure (PAP) and pulmonary capillary wedge pressure. Hemodynamic parameters (systemic arterial pressure , PAP, and pulmonary capillary wedge pressure) and ventilatory parameters (plateau airway pressure [Plat AwP] and compliance [Compl]) were continuously monitored and stored on a computer.

After a stabilization period, pigs were sacrificed by inducing ventricular fibrillation with a subxyphoidal needle puncture using a square-pulse generator (amplitude range +15 to −15 V, current <300 mA, and frequency 50 Hz). After cardiac arrest, the endotracheal tube was disconnected from the ventilator and left open to the air. According to the study protocol pigs were heparinized or not.

The cadavers were left untouched for 1 h at room temperature. Lung temperature was measured *via* a probe in the endotracheal tube and the rectal temperature was monitored.

All animals received humane care in compliance with the Principles of Laboratory Animal Care, formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996 (NIH Publication No. 85–23, Revised 1996). The study was approved by the Institutional Review Board on animal research at the KU Leuven.

2.3. Preservation of the heart–lung block

Two chest drains were inserted in each pleural cavity after 1 h of warm ischemia. Lungs were then cooled with cold saline in a closed circuit. *Via* the deep drains, cold saline solution was infused and continuous recirculated with a roller pump from a reservoir placed in an ice basket. The system was filled with approximately 6 L of cold saline. To ensure that the lungs were well immersed in the fluid, the superficial drains were connected to an overflow system of 5 cmH_2O. The temperature of the lungs was measured *via* a probe in the endotracheal tube and the rectal temperature was monitored. After 2.5 h of topical cooling, sternotomy was performed. The thymic tissue was excised, pericardium and pleural cavities were widely opened, and lungs were inspected. The pulmonary artery, ascending aorta, and caval veins were encircled. Gross microthrombi in the pulmonary artery and left atrium were evacuated as much as possible. In [C] and [H] groups, lungs were explanted without flush. In [R] and [HR] groups, the left atrium was cannulated (MOD Cannula 18 Fr; International Medical Products NV/SA, Brussels, Belgium) through a purse string and the lungs were flushed in retrograde manner with 50 mL/kg Perfadex at room temperature (18°C) buffered with Trometamol (0.3 mL/L, 2 g/5 mL; Addex-THAM, Kabi, Uppsala, Sweden) and $CaCl_2$ (0.6 mL/L, 11mEq). The caval veins were ligated and the ascending aorta was clamped. The inferior caval vein was incised for venting of the heart. During the flush, ventilation was restarted with a low tidal volume and a low frequency to avoid cold lung injury related to mechanical stress. The lungs were kept on ice during the 30 min of preparation.