ORIGINAL ARTICLE

Steroids can reduce warm ischemic reperfusion injury in a porcine donation after circulatory death model with *ex vivo* lung perfusion evaluation

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SUMMARY

Donation after circulatory death (DCD) is being used to increase the number of transplantable organs. The role and timing of steroids in DCD donation and *ex vivo* lung perfusion (EVLP) has not been thoroughly investigated. In this study, we investigated the effect of steroids on warm ischemic injury in a porcine model (n = 6/group). Following cardiac arrest, grafts were left untouched in the donor (90-min warm ischemia). Graft function was assessed after 6 h of EVLP. In the MP group, 500 mg methylprednisolone was given prior to cardiac arrest and during EVLP. In the CONTR group, no steroids were added. Median lung compliance (13 ml/cmH_20) was significantly better preserved in the CONTR group than in the MP group (30.5 ml/cmH_20). Also, median wet-to-dry weight (6.11 vs. 6.94) and CT density (182.5 vs. 352.9 g/l) were significantly better in the MP group than in the CONTR group, respectively. There was no difference in oxygenation and pulmonary vascular resistance. Perfusate cytokine analysis showed a significant reduction in IL-1β, IL-8, IFN-α, IL-10, TNF-α, and IFN-γ in MP. Cytokines in bronchoalveolar lavage were not decreased except for IFN-gamma. We demonstrated that warm ischemic injury in DCD donation can be attenuated by steroids when given prior to warm ischemia and during EVLP. Ethical context of donor preconditioning should be discussed further.

Transplant International 2016; 29: 1237–1246

Key words
animal models, donation, donation after circulatory death, donor management, *ex vivo* lung perfusion, extended donor pool, experimental transplantation, ischemia reperfusion injury, organ preservation and procurement

Received: 30 March 2016; Revision requested: 27 April 2016; Accepted: 28 July 2016

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doi:10.1111/tri.12823

Introduction

Lung transplantation remains the only life-saving treatment option for patients suffering from end-stage pulmonary disease. Due to its success, lung transplant programs worldwide are increasing with over 4000 lung transplants procedures performed annually [1]. However, there is an ongoing disparity between the number of patients on the waiting list and the number of good quality donor organs for transplantation. This leads to increased waiting times and a persistent mortality on the waiting list as high as 15% [2].

Because of this organ shortage, other sources of organ recovery besides the classical brain-dead donor (DBD) are being addressed nowadays. Over the years, organ donation after circulatory death (DCD) has been reintroduced in transplant programs [3–5] with a marked increase since 2001. On average, only 7% of all lung grafts are derived from DCD donors according to the latest registry analysis; however, in some programs this reaches up to 32% [3]. Generally, DCD donation is classified into two main categories and further subdivided following the Maastricht classification [6]. In uncontrolled DCD donation, the patient is found with circulatory arrest, is dead upon arrival, or dies after unsuccessful resuscitation. Controlled DCD donors represent patients who die after a switch off of mechanical, ventilation, or organ perfusion-supported therapies or when circulatory arrest occurs prematurely in a DBD donor. To optimize lung preservation and limit the impact of warm ischemic injury, several strategies have been explored. Topical cooling by insertion of chest tubes can be applied in the controlled setting, but is mostly used in uncontrolled DCD donation [7,8]. In controlled donation, the current clinical practice includes rapid flush perfusion (antegrade in the donor and retrograde at the back table after organ recovery) [9]. Pre-arrest interventions are mainly limited to heparinization if legally authorized. This can theoretically be beneficial as confirmed by experimental data [10]. However, clinical studies comparing strategies with and without heparin are lacking. In fact, several centers report good outcome without pre-arrest heparinization [11]. These centers do use a retrograde flush in its heparin-free scenario which also seems protective [12].

In all scenarios, DCD organs suffer from a variable period of warm ischemia which could lead to increased ischemia–reperfusion injury and a reduction in organ quality. The tolerable length of this warm ischemic interval for lungs can be extended up to 60–90 min [13,14]. To assess organ quality of lungs donated by a DCD donor prior to transplantation, *ex vivo* lung perfusion (EVLP) has been developed [15]. With this technique of machine perfusion, lungs are perfused by a pump and ventilated under normothermic conditions. During EVLP, lungs can be physiologically evaluated and, nowadays, new imaging techniques can even be applied to fully assess the organ of previously unknown quality [16]. Since the introduction of EVLP in 2001 by Steen *et al.* [17], there is the actual potential to evaluate the donor organ prior to transplantation. This is especially recommended in uncontrolled DCD donation programs where outcomes are better when EVLP is applied [8]. Currently, in only 12% of controlled DCD, EVLP is clinically applied [3]. Some groups do report better outcome in controlled DCD donation [18] when lungs have been perfused and evaluated on EVLP. Therefore, it may be advisable to consider this technique as a platform to assess the risk for severe ischemia–reperfusion injury (IRI) to decide on the optimal preservation strategy based on physiological evaluation. This opens up the ability to even recondition donor lungs of unknown or inferior quality prior to transplantation. Hereby, an increase in available donor organs with optimal quality is expected [19,20].

Steroids are among the most potent anti-inflammatory and immunosuppressive agents. In the airways, they bind to the glucocorticosteroid receptors which are ubiquitously expressed in all cells throughout the airways. After translocation to the cell nucleus, they inhibit nuclear factor kappa B (NFκ-B) activation followed by blockage of pro-inflammatory genes [21–23]. Therefore, steroids are of particular interest in ischemia–reperfusion injury remodeling. They are already a component of the perfusate used in the majority of EVLP protocols [15]. However, the exact role of steroids during EVLP has never been elucidated and comparative data of EVLP with and without methylprednisolone is lacking. Besides *ex vivo* administration of steroids, these immunomodulatory drugs can also be administrated to the donor. Most brain-dead patients are now treated with steroids before procurement of the organs. The rationale to add steroids in the donor is to block the upregulation of several pro-inflammatory cytokines during the onset of brain death and improvement of hemodynamic instability following adrenal insufficiency [24]. The evidence, however, is not robust based on a recent meta-analysis [25]. In addition, besides these potential benefits in DBD donors, their role in warm ischemic injury and DCD donation has never been investigated. Nevertheless, over 90% of centers using DCD organs

Transplant International 2016; 29: 1237–1246
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report that steroids are applied prior to circulatory arrest [3].

We aimed to investigate the role of steroids in DCD lung donation to protect against warm ischemia–reperfusion injury. Therefore, in this study, we hypothesized that administration of steroids prior to onset of warm ischemia and during EVLP has a beneficial impact on pulmonary graft function.

Methods

This experimental study was performed in compliance with the Principles of Laboratory animal care published by the National Institute of Health Volume 25, No. 28 (revised 1996). Local ethics approval was obtained at the research institute (NTS P043/2014).

Donor procedure

Domestic pigs Topig 20 (mean 40.75 kg) were divided into two groups ($n = 6$/group). Animals were anesthetized with an intramuscular injection of 5 mg/kg Zoletil 100 (Virbac, Carros, France) and 3 mg/kg Xyl-M 2% (VMD, Arendonk, Belgium). Anesthesia was maintained using 10 mg/kg/h propofol, 20 μg/kg/h fentanyl, and intermittent boli of pancuronium 2 mg for muscle relaxation. Animals were intubated with a 7.0-mm endotracheal tube and ventilated (Aestiva 3000; GE Healthcare Europe GmbH, Little Chalfont, UK) with a tidal volume (TV) of 8 ml/kg, positive end-expiratory pressure (PEEP) of 5 cmH_2O and FiO_2 of 30%. Respiratory rate (RR) was adjusted to the end-tidal carbon dioxide ($ETCO_2$) (45–55 mmHg). Blood pressure was monitored invasively in the right carotid artery. All animals died of cardiac arrest which was induced by direct electrical stimulation of the myocardium with an electrical pulse generator that led to ventricular fibrillation. Animals were disconnected from the ventilator when cardiac arrest was induced. Prior to cardiac arrest, all animals were heparinized with 300 IU/kg. In group 1, 500 mg Solu-Medrol (Pfizer, Brussels, Belgium) was given prior to induction of ventricular fibrillation (MP group). In group 2, no steroids were administered to the donor animal (CONTR group).

Following cardiac arrest in the donor, grafts were left untouched in the deceased donor for 90 min after which they were flushed antegradely with 50 ml/kg cold thromethamol-buffered OCS solution (Transmedics, Andover, MA, USA). The heart–lung block was excised and a retrograde flush (1L thromethamol-buffered OCS solution) was performed at the back table. Lungs were instrumented on ice for a short period of time (73.2 ± 7.5 min), while the XVIVO (Göteborg, Sweden) cannulas were secured in the pulmonary artery and atrial cuff. An 8.0-mm ET tube was secured in the trachea. The donor procedure was performed as previously described [26].

Ex vivo lung perfusion

After a 1-h rewarming period and slow increase in the flow to 40% of the estimated cardiac output (calculated as 100 ml/kg), lungs were further perfused and evaluated for 6 h in total. Lungs are perfused with an acellular albumin containing dextran solution. The production of the perfusate and technique of EVLP are performed as described previously [26]. In the CONTR group, no steroids were added to the perfusate. In the MP group, 500 mg Solu-Medrol ® (Pfizer) was added to the perfusate to continue the steroid exposure to the preconditioned grafts in the MP group in order to investigate the maximal effect of steroids to DCD grafts.

During 6 h of EVLP, we monitored dynamic airway compliance (Compl), oxygenation (PaO_2/FiO_2) and pulmonary vascular resistance (PVR) hourly. We analyzed end-experimental parameters only to dichotomize between acceptable and nonacceptable lungs.

Tissue sampling

At the end of the experiment, tissue samples were taken for histological evaluation and wet-to-dry weight (W/D) ratio calculation (after 48 h in the oven at 80 °C). Pathology samples are scored by a blinded pathologist for neutrophilia, congestion and presence of eosinophils. Bronchoalveolar lavage with two times of 30 cc saline 0.9% was performed in the right middle lobe. Pooled fractions were returned and the supernatant was analyzed with a porcine multiplex ELISA kit for IL-1β, IL-4, IL-8, IL-10, IFN-γ, IFN-α, and TNF-α according to the manufacturer's protocol (Thermo Fisher Scientific Inc, Waltham MA, USA). Also perfusate samples from the end of the experiment were analyzed with the same ELISA analysis. The left lung was inflated at 25 cmH_2O, frozen solid in the fumes of liquid nitrogen, and scanned with Siemens Somatom CT scanner. Lung mass, volume, and density were measured on the basis of the CT scan, using imaging software (Horos™) in which the lung is manually delineated and the number of voxels and mean density of the voxels within the volume are determined [27].

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Statistical analysis

All data are expressed as median with IQ range when depicting physiological variables in time or as a scatter plot with median and IQ range when comparing variables at the end of the experiment (GRAPHPAD PRISM 4; GraphPad Software Inc, La Jolla, CA, USA). Permutation tests were conducted in R (R Foundation, Vienna, Austria) using the "coin" package to compare data at the end of EVLP. Baseline parameters of the donor animals are described as median (25% QI–75% QI) and are analyzed with the same permutation test.

In cases where lungs could not sustain the full 6 h of EVLP, data points recorded in the next hours after the premature end of EVLP were considered the same as the last data point available to allow comparison at all evaluation points. Therefore, at the end of EVLP the last available data point is included for the statistical analysis. Graft survival on EVLP is analyzed with a log rank test in GRAPHPAD PRISM 4 (GraphPad Software Inc).

Results

Groups

Baseline parameters are illustrated in Table 1.

Functional assessment of pulmonary grafts during EVLP

Figure 1(a) depicts the change in dynamic airway compliance over time (median – IQ range). It is similar at the onset of evaluation (starting after 1 h of EVLP). After the first recruitment maneuver at 1.5 h of perfusion, compliance increased in both groups followed by a gradual decrease. When comparing the dynamic airway compliance at the end of EVLP (Fig. 1b), we noted that it was significantly better preserved in the MP group (median Compl 13 ml/cmH_20 in the CONTR group versus 30.5 ml/cmH_20 in the MP group; $P = 0.0304$).

Figure 1(c) depicts the change in oxygenation (evaluated by PaO_2/FiO_2) over time (median – IQ range). PaO_2/FiO_2 decreased in both groups and was also not significantly different when comparing it at the end of the experiment (median PaO_2/FiO_2 486.7 in the CONTR group versus 430.4 in the MP group; $P = 0.5887$) (Fig. 1d).

Figure 1(e) depicts the change in pulmonary vascular resistance (PVR) over time (median – IQ range). PVR is low in both groups at the first evaluation moment (1 h perfusion). During the experiment, PVR slowly increases in both groups. When comparing PVR at the end of EVLP (Fig. 1f), we observed a similar PVR in both groups (median PVR 473.7 dynes $\times$ s $\times$ cm^{-5} in the CONTR group versus 430.4 dynes $\times$ s $\times$ cm^{-5} in the MP group; $P = 0.8182$).

For all experiments in the MP group, grafts could be perfused for 6 h. In the CONTR group however, there was a dropout of three experiments where perfusion was ended on 3.75, 4.0 and 4.5 h, respectively, due to excessive edema formation (Fig. 2). The superior survival of the grafts in the MP group nearly reaches significance ($P = 0.055$).

Assessment of pulmonary edema

A high W/D weight (median 6.94) is observed in the CONTR group and a low W/D weight (median 6.11) in

Table 1. Baseline parameters of the donor animals. Data are presented as median (25–75% IQR); *P*-value permutation test.

	CONTR	MP	*P*-value
Donor			
Weight (kg)	43 (39–46)	40 (39–46)	0.05
TV (ml/kg)	7.9 (7.7–8.0)	7.9 (7.8–8.0)	0.79
HR (bpm)	102 (88–119)	100 (72–114)	0.46
MAP (mmHg)	92 (81–103)	86 (72–101)	0.43
Compl (ml/cmH_20)	28.5 (27.0–31.0)	30 (27–33)	0.45
P/F (mm Hg)	427 (410–452)	453 (413–495)	0.22
Hct (%)	33.7 (31.3–35.9)	35.3 (33.3–39.9)	0.14
WBC (109/l)	14.5 (12.4-17.3)	18.2 (11.8–22.4)	0.26
Neutrophils (%)	37 (24–43)	41 (32–59)	0.23
Neutrophils (109/l)	5.9 (3.0–6.6)	6.5 (5.3–10.4)	0.12
CIT (min)	77 (70–85)	70 (62.5–74.5)	0.06

TV, tidal volume; HR, heart rate; MAP, mean arterial pressure; Compi, dynamic airway compliance; P/F, partial arterial oxygen pressure over fractional inspired oxygen ratio; Hct, hematocrit; WBC, white blood cell count; CIT, cold ischemic time.

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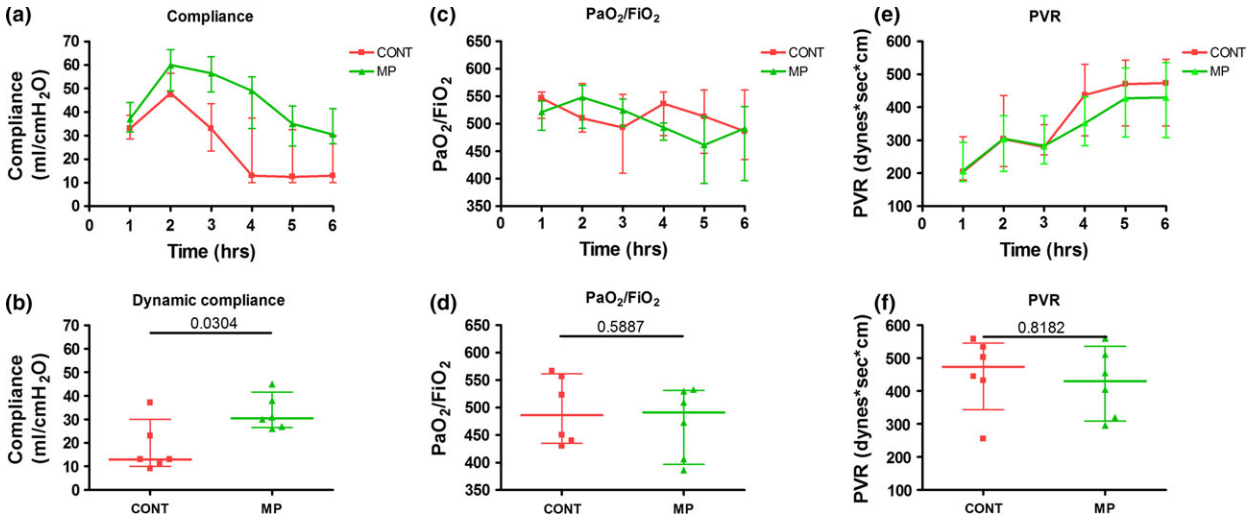

Figure 1 Dynamic airway compliance, oxygenation (PaO_2/FiO_2), and pulmonary vascular resistance are depicted during the 6 h of *ex vivo* lung perfusion in (a, c, e), respectively (median – IQ range), for both groups. Compliance, oxygenation, and pulmonary vascular resistance (PVR) are depicted at the end of *ex vivo* lung perfusion (EVLP) (scatter plot median – IQ range) in (b, d, f), respectively. A permutation test shows significantly better airway compliance at the end of EVLP in the MP group ($P = 0.0304$). All other parameters are not significantly different.

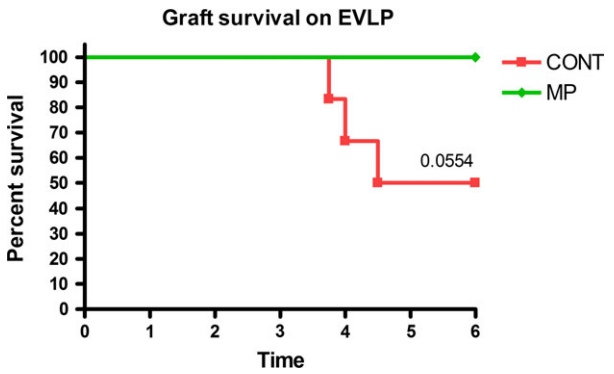

Figure 2 Survival of the lung graft on *ex vivo* lung perfusion (EVLP) was ended when 1000 ml of perfusate was transformed in lung edema and ventilation was impossible. There was a trend in better graft survival in the MP group that only just failed to reach significance (log rank test $P = 0.055$).

the MP group. W/D weight ratio is significantly lower ($P = 0.0200$) in the group that received methylprednisolone (Fig. 3a). Density measurements on CT scan confirmed this excess of extravascular lung water accumulation and showed a significantly higher ($P = 0.0022$) density in the CONTR group (median 352.9 g/l) compared with the MP group (median 182.5 g/l) (Fig. 3b). Septal thickening and severe lung edema can clearly be visualized in the CONTR group (Fig. 3c) in comparison with the MP group (Fig. 3d).

Histology

Histological analysis did not reveal any significant differences in congestion ($P = 0.5798$), neutrophil invasion ($P = 0.1252$) and membrane disruption ($P = 0.2077$) compared with the CONTR group.

Immunological evaluation

Porcine multiplex analysis of the perfusate sample at the end of EVLP (Fig. 4) showed a median IL-1B level of 124.6 pg/ml in the CONTR group versus 39.8 pg/ml in the MP group ($P = 0.0022$); a median IFN-alpha level of 94.6 pg/ml in the CONTR group versus 0.66 pg/ml in the MP group ($P = 0.0037$); a median TNF-alpha level of 3181 pg/ml in the CONTR group versus 225.8 pg/ml in the MP group ($P = 0.0081$); a median IL-10 level of 94.6 pg/ml in the CONTR group versus 0.66 pg/ml in the MP group (0.0037); and a median IFN-gamma level of 2.83 pg/ml in the CONTR group versus 0.08 pg/ml in the MP group ($P = 0.0050$). IL-8 was above detection limit in the CONTR group (highest standard depicted), but low in the MP group (median 45.32 pg/ml). IL-4 was below the detection limit.

Multiplex analysis of the BAL fluid at the end of EVLP showed a median IL-1B level of 36.45 pg/ml in the CONTR group versus 34.21 pg/ml in the MP group ($P = 0.2876$); a median IFN-alpha level of 0.36 pg/ml in the CONTR group versus 0.38 pg/ml in the MP group ($P = 0.5582$); a median TNF-alpha level of 233.8 pg/ml in the CONTR group versus 105.4 pg/ml in the MP group ($P = 0.1727$); and a median IL-8 level of 174.2 pg/ml in the CONTR group versus 61.8 pg/ml in the MP group ($P = 0.0649$). IFN-gamma was

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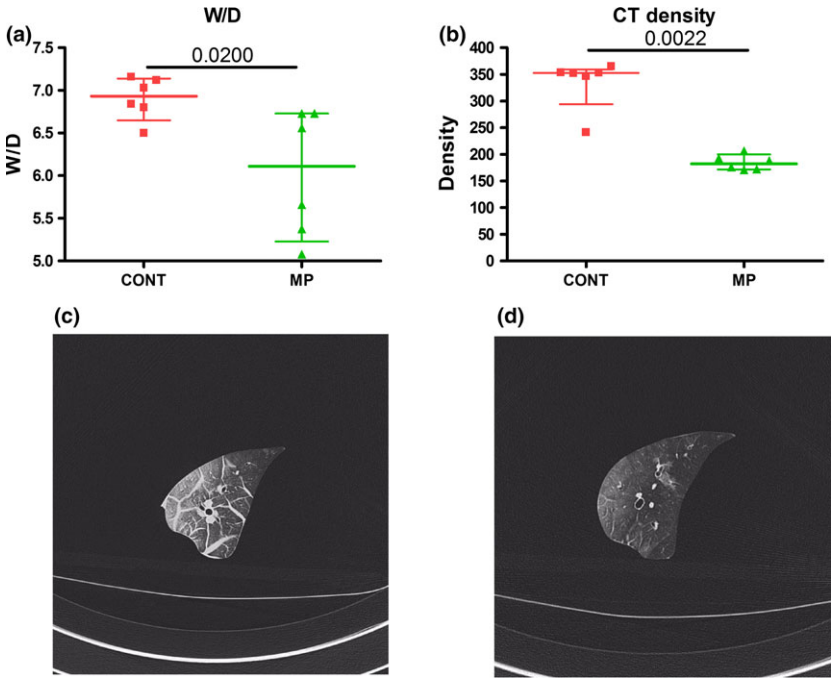

Figure 3 (a) Wet-to-dry weight is depicted in a scatter plot with median – IQ range. A permutation test of the wet-to-dry weight ratio (W/D) shows significantly less lung edema formation in the MP group ($P = 0.0200$). (b) Density measured on CT scan is depicted in a scatter plot with median – IQ range. A permutation test shows a significantly higher density measurement on CT scan analysis in the CONTR group compared with the MP group ($P = 0.0022$). (c) CT scan of left lower lobe in the CONTR group. (d) CT scan of left lower lobe in the MP group.

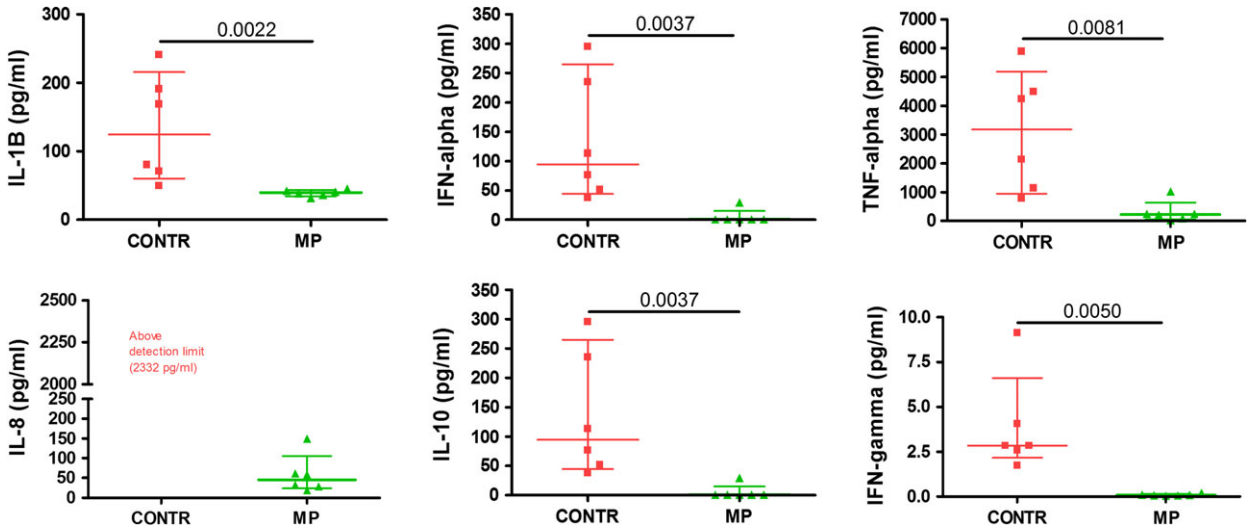

Figure 4 Porcine multiplex analysis of the perfusate sample at the end of *ex vivo* lung perfusion (EVLP). IL-1B, IFN-alpha, TNF-alpha, IL-10, and IFN-gamma are all significantly lower in the CS group. IL-8 was above detection limit in the CONTR group, but low in the CS group. IL-4 was below detection limit and is not depicted. Data points are depicted in a scatter plot with median – IQ range and the resulting *P*-value of the permutation test.

significantly lower ($P = 0.0157$) in the MP group (median 0.05 pg/ml) compared with the CONTR group (median 0.1 pg/ml). IL-10 and IL-4 were both below the detection limit.

Discussion

We report our experimental findings on the role of steroids in a DCD model of organ donation. To our

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knowledge, this is the first experimental report on the use of steroids in a DCD donor. We demonstrated that administration of steroids prior to warm ischemia and during EVLP evaluation significantly improved lung function, lung edema and reduced a subset of inflammatory markers.

By giving steroids to the donor prior to the onset of warm ischemia and further exposure to steroids during EVLP, we observed improved lung compliance at the end of EVLP. Pulmonary vascular resistance and oxygenation did not differ between both groups. However, it has previously been advocated that compliance is the best parameter to predict donor lung quality [28,29] as this parameter directly reflects the impact of fluid extravasation in the lung. Also, physiological acceptance criteria for transplantation after EVLP are not yet been agreed upon and other groups do advocate the use of oxygenation and pulmonary vascular resistance as the best evaluation parameters with excellent results after transplantation [30]. W/D is still the golden standard for estimation of lung edema, and in our experimental study, it was significantly lower in the MP group. This could be further validated by a lower density measurement in the methylprednisolone group on CT scan. The latter provides assessment of the whole lung surface, while a biopsy provides information only on a small portion of the tissue. Implementation of CT scanning might be considered as a valuable noninvasive tool to measure pulmonary edema.

The cytokine expression profile of lungs in both groups was represented by evaluating cytokines in both the circulating perfusate and BAL (at the end of EVLP). Administration of steroids to the donor in addition with exposure to steroids during EVLP resulted in a decreased level of cytokine production and release, especially in the perfusate. Also, this reflects a reduced organ inflammation, but the role of cytokine expression on EVLP is still largely unknown [31]. It might be that a different pattern of cytokines is expressed during *ex vivo* organ perfusion that does not completely reflect the *in vivo* reperfusion situation. We used an acellular perfusate and the reperfusion injury observed during our setup is mainly driven by resident leukocytes in the pulmonary graft. This expressed cytokine panel also suggest an important role for macrophage secretion. The observation that the anti-inflammatory cytokine IL-10 was also significantly reduced indicates that we should better look at the balance between pro- and anti-inflammatory mediators, rather than the absolute concentration.

Early outcome after lung transplantation is mainly impaired by the occurrence of severe primary graft dysfunction (PGD) driven by ischemia–reperfusion injury and occurs in up to 30% of lung transplant recipients [32,33]. Despite better supportive treatment options such as extra-corporal membrane oxygenation [34] to limit early mortality from severe PGD, this syndrome has a significant impact on long-term outcome with an increased 90-day and 1-year mortality after severe PGD at 72 h after lung transplantation [33]. Also, there is an increased risk to develop bronchiolitis obliterans syndrome (BOS) [32,35–37] following high-grade primary graft dysfunction.

In addition, the use of DCD organs itself seems to be an increased risk factor for PGD. However, similar short- and long-term outcomes between DBD and DCD donors have been reported [38–40]. Therefore, it is of great interest to limit primary graft dysfunction after lung transplantation with a specific strategy such as steroid administration. The possible benefit of steroid administration is already been highlighted in brain-dead organ donation [25,41]. That is, steroids can suppress the cytokine release during the catecholamine storm and improve hemodynamic stability in adrenal insufficient patients [25,42]. Controlled DCD donors suffer an agonal phase that is unpredictable, prior to circulatory arrest. This agonal phase can add a large variability to the injury and is difficult to standardize. In a previous study [43], we have investigated the impact of different modes of death in DCD donation. We could identify that hypoxic arrest was more detrimental to the graft quality [44]. In our current study, we have chosen to work with a standardized warm ischemic porcine DCD model, with immediate onset of the warm ischemic interval by induction of ventricular fibrillation and disconnection of the ventilator (in a paralyzed animal). In this way, we could better standardize the warm ischemic injury (still the most important component of IRI in DCD donation). The effect of steroids in a controlled DCD model induced by hypoxic arrest with variable periods of warm ischemia is also an interesting study to conduct in the future.

The major limitation of this study is the absence of a control group where steroids are used only in the donor animal. However, steroids are included in all EVLP protocols without convincing evidence for a beneficial effect on PGD. Based on previous preliminary data in our laboratory, we are confident that steroid administration postinjury during EVLP only, cannot reverse warm ischemic injury. This is also

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shown from other research experiments where steroids are applied in the perfusate in both control and treatment groups [45–47]. Therefore, we believe that it is the administration of steroids to the DCD donor prior to circulatory arrest that is important for optimal organ preservation to alleviate warm ischemic damage. The administration window of preconditioning and preservation strategies is still largely unknown. In order to avoid missing any positive effect by focusing on a narrow window, we chose to expose the grafts to steroids throughout the whole experiment. Of course, further experiments should now be designed to elucidate the role of the pre-arrest donor treatment or treatment of the graft during EVLP only. Also, our findings need to be validated in a transplant model.

In case of donation after brain dead (DBD), the advantage of using steroids has been investigated previously [25]. However, the role of steroids in DCD donation has never been investigated. The reason is twofold: firstly, DCD donation has only recently become of higher interest and research in donor management of the DCD donor is limited and difficult to design. Secondly, the dead donor rule impedes on any intervention in the patient awaiting therapy withdrawal (controlled DCD donor) [48]. However, pre-arrest therapies such as the use of heparin have been widely adopted in various European countries to be used in DCD protocols to improve organ function [3,10]. Even though a relation between the administration of heparin and the acceleration of the dying process has not been investigated or demonstrated so far, we do not know whether there is a relation between the dying process or length of the agonal phase and the administration of high-dose glucocorticoids. This opens up the discussion about expanding donor management to DCD, as it is more and more applied in DBD programs. As steroids are not harmful to patients, we believe that steroid administration to DCD donors should be considered. Despite this ethical issue, the latest report of the DCD registry within the International Society of Heart and Lung Transplantation noted that already over 90% of the participating centers give steroids to the DCD donor prior to declaration of dead [3]. Unfortunately, there are no data available on the doses, frequency, and timing of steroid administration in the DCD donation process. We therefore do not know whether steroids were administered during the ICU admission as a treatment to reduce cerebral edema, or whether it was administered to optimize donor organ quality. If these steroids would have been administered intentionally to optimize donor quality, this course of action coincides with the dead donor rule as therapy is given to a patient who is not declared dead yet. Some believe that this policy could bring harm to transplant programs. However, others believe that once the decision for switch off and organ donation has been made, one can go forward with donor management and optimization protocols which should be performed by an independent team to avoid any conflict of interest or harm to the donor. We believe that our findings should further be embedded in an ethical discussion to decide whether pretreatment in a DCD donor is ethically and legally acceptable. Donor management of a DCD donor would however be most feasible with interventions that are beneficial when applied only just prior to circulatory arrest (as shown in these experiments). This avoids implementation of complex and time-consuming management protocols prior to the controlled DCD procedure.

We conclude that administration of steroids to a DCD donor and during *ex vivo* lung perfusion attenuates warm ischemia–reperfusion injury. The role of steroids during *ex vivo* lung perfusion only should be the subject of future research. In addition, a study on the effect of steroids administered only to the donor in a controlled DCD model with hypoxic arrest will also add knowledge in the future. We advocate the use of steroids in clinical DCD programs worldwide, with caution to further introduce preconditioning strategies prior to declaration of death in organ donation programs.

Authorship

AM and AN: designed the experimental study and wrote the manuscript. AM and MB: performed the research and collected all data. SEV: contributed to the analysis of tissue samples and CT scans. AM: analyzed the data. GMV, RV, BMV and DVR: helped designing the study and did the final review of the manuscript.

Funding

This study was funded by the Clinical Research Fund of the University Hospitals Leuven (Leuven, Belgium).

Conflict of interest

The authors have declared no conflicts of interest.

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Acknowledgements

The authors thank E.K.V. for scoring of all histological samples and thank D.S. and S.C. for performing the MULTIPLEX cytokine analysis. Finally, Ms. Nicole Jannis is greatly thanked for her help in coordinating all experiments and her expert help during the experiments.

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