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# Argon and xenon ventilation during prolonged *ex vivo* lung perfusion

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ABSTRACT

*Background:* Evidence supports the use of *ex vivo* lung perfusion (EVLP) as a platform for active reconditioning before transplantation to increase the potential donor pool and to reduce the incidence of primary graft dysfunction. A promising reconditioning strategy is the administration of inhaled noble gases based on their organoprotective effects. Our aim was to validate a porcine warm ischemic lung injury model and investigate post-conditioning with argon (Ar) or xenon (Xe) during prolonged EVLP.

*Methods:* Domestic pigs were divided in four groups ($n = 5$ per group). In the negative control group, lungs were flushed immediately. In the positive control (PC) and treatment (Ar, Xe) groups, lungs were flushed after a warm ischemic interval of 2-h *in situ*. All grafts were evaluated and treated during normothermic EVLP for 6 h. In the control groups, lungs were ventilated with 70% $N_2$/30% $O_2$ and in the treatment groups with 70% Ar/30% $O_2$ or 70% Xe/30% $O_2$, respectively. Outcome parameters were physiological variables (pulmonary vascular resistance, peak airway pressures, and $PaO_2/FiO_2$), histology, wet-to-dry weight ratio, bronchoalveolar lavage, and computed tomography scan.

*Results:* A significant difference between negative control and PC for pulmonary vascular resistance, peak airway pressures, $PaO_2/FiO_2$, wet-to-dry weight ratio, histology, and computed tomography-imaging was observed. No significant differences between the injury group (PC) and the treatment groups (Ar, Xe) were found.

*Conclusions:* We validated a reproducible prolonged 6-h EVLP model with 2 h of warm ischemia and described the physiological changes over time. In this model, ventilation during EVLP with Ar or Xe administered postinjury did not improve graft function.



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## 1. Introduction

Lung transplant programs are being hampered by organ shortage due to a low recovery rate of donor lungs from multiorgan donors [1]. Successful efforts have been made to increase the donor pool including the use of donors after circulatory death (DCD) and extended criteria donors [2,3]. However, further expansion with acceptable grafts is still requisite. In addition, the problem of primary graft dysfunction, resulting from ischemia–reperfusion injury (IRI), decreases early posttransplant outcome [4,5]. To enlarge the donor pool and improve outcome, active resuscitation of donor organs before transplantation is a promising strategy.

Normothermic *ex vivo* lung perfusion (EVLP) is entering the clinical reality as a tool for graft evaluation and preservation [6]. This technique was developed to evaluate DCD donor lungs before transplantation [7]. EVLP was further successfully applied to assess and recruit previously rejected organs [8]. Currently, research is investigating the potential to actively recondition lung grafts with EVLP [9], serving as a platform to stimulate repair mechanisms while organs are metabolically active. Prolonged and stable perfusion times are essential conditions for EVLP rehabilitation, which can be extended up to 12 h [10].

Inhalational therapy for lung rehabilitation is an excellent administration route, and research on inhalational therapy such as carbon monoxide [11] and hydrogen therapy [12] has already been investigated. However, the effects of noble gases on IRI have not been investigated so far. Noble gases, including argon (Ar) and xenon (Xe), are chemically inert but exhibit biological effects [13,14]. Various *in vitro* and *in vivo* injury models in the brain [15,16], myocardium [17] and kidneys [18,19] have shown a protective effect of Ar and Xe attributed to antiapoptotic and anti-inflammatory properties [20–25]. The problem with Xe is its scarcity (0.9 ppm), whereas in contrast, Ar is the third most abundant gas in the earth's atmosphere (9300 ppm). Therefore, the use of Ar treatment could be of higher economic benefit.

Potential organ-protective effects on pulmonary grafts have not been explored and IRI might be a perfect target for noble gas treatment. A major advantage is the possible administration of these gases through ventilation. In addition, higher concentrations in the gas phase can be administered during EVLP without additional risk for the recipient.

This study aimed to investigate the potential of pulmonary allograft reconditioning using *ex vivo* noble gas treatment during 6 h of normothermic EVLP in a 2-h warm ischemic injury model.

## 2. Methods

### 2.1. Animals

Domestic male pigs (Topigs 20, 36–42 kg) were used. Local ethical approval was obtained at the research institute (NTS P043/2014). 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 and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996).

### 2.2. Animal anesthesia and baseline

Anesthesia was induced by an intramuscular injection with 5-mg/kg Zoletil 100 (Virbac, Carros, France) and 3-mg/kg XYL-M 2% (V.M.D., Arendonk, Belgium). Muscle relaxation and analgesia were maintained with 2-mg pancuronium and 20 μg/kg/h of fentanyl. Continuous intravenous infusion of 10-mg/kg/h propofol was used for anesthesia maintenance. Animals were intubated with a 7.0-mm endotracheal tube and ventilated (Aestiva 3000; GE Healthcare Europe GmbH, Little Chalfont, United Kingdom) with a tidal volume (TV) of 8 mL/kg, positive endexpiratory pressure (PEEP) of 5 cm $H_2O$, and $FiO_2$ of 30%. Respiratory rate was adjusted to $ETCO_2$ (45–55 mm Hg). Invasive blood pressure was monitored. Animals received 1-g cefazolin, 500-mg Solu-Medrol (Pfizer, Brussels, Belgium) and 300 IU/kg of heparin.

### 2.3. Study groups

Animals were divided in four groups ($n = 5$ per group): negative control (NC), positive control (PC), Ar group, and Xe group.

In the PC and treatment groups, circulatory and respiratory arrest was induced by myocardial fibrillation with an electrical pulse generator and ventilator disconnection. Animals were left untouched at room temperature (warm ischemic interval of 120-min *in situ*). In the NC group, no warm ischemia was induced and lungs were procured immediately.

### 2.4. Procurement of donor lungs

A median sternotomy was performed after baseline assessment in NC, and 15 min before completion of warm ischemia in PC, Ar, and Xe. The pulmonary artery and caval veins were encircled, and a purse-string was sutured on the right ventricular outflow tract to secure the 20-Fr flush cannula in the pulmonary trunk. After inflow occlusion, lungs were cold flushed (4°C) in an antegrade way with 50-mL/kg tromethamol-buffered Perfadex (Xvivo Perfusion AB, Goteborg, Sweden). To optimize flush conditions, lungs were protectively ventilated (inspiratory pressure of 15 cm $H_2O$ and PEEP of 5 cm $H_2O$). After explantation, an additional retrograde flush (1 L) was performed, and the lungs were cannulated with the XVIVO Lung Cannula Set with closed atrium. An endotracheal tube of 8.0 mm was secured in the trachea.

### 2.5. Perfusate

The perfusate (1.5 L) was composed of tromethamol-buffered Perfadex with 70 g/L of human albumin (C.A.F.-D.C.F., Neder-Over-Heembeek, Belgium). Also, 2.5-g glucose, 1-g cefazolin, 500-mg Solu-Medrol, 50-mEq sodium bicarbonate, 0.18-g calcium, and 30 IU of insulin were added. Baseline samples of the priming solution were analyzed.