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Ex Vivo Assessment of Porcine Donation After Circulatory Death Lungs That Undergo Increasing Warm Ischemia Times

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Background. Increased utilization of donation after circulatory death (DCD) lungs may help alleviate the supply/demand mismatch between available donor organs and lung transplant candidates. Using an established porcine DCD model, we sought to determine the effect of increasing warm ischemia time (WIT) after circulatory arrest on lung function during ex vivo lung perfusion (EVLP). **Methods.** Porcine donors (n = 15) underwent hypoxic cardiac arrest, followed by 60, 90, or 120 minutes of WIT before procurement and 4 hours of normothermic EVLP. Oxygenation, pulmonary artery pressure, airway pressure, and compliance were measured hourly. Lung injury scores were assessed histologically after 4 hours of EVLP. **Results.** After EVLP, all 3 groups met all the criteria for transplantation, except for 90-minute WIT lungs, which had a mean pulmonary artery pressure increase greater than 15%. There were no significant differences between groups as assessed by final oxygenation capacity, as well as changes in pulmonary artery pressure, airway pressure, or lung compliance. Histologic lung injury scores as well as lung wet-to-dry weight ratios did not significantly differ between groups. **Conclusions.** These results suggest that longer WIT alone (up to 120 minutes) does not predict worse lung function at the conclusion of EVLP. Expanding acceptable WIT after circulatory death may eventually allow for increased utilization of DCD lungs in procurement protocols.

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Over the past decade, lung transplant volume in the United States increased by 66%, from 1405 transplants in 2006, to 2327 transplants in 2016. Unfortunately, waitlist additions have increased at a similar rate, with 2789 new registrations in 2016.[1] Various centers are now performing transplants using Maastricht category III controlled donation after

circulatory death (DCD) lungs, in an effort to maximize the number of transplants performed.[2] As the supply of traditional donation after brain death lungs remains stagnant and waitlist mortality continues to rise, DCD lungs are becoming an important source of additional organs.[3] Studies to date suggest that outcomes with Maastricht category III DCD lungs are similar to outcomes with donation after brain death lungs.[4-6] Even with inclusion of DCD lungs in transplant protocols, overall utilization remains low, and the supply of acceptable organs has not fulfilled the ever-increasing demand.[7,8]

Uncontrolled DCD lungs (Maastricht categories I and II) are another potential source of organs but are currently not utilized for transplantation in the United States due to ethical concerns, logistical barriers, and graft function uncertainty.[8,9] Warm ischemia time (WIT) varies greatly depending on whether the patient dies in the hospital or out in the community, and whether or not adequate, timely cardiopulmonary resuscitation is performed. With the availability of ex vivo lung perfusion (EVLP) as a platform for graft assessment and potential rehabilitation, uncontrolled DCD lungs may one day become a valuable source of transplantable organs.[8]

Our laboratory has experience studying DCD lung transplantation using a porcine model of hypoxic cardiac arrest.[10] Using this model, we have evaluated the effect of cold ischemia time before and after EVLP, ventilation strategies during EVLP, and ex vivo lung rehabilitation with adenosine receptor pathway modulation.[11-15] Building on our previous

work, the objective of the current study was to determine the effect of increasing WIT after circulatory arrest on lung function during EVLP. We anticipate that EVLP will be standard practice for the assessment of uncontrolled DCD lungs before transplantation, allowing use of these lungs to become a reality. Therefore, we sought to understand how differences in WIT affect commonly used lung function parameters for determining whether or not to transplant lungs at the conclusion of EVLP. We hypothesized that DCD lungs exposed to increasing WIT up to 120 minutes after circulatory arrest would still meet transplant criteria after 4 hours of normothermic EVLP.

MATERIALS AND METHODS

Animals

Mature domestic swine of both sexes (27-34 kg) were used. All animals received humane care in compliance with the 2011 *Guide for the Care and Use of Laboratory Animals, 8th edition* as recommended by the U.S. National Institutes of Health. The study was reviewed and approved the University of Virginia Animal Care and Use Committee.

Study Design

Animals were randomized to 3 groups that underwent 60 (60 WIT, n = 3), 90 (90 WIT, n = 6), or 120 (120 WIT, n = 6) minutes of WIT after hypoxic arrest. Subsequently, all lungs underwent 4 hours of normothermic EVLP.[16] Exclusion criteria according to the Toronto protocol were used in this study to determine transplant suitability: partial pressure of oxygen (PO_2) to fraction of inspired oxygen ratio (FiO_2) < 400 mm Hg, greater than 15% increase of pulmonary artery (PA) pressure, and greater than 15% deterioration of airway pressures and compliance.[17] Group 60 WIT served as the control arm of the study based on previous data from our laboratory showing reasonable ex vivo lung function after hypoxic cardiac arrest and 60 minutes of warm ischemia.[10]

Hypoxic Cardiac Arrest

Animals were anesthetized (intramuscular injection of 6 mg/kg tiletamine/zolzazepam and 2 mg/kg xylazine), intubated, weighed, and ventilated (3% isoflurane; tidal volume, 8 mL/kg; respiratory rate, 18-20 breaths/min; positive end-expiratory pressure, 5.0 cmH$_2$O, and 1.0 FiO$_2$), as previously described.[18] Systemic heparin was not administered. A baseline arterial blood gas (ABG) sample was obtained and continuous electrocardiogram monitoring initiated. The endotracheal (ET) tube was clamped with the lungs inflated to half of tidal volume to initiate hypoxia. Every 1 to 2 minutes thereafter, an ABG sample was obtained to characterize the physiologic changes taking place during hypoxia. pH, partial pressure of arterial carbon dioxide ($PaCO_2$), partial pressure of arterial oxygen (PaO_2), bicarbonate level (HCO_3), base excess, and lactic acid concentration were recorded with each sample. Mean arterial pressure (MAP) and heart rate were recorded continuously until asystole, at which point the WIT began.

Donor Lung Procurement

At completion of the prescribed WIT, ventilation was resumed at the prearrest settings. The thorax was accessed via median sternotomy, the pericardium and pleura were incised, and the main PA was cannulated with a cardioplegia cannula (Terumo Heart, Ann Arbor, MI). Prostaglandin-E1 (500 μg; Pfizer, New York, NY) followed by 1.5 L of 4°C Perfadex (XVIVO Perfusion, Englewood, CO) with 15 000 IU heparin (Hospira Inc., Lake Forest, IL) was flushed through the lungs and drained via left atrial (LA) appendage atriotomy. The venae cavae were ligated, and the lungs were cooled with topical ice slush. After completion of the flush, the trachea was again clamped with the lungs inflated to half of tidal volume and the heart-lung bloc procured.

Lung Preparation and EVLP

After back-table removal of the heart and preparation of the trachea, main PA, and LA cuff, an ET tube and yellow and green cannulas (XVIVO Perfusion, Englewood, CO) were sutured into place to set up a closed atrial system, as described by the Toronto group.[16,19] The lungs were maintained cold on a bed of ice during preparation. A retrograde flush was performed with 500 mL of 4°C Perfadex (at a height of 90 cm above the lungs) before placing the lungs on the EVLP circuit. The target cold ischemic period from end of WIT to initiation of EVLP was 1 hour.

EVLP was performed as previously described.[10] The circuit was primed with 2 L of acellular Steen Solution (XVIVO Perfusion, Englewood, CO) at room temperature, supplemented with 500 mg cefazolin (APP Pharmaceuticals, Schaumburg, IL), 500 mg methylprednisolone (Pfizer, New York, NY), and 10 000 IU heparin.[16,17] The lungs were transferred to the EVLP chamber and flow was initiated through the LA cannula to remove air from the pulmonary vasculature and PA cannula. The inflow tubing was then connected to the PA cannula and forward flow was initiated at 0.2 L/min. Over the first 30 minutes, the perfusate was warmed to 37°C using a heat exchanger. At 32°C, ventilation was initiated (tidal volume 8 mL/kg, respiratory rate 8 breaths/min, positive end-expiratory pressure 5.0 cmH$_2$O, FiO$_2$ 0.21). Over the subsequent 30 minutes, flow was increased to a target rate of 40% of estimated cardiac output (100 mL/kg donor body weight), which was achieved in all experiments. A tri-gas mixture (86% nitrogen, 8% carbon dioxide, 6% oxygen) was used to deoxygenate the perfusate with a target inflow partial pressure of carbon dioxide (PCO_2) of 35-45 mm Hg. LA pressures were maintained between 2 and 5 mm Hg by adjusting the height of the perfusate reservoir.

Lung Function Assessment

EVLP was run continuously for 4 hours. At each hour, lung recruitment to a peak airway pressure of 20 cm H$_2$O was performed and the FiO$_2$ was increased to 1.0 for 15 minutes. Inflow (PA) and outflow (LA) perfusate samples were obtained to measure the functional partial pressure of oxygen (PO_2). Peak and plateau airway pressures (cm H$_2$O) were recorded after lung recruitment and used to calculate dynamic and static compliance (mL/cm H$_2$O). After 4 hours, the lungs were removed from the circuit and flushed antegrade with 500 mL of 4°C Perfadex.

Pulmonary Edema

Three samples of fresh tissue (from the same anatomic locations on each set of lungs) were obtained at the end of EVLP and weighed individually. After desiccation in a vacuum oven, dry tissue weights were obtained and wet-to-dry weight ratios calculated. For each set of lungs, all 3 sample
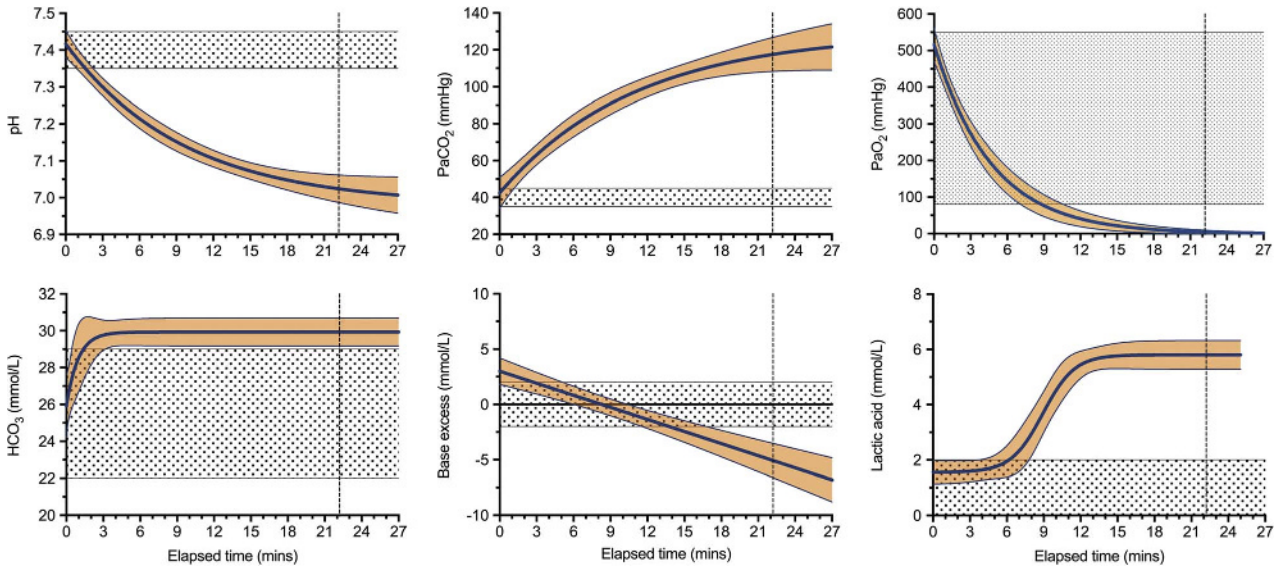
FIGURE 1. Physiologic changes during hypoxia from ET tube clamp to circulatory arrest. pH, $PaCO_2$, PaO_2, HCO_3, base excess, and lactic acid are shown. The horizontal shading indicates normal ranges for each parameter. The vertical dashed line indicates the mean time of death (22.2 minutes). Curves are shown with 95% confidence interval.

ratios were averaged to obtain 1 wet-to-dry weight ratio per set of lungs to assess overall edema accumulation.

Histology

The lower lobe of each left lung was inflation-fixed in formalin (20 cm H_2O) overnight. Four tissue samples per lung were obtained, paraffin-embedded, and sectioned. Lung sections from each sample were stained with hematoxylin-eosin (H&E) and used to calculate an average lung injury severity score. H&E sections were evaluated by a masked pathologist, and lung injury severity scores were based on polymorphonuclear cells per $40\times$ high-powered field (0, $\leq$ 5; 1, 6-10; 2, 11-20; 3, $\geq$ 20), alveolar edema (0 $\leq$ 5%, 1 = 6-25%, 2 = 26-50%, 3 $\geq$ 50%), and interstitial inflammation (0 = none, 1 = minimal, 2 = moderate, 3 = severe), as previously described.[15]

Statistical Analysis

Statistical comparisons between groups were conducted using 1-way analysis of variance with Bonferroni's correction for multiple comparisons (60 WIT vs 90 WIT, 60 WIT vs 120 WIT, 90 WIT vs 120 WIT) or Student t test depending on the number of groups in each analysis. Data are reported as mean ± standard error of the mean, with P value for

significance of 0.05. Linear and nonlinear (exponential, sigmoidal, and polynomial) regression were used to generate best-fit lines with 95% confidence interval bands for data shown in Figures 1 and 2. All 4 models were compared with goodness of fit testing to determine which model best represented each dataset. All statistical analyses were performed using Prism 7 (GraphPad Software, La Jolla, CA).

RESULTS

Donor Animal Characteristics and Elapsed Time

Donor animal characteristics and the elapsed time at each stage of the experiment are shown in Table 1. There were no significant differences between groups in terms of donor animal weight, baseline PaO_2/FiO_2, or elapsed time from ET tube clamp to asystole, with a mean weight for all animals of 30 ± 0.7 kg, baseline PaO_2/FiO_2 of 494 ± 22 mm Hg, and time from clamp to asystole of 22.2 ± 1.7 minutes. Since groups were randomized based on duration of WIT, there were significant differences in time from ET tube clamp to cold flush, time from ET tube clamp to initiation of EVLP, and total elapsed time from ET tube clamp to completion of EVLP.
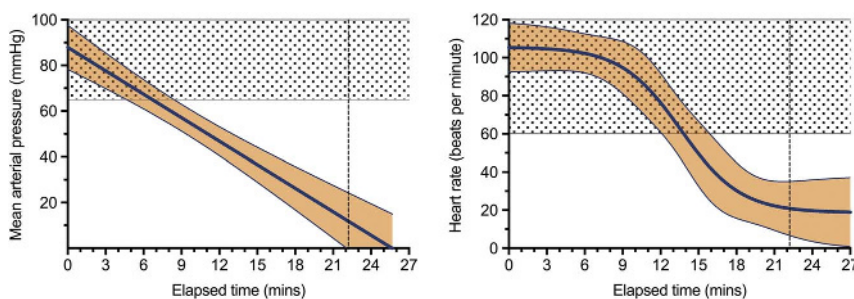

FIGURE 2. MAP and heart rate during hypoxia from ET tube clamp to circulatory arrest. The horizontal shading indicates normal ranges. The vertical dashed line indicates the mean time of death (22.2 minutes). Curves shown with 95% confidence interval.

TABLE 1.

Donor animal characteristics and elapsed time during experiment

	60 WIT (n = 3)	90 WIT (n = 6)	120 WIT (n = 6)	*P*
Donor characteristics				
Animal weight, kg	29.2 ± 1.2	29.8 ± 1.0	30.6 ± 1.5	0.78
Initial PaO_2/FiO_2	494 ± 54	509 ± 23	481 ± 46	0.87
Female: n (%)	2 (66)	5 (83)	4 (66)	0.77
Elapsed time, min				
ET tube clamp to asystole	20 ± 0	24.5 ± 3.4	21.0 ± 2.6	0.57
Warm ischemia	60	90	120	—
ET tube clamp to cold flush	80 ± 0.5	114.5 ± 3.4	141 ± 2.6	<0.0001
Cold ischemia, min				
Cold flush to lungs out	15 ± 0.6	19.8 ± 1.3	18.8 ± 1.2	0.10
Lungs out to EVLP start	48.7 ± 1.9	45 ± 3.1	44 ± 5.1	0.78
Total cold ischemia	63.7 ± 1.3	64.8 ± 3.9	62.8 ± 4.5	0.94
Time before EVLP, min	144 ± 1.3	179 ± 4.0	204 ± 6.1	<0.0001
EVLP, min	240	240	240	—
Total elapsed time, min	384 ± 1.3	419 ± 4.0	444 ± 6.1	<0.0001

Groups compared using 1-way analysis of variance. Mean ± standard error of the mean reported.

Physiologic Changes During Hypoxia

The ABG samples were obtained every 1 to 2 minutes during hypoxia. Changes over time from ET tube clamp to asystole for each parameter are shown in Figure 1. Changes in MAP and heart rate are shown in Figure 2. The normal range is indicated with horizontal shading to identify the time at which the parameter became abnormal. The mean time of death was 22.2 minutes (vertical line, Figures 1 and 2), which displays how abnormal each parameter was on average upon initiation of the prescribed warm ischemia period. Within 3 minutes of inducing hypoxia, pH, $PaCO_2$, HCO_3, and base excess were all abnormal. After 6 minutes, MAP and lactic acid levels were abnormal, and within 8 minutes, PO_2 values fell below the normal range. Heart rate fell below the normal range at 14 minutes.

Oxygenation Capacity and PA Pressures During EVLP

Mean PO_2/FiO_2 at each hour during EVLP is shown in Figure 3A. Final PO_2/FiO_2 after 4 hours of EVLP was not significantly different between groups (60 WIT: 518.3 ± 33.7 vs 90 WIT: 477.2 ± 31.7 vs 120 WIT: 427 ± 36.2 mm Hg, *P* = 0.28), with all 3 meeting the oxygenation threshold for
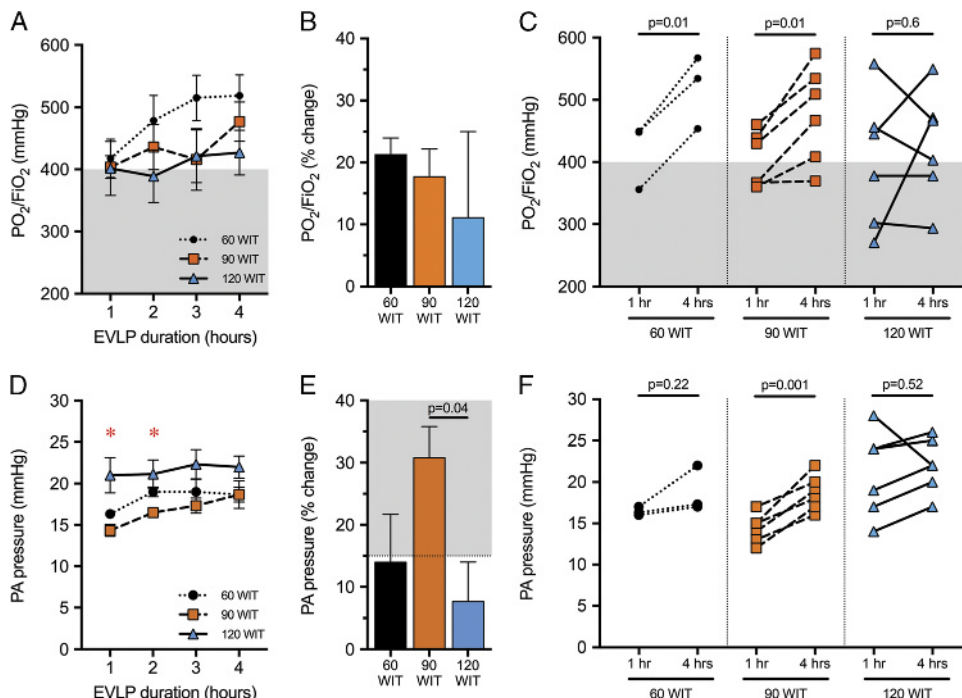

FIGURE 3. Changes in oxygenation and PA pressure during EVLP. A, Hourly PO_2/FiO_2 during EVLP. B, Mean percent change of PO_2/FiO_2. C, Change in PO_2/FiO_2 for each animal per group. D, Hourly PA pressure. E, Mean percent change of PA pressure. F, Change in PA pressure for each group. Groups 60 WIT, 90 WIT, and 120 minutes WIT, lungs procured after hypoxic cardiac arrest and 60, 90, or 120 minutes of warm ischemia.

transplant suitability (final $PO_2/FiO_2 > 400$ mm Hg).[17] Oxygenation capacity increased during EVLP for all groups, with no significant difference between groups in percent change of PO_2/FiO_2 (Figure 3B). EVLP significantly improved the oxygenation capacity of 60 WIT ($P = 0.01$) and 90 WIT ($P = 0.01$) lungs, but did not for 120 WIT lungs ($P = 0.6$) (Figure 3C). The PA PCO_2 levels were within the target range of 35 to 45 mm Hg for all animals, with no difference between groups (60 WIT: 41.1 ± 0.9 vs 90 WIT: 40.7 ± 0.5 vs 120 WIT: 42.1 ± 0.5 mm Hg, $P = 0.15$).

Mean PA pressures during EVLP were different between groups at hours 1 ($P = 0.02$) and 2 ($P = 0.04$), but not at the completion of EVLP (60 WIT: 18.7 ± 1.7 vs 90 WIT: 18.7 ± 0.9 vs 120 WIT: 22 ± 1.3 mm Hg, $P = 0.12$) (Figure 3D). Pulmonary artery pressure increased during EVLP for all 3 groups. The percent change remained below the exclusion criteria of 15% for groups 60 WIT ($14 \pm 7.7\%$) and 120 WIT ($7.7\% \pm 6.4\%$), but not for group 90 WIT ($30.8 \pm 5.0\%$, $P = 0.04$) (Figure 3E). Final PA pressures were significantly higher compared to starting PA pressures for group 90 WIT ($P = 0.001$) (Figure 3F). LA pressures were maintained within the target range for all groups (60 WIT: 2.3 ± 0.5 vs 90 WIT: 2.5 ± 0.3 vs 120 WIT: 2.8 ± 0.2 mm Hg, $P = 0.5$).

Airway Pressures During EVLP

There were no significant differences in plateau or peak airway pressures between groups at each hour of EVLP (Figure 4A and D). The percent change in airway pressures was also not different between groups, with all groups having mean changes in plateau and peak airway pressures that fell below the exclusion criteria of 15% (Figure 4B and E). Final airway pressures were not significantly different from starting airway pressures in all 3 groups (Figure 4C and F).

Compliance During EVLP

Static and dynamic compliance at each hour of EVLP were not significantly different between groups (Figure 5A and D). Percent change in static compliance (60 WIT, $-11.4\% \pm 5.4\%$ vs 90 WIT, $2.6\% \pm 11.3\%$ vs 120 WIT, $25.2\% \pm 23.2\%$; $P = 0.43$) and dynamic compliance (60 WIT, $-10.7\% \pm 8.3\%$ vs 90 WIT, $4.0\% \pm 12.2\%$ vs 120 WIT, $15.6\% \pm 16.3\%$; $P = 0.53$) were not significantly different between groups, with all groups having mean changes within the acceptable limit for transplant suitability (<15% deterioration) (Figures 5B and E). Ex vivo lung perfusion did not significantly change static and dynamic compliance values for any group (Figures 5C and F).

Pulmonary Edema After EVLP

Lung wet-to-dry weight ratios were calculated to assess pulmonary edema. There were no significant differences between groups in wet-to-dry weight ratios (60 WIT, 6.3 ± 0.5 vs 90 WIT, 7.1 ± 0.7 vs 120 WIT, 7.4 ± 0.3, $P = 0.36$).

Histologic Assessment After EVLP

Composite lung injury severity scores were not significantly different between groups (60 WIT, 2.1 ± 0.5 vs 90 WIT, 2.6 ± 0.7 vs 120 WIT, 3.8 ± 0.3, $P = 0.14$) (Figure 6); however, lung injury severity scores did rise somewhat as WIT increased.

DISCUSSION

Using a porcine DCD model, the present study sought to evaluate the effect of warm ischemia after circulatory arrest
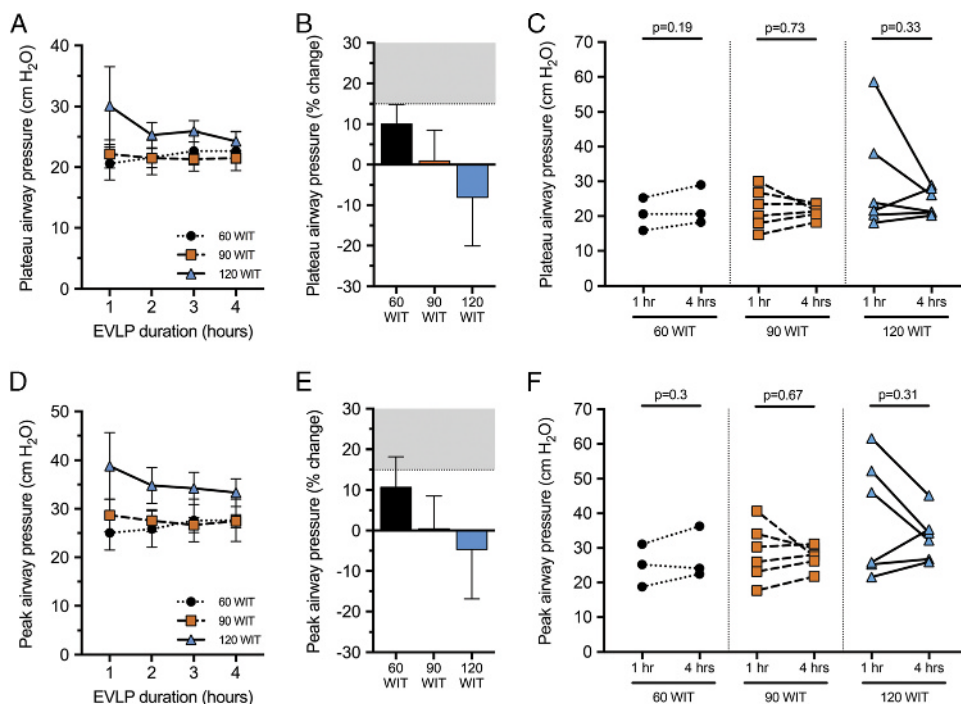

FIGURE 4. Changes in airway pressures during EVLP. A, Hourly plateau airway pressures. B, Mean percent change in plateau airway pressure. C, Change in plateau airway pressures for each animal per group. D, Hourly peak airway pressures. E, Mean percent change in peak airway pressure. F, Change in peak airway pressures for each animal per group. Groups 60 WIT, 90 WIT, and 120 minutes WIT, lungs procured after hypoxic cardiac arrest and 60, 90, or 120 minutes of warm ischemia.

FIGURE 5. Changes in compliance during EVLP. A, Hourly static compliance. B, Mean percent change in static compliance. C, Change in static compliance for each animal per group. D, Hourly dynamic compliance. E, Mean percent change in dynamic compliance. F, Change in dynamic compliance for each animal per group. Groups 60 WIT, 90 WIT, and 120 minutes WIT, lungs procured after hypoxic cardiac arrest and 60, 90, or 120 minutes of warm ischemia.
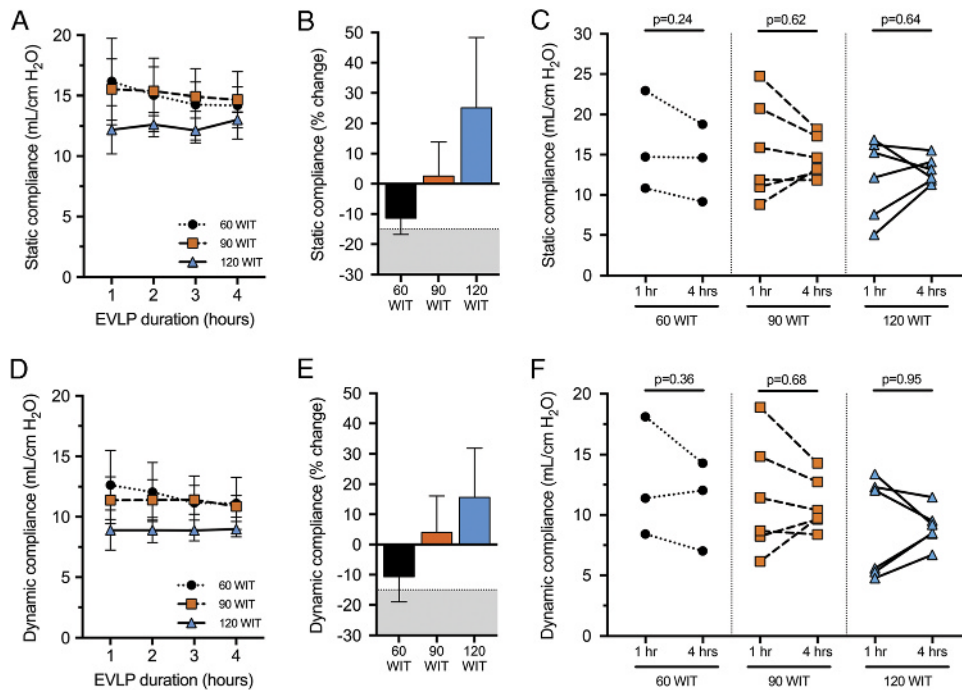
on ex vivo lung function. After confirming asystole, donor lungs were exposed to 60, 90, or 120 minutes of warm ischemia followed by 4 hours of EVLP. Transplant suitability was determined at the completion of EVLP using the Toronto exclusion criteria ($PO_2/FiO_2 < 400$ mm Hg, >15% increase in PA pressure, and >15% deterioration of airway pressures and compliance).[17,20] All 3 groups (60 WIT, 90 WIT, and 120 WIT) met the criteria for oxygenation, with no significant difference between groups in final PO_2/FiO_2. Pulmonary artery pressure increases were acceptable for transplantation in groups 60 WIT and 120 WIT, but not in group 90 WIT (30.8% ± 5.0%). All 3 groups met the transplant criteria for acceptable changes in airway pressures and compliance. There were also no significant differences between groups in edema accumulation or histologic lung injury severity scores. These results suggest that DCD lungs with WIT up to 120 minutes still meet transplant criteria after 4 hours of EVLP.

In recent years, many institutions both in the United States and internationally have expanded their procurement protocols to accept Maastricht category III "controlled" DCD lungs.[5,21] These patients undergo planned withdrawal of treatment, often in the operating room, with transplant teams nearby who begin organ procurement shortly after confirmation of death.[8] Outcomes after lung transplantation with "controlled" DCD lungs have been comparable to outcomes with heart-beating donors.[22,23] Although the addition of EVLP has not been shown to improve survival after transplantation with DCD lungs, there is potential that EVLP may allow for inclusion of DCD lungs with longer WITs that would otherwise be rejected.[24,25] "Uncontrolled" Maastricht category I and II donors have yet to be incorporated into routine transplant protocols due to associated logistic, physiologic, and ethical challenges, including timely notification of the procurement team, sufficient communication with family members, and proper management of the donor.[26]
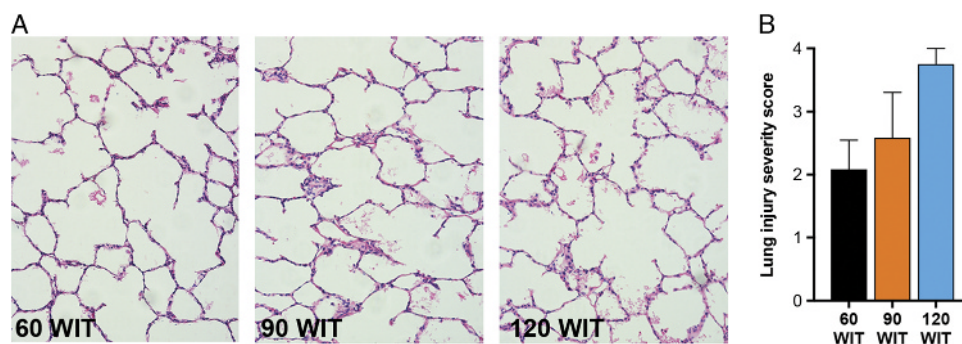

FIGURE 6. Lung histology at the end of EVLP. A, Representative H&E sections (20× magnification). B, Lung injury severity scores. Groups 60 WIT, 90 WIT, and 120 minutes WIT, lungs procured after hypoxic cardiac arrest and 60, 90, or 120 minutes of warm ischemia.

One center in Spain was able to navigate the challenges associated with patients who die outside of the hospital and have reported their experience.[27-29]

For the present study, we sought to better understand the associated physiologic challenges after hypoxic cardiac arrest using our established porcine model of lung procurement. Although this model does not reflect all the various pathways that can lead to circulatory arrest, it does parallel donors who suffer respiratory arrest, which includes prolonged hypoxia from pulmonary disease and acute respiratory emergencies from airway obstructions and drowning. Published data in a porcine lung transplant model show that premortem hypoxia is associated with significant deterioration in graft function.[30]

The present study monitored all animals with frequent ABGs and continuous cardiac monitoring during the hypoxic period to better characterize the physiologic changes associated with this model. Certain parameters (pH, $PaCO_2$, HCO_3, and base excess) became abnormal within a few minutes of hypoxia while others took longer (MAP, lactic acid levels, PO_2, and heart rate). We believe these data are valuable in helping to understand the quality of lungs that can be expected after certain types of death. Lungs exposed to warm ischemia after hypoxic cardiac arrest will likely function differently compared with lungs after sudden cardiac arrest.

After exposure to increasing amounts of warm ischemia, lungs from all 3 groups met all transplant criteria after 4 hours of EVLP, except the 90 WIT group on 1 parameter (PA pressure increase >15%). Additionally, when oxygenation capacity, airway pressures, and compliance were compared between groups, there were no significant differences after 4 hours of EVLP. This finding highlights the value of EVLP assessment of lungs before determining transplant suitability, as it appears that increasing WIT alone (up to 120 minutes) does not predict lung function after EVLP. Additional studies are necessary to determine a defined upper limit of acceptable WIT. It likely will be prudent to assess all potential donor lungs (within a large window of warm ischemic exposure) via EVLP to determine transplant suitability.

In the present study, EVLP provided a useful platform for assessing graft quality but did not confer much in terms of reconditioning. Ex vivo lung perfusion significantly improved oxygenation capacity for groups 60 WIT and 90 WIT, but not for group 120 WIT. Pulmonary artery pressures were significantly worse at the completion of EVLP for group 90 WIT, which was unexplainable but could be related to air trapping, vasoconstriction due to decreased organ temperature, or persistent clot burden. Although airway pressures and compliance did not differ significantly different between groups, group 120 WIT appeared to benefit the most with static compliance improvements of 25.2% ± 23.2% and dynamic compliance improvements of 15.6% ± 16.3%. Despite the lack of a statistical difference in improvement between hours 1 and 4 of EVLP for most parameters, all parameters at the completion of EVLP except for PA pressures in group 90 WIT were within acceptable limits for transplantation. Additionally, supporting data such as pulmonary edema and histologic lung injury severity scores were not different between groups. Although the definitive test will be lung function after transplantation, it appears that increasing WIT alone does not worsen lung function after 4 hours of EVLP.

Although Maastricht category I and II lungs comprise a potential solution to the supply-demand mismatch, there are significant hurdles that must be overcome before they can become an acceptable, routine option.[31] Heparin was not administered to animals in the present study, as any premortem interventions will likely not be possible in an "uncontrolled" donor scenario. Clot within the pulmonary vasculature was present in most donor lungs procured after 90 and 120 minutes of warm ischemia, but was able to be flushed out with Perfadex. Published data on nonheparinized category III lungs is encouraging, but WITs are shorter under those circumstances.[22,32] Other uncertainties include the cause of death (eg, respiratory arrest, cardiac arrest, exsanguination) and any resuscitation efforts that are attempted (eg, rescue breathing, chest compressions). This variability in patient presentation makes EVLP a near necessity to fully assess graft function and potentially rehabilitate damaged organs. Our laboratory and others have evaluated various treatment options during EVLP that may help recondition injured lungs.[12,33-35] As these therapies are developed, the likelihood increases that we may one day have protocols that allow for routine, successful transplantation of Maastricht category I and II lungs.

The present study does have limitations. First, although this study was designed to set a baseline for acceptable warm ischemic injury by testing ex vivo lung function, the findings are limited because the organs were not subsequently transplanted into recipient animals and reperfused. The effect of ischemia-reperfusion injury after transplant on graft function will be an important next step of investigation. Second, there is inherent variability present in large animal studies. Our study was limited to n = 3 for the 60 WIT group and n = 6 for the 90 and 120 WIT groups, and it is possible that the use of higher numbers of animals per group could have revealed small but significant differences between groups in some parameters (eg, histologic lung injury scores). Based on previous work from our laboratory using 60 minutes of warm ischemia after hypoxic cardiac arrest, the number of animals randomized to group 60 WIT was limited to 3.[10] Third, our study was also limited by the hypoxia method used to induce cardiac arrest; however, it provided consistent, reproducible injury. Finally, our findings are limited by not having a group that failed to meet transplant criteria after EVLP, which would have helped define the upper limit of acceptable WIT. Based on increasing variability in the data for the 120 WIT group, especially PO_2/FiO_2 and PA pressures (Figure 3) and airway pressures (Figure 4), we speculate that 120 minutes of WIT may be approaching the upper limit of acceptability.

In conclusion, for DCD donors to become a routinely utilized source of lungs for transplantation, understanding the effect of increasing WIT after different types of death is necessary. The present study shows that DCD lungs exposed to WIT up to 120 minutes after hypoxic cardiac arrest still meet transplant criteria after 4 hours of normothermic EVLP. As the field of lung transplantation continues to evolve to meet the ever-growing demand for organs, Maastricht category I and II lungs DCD lungs may eventually become acceptable for transplantation in conjunction with routine assessment by EVLP.

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