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An experimental study of the recovery of injured porcine lungs with prolonged normothermic cellular *ex vivo* lung perfusion following donation after circulatory death

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SUMMARY

Donation after circulatory death (DCD) is an underused source of donor lungs. Normothermic cellular *ex vivo* lung perfusion (EVLP) is effective in preserving standard donor lungs but may also be useful in the preservation and assessment of DCD lungs. Using a model of DCD and prolonged EVLP, the effects of donor warm ischemia and postmortem ventilation on graft recovery were evaluated. Adult male swine underwent general anesthesia and heparinization. In the control group ($n = 4$), cardioplegic arrest was induced and the lungs were procured immediately. In the four treatment groups, a period of agonal hypoxia was followed by either 1 h of warm ischemia with ($n = 4$) or without ($n = 4$) ventilation or 2 h of warm ischemia with ($n = 4$) or without ($n = 4$) ventilation. All lungs were studied on an EVLP platform for 24 h. Hemodynamic measures, compliance, and oxygenation on EVLP were worse in all DCD lungs compared with controls. Hemodynamics and compliance normalized in all lungs after 24 h of EVLP, but DCD lungs demonstrated impaired oxygenation. Normothermic cellular EVLP is effective in preserving and monitoring of DCD lungs. Early donor postmortem ventilation and timely procurement lead to improved graft function.

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Key words
cellular preservation, donation after circulatory death, *ex vivo* lung perfusion, lung transplantation

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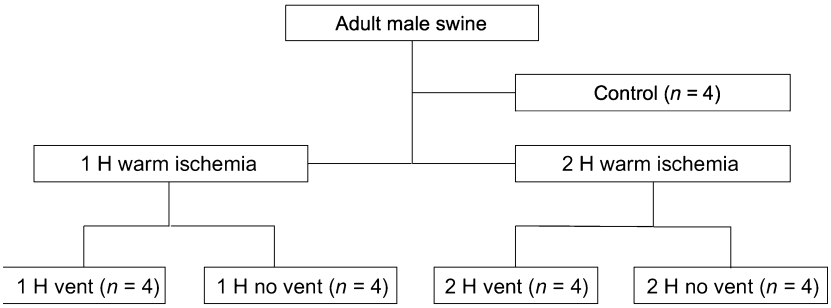

Figure 1 Diagram of study design. A total of 20 swine were divided into one control group and four treatment groups based on different strategies for warm ischemic downtime and postmortem ventilation. The four distinct treatment groups were: *1H vent* (1 h of warm ischemia with ventilation), *1H no vent* (1 h of warm ischemia without ventilation), *2H vent* (2 h of warm ischemia with ventilation), and *2H no vent* (2 h of warm ischemia without ventilation).

Introduction

Fewer than 2% of lung transplants in the USA are performed using donation after circulatory death (DCD) lungs, despite a persistent shortage of donor organs [1]. In selected patients with severe irreversible illness requiring continuous life-sustaining therapy, DCD allows family to authorize organ recovery, which follows compassionate extubation and spontaneous cardiac arrest in an operating room [2]. Cardiac arrest often follows quickly, minimizing organ exposure to atelectasis and warm ischemia. However, the time to arrest can be prolonged, leading to an hour or more of hypoventilation and shock [3,4]. The unpredictability of this process and the inability to evaluate donor lungs following procurement have limited the proliferation of DCD in lung transplantation.

Blood-based normothermic *ex vivo* lung perfusion (EVLP) reduces cold ischemia compared with standard ice preservation and is reported to reduce the incidence of primary graft dysfunction (PGD) in standard donor lungs [5,6]. Experience with this platform in extended-criteria donors, including DCD, is growing in human clinical trials [7,8]. Acellular EVLP platforms have been used for short durations to study the effects of DCD in animal models; most suggest evidence of early lung failure gradually improves with EVLP, even in lungs exposed to 4 h of pre-EVLP ischemia [9–14].

Our group has demonstrated that lungs can be maintained on a blood-based normothermic EVLP platform for 24 h, allowing comprehensive monitoring and possible recovery [15]. With the current study, we utilized this model to explore the effects of DCD-induced atelectasis and ischemia on recovery of lung mechanics and hypothesize that prolonged blood-based EVLP will be effective in mitigating DCD-associated lung injury.

Materials and methods

Animal model

Twenty castrated adult male Yorkshire swine (average weight 81.9 kg) were randomly divided into five groups of four swine each, including a control group (Fig. 1). The four treatment groups represented a spectrum of DCD injury, based on varying scenarios of cardiac arrest, warm ischemia, and postmortem mechanical ventilation. This protocol was approved by our local Institutional Animal Care and Use Committee.

Cardiac arrest and lung procurement

In accordance with our institutional guidelines, anesthesia was induced using an intermuscular injection of 5–7 mg/kg tiletamine/zolazepam, followed by an intravenous bolus of 5–7 mg/kg methohexital, and was maintained using endotracheal intubation and isoflurane (1.5 minimal alveolar concentration) in all study animals, which were ventilated with a 6–8 ml/kg tidal volume and 5 mmHg positive end-expiratory pressure (PEEP). Baseline arterial blood gas (ABG) measurements were obtained. Median sternotomy was performed and both pleural spaces entered. Once the absence of gross pulmonary abnormalities was confirmed, 30 000 U of heparin were administered and allowed to circulate for 3 min.

In the control group ($n = 4$), cardiac arrest was induced by aortic clamping and antegrade delivery of chilled St. Thomas Hospital cardioplegic solution via an aortic root cannula [16]. The lungs were flushed antegrade via the main pulmonary artery (PA) with 2 l of chilled OCS Lung solution (TransMedics Inc., Andover, MA, USA), with venting through the left atrial appendage (50 mg nitroglycerin in the first liter). Topical slush was

applied and ventilation was continued during the antegrade flush. During flush and cardioplegia delivery, 1.5–2 l of autologous whole blood was drained from the inferior vena cava (IVC) using a 32F venous cannula, tubing, and a reservoir bag (TransMedics Inc.). The heart was then excised, followed by the lungs en bloc. One liter of OCS Lung solution was delivered via the pulmonary vein ostia (250 ml to each) as a retrograde flush.

Cardiac arrest was achieved in the four treatment groups ($n = 16$) using a prolonged expiratory hold, followed by a 15-min "no-touch" period of agonal hypoxia, regardless of the time to arrest. At the end of this period, whole blood was collected from the IVC and the chest was temporarily closed. These swine then underwent either 1 or 2 h of warm ischemia, with or without postmortem mechanical ventilation (3–4 ml/kg tidal volume, 5 mmHg PEEP, 100% FiO_2). The four treatment groups were labeled as follows: the *1H vent* group (1 h of warm ischemia with ventilation), the *1H no vent* group (1 h of warm ischemia without ventilation), the *2H vent* group (2 h of warm ischemia with ventilation), and the *2H no vent* group (2 h of warm ischemia without ventilation). At the end of the treatment period, the chest was reopened and ventilation resumed (in unventilated animals). The lungs were flushed antegrade, procured, and flushed retrograde. The study design is summarized in Fig. 1.

Ex vivo lung perfusion

Following procurement and retrograde flush, the trachea and main PA of the bilateral lung block were instrumented using dedicated OCS Lung cannulae (TransMedics Inc.). The OCS Lung device (TransMedics Inc.) was primed with 1600 ml autologous whole blood and 700 ml OCS Lung solution, which were first passed through a leukocyte filter. Standard additives (1 g cefazolin, 200 mg ciprofloxacin, 200 mg voriconazole, 500 mg methylprednisolone, 1 vial multivitamins, 20 IU regular insulin, 4 mg milrinone, 20 mEq $NaHCO_3$, 50 mg nitroglycerin, and 50% dextrose) were infused into the circuit.

The donor lungs were placed in the perfusion chamber and the trachea and PA cannulae were connected to the OCS Lung. The flow was gradually increased to a rate of 1.5–2.0 l/min while warming the lungs to 37 °C. Ventilation was initiated with 6–8 ml/kg tidal volume and 5 mmHg PEEP once the temperature reached 34 °C. By 30 min of perfusion, all lungs were warm and ventilating appropriately. Using integrated sensors, the following variables were measured continuously during 24 h of EVLP: mean pulmonary artery pressure (mPAP), pulmonary vascular resistance (PVR), peak airway pressure (PAWP), tidal volume, PEEP, temperature, hematocrit (HCT), arterial (SaO_2), and venous (SvO_2) oxygen saturation. Dynamic compliance (C_{dyn}) was calculated post hoc using the formula:

$$\left(C_{dyn} = \frac{\text{tidal volume}}{\text{PAWP-PEEP}}, \frac{(\text{ml})}{(\text{mmHg})}\right)$$

During periods of prolonged preservation ("preservation mode"), the pump flow was maintained at 1.5–1.75 l/min. In accordance with clinical practice, the circuit oxygenator and ventilator was supplied with a gas mixture of 12% O_2, 5.5% CO_2, and balanced N_2.

Dedicated assessments of lung function were performed at 0, 2, 4, 6, 8, and 24 h of EVLP. Immediately before each of those time points, the flow was gradually increased from 1.5–1.75 to 2.5–3.0 l/min. Once the goal flow was reached, the oxygenator was washed out with a gas mixture of 6% CO_2 and balanced N_2. This supplied deoxygenated blood to the lungs, which were then ventilated with room air, allowing measurement of their native capacity for gas exchange. After 2 min in this "assessment mode," a blood sample was drawn for ABG and serum cytokine analysis and preservation mode was restored. PaO_2:FiO_2 (P:F) ratio was calculated manually. Flexible bronchoscopy was performed after each assessment. Supplemental $NaHCO_3$ and glucose were administered as needed based on the results of real-time blood sample analysis to maintain a serum bicarbonate of 20 mmol/l and a serum glucose of 120 mg/dl.

Measurement of pulmonary edema

The relative development of pulmonary edema was measured using lung block weights (pre- and post-EVLP), hematocrit (continuously measured during EVLP), and overall reservoir volume replacement (RVR, calculated after EVLP). Increases in weight, HCT, and RVR were indicative of edema, which was further assessed qualitatively by gross inspection and by serial flexible bronchoscopy.

Measurement of inflammatory expression

Aliquots of the perfusate were assessed for cytokine release in all groups. Levels of IL-1b, IL-6, IL-8, and IL-10 were measured on the Luminex platform (Luminex Corporation, Austin, TX, USA) using bead sets from EMD Millipore (Billerica, MA, USA).

Histologic assessment of lung injury

Following EVLP, samples of lung were formalin-fixed and embedded in paraffin (Sigma, St. Louis, MO, USA). Sections were cut to a thickness of 6 μm, incubated at 56 °C overnight, and stained with hematoxylin and eosin. Microscopic evaluation of tissue specimens chosen from the right upper lobe and left lower lobe of each set of study lungs was performed by a single investigator (A.P.-M.). Displayed images were captured using an Olympus BX51 microscope using a 10× objective (100× final magnification) and numerical aperture of 0.40 (Olympus Corporation, Tokyo, Japan) and an RT Spot camera using SPOT ADVANCED version 4.6 software (SPOT Imaging, Sterling Heights, MI, USA).

Statistical analysis

All groups were compared using hourly measurements of C_{dyn}, HCT, SaO_2, SvO_2, PVR, and mPAP and all available measurements (at 0, 2, 4, 6, 8, and 24 h) of P:F ratio over 24 h. Each analysis used a mixed linear model implemented in SAS's MIXED procedure (v.9.4; SAS Institute Inc., Cary, NC, USA), with an exponential correlation structure (SP(EXP) in the MIXED procedure's RANDOM statement) to account for temporal dependence between measurements on a pair of lungs. Based on preliminary findings, some outcomes were transformed before analysis: The common logarithms of PVR, mPAP, SvO_2, and P:F ratio were analyzed, and the arcsine square root transformation was used for SaO_2. C_{dyn} and HCT were not transformed. Adjusted averages (accounting for missing observations, when they existed) and standard errors at each time point were calculated on the transformed scale. Plots display adjusted averages and plus or minus one standard error, backtransformed to the original measurement scale.

Statistical tests assessed (i) differences between all groups in group averages over all time points ("group" effects in Table 3); (ii) differences between all groups in the pattern over time ("interaction" effects in Table 3); and (iii) pairwise group differences. Pairwise tests compared each pair of groups using values at 8 and 24 h, and also using the change from baseline to 8 h and the change from 8 to 24 h. Baseline was defined as 0 h for P:F ratio and 1 h for all other outcomes. *P*-values were not adjusted for multiple comparisons. Numerical data are presented in the text as mean ± standard deviation.

Results

All procurements and EVLP runs were technically successful. The interval between cross-clamp and reperfusion on the OCS Lung was less than 30 min all individual studies.

Physiologic assessment during EVLP

Perfusion

Goal flow rates were achieved in all individual studies without undue rises in PVR or mPAP in both preservation (1.5–1.75 l/min) and assessment modes (2.5–3.0 l/min). The volumes of glucose and $NaHCO_3$ supplementation did not significantly differ between the five groups. The initial (0 h) serum lactate level was significantly elevated in the *2H no vent* group compared with the other four groups ($P < 0.0001$), but were similar among the other four groups.

Pulmonary physiology

Hemodynamic and ventilatory measures were compared over 24 h of EVLP, broken into three comparisons: baseline to 8 h (to simulate the current clinical standard for EVLP), 8–24 h (to simulate prolonged preservation), and global effects of group assignment and time on EVLP on the outcome measures (Fig. 2). Initial PVR and mPAP were similar across all five groups. The *1H no vent, 2H vent*, and *2H no vent* groups had the lowest (worst) initial C_{dyn} compared with the control and *1H vent* groups (Fig. 2).

After 8 h of EVLP, only the *2H vent* group had significantly elevated PVR and mPAP compared with control. The lower baseline C_{dyn} values of the *1H no vent, 2H vent*, and *2H no vent* groups persisted at this time point (Fig. 2a–c, Table 1a). Changes in PVR, mPAP, and C_{dyn} from baseline did not meaningfully differ from that of control over this period (Table 1b). The results of pairwise comparisons of the noncontrol groups at 8 h according to point values and the changes from baseline are presented in Tables S1a and b.

Most significant differences between groups in PVR, mPAP, and C_{dyn} between groups disappeared between 8 and 24 h of preservation, except for the *1H no vent* group, which had significantly lower C_{dyn} and mPAP at 24 h compared with control (Fig. 2a–c, Table 2a). However, the overall change in PVR, mPAP, and C_{dyn} between 8 and 24 h was small and similar to control, except for the significant reduction in PVR and mPAP

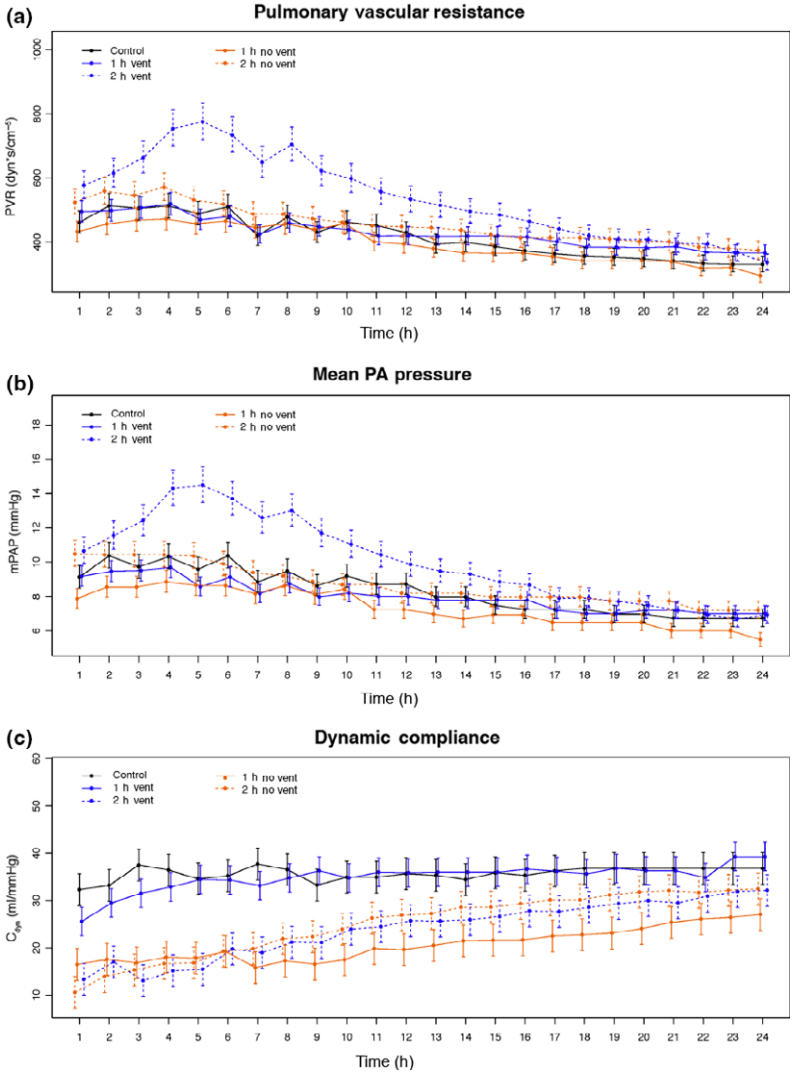

Figure 2 Physiologic parameters measured during *ex vivo* lung perfusion (EVLP). Note improvement of (a) PVR, (b) mPAP, and (c) C_{dyn} to control levels over 24 h in all groups. C_{dyn}, dynamic compliance; mPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance.

in the *2H vent* group over this time (Fig. 2a–c, Table 2b). The results of pairwise comparisons between the noncontrol groups according to the point values at 24 h and changes from 8 to 24 h are presented in Table S2a and b. Global effect analysis of PVR, mPAP, and C_{dyn} demonstrated that each was affected both by group assignment and time (Table 3).

Pulmonary edema

Pulmonary edema that developed before and during EVLP was proportional to the severity of DCD insult. Pre-EVLP weights of lung blocks in the *1H no vent*, *2H vent*, and *2H no vent* were higher than those in the control and *1H vent* groups, indicating *in situ* development of pulmonary edema in more injured groups. Post-EVLP weights in these groups were higher compared with both their pre-EVLP weights and the post-EVLP weights in the control and *1H vent* groups (Fig. 3a). Greater RVR was required in each of the treatment groups compared with controls (Fig. 3b). Starting hematocrit was higher in all of the treatment groups compared with control and remained significantly elevated after 8 h of EVLP in the *1H vent* and *2H no vent* groups, a disparity that persisted over the remainder of the study period (Tables 1a and 2a, Fig. 3c). Differences in hematocrit were significantly influenced by both time and group assignment (Table 3).

Oxygenation

The initial (0 h) P:F ratios were lower in lungs subjected to more severe DCD injury (334 ± 75 in the control group versus 213 ± 32 in the *2H no vent* group). The P:F ratio in the control group increased significantly over the first 8 h relative to all treatment groups over this interval (Table 1a, Fig. 4). P:F

Table 1. (a) Pairwise point comparisons versus control at 8 h. (b) Pairwise comparisons of change from baseline versus control at 8 h.

Outcome	Control	1H Vent	*P*-value (versus control)	1H No Vent	*P*-value (versus control)	2H Vent	*P*-value (versus control)	2H No Vent	*P*-value (versus control)
(a)									
Vascular resistance (dyn s/cm^5)	477.97	459.52	0.696	457.09	0.674	704.04	*<0.001*	486.97	0.86
Mean PA pressure (mmHg)	9.49	8.75	0.401	8.65	0.365	13.00	*0.002*	9.19	0.754
Dynamic compliance (ml/mmHg)	36.51	34.77	0.701	17.23	*<0.001*	21.16	*0.001*	21.93	*0.002*
Hematocrit (%)	17.00	24.40	*<0.001*	17.25	0.907	20.75	0.079	28.25	*<0.001*
SaO_2 (%)	93.09	93.29	0.951	85.34	0.06	84.07	*0.032*	90.48	0.057
SvO_2 (%)	94.43	90.28	*0.041*	85.32	*<0.001*	84.43	*<0.001*	90.39	0.057
P:F ratio (mmHg)	483.84	287.21	*0.019*	206.06	*<0.001*	222.59	*<0.001*	223.56	*<0.001*
(b)									
Vascular resistance (dyn s/cm^5)	11.50	−34.00	0.29	21.75	0.84	148.50	0.126	−35.00	0.32
Mean PA pressure (mmHg)	0.25	−0.40	0.406	0.75	0.612	2.75	0.142	−1.25	0.117
Dynamic compliance (ml/mmHg)	4.23	9.21	0.222	0.75	0.418	7.85	0.399	11.35	0.098
Hematocrit (%)	0.50	0.00	0.777	−3.50	*0.032*	−0.50	0.591	−1.75	0.227
SaO_2 (%)	−1.73	2.88	0.059	1.88	*0.377*	0.80	0.392	15.25	*<0.001*
SvO_2 (%)	−1.45	1.48	0.162	2.47	*0.293*	2.67	*0.043*	17.33	*<0.001*
P:F ratio (mmHg)	157.14	50.00	0.142	−91.07	*0.017*	−15.12	0.089	10.60	0.228

H, hours; mPAP, mean pulmonary artery pressure; PA, pulmonary artery; P:F, PaO_2:FiO_2; SaO_2, systemic arterial oxygen saturation; SvO_2, systemic venous oxygen saturation.

Pairwise point comparisons of (a) absolute values and (b) differences from baseline versus control between 0 and 8H. For mPAP, VR, SaO_2, SvO_2, and PF, hypothesis tests were conducted on transformed scales, and so do not reflect direct comparison of the values shown. Statistically significant *P*-values shown in italics.

Table 2. (a) Pairwise point comparisons versus control at 24 h. (b) Pairwise comparisons of change from 8 h versus control at 24 h.

Outcome	Control	1H Vent	*P*-value (versus control)	1H No Vent	*P*-value (versus control)	2H Vent	*P*-value (versus control)	2H No Vent	*P*-value (versus control)
(a)									
Vascular resistance (dyn s/cm^5)	330.60	365.34	0.321	295.53	0.291	336.12	0.876	374.37	0.242
Mean PA pressure (mmHg)	6.74	7.00	0.69	5.48	*0.043*	6.93	0.782	7.20	0.512
Dynamic compliance (ml/mmHg)	36.78	39.27	0.582	27.03	*0.041*	32.12	0.327	32.44	0.361
Hematocrit (%)	16.75	23.40	*0.001*	17.50	0.725	17.25	0.815	24.50	*<0.001*
SaO_2 (%)	93.63	94.02	0.898	88.13	0.152	88.82	0.203	92.64	0.772
SvO_2 (%)	94.95	91.11	*0.038*	88.30	*0.004*	89.50	*0.011*	92.82	0.22
P:F ratio (mmHg)	524.93	372.56	0.102	209.65	*<0.001*	252.41	*0.001*	234.21	*<0.001*
(b)									
Vascular resistance (dyn s/cm^5)	−148.50	−97.20	0.277	−166.25	0.616	−390.50	*0.006*	−112.75	0.435
Mean PA pressure (mmHg)	−2.75	−1.80	0.344	−3.25	0.391	−6.50	*0.032*	−2.00	0.46
Dynamic compliance (ml/mmHg)	0.28	4.49	0.429	9.80	0.091	10.96	0.058	10.51	0.069
Hematocrit (%)	−0.25	−1.00	0.748	0.25	0.839	−3.50	0.187	−3.75	0.156
SaO_2 (%)	0.55	0.34	0.951	2.80	0.677	5.60	0.419	2.20	0.7
SvO_2 (%)	0.53	0.52	0.98	3.00	0.69	7.60	0.361	2.68	0.551
P:F ratio (mmHg)	25.40	13.02	0.551	9.29	0.831	28.33	0.883	12.26	0.908

H, hours; mPAP, mean pulmonary artery pressure; PA, pulmonary artery; P:F, PaO_2:FiO_2; SaO_2, systemic arterial oxygen saturation; SvO_2, systemic venous oxygen saturation.

Pairwise point comparisons of (a) absolute values and (b) differences from baseline versus control between 8 and 24H. For mPAP, VR, SaO_2, SvO_2, and PF, hypothesis tests were conducted on transformed scales, and so do not reflect direct comparison of the values shown. Statistically significant *P*-values shown in italics.

Table 3. Global effect testing.

Outcome	Effect	*P*-value
Vascular resistance	Time	*<0.0001*
	Group	*0.0139*
	Interaction	0.1411
Mean PA pressure	Time	*<0.0001*
	Group	*0.0088*
	Interaction	*0.0236*
Dynamic compliance	Time	*<0.0001*
	Group	*0.0015*
	Interaction	0.1869
Hematocrit	Time	*0.0003*
	Group	*<0.0001*
	Interaction	0.264
SaO_2	Time	*<0.0001*
	Group	0.1719
	Interaction	*<0.0001*
SvO_2	Time	*<0.0001*
	Group	*0.0082*
	Interaction	*<0.0001*
P:F ratio	Time	0.775
	Group	*0.0004*
	Interaction	0.7805

PA, pulmonary artery; P:F, PaO_2:FiO_2; SaO_2, systemic arterial oxygen saturation; SvO2, systemic venous oxygen saturation.

Global effect testing. Only *P*-values displayed. Statistically significant values in italics.

remained stable in each of the treatment groups except for *1H no vent*, which decreased between baseline and 8 h (Table 1b). Except for the *1H vent* group (P:F 404 ± 209), P:F remained significantly lower than control (P:F 541 ± 170) at 24 h in all treatment groups; the lowest values were seen in the *1H no vent* group (216 ± 82, Table 2a, Fig. 4). Overall, P:F ratio was significantly influenced by group assignment but not by time (Table 3).

Lung injury and inflammatory expression

Gross injury

Before the initiation of EVLP, we observed progressive parenchymal abnormalities (e.g., superficial punctate hemorrhage, patchy contusion, and interlobular edema) proportional to the severity of DCD injury (longer warm ischemia, no ventilation). After 24 h of EVLP, most hemorrhagic abnormalities and atelectasis had resolved in all four treatment groups, although parenchymal edema persisted in the *1H no vent*, *2H vent*, and *2H no vent* groups. Representative samples of the initial (0 h) and final (24 h) gross appearance of swine lungs in our study are depicted in Figure S1.

Histologic injury

No consistent relationship could be detected between the extent of warm ischemic/atelectatic insult and histologic abnormalities after 24 h of EVLP. Microscopic appearance of lung tissue samples was associated primarily with the gross appearance of the lung

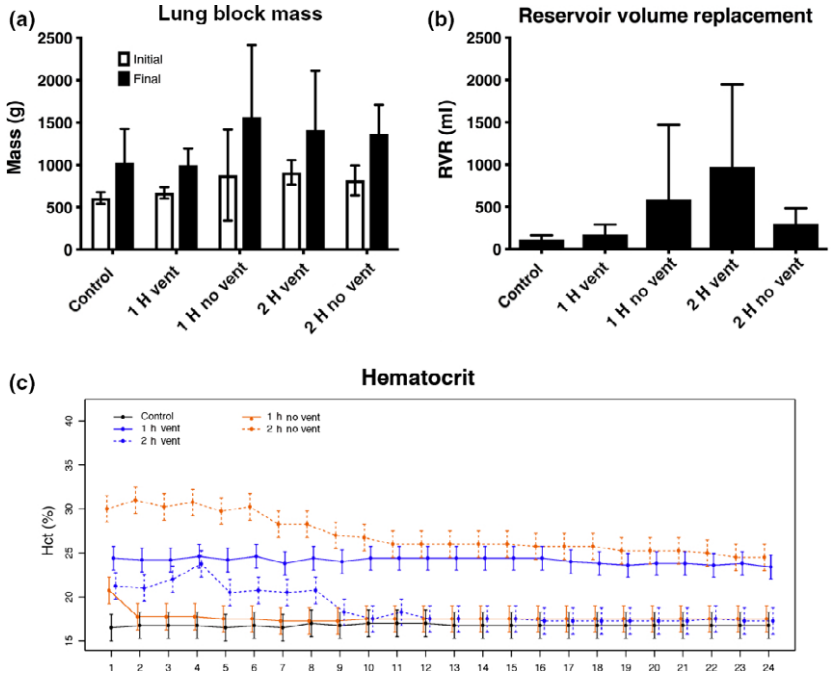

Figure 3 Measurement of pulmonary edema before and after 24 h of EVLP. (a) Pre- and post-EVLP lung block weights were higher in more severely injured lungs (longer warm ischemia, no ventilation). (b) More severely injured lungs generally required greater RVR. (c) Perfusate hematocrit was elevated in all treatment groups at baseline but normalized to control levels in some groups. EVLP, *ex vivo* lung perfusion; RVR, reservoir volume replacement.

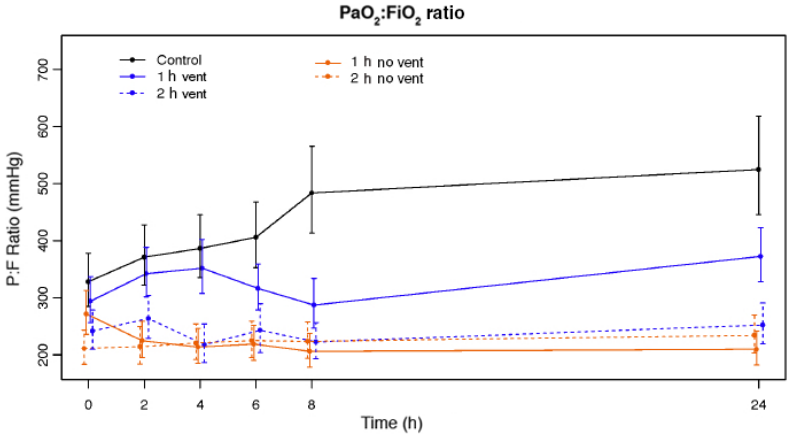

Figure 4 Changes in oxygenation (measured by P:F ratio) during 24 h of EVLP. EVLP, *ex vivo* lung perfusion; P:F: PaO_2:FiO_2.

parenchyma. Samples taken from grossly hemorrhagic portions of lung demonstrated microscopic alveolar hemorrhage, while samples taken from normal-appearing areas appeared histologically normal (Figure S2).

Cytokine expression

Perfusate expression of both proinflammatory (IL-1b, IL-6, and IL-8) and anti-inflammatory (IL-10) cytokines generally increased throughout EVLP (Figure 5). Expression of IL-6, IL-8, and IL-10 at 24 h increased across groups proportional to severity of DCD injury.

Discussion

This study provides novel insight into the recovery of lung mechanics after DCD over 24 h using continuous monitoring with a blood-based EVLP platform. We evaluated the relative contributions of the two fundamental components of DCD-associated injury: atelectasis (from hypoventilation) and ischemia (from hypotension/shock). Lungs with even severe injury demonstrate improvements in hemodynamics, compliance, and gross morphology after 24 h of EVLP. However, fixed gas exchange deficits were evident if the ischemic interval exceeded 1 h or if postmortem ventilation was withheld.

The impact of the donor ischemic interval is clearly demonstrated. Longer ischemic time was associated with greater initial impairment in pulmonary hemodynamics and compliance, greater inflammation, worse gross morphology, and increased edema. This was seen in both ventilated and nonventilated lungs, consistent with other groups demonstrating progressive lung injury in animal models exposed to longer ischemic intervals [9,10]. Egan *et al.* [11,12] demonstrated acceptable post-transplant function in canine models of single and bilateral lung transplantation without EVLP from donors subjected to up to 4 h of warm ischemia, but the best performance was observed in animals down only 1–2 h.

Consistent with other groups, we also confirm the importance of early ventilation in DCD [9,10,17]. The *1H vent* lungs performed almost identically to controls by 8–24 h of EVLP. Conversely, the *1H no vent* lungs had the greatest fixed deficits in oxygenation. However, with 2 h of donor ischemia, the effects of ventilation were dampened and at times paradoxical. There was evidence of greater edema in the *2H no vent* group compared with the *2H vent* group. However, expression of IL-1b and IL-6 was actually higher in the *2H vent* group compared with the *2H no vent* group. Also, PVR and PAP became worse during the first 8 h of EVLP in the *2H vent* group compared with the *2H no vent* group. These findings suggest that longer periods of warm ischemia may lead to injury of the pulmonary vasculature and surrounding tissue that may then be aggravated by ventilation. Collectively, our data suggest that both ischemia and atelectasis can lead to lung injury and the combination of the two will cause unpredictable, but generally poor, early graft function.

Ex vivo lung perfusion is a promising modality for evaluating the effects of DCD-induced lung injury and several investigators have described its use with acellular perfusates for this purpose in animal and human settings [18]. Nakajima *et al.* [13] demonstrated good post-transplant function in DCD canine lungs subjected to 4 h of warm ischemia followed by 3.5 h of acellular EVLP. Machuca *et al.* reported 32 clinical transplants using DCD lungs after acellular EVLP. These recipients experienced a shorter hospital stay, less PGD, and shorter duration of postoperative ventilation compared with recipients of non-EVLP DCD lungs [19].

Suzuki *et al.* [20] reported a successful DCD transplant after EVLP using a donor who suffered multiple episodes of cardiac arrest after severe penetrating

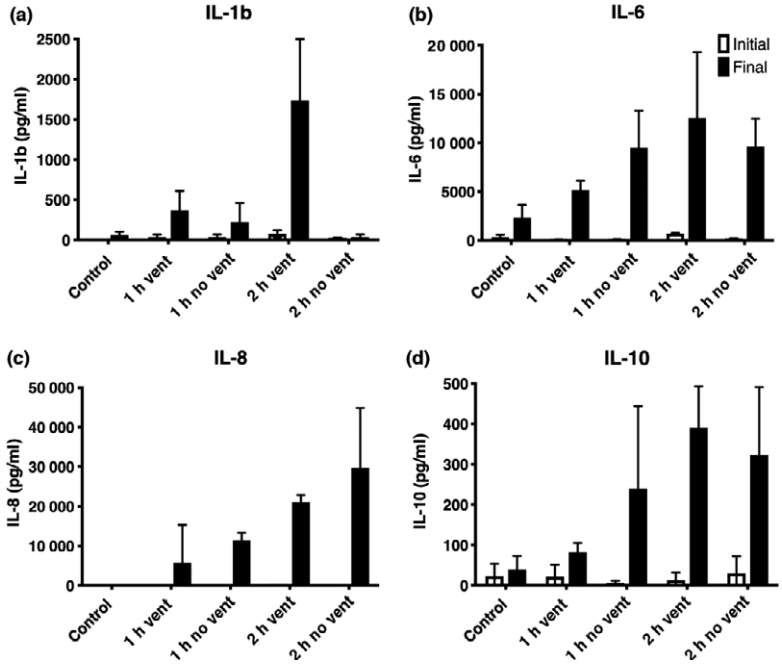

Figure 5 Cytokine expression before and after EVLP. Serum levels of IL-6, IL-8, and IL-10 at 24 h correlated positively with injury severity. EVLP, *ex vivo* lung perfusion; IL, interleukin.

trauma. The lungs demonstrated steady improvement in airway pressure and PVR, as well as good gas exchange, over 3 h of EVLP and were subsequently transplanted.

Data describing the use of blood-based EVLP in the DCD setting are limited. Bozso *et al.* [8] reported good early results of three lung transplants using DCD donors after 80–150 min of blood-based EVLP. The EXPAND trial is currently evaluating the use of blood-based EVLP with extended-criteria donors, including DCD [7]. Our group recently showed that autologous whole donor blood can be used in these systems to significantly improve the stability of lungs on EVLP [15]. The current study is the first to use blood-based EVLP to evaluate the functional recovery of DCD lungs over 24 h.

In our swine model, PVR, mPAP, and C_{dyn} all improved over 24 h of EVLP in all lungs in all five groups, regardless of antecedent insult. The significant combined effects of time on EVLP and injury severity on recovery suggests that a prolonged interval (>8 h) on EVLP may be necessary to ameliorate the effects of DCD-related injury. Despite this, only lungs in the control and in the *1H vent* groups consistently demonstrated transplantable P:F ratios. The combination of severe edema and poor oxygenation with normal PAP and increased inflammatory expression in more injured lungs is analogous to PGD related to ischemia–reperfusion injury (IRI) following implantation. The current clinical management of PGD is generally supportive, consisting primarily of low-tidal volume ventilation over an extended period, similar to the model of prolonged EVLP we describe [21]. Combined with our finding that differences in oxygenation were a function of severity of DCD insult and not time of on EVLP, we conclude that our model of prolonged EVLP is capable of reversing pulmonary vasoconstriction and atelectasis related to DCD but does not correct the epithelial and endothelial dysfunction resulting from IRI [21].

What can be done prior to donation and during EVLP to mitigate the effects of prolonged donor ischemia and effects of atelectasis after transplant? Possible interventions include topical hypothermia and donor ECMO [22–25]. However, many postconditioning therapies have the potential to reverse injury and further improve lung function on EVLP. These include intrabronchial delivery of surfactant and/or ß$_2$-adrenergic receptor agonists, addition of mesenchymal stem cells or adenosine A2A receptor agonists to the perfusate, and the use of adenoviral vectors for gene therapy [26–31].

Limitations

Some aspects of our model could be a challenge to translate to the clinical environment, particularly in the setting of uncontrolled DCD (uDCD) [2]. First, heparin was administered prior to cardiac arrest. A strategy of postmortem heparin administration was reported by

Fernández *et al.* and was also described in a recent review of DCD lung donation by Erasmus *et al.* [2,25]. Moreover, Van De Wauwer *et al.* [32] demonstrated, also in a porcine uDCD model and using EVLP, that retrograde flush via the pulmonary veins before cardiac arrest had a greater protective effect than postmortem heparinization during functional assessment using EVLP. Wallinder *et al.* [33] demonstrated similar findings.

Second, autologous donor whole blood was used as a major component of the perfusate. Blood was collected after cardiac arrest without issue in our model. We believe this would also be the case in a clinical setting, as demonstrated by Fernández *et al.* [25], who relied on large-volume blood collection as a component of assessment of donor gas exchange after arrest.

Third, hypothermic lung preservation during the DCD interval may have allowed longer or better-tolerated ischemic intervals. In a swine model of uDCD and topical cooling by pleural lavage with saline slush, Steen *et al.* [22] demonstrated adequate post-transplant function after 6 h of static preservation *in situ*. This was omitted from the current study to focus on the isolated effects of warm ischemia and postmortem ventilation.

Fourth, the translatability of our results to human lungs, which may be more resistant to warm ischemia than swine lungs, is unclear. Furthermore, standard transplant criteria (P:F ratio >300 with normal morphology and hemodynamics) were extrapolated from clinical practice as a determination of suitability for transplantation. It is possible that lower P:F ratios would allow reasonable clinical outcomes. A chronic survival model of post-EVLP swine transplant would allow better characterization of acceptable threshold parameters.

Finally, to characterize the separate effects of ischemic time and postmortem ventilation, available animals were divided so that the number of subjects in each group ($n = 4$) is relatively small. This should be considered when interpreting these results.

Conclusions

Normothermic blood-based EVLP offers a unique look at the mechanics of lung recovery over 24 h after a stepwise increase in severity of DCD insult. In a swine model, we found that ≤2 h of warm ischemia did not affect recovery of hemodynamic or ventilatory parameters. Early postmortem ventilation maximized recovery. However, EVLP could not reverse impairments in gas exchange in severely injured lungs. Further studies are required to extend the acceptable ischemic interval and to clarify the EVLP measurements that have the greatest effect on transplant outcomes.

Authorship

JRS: Intellectual conception of project, extensive literature review, data collection, data analysis, figure and table construction, principal composition of manuscript, manuscript editing, final approval of manuscript. LMM: Intellectual conception of project, data collection, data analysis, manuscript editing, final approval of manuscript. PAI: Intellectual conception of project, data collection, manuscript editing, provision of study animals and laboratory space, final approval of manuscript. RZB: Data analysis, figure and table construction, manuscript editing, final approval of manuscript. HH: Data analysis, sample processing, manuscript editing, final approval of manuscript. TLI: Intellectual conception of project, data collection, manuscript editing, final approval of manuscript. BH: Data collection, manuscript editing, final approval of manuscript. AP-M: Intellectual conception of project, data collection, data analysis, partial construction of figures, manuscript editing, final approval of manuscript. GL: Intellectual conception of project, data collection, data analysis, manuscript editing, final approval of manuscript.

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Conflicts of interest

The authors have declared no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Gross comparison of porcine lungs before (0H) and after (24H) prolonged EVLP.

Figure S2. Representative histologic sections from (a) grossly normal and (b) grossly hemorrhagic segments of porcine lung after 24 h of EVLP.

Table S1. (a) Pairwise point comparisons of noncontrol groups at 8 h of EVLP. (b) Pairwise comparisons of change from baseline between noncontrol groups at 8 h of EVLP.

Table S2. (a) Pairwise point comparisons of noncontrol groups at 24 h of EVLP. (b) Pairwise comparisons of change from 8 h between noncontrol groups at 24 h of EVLP.

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