Extending Function and Applications of Isolated Cardiopulmonary Systems

A DISSERTATION
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Acknowledgements

My sincerest thanks to my advisor Paul who was instrumental in bringing my efforts to bear on this work. Thanks also to my patient family and to the former and current staff, graduate students, collaborators, and volunteers at the Visible Heart Laboratory who lent me their time and diligence. Your efforts made this work both possible and enjoyable.

Dedication

To my constant companions Loki and Zoey who sacrificed so much time in my pursuit of these studies.

Thesis Abstract

The use of isolated mammalian hearts has a history that is responsible for a staggering amount of the basic physiological knowledge we have about the cardiovascular system and is a primary gateway between ideas and clinical treatment. Advances in cardiac physiology, surgery, transplantation, pacing, defibrillation, ablation, and pharmacology are derived from this area of research. The work outlined here takes identified issues with experimental preparations, as well as clinical applications, to investigate solutions and directions for their systematic address. Extending the utility and window of viability of the isolated heart and lungs has resulted in clinically applicable advances in drug treatments and assessment tools. Most importantly though, it has the potential to expand the population of acceptable donor organs where there is immediate need and continuous shortfall in supply.

My thesis consists of chapters which progress in translational application, making use of novel and comprehensive ways of controlling and investigating isolated cardiovascular systems.

In the first chapter, the Visible Heart® preparation is used to replicate and extend a classic temperature experiment in the large isolated porcine heart. This chapter also addresses the clinical applications of optimizing heart function with emerging isolated heart transportation devices; making the best use of efforts to assess and maintain the heart for transplantation. This is followed in chapters 2 using the Visible Heart® system to assess therapeutic drug delivery for treating atrial fibrillation and again preserving a heart's function for transplantation.

The advancement of the isolated heart preparation is further driven by procedural concerns with cryo-ablation technologies to include functional lungs. This comprehensive system is used on actual human heart lung-bloc combinations for investigative purposes and required its own set of unique engineering solutions to produce a viable test platform.

It is also this evolution of the isolated heart preparation that was a significant factor in bringing the Lung Organ Care System (OCS™) to the Visible Heart Laboratory as a unique research tool. As a commercial device, the OCS™ device seeks to replace the storage-on-ice standard of care with warm and ventilated perfusion of the lungs independent of the heart. As a laboratory instrument it has allowed new opportunities for investigating both basic lung physiology as well as providing lessons that are clinically applicable. The completely novel thermal monitoring of the lungs in this isolated state are discussed in Chapter 5 which investigates thermal tools and profiling of lung damage for the first time. This provides a whole new paradigm for emerging lung and general organ assessment directly relating identified injury states, overall lung function, and recovery/damage profiles that may help physicians make better use of precious donor lungs.

In extending the use of the isolated lungs to an underutilized population of donors, the final chapter, Chapter 6, demonstrates for the first time a controlled study and injury model for donation after cardiac death (DCD). With modification to the current clinical use protocol for the OCS™ device, the viability window for injured lungs is shown to be nearly tripled. The impact of demonstrating viable DCD lungs on this system is the

potential to *greatly* expand the number of lungs for transplantation, which would be invaluable to many currently on a long wait list.

My thesis work has produced stable isolated cardio-vasculature systems with direct impact on the design of devices, investigation, therapy and monitoring in the pursuit of bettering the standard of care and expanding the availability of the organs for transplantation. It provides new and unique combinations of heart and lungs tailored to the investigative necessity in human anatomy and a more comprehensively described large mammalian model for anatomy, physiology and acute injury.

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Chapter 1 : Temperature Modulation and Optimization of the Isolated Heart

Preface

The human recovered donor heart is routinely being used clinically for transplantation and yet the question of how best to preserve the isolated functional heart remains is a surprisingly open question. Current technology seeks to limit the ischemic period and continuously monitor an array of indicators which in turn indicate an administrations of drugs and treatments. There is however a gap in the understanding of function in the isolated heart which is becoming a standard of care that bridges the time between donors and recipients.

The new array of sensors and capabilities that I have added to the Visible Heart® apparatus aim to better sustain the heart and lung preparations has also allowed new insight into the basic physiology of the heart. Starting with extensive temperature monitoring capabilities, and joining oxygen sensors paired with invasive pressure monitoring of all the chambers of the heart, we re-engineer in this chapter one of the most basic (and among the first) experiments of the isolated heart. That is the physiological response to temperature variation, classically linking a decrease in temperature to a decrease in the heart rate. Our results demonstrate this classic results of basic cardiac physiology with decreasing heart rate and oxygen consumption with the lowering of temperature.

This work uniquely combines the Visible Heart® system's unique blood-less preparation and new metric tracking performed as a *continuous* relationship between comprehensive temperature, heart rate, oxygen levels, and five chamber monitoring on a large mammalian heart as an excellent analog for the human heart. This data has never been collected and reported so comprehensively and under such dynamic conditions. It has also never been done in a better model for the translation to human heart physiology much less the emerging clinical devices that will make use of the optimization this work demonstrates at sub-normothermic preservation temperatures.

The result is clinically applilcable indications that isolated cardiac function is optimized around 3-4°C below the normothermic range, where the oxygen demand is reduced without apparent compromises to function which follow any further lowering of the temperature. A functional sub-normothermic temperature preservation method is likely the optimal preservation method for the heart as functional transplant technologies can take advantage of this new information.

I am responsible for the data collection, analysis, manuscript preparation, and system design, programming, construction and operation contributing to this work.

Induced Function Modulations of an Isolated Large Mammalian Heart Model

Brian T. Howard, M.S., Paul A. Iaizzo, Ph.D.

Summary

Here we describe a comprehensively monitored isolated heart model, which demonstrates optimization of isolated cardiac functions under mild hypothermic conditions: obtained results may have direct clinical implications to emerging heart preservation methods for transplantation. Metrics of cardiac function were tracked as continuous variables during temperature changes between about 31-39°C; eliciting well defined reductions in metabolic demands and heart rate modulations. A functional optimization was observed with hemodynamic monitoring for the isolated heart model at about 34.7 ± 0.9 °C (N=13). Additionally, we further explored the active pacing of these isolated hearts as a means to remove the ability of the heart rate to be modulated in response to temperature fluctuations, which can allow us insights as to intrinsic contractile alterations. The observations described here should have useful insights for both individuals utilizing heart preservation systems and those using isolated heart models for pre-clinical device testing.

Keywords:
Isolated heart, Hemodynamic Responses, Mild Hypothermia, Optimization, Transplantation, Pacing

Background

Changing the operating temperatures of a functional isolated heart is a classic physiology experiment [6]: i.e., ever since the 1880's when Henry Martin demonstrated the need for coronary perfusion in maintenance of the isolated mammalian heart/lung bloc [7] and later in 1895 when Langendorf further isolated the heart from the lungs [8]. For over a century since, isolated heart experiments have yielded enormous amounts of critical information relative to the physiology of heart, as well as for the known safeties and efficacies of employed drugs and medical devices [9-19].

The utilization of isolating heart models and specifically their reanimation continues to be of primary interest relative to assessment function prior to transplantation (e.g., in the TransMedic's Organ Care™ System) [1]. Further, cardiac temperature modulation in itself is commonly being defined as an additive therapy in such approaches [2], yet despite over a 120 years of isolated heart work detail analyses of such, have been lacking .

Since 1997, the Visible Heart® Laboratory has utilized a unique large mammalian heart model [25] elucidated the role of various pharmacological agents on hemodynamic responses and provided for novel direct imaging of functional cardiac anatomy [26, 27, 28]. Importantly, this isolated heart model lacks: 1) autonomic innervation, 2) systemic effects, 3) metabolism of administered agents, and the chemical pathways inherent to blood sustained preparations. In other words, pharmacological and/or thermal responses of reanimated hearts are those elicited purely borne of the heart. Here, we utilized the Visible Heart® methods with cyclic temperature modulation of porcine heart specimens

to establish characteristic temperature responses as a more appropriate analog for human heart preservation. Concurrent measurements of the dissolved oxygen, thermal profile and complete hemodynamics provided us with a more predictable and modifiable isolated human heart analog.

Methods

Porcine hearts (N=16) were isolated according to previously established methods [26]. Each preparation was maintained at an approximate baseline 37.0 °C as measured by a primary reference needle probe centered transmuraly in the left ventricular free wall. The thermal condition of the heart was additionally monitored by up to ten different thermocouples and/or with an external thermal video camera.

Pressure sensing lumen catheter were placed directly into ventricular heart chambers and the sustaining vasculature to give a complete, five chamber hemodynamic picture of each heart. This allows for a quantitative assessment of a given heart's function which respective temperature modulations. From an equilibrium state of at least 37.0 °C, the perfusion system and the given heart were allowed to cool gradually towards the ambient temperature over the course of 20-25 minutes and data continuously recorded. Note, functions were also assessed during the process of re-warming each heart, i.e., prior to any subsequent cooling trials being attempted: i.e., data were only collected from a study in which the viability of a given heart was considered to be sustained. Functional performance data were matched between the relative cardiac heart

rates (as could be calculated independently from each chamber's pressure data and the ECG), to the temperature data.

When pacing was employed, the hearts were paced at a rate at least 10 bpm above the intrinsic rate and it was performed with a pacing lead (Streamline™ bipolar temporary myocardial pacing lead, 6495, Medtronic PLC, Minnesota, Fridley, USA) placed within the left ventricular myocardium and utilized in a VOO mode (2090 programmer, Medtronic)

Placement of Thermocouple probes

The general format for the thermal tissue monitoring included two needle probes placed in the left ventricular freewall (1,2) (Figure 1-1) which were subsequently used for the reference temperatures; one needle probe in the right ventricular freewall (3); a passive flow thermocouple in each of the ventricular volumes (4,5), and surface temperature probes approximately medial on the right ventricular freewall (6) and the second placed on the left atrial tissue between the pulmonary veins held against the supporting foam piece (7). Additional thermocouples were employed to monitor the ambient temperature as well as those in the pre-load, and afterload buffer chambers.. All thermocouples employed, were T-type and data were collected using a Iso-Thermex 256 acquisition system (Columbus Instraments, Columbus, Ohio, USA) controlled through a custom LabView program (National Instruments, Austin, Texas).

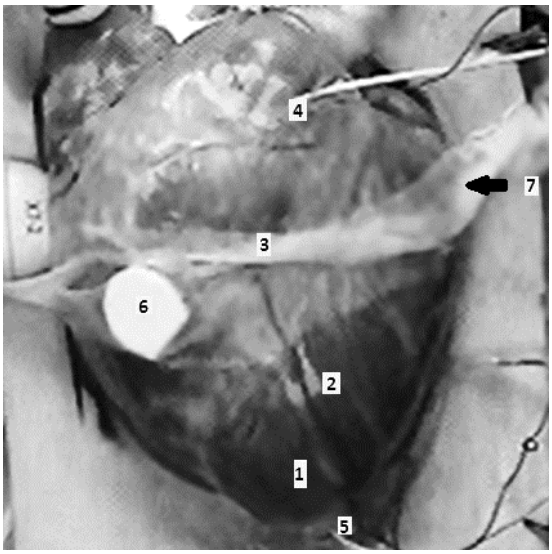


Figure 1-1: Shown are the 7 standard site locations of temperature probe placements (right). Temperatures of the heart muscle and perfusion fluid were monitored during temperature variations: and observed to maintain good agreement to within +/- 0.5°C, providing high confidence in treating any single measured temperature value as characteristic of the large mammalian heart through these temperature changing trials.

Statistical Methodology

The values here are reported as means ± standard deviations. Linear regressions were performed on the data windowed to our reported ranges.

Results

As a given heart was induced to cool, the relative heart rates were observed to decrease. Temperature variation between the direct probes (1,2,3,6, & 7 in Figure 1-1) usually stayed within less than half a degree of difference for the cooling phase. The additional probes and thermal monitoring served mainly as strong verification of the

ventricular tissue temperatures used for reference against the 5 second averaging of the heart rates, for investigative purposes.

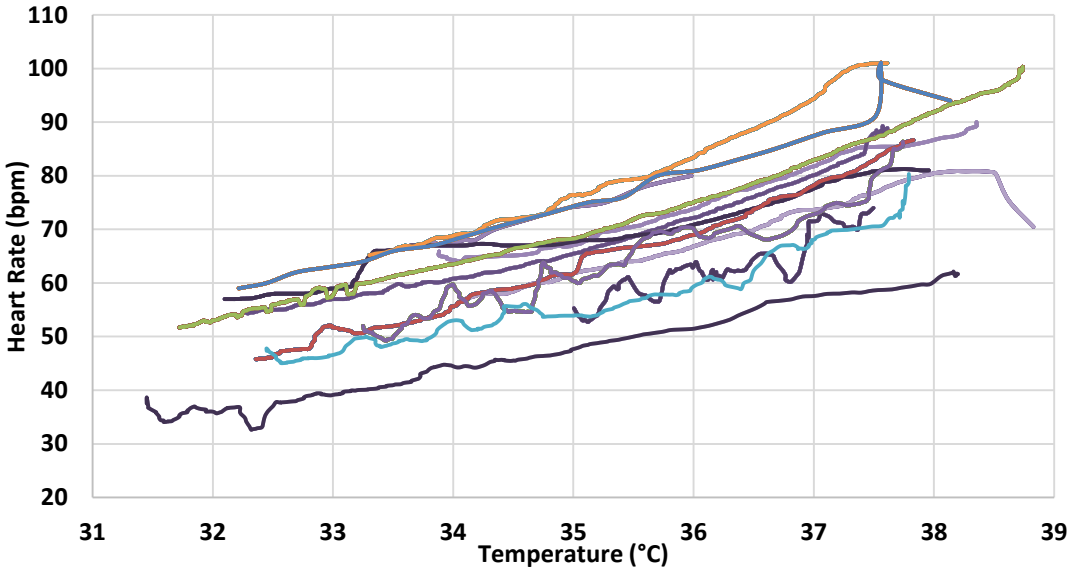


Figure 1-2: Tracking of individual cooling experiments which take between 20-25 minutes. Each line is raw temperature data collected from individual experimental runs.

Taken altogether with a linear approximation of each trial's temperature dependence in these 16 cooling trials, the heart rate in these isolated swine hearts decreased at a rate of 5.89 ± 1.17 bpm/°C.

Here we note a relative linear relationship for each of our 16 cooling trials: averaged together they provided us an empirical measure of the heart rate change with temperature of 5.89 ± 1.17 bpm/°C: i.e., within the previously reported ranges. We also note a minor secondary trend showing dependence on the initial heart rate (Figure 1-3) making a modification of that rate by about 1.5 bpm/°C for every 10 bpm change in initial rate.

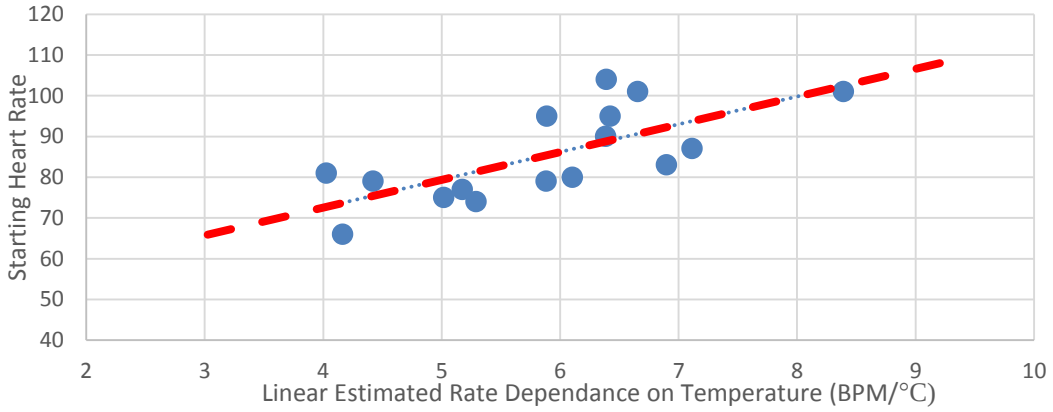


Figure 1-3: The degree to which the heart rate is modulated by a temperature change appears to have a loose secondary dependence on the initial heart rate at the beginning of the temperature modulation experiment; changing faster by about 1.5 BPM/°C for every 10 BPM increase in initial heart rate (R^2 = 0.51).

Temperature (°C)	Heart Rate					
39	64	90	116	142	168	194
38	62	85	108	131	154	177
37	60	80	100	120	140	160
36	58	75	92	109	126	143
34	54	65	76	87	98	109
32	49	55	60	65	70	76
30	45	44	44	43	43	42
37	60	80	100	120	140	160
Estimated bmp/°C	2.2	5.1	8.0	11.0	13.9	16.8

Table 1-1: Tabulated estimation of heart rate based on the initial heart rate at nominal (37°C) temperature and the change in temperature. This reference table summarizes the experiments done with large mammalian (porcine) hearts. Correlation to human data would be easily tested against with

The rate modulation of the heart with temperature is important but not the only thing that is changed with the temperature. As a heart was cooled from the ambient temperature, the contractions of the heart became initially become stronger (Figure 1-4).

It is also acknowledged that the reduction of temperature results simultaneously in a lower oxygen demand which we have confirmed with in-line dissolved oxygen monitoring (Figure 1-5). These increases in function persisted only until a certain point in the cooling process, peaking for these specimens at approximately 34.71 ± 0.89 °C (N=13) and again during rewarming at 34.19 ± 1.78 °C (N=10).

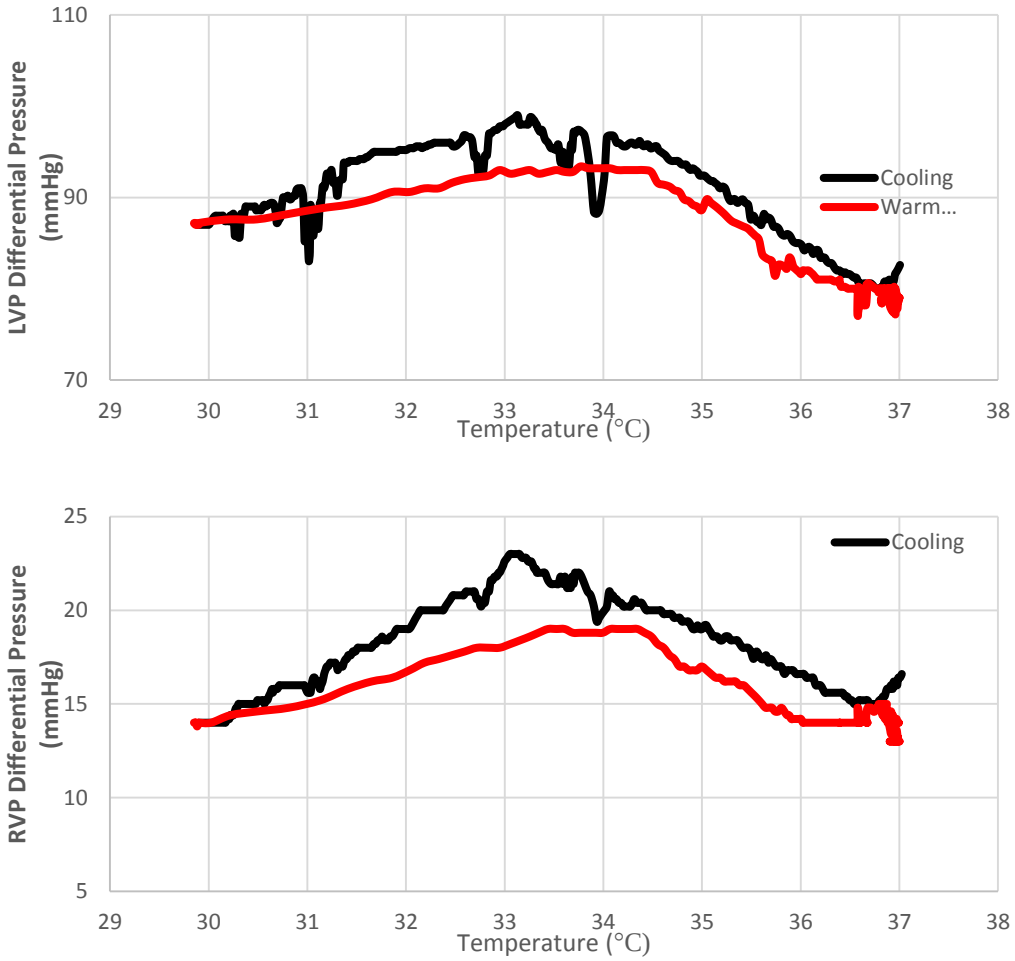


Figure 1-4: Example trial showing the direction of the warming/cooling has a noticeable effect in achieving the maximum differential pressure. The difference between the heating and cooling trials was primarily the time taken to achieve the temperature change.

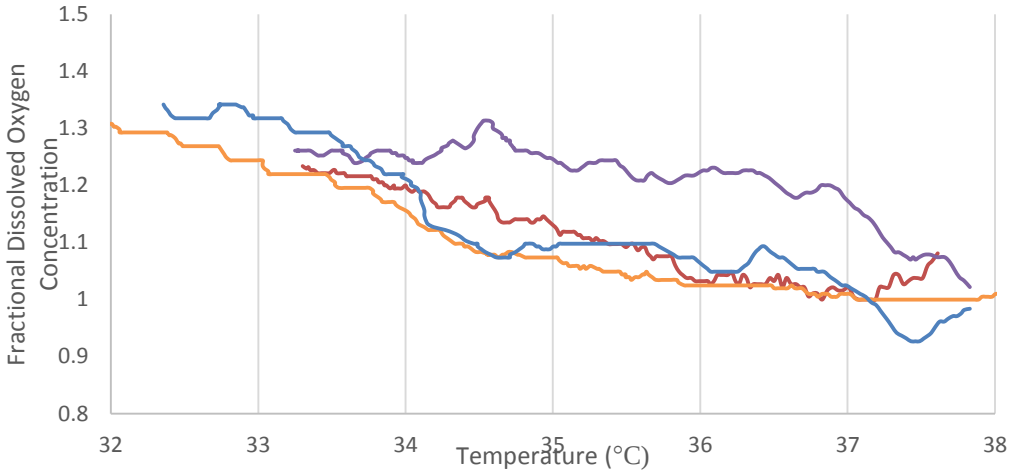


Figure 1-5: Fractional Oxygen Content of the perfusing solution as a function of the decreasing temperature roughly shows about a 5% savings in the oxygen demand per degree although the system itself is not necessarily physiological in this regard being naturally ischemic from the beginning.

During these cooling trials, the relative rates were much slower and consequently lends itself better to a quasistatic approximation: i.e., taking approximately 20-25 minutes to achieve the minimum temperature. The warming of the heart utilizing our Visible Heart setup, typically required only 5-10 minutes. Temperature and pressure data were fit with quadratic approximations, to estimate the temperature corresponding to the optimal pressure generation.

Pacing

Several interesting phenomenon were also observed through depriving the isolated heart of the ability to regulate the intrinsic rate relative to a change in temperature. First the temperature at which pacing becomes ineffective/intermittent can

be identified (Figure 1-6) below about 33 °C, an aspect of the conduction system that is lost when reducing the heart to a study of cooling effects on isolated muscles [21,24].

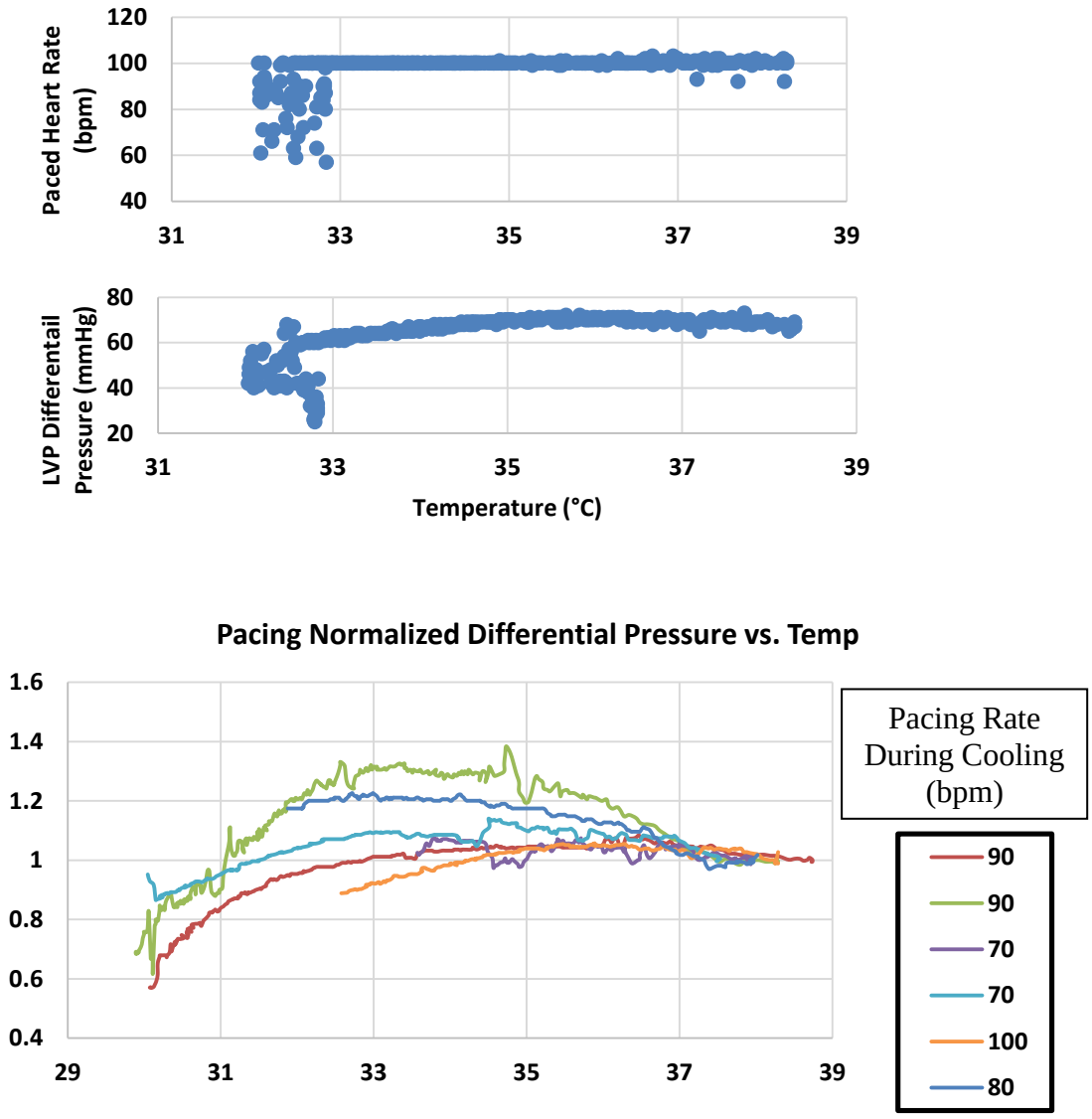


Figure 1-6: Heart pacing is seen here for an example trial to be intermittent on the high end where it may compete with sinus rhythm and on the low end, a floor just below 33°C shows the intermittent failure to capture pacing.

Secondly we observe that even with pacing the optimization of ventricular function is seen to increase and peak with the reduction in temperature though other considerations are present (Figure 1-6). Thirdly the act of overpacing in this model is also an exercise in time. With or without pacing the temperature drop occurs in the same approximate time period but pacing can sustain a larger demand on the heart throughout the process, posing the possibility of exhausting the heart's energy stores before a proper account of its temperature response is measured.

Discussion:

Here we utilized an isolated working heart swine model to revisit the
The highly controlled large mammalian (porcine) model described here, and often used as an appropriate human heart analog, shows physiological response consistent with conventional knowledge and an optimization of function that are of clinical utility.

The sub-normothermic performance of this large mammalian heart demonstrates optimized function at 34.71 ± 0.89 °C corresponding to a lower overall oxygen demand as compared with normothermic conditions. The operation of a heart within this temperature range may be the best assessment of the organ's potential function following transplantation. Also done in an isolated and acellular preparation, this response is free from nerve or chemical responses, demonstrating a 5.89 ± 1.17 bpm/°C rate-temperature dependence along with a dependence of this rate on the initial intrinsic pace; comparable with such measurement of the in-situ human response in hyperthermic conditions observed by Jose et.al. [29] placing human heart rate modulation at 7.1 bpm/°C.

The consistent insult that occurs during transplant procedures is ischemia which has classically been combated with a lowering of the heart's temperature via preservation on ice: this reduces the myocardial metabolic demands until normal conditions can be restored in a recipient patient. The idea of perfused and hypothermic isolated organ preservation is not new [3] but perfusion systems for transplant organ transportation are now becoming more familiar in many cardiac centers of excellence. A recent move away from ice preservation for organ recovered follows from the needs for assessing function, minimizing ischemic damage and potentially extending the window between recovery and transplantation. The Organ Care™ System for hearts recently completed the PROCEED II trial [2,4] and the system is being used in the laboratory for the investigation of recovering hearts after cardiac death [5]. There remains a need to evaluate the utility of mild hypothermia in such circumstances in place of establishing purely normothermic conditions with emerging ex-vivo perfusion systems. We consider here that the optimal parameters for perfusion are far from consensus and remain to be better characterized, as we attempt to begin doing in this study.

The effects of temperature modulation relative to ischemic protection of the heart have been well explored as it relates to ischemic protection in non-isolated small animal models [20]. Trials which have described the hemodynamic function as it relates to temperature have also historically prescribed temperature setpoints for comparison [21, 22, 23, 20]. More specifically relative to our study, the porcine model has been considered as a viable human analog, yet most such previous studies have had an in-situ

focus: i.e., on the efficacy of cooling methods [23] rather than on resultant cardiac function. It should be noted that there has also been reported inestigations on isolated myocardium; comparing thermal responses of swine versus human tissues and these provide some useful insights as to thermal effects [24,21].

The limits of the model lie in the preparation's natural global ischemia and was performed with healthy porcine specimens. For the same reason the ischemic condition and the well explored area of porcine cardiac injury models lends itself directly to application with isolated heart preparations for transplantation. This model is a basis for finding new ways to extend the window of viability and recover or improve organ function and patient outcomes.

More rigorous attempts have been made at the numerical characterization of this temperature/frequency relationship (Starling [6], Barcroft and Izquiedro [1]), often limiting to the relevant temperature ranges and working from other animal models. The method employed here is a more appropriate animal model and goes beyond previous studies of mild hypothermia limited by animals (and hearts) that are small for a human analog, non-isolated, focused on neurological or method efficacy, and/or prescribe temperature set-points which omit the real time effects [32, 33, 34, 35, 36, 20, 23, 30, 22, 31, 21, 24]. Further, data collected during the re-warming phases appears predictably hysteretic with respect to the function but rate dependence still corroborates the measured values from the cooling phase.

Conclusions

The thermal response from an isolated large mammalian heart is reported here with a time course and level of detail that has not been approached by previous investigations and the result is a better understanding of the functional response of the heart and direct application to emerging clinical technologies. Maintenance of isolated organs at sub-normal temperatures (several degrees in contrast to current ice storage) with the aid of perfusion systems could prove an improvement to standard of care during events such as transplantation. It is also valuable information for research purposes where in the immediate future, this methodology can also be extended directly to (and verified by) application with the isolated human heart research being done by the Visible Heart Lab in an ongoing collaboration [28].

Chapter 2 : Assessment of pharmacological agents

Preface

In the course of surgical intervention there exists the opportunity to treat or prevent either ischemia and reperfusion injury or incidences of induced arrhythmias such as atrial fibrillation (AF). Furthermore, the mitigation of AF post-surgery/transplantation has the potential to greatly assist in the recovery of patients. It is well documented that the application of pharmaceutical agents can also improve the functional performance of the heart. The Visible Heart® methods has served as a platform to assess the function of multiple pharmaceutical agents for such applied translational applications.

In previous work from our laboratory, several pharmacological agents have been shown to be individually beneficial for these purposes, but the potential additive benefits of combining the agents is assessed here. My specific contribution to this work spans developing the methodology which is described in an accepted video publication as well as the extensive data collection and analysis of current and historic data. It is on the basis of this analysis and both my assessment and recommendation that the best performing combination have begun further assessment for their potential benefits on the various lung injury models as described in Chapter 5-6.

I am responsible for the data collection, data analysis, methodology development and manuscript preparation of this work. Currently this paper is awaiting further submission for publication consideration.

Investigation of Combined Pericardial Delivery of Agents for Mitigation of Atrial Fibrillation and Ischemic Protection: Preconditioning for Open Heart Surgical and Transplant Applications

Brian Howard, MS, Tinen Iles, MS, Steve Howard, PhD, Chris Rolfes, PhD, Nate Menninga, Paul Iaizzo. PhD

Summary

Treatment of atrial fibrillation is addressed in a combined cardiac surgical and post-transplant model using large porcine specimens. Omegaven, Tauroursodeoxycholic Acid (TUDCA), and Docosahexaenoic Acid (DHA) which have previously shown positive effects in this isolated model are administered in several combinations to the pericardial space as a convenient and potentially effective method of improving protection of the heart against atrial fibrillation as well as improving the post-transplantation hemodynamic function. As measured against a control group, the combination of TUDCA and Omegaven demonstrated significantly improved hemodynamic performance. That combination and that containing only Omegaven with DHA showed an increased propensity for terminating and time sustained in AF; the combination of Omegaven and DHA possibly doing so through the elongation of measured refractory periods in the atrial tissue.

Keywords:
Atrial Fibrillation, AF, Omegaven, DHA, Docosahexaenoic Acid, TUDCA, Tauroursodeoxycholic Acid, transplantation, cardiac surgery, Visible Heart®.

Introduction

Atrial Fibrillation (AF) is a substantial burden on the heart and is often observed following cardiac procedures including transplantation. AF is cited as the most common complication following cardiac procedures, producing longer, more expensive hospitalizations[1,2,4,6] even with new, less invasive technologies [3,5]. Simultaneously, there is justification for long term benefits to the heart from positive dietary choices and a host of protective agents that have yet to be thoroughly assessed for their protective benefits with regards to AF and cardiac function.

Following a pericardial delivery method similar to that which was recently published (7) we investigated the combined use of three agents including Omegaven, and Docosahexaenoic Acid (DHA), and Tauroursodeoxycholic acid (TUDCA) (T) as treatments for AF and protective benefits manifest in better transplant performance.

Omegaven (O) is a fatty acid emulsion (particularly Omega-3 fatty acids) currently used as a nutritional supplement. Docosahexaenoic Acid (D) has been shown benefits previously to improve ischemic protection by itself [14] and also to possibly reduce inflammation markers in pediatric surgery [9]. Synthesized Tauroursodeoxycholic acid or TUDCA is the hydrophilic component of Ursodeoxycholic acid (UDCA) found to be upregulated in hibernating bears. and the subject of current efficacy trials for Cholestatic Liver Disease [8], and show cognitive protection benefits in mice as well [11]. Importantly TUDCA has shown the potential as a cardio protectant in limited models [10, 12] and is uniquely assessed here in an ischemic large mammalian model.

To assess the effectiveness of each agent in the pericardial delivery an array of data was collected in a porcine model between phases of *in situ* operation and then transplant/reanimation using the Visible Heart® system [13]. The target measurements *in situ* include the refractory periods of the atria, ventricle, and AV node, baseline measurement of the hemodynamics, and total AF burden is assessed from measuring the time spent in fibrillation following electrically induced AF. Following procurement and reanimation on the Visible Heart™ system, hemodynamics are tracked over the course of an hour with periodic monitoring of the glucose and lactate levels as a measure of the heart's relative performance.

Methods

The model makes use of porcine specimens as analogs for human anatomy and physiology. Using 70-90 kg Yorkshire X porcine, the general cardiac specimen well approximates a normal adult human heart size (300-500g). The animal is anesthetized, pressure monitoring is placed for all four chambers of the heart as well as a branch of the femoral artery; pacing leads are placed in the right ventricle and atrium. A medial sternotomy is then performed to expose the heart as would be done clinically during open heart procedure or organ recovery for transplantation.

Once the heart is exposed via the medial sternotomy the pericardium is opened and suspended to form a cradle. A pericardial unipolar lead is inserted into the left atrial appendage and a pericardial bi-polar lead is placed through the myocardium of the left

ventricle. A soft flexible injection catheter is placed into the pericardial cradle in preparation for administration of assigned treatment.

The implanted pacing leads are connected to a Medtronic GEM III pacemaker. The pacemaker is used in conjunction with a Medtronic 2090 programmer to run a programmed electrical stimulation (PES) protocol to determine the refractory periods of the atrial, ventricular, and AV node. The output from the pacemaker along with the electrical waveforms generated by the pericardial leads in the left atria and left ventricle are recorded on a St. Jude ESI machine. Here the waveforms are synced and lined up such that the electrical activity from the atria and ventricles are individually represented by the electrical signal from their respective pericardial leads. The sensing from the pacemaker can be observed to mirror those atrial and ventricular contractions.

Timeline

Timeline ---->																	
	Constant Hemodynamic Monitoring											Constant Hemodynamic Monitoring			
	In Situ									Reanimation		In Vitro			
	Baseline:			0 min	30 min		60 min			70-200 min		0-60 min	20 min	40 min	60 min
Anesthesia	Instrumentation	Normalization Period	Refractory Period Measurement	AF induction	Echocardiograph recordings	Administration of Treatment	Refractory Period Measurement	AF induction	Refractory Period Measurement	AF induction	Cardioplegia and Heart Removal	Reanimation on Visible Heart®	Air Blood Gas Samples of CS	LV short axis echo	LV short axis echo	LV short axis echo

Figure 2-1: Procedural timeline proceeding right to left assessment of AF is relegated to In Situ measurements while post transplantation In Vitro is primarily tasked with assessing the hemodynamic performance.

Why refractory periods?

The refractory period is simply the time that a cardiac myocyte needs to reset itself in order to be ready to contract again. This is an important value as much of the chaotic, asynchronous contractions of the atrial tissue as is characterized by AF rely on continuous conduction of those errant electrical signals in order to propagate. Consequently if a cell is still unable to contract (i.e. within the refractory period) when an errant signal is encountered, the supposition is that the AF propagating signal will end there instead of being passed along as may have been done by a cell which had reset itself to contract much quicker.

Measuring Refractory Periods

To determine the refractory period an eight pulse burst pacing is initiated at 150 bpm (400ms spacing) with a time variable ninth pulse which whose individual period is slowly decreased until the time between the eighth and ninth pulse of the train is within the refractory period. This is consequently characterized by a loss in activation of the surrounding tissue. This pacing protocol is done with each of the implanted leads on the right side, first assessing the atria and then the ventricles.

The first pacing protocol is run in the right atrium. As the timing of the ninth pulse is reduced, eventually the corresponding activation in the ventricle will lapse. The activation may still be observed in the atrium, but the ventricles are not receiving the

electrical activation. This does not necessarily mean that the timing of the ninth pulse has been reduced to within the refractory period of the ventricle, but rather that the cells responsible for transmitting the signal between the chambers, that is the atrial-ventricular (AV) node, are within their refractory period. The timing is further reduced until there is no final pulse activation in the atrium as well. This is recorded to be the refractory period of the atria. This protocol is immediately repeated with an initial eight pulse spacing equal to 200 bpm (300ms spacing) followed by repetition of both initial spacing in the pacing protocol for the implanted ventricular pacing lead. However from the ventricular burst pacing protocol only the ventricular refractory periods are measured.

Induction of Atrial Fibrillation

Following measurement of the refractory periods, the AF burden is determined by inducing AF using burst pacing through the pericardial lead on the left atrial appendage. A two second administration of 2ms, 5.5-6.5V, square wave at 200Hz reliably induced AF in the porcine model. The episode of AF would typically resolve itself within 10-60 seconds, returning to a normal sinus rhythm. The time in AF was measured from the initial triggering of the burst pace to the first atrial beat of normal sinus rhythm. This was repeated ten times except in the cases where a period of induced AF was sustained for more than 60 seconds. In those cases, no further shocks/AF was administered and the incidence of AF was given up to ten minutes to resolve. If not

resolved within the ten minutes, intervention in the form of a 5J (cardioversion) defibrillation shock was administered to the atria via internal paddles.

Administration of Agents

A syringe pump is used to administer 10mL of the treatment or control over 10 minutes. A piece of gauze is placed over the top of the exposed heart and tucked down into the sides of the pericardial cradle and serves to effectively wick the solution over the top of the heart. This is done to ensure both more complete administration of the treatment to the heart and as a practical consideration which is that a piece of gauze would be readily accessible and easy to use in an operation setting. Subsequent measurements of tissue refractory periods and AF induction were made at 30 and 60 minutes following administration of agents.

Hearts were cardioplegied, isolated,, and reanimated on the Visible Heart™ system within 70-120 minutes where hemodynamics were monitored and the perfusing fluid was sampled at 5 minute intervals for lactate and glucose levels from the coronary sinus.

One experienced surgeon was responsible for all surgical procedure in all animals, from the placement of leads/catheters to reanimation of the heart on the Visible Heart™ system. This was done for consistency and removing bias from in the model as they were additionally blinded to the treatment/control.

Results

Preliminary assessments following the same methodology over a longer 90 minute course were undertaken with some of the component treatments (DHA, and Omegaven) and a motivational factor for their assessment in this combined treatment study. They are shown here against normal (saline) controls and negative (EtOH) alcohol controls (Figure 2-2). The Omegaven and DHA were both shown to decrease the time for which induced AF was sustained.

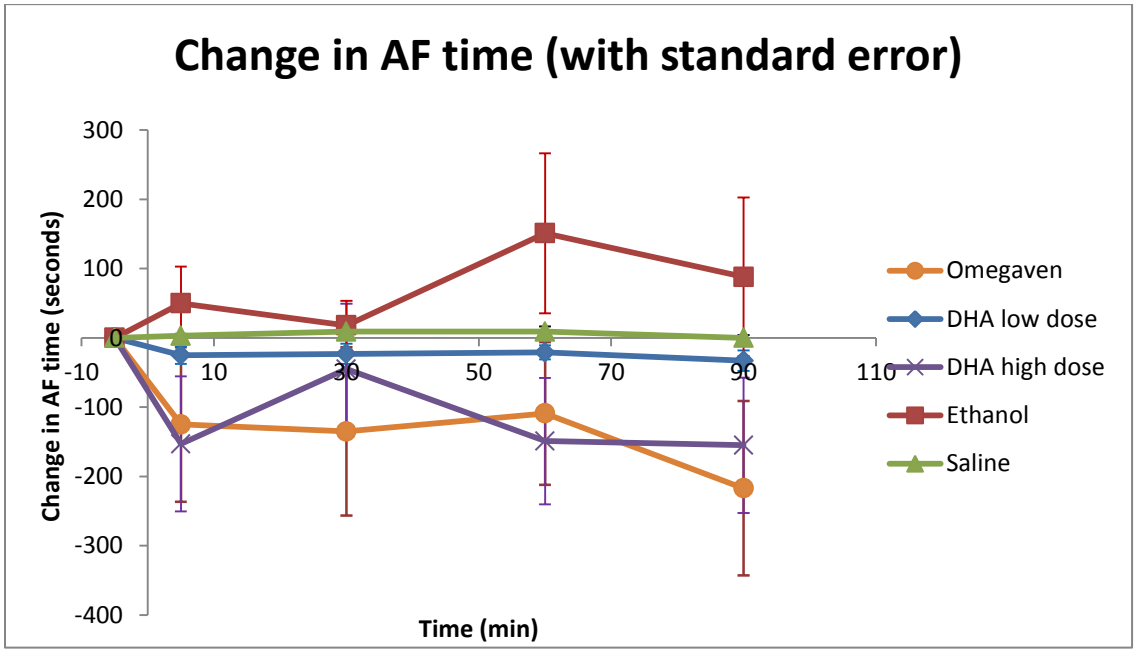


Figure 2-2: Comparison of sustained AF time (up to 10 times induced per time period) as a function of dosing with DHA, Omegaven, and two control classes; negative Ethanol control, and neutral saline control.

Limiting the time which an animal is allowed to sustain atrial fibrillation presents some challenges with data collection. We report here both the total average time it took for the hearts to come out of induced AF (Figure 2-3) as well as the number of times that hearts successfully recovered from AF within the allotted one minute period (Figure 2-4).

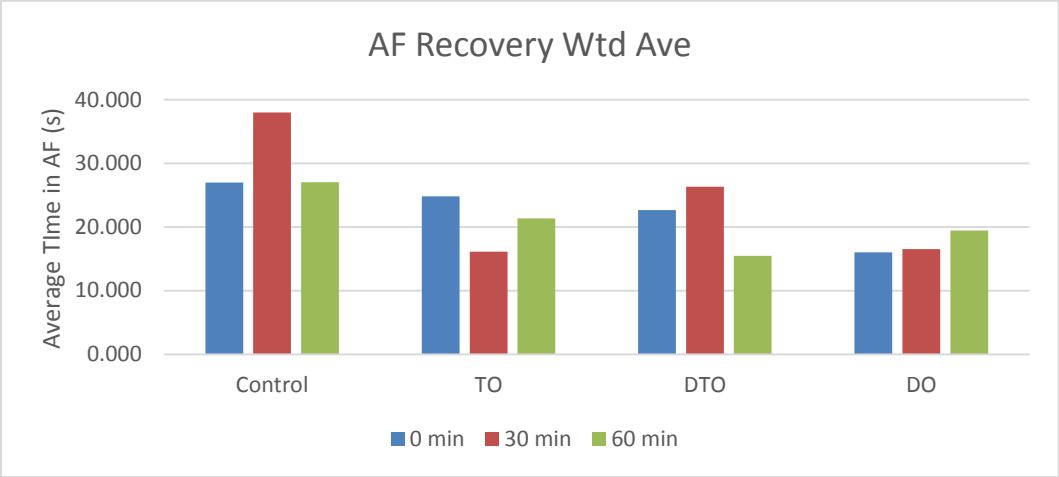


Figure 2-3: The amount of time that AF was sustained with combined treatments at each time point.

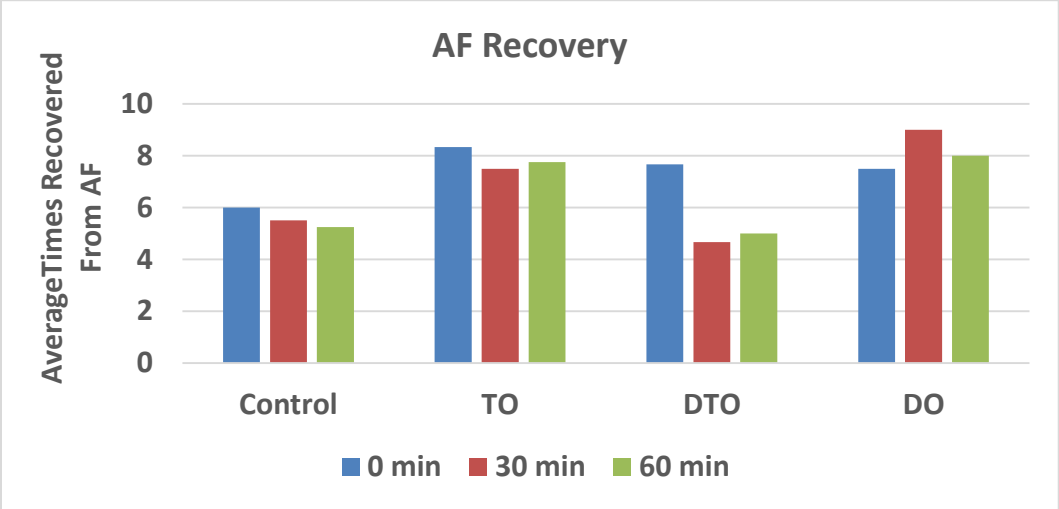


Figure 2-4: The average number of times that hearts recovered from induced AF also suggests a positive benefit from combinations of Omegaven combined with TUDCA (TO) and DHA (DO) on a sustained basis.

Refractory Periods

A proposed mechanism for AF protection is the ability of an agent to extend the effective refractory period of the cardiac tissue. Refractory periods were measured in situ during the hour following administration using a Medtronic 2090 operating a GEMIII to run independent EP study protocols on ventricular and atrial pacing leads to determine

the refractory periods in the 1) Atria, 2) Atrioventricular node (AVN), and 3) Ventricular tissue.

The protocol paces the atria for eight beats at 400ms initial pacing interval with a reducing ninth beat interval; repeated each time with an initial 300ms pacing interval. The difference between 300 and 400ms initial intervals was observed to make a significant difference in the pacing refractory period measurements with the exception of the atria, which only approached significance (Figure 2-5).

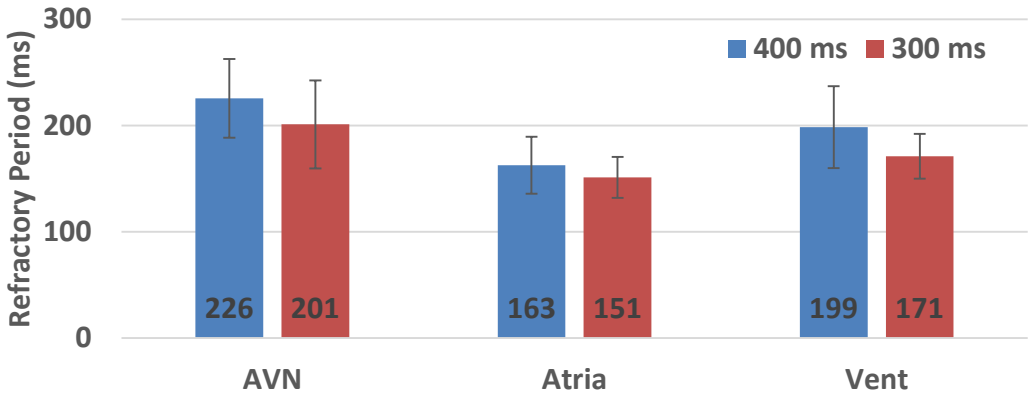


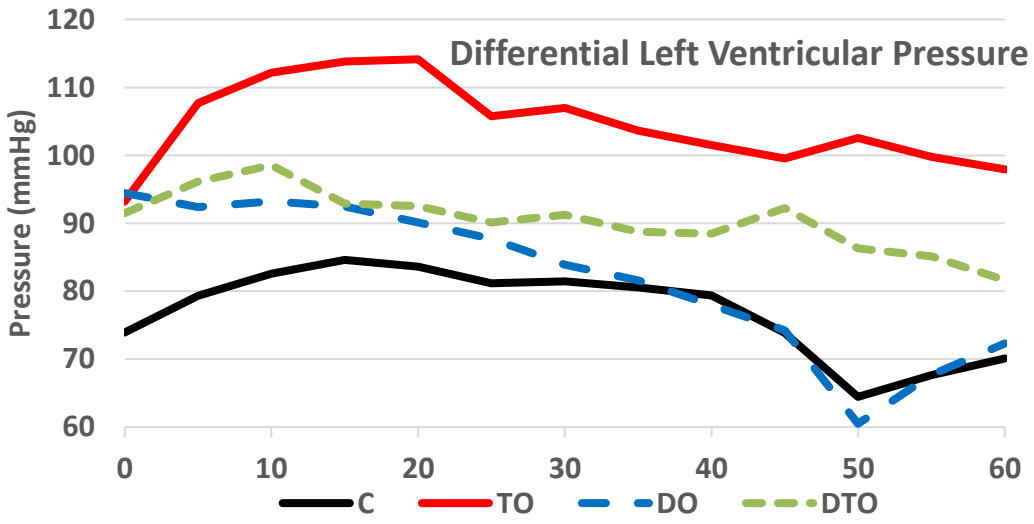
Figure 2-5: Refractory period data over the entire untreated population shows significant dependence on the timing of the initial train in the AV node and Ventricular myocardium with a similar reduction in the refractory period mirrored in the Atrial tissue (p=0.033, 0.0095, and 0.085 respectively).

Importantly, none of the treatments showed significant modification of the refractory periods with the shorter initial train (300ms spacing). All significance variation happened with an initial timing of 400ms). Additionally a baseline group (B) was established from all untreated samples which include all of the initial samples from trials without regard to their treatment. As verification, the saline treated control group showed no significant deviation from the B group at any time-point (including 60 minutes). This was also true for ALL treatment groups at their baseline (untreated) time point.

The TUDCA-Omegaven (TO) treated group did NOT CHANGE the refractory periods significantly from the B group in any of the cardiac tissue. The DHA-TUDCA-Omegaven (DTO) treated group DECREASED the refractory periods in the AV node and Atrial tissue with respect to the B group (p=0.055 and 0.040 respectively). The DHA-Omegaven (DO) treated group INCREASED the refractory periods in the AV node and Ventricular tissue with respect to the B group (p=0.005 and 0.037 respectively).

Hemodynamic Performance

The heart is reanimated on the Visible Heart™ system and hemodynamics is continuously monitored. In fully physiologically accurate operational mode, the averaged differential pressures of the ventricles are shown in Figure 2-6.



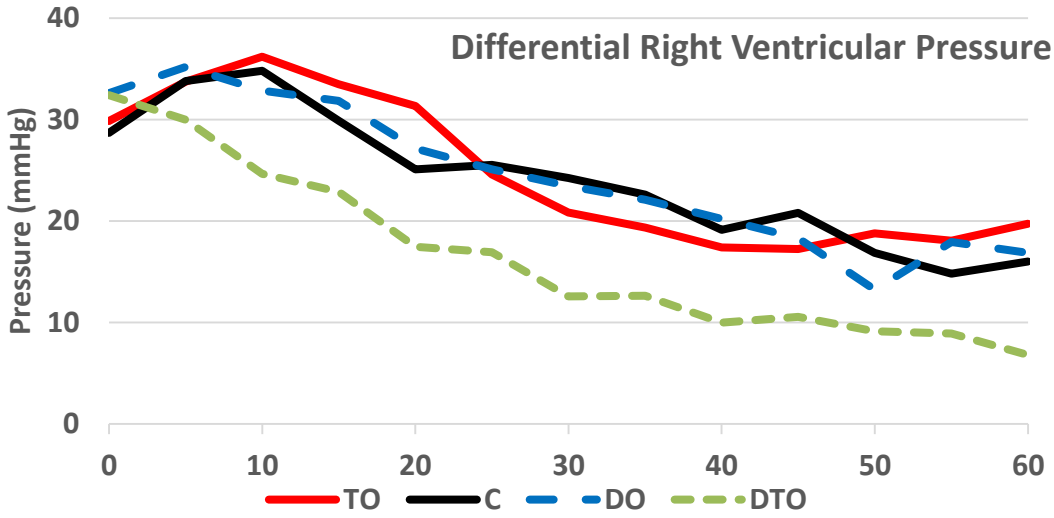


Figure 2-6: Following reanimation of the heart, hemodynamics was monitored over the course of an hour. Shown here are the differential pressure trends in the Left (top) and Right (bottom) Ventricles.

Treatment with any of the combinations showed improvement on the initial pressure generation in the left ventricle with significantly better performance at the hour endpoint with the TO combination and favorable results for DTO as well.

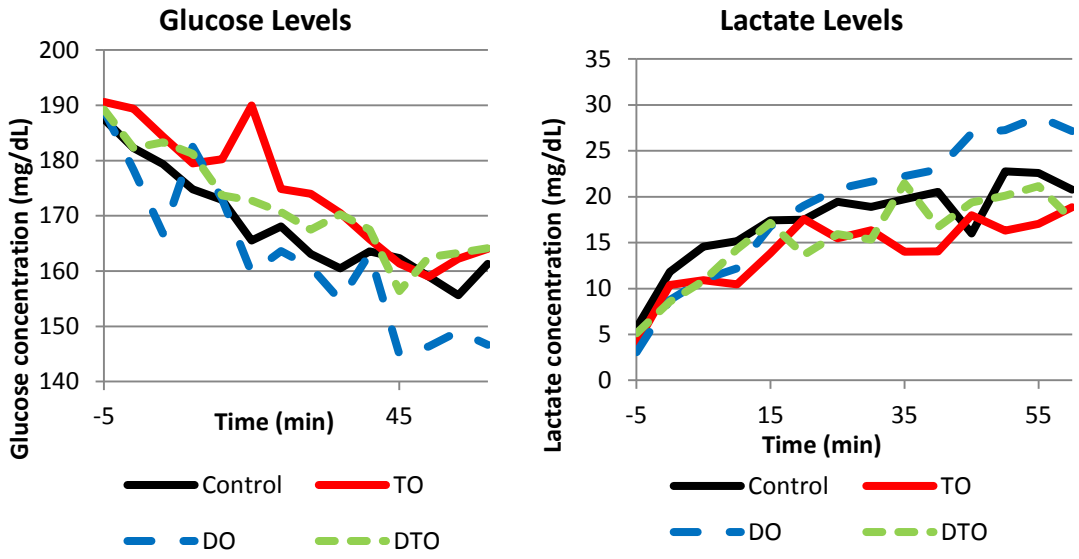


Figure 2-7: Glucose is observed to decrease and lactic acid increase in all cases, showing no statistical difference for this limited population.

The DO combination trends may suggest better cellular metabolism with a larger consumption of the available glucose level and increased lactic acid production (Figure 2-7) but the endpoints are not statistically significant for the sample size.

Discussion

On the basis of the hemodynamic profiles, it is clear that a treatment in any case is initially preferred by the transplanted heart in this large mammalian model. Of those treatments, Omegaven was the consistent base. Those treated additionally with TUDCA sustained improved function as compared with control hearts or those treated with the DO combination.

The results concerning in situ AF protection were mixed. Combinations of TO and DO are associated with an increased propensity to exit AF, while the protective mechanism is only associated with an increased refractory period in the absence of TUDCA (in the DO combination). But all treatments are observed to spend less total time in AF.

The simple treatment of these agents show promise in the ability to provide high doses without risking gross systemic effects can be done in a short time period and concurrent with other procedures, offering both some potential AF protection and gross hemodynamic performance benefits. Worth re-iteration is the large mammalian model presented here on the Visible Heart™ system is an ischemic model meaning that the initial improvement over baseline may be attributable to improved resistance to the

ischemic insult of isolation or cardiac arrest, and further agent benefits may be considered protective against the induced perpetual ischemic condition.

This is a clinically translatable treatment and model for such agent assessments. In fact, current derivatives of these testing methods are being used as a baseline research model.

Chapter 3 : Isolated Heart and Heart Lung Combinations

Preface

In 2013 the Visible Heart® Laboratory began reanimating the heart while maintaining native and functional lung anatomy (heart-lung bloc preparations). This addition of lungs to the Visible Heart® preparation was initially driven by the need for better imaging of functional pulmonary vein anatomies. The pulmonary veins play a very important role in the presentation of atrial fibrillation (AF) and thus are key targets for a lot of different cardiac devices and procedures to treat AF. The paper in this chapter outlines the primary results which were obtained from multiple reanimations of human heart and heart/lung combinations. Described here are the expanded capabilities of the Visible Heart® apparatus/methods and made possible by my engineering contributions.

The inclusion of lungs on the isolated preparation is not unique, in fact the very first successful mammalian heart preparations were done by isolating the heart and lung together from the rest of the systemic circulation in an animal using the lungs as a tool to deliver oxygen to the heart being studied. Henry Martin was the first to successfully do this and thus solved the problem of requiring perfusion of the coronary arteries after isolation of the heart. His work which was built upon by Oscar Langendorf who further isolated the heart from the lungs altogether. Technology has advanced considerably in the time since and in removing the blood as well, this work comes full circle to include the lungs not as an oxygen delivery tool, but as a direct subject of study.

The primary of advantage of reanimating heart/lung blocs (both swine and human) with the Visible Heart® methods is that it allows for the visualization of the

device-tissue interfaces. This setup was almost immediately employed at the Visible Heart Lab for precisely that in the case of the next generation of cryo-ablation devices; i.e., investigating ice-ball formation/dissipation and handling characteristics in tortuous anatomy. This required a high degree of modifications to the Visible Heart® apparatus for which was my primarily responsibility.

My engineering with this system puts in place reliability fail-safes, using a new array of sensors (pH, O2, temperatures, fluid levels/pressure, fluid color, etc.), custom pneumatic pinch valves, and automated control routines built into ergonomic and ease of use considerations such as the double touch-screen controls, with continuous data recording and synchronization. Because of my work this system is capable running an array of experiments by itself and maintaining a heart/lung preparation for as long as the heart is able to function with minimal if any intervention.

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The Novel In vitro Reanimation of Isolated Human and Large Mammalian Heart-Lung Blocs

Running Head: Reanimation of Isolated Heart-Lung Blocs

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Summary

Background

In vitro isolated heart preparations are an invaluable tool for the study of cardiac anatomy, physiology, and device testing. Such preparations afford investigators a high level of control, independent of host or systemic interactions, and high throughput if desired. Here we present that isolated human and swine preparations with the lung(s) attached are particularly valuable for the study of device-tissue interaction and anatomy. Additionally we detail our laboratory's experience with developing these methods for heart/lung bloc studies

Methods

Four human and 18 swine heart-lung preparations were procured using techniques analogous to those of cardiac transplant. Specimens were then rewarmed and re-perfused using modifications of a closed circuit, isolated, beating and ventilated heart-lung preparation. Positive pressure mechanical ventilation was also employed, and pericardial defibrillation was applied to elicit native sinus rhythm after rewarming. Videoscopy, fluoroscopy, ultrasound, and infrared imaging were performed for anatomical and experimental study.

Results

Systolic and diastolic pressures observed for human and swine specimens, respectively, were 68/2±11/7 and 74/3±17/5 mmHg, with heart rates of 80±7 and 96±16 bpm. High

resolution imaging within functioning human pulmonary vasculature was obtained among other anatomies of interest.

Conclusions

We report the first dynamic images of the pulmonary vasculature during cardiopulmonary function in isolated reanimated heart-lung blocs. This experimental approach provides unique in vitro opportunities for the study of novel medical therapeutics applied to both human and large mammalian heart-lung specimens.

Introduction

In vitro isolated heart preparations have been a cornerstone of cardiac research since Langendorff's original methodology was described in the 1890s [1]. The benefits of isolated heart research are numerous and can be remarkable depending on the investigator's goal. Isolated hearts offer a high degree of control over the system including, but not limited to: perfusate selection, flow control, and pre- and after-load variability. For a thorough historic summary of these experimental models the reader is referred to a review by Hill *et al.* [2]. Furthermore, numerous pharmacological studies using such approaches can help elucidate the direct action of agents on the isolated cardiac tissues, i.e., while avoiding systemic interactions of other agents or breakdown products (e.g., cardiac-nervous system, hepatic metabolism, etc.) [3].

Additionally, high-throughput cardiac perfusion systems can be designed, or now even purchased off the shelf, in which multiple small mammalian hearts can be experimented on simultaneously. Isolated heart preparations have garnered notable insights to mechanisms of arrhythmias [4] and have been reviewed elsewhere [5]. Depending upon the system configuration, a wide range of equipment and modalities are available to the investigator including: electrophysiological monitoring and stimulus, ultrasonography, ultrasonic stimulation, fluoroscopy, infrared thermography, direct visualization via videoscopes, and anatomical mapping systems. Furthermore, the utilization of large mammalian isolated hearts allows for critical preclinical testing of device-tissue interactions in an environment highly similar to actual human anatomy and physiology, if

the proper animal model is selected for investigation [6]. Comparative imaging of normal versus pathologic conditions, or interspecies comparisons, to determine optimal approaches, models, and designs is critical to development of novel therapeutics [7]. To the medical device designer, engineer, or clinician, these insights have proven to be of high educational value [8, 9].

Despite isolated heart preparations being a valuable tool, proper anatomical relationships can be compromised when the lungs are removed. In particular, the pulmonary veins and their native ostia are of interest in the context of pulmonary vein isolation ablation treatments for atrial fibrillation. Heart-lung preparations have been utilized previously to elucidate the release of atrial natriuretic peptide [10] and expand the pool of lung transplants to non-beating donors [11]; they have also been used in numerous pharmacologic studies. Interestingly, the first heart-lung preparations are often attributed to Knowlton and Starling [12], however their work acknowledges the methods of Martin [13] which were presented in lecture at Johns Hopkins in 1883. The first publication by Martin of his heart-lung bloc preparation was released in 1881 [14], therefore predating Langendorff's work by fourteen years. In short, this preparation cannulates in situ the superior vena cava and one of the branches coming off the aortic arch. A closed loop is created by which pressure can be monitored, a compliance chamber is incorporated and pre- and after-loads are varied.

It is also possible for human hearts from non-viable organ donors to be successfully reanimated using an isolated experimental apparatus [2]. The Visible Heart® methods have been previously described by our laboratory [15], but more recently we have expanded these novel experimental approaches to incorporate whole large mammal heart-lung blocs, including both human and swine studies. To the authors' knowledge, this is the first description of large mammalian heart-lung blocks being used to achieve dynamic imaging in the pulmonary vasculature.

Material and Methods

The isolated heart-lung technique developed by our laboratory has been used successfully to reanimate human and swine hearts with right, left, or both lungs attached and functioning. Swine studies were approved by the Internal Animal Care and Use Committee at the University of Minnesota. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals. Human hearts were approved for study by the Human Subject Committee Internal Review Board. Consent for use of the hearts for research purposes was received from the donors' family members before explantation via LifeSource (St. Paul, MN, USA).

The detailed procurement procedure has been described previously [2, 15]. Briefly, a median sternotomy was performed and an aortic root cannula implanted for delivery of cardioplegia. The inferior vena cava (IVC) was ligated and, just prior to cardioplegia delivery, the IVC for human preparations was removed with the liver if it was being

recovered for transplant, and the superior vena cava (SVC) and aorta were cross-clamped. Cardioplegia was then delivered under pressure to rapidly cool and arrest the heart. The heart and lungs were then dissected and the heart-lung bloc removed by transection of the major vessels, trachea, and esophagus. The human specimens were then transported on ice to the laboratory within 6 hours of cross-clamp. An analogous procedure was performed on swine hearts in our laboratory (mean animal weights of 84±14kg; n=18). We have typically performed these studies with just one lung attached, but the method has been recently adapted to include both lungs.

Upon arrival of human (or after explantation of swine) specimens, hearts were placed in an ice slurry of modified Krebs-Henseleit buffer while cannulation of the great vessels was performed (i.e., IVC, SVC, and aorta). If a one-lung preparation was desired, the left/right pulmonary veins and artery were dissected from the left/right lung, and the lung was removed. These vessels were cannulated as well, and a hemostasis valve was fitted for access. If both lungs were desired in the preparation, the pulmonary trunk was cannulated to allow control of the buffer flow, either directing all flow through the lungs or allowing some flow to the reservoir (i.e., a parallel path through the lungs and to the reservoir). An intubation tube was placed in the trachea and connected to a ventilator to control flow through the airway. Preparations were ventilated at a respiration rate of 11-15 per minute and a volume of 150-250 milliliters per lung.

The heart-lung blocs were then connected to the apparatus described in detail previously [15] that was adapted for such use. A schematic of this system can be found in

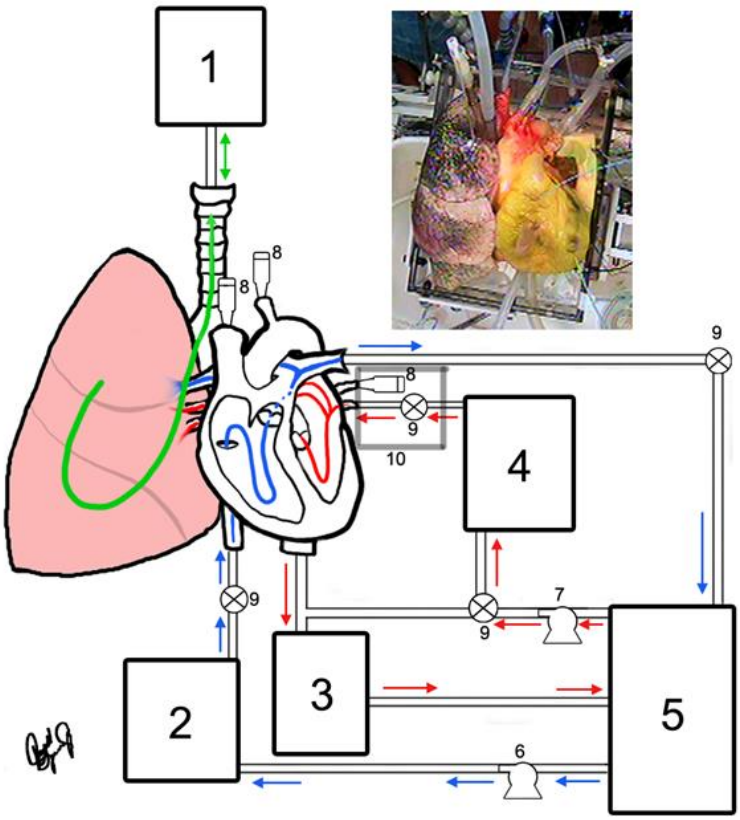

Figure 3-1. The system was altered to vary the aforementioned parameters of other isolated heart research systems and functioned in either partial or four-chamber working mode. Partial working mode is similar to a Langendorf apparatus function, but fluid flow continues through an isolated lung. The system utilized a cardiovascular bypass oxygenator and heated water jacketed fluid reservoirs to maintain the proper physiologic environment. Seven to eight liters of modified Krebs-Henseleit buffer were contained in the system, and buffer changes of approximately four liters were performed regularly to wash out metabolites and maintain visualization as desired.

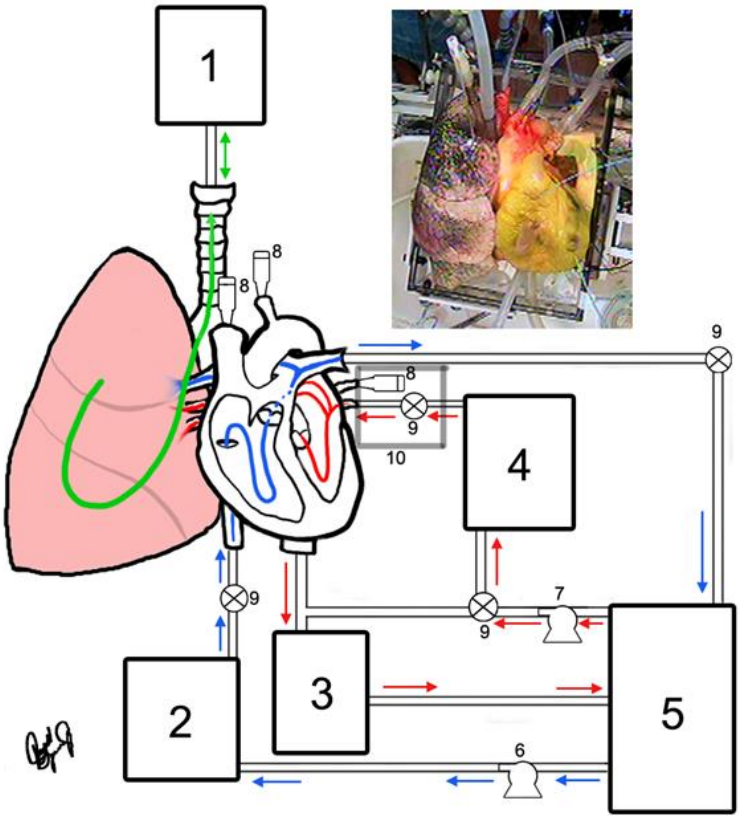

Figure 3-1: (Top right) External view of human heart 277 in systole and attached to the system. (Center) Flow diagram for a functional heart and lung reanimation consisting of: (1) a respirator connected to the cannulated trachea and thus attached to the lung(s), (2) a pre-load chamber for the right side of the heart, (3) an aortic after-load chamber which mimics the resistance that the left ventricle works against, (4) a left pre-load chamber employed when only one lung is present, (5) an oxygenator reservoir for pooling fluid expelled by any cannulated branch of the pulmonary artery, (6 & 7) fluid pumps to maintain the pre-load pressures, (8) hemostasis valves that allow access for delivery of cameras, instruments, and assorted devices, (9) valves that may also be used to redirect flow as physiologically appropriate, while (10) cannulation of the pulmonary vein(s) are shown here for a right lung preparation, but are absent or translated when either both lungs or the left alone respectively are used.

Once the specimen was re-warmed to 37°C, dobutamine was added to the system and the heart was defibrillated with 34 joules of energy supplied by a programmer-analyzer unit (#88345 Medtronic, Inc., Minneapolis, MN, USA) via a pair of external patches (#6721, Medtronic, Inc.) placed epicardially above and below the ventricles. These hearts

generally began beating in native sinus rhythm after a single defibrillation. It should be noted that one human heart developed heart block at two hours post-reanimation, and was then paced by a temporary pacing lead at 60 beats per minute; all specimens could be paced as desired. Hemodynamics of the left and right ventricle were recorded by Utah Medical pressure transducers (Model DPT-200, lot#1101991, Midvale, UT, USA) via water columns from venogram balloon tipped catheters (Attain 6215, Medtronic, Inc.).

High-resolution Olympus commercial endoscopes (Model 1V8200T, Model 1V8420, Center Valley, PA, USA) were then placed within these heart and/or lungs to capture functional anatomy. To our knowledge, these are the first images of the pulmonary veins and arteries within the lung of functioning human heart-lung blocs.

Results

Using this novel experimental approach, eighteen swine and five human heart-lung blocs were successfully reanimated. Hemodynamic functioning of these in vitro reanimated specimens was augmented by the delivery of inotropic agents and/or by increased dosing with extracellular calcium. Prior to heart recovery, the mean heart rate and blood pressure for the swine were: 91±13 beats per minute and 105/56±13/9 mmHg, respectively. Table 3-1 provides partial cardiac medical histories for the organ donors from which the human hearts were recovered. Table 3-2 provides the relative hemodynamic performance data for these reanimated heart-lung preparations after hemodynamic stabilization post-defibrillation.

Table 3-1: Summary of donor information and hemodynamic status prior to organ recovery

Human Specimens							
Specimen	Gender	Age (yrs.)	Weight (kg)	Cause of Death	HR (bpm)	BP (mmHg)	CVP (mmHg)
HH 277	M	60	113.4	Head trauma	71	105/61	15
HH 284	F	78	54.4	CVA	103	118/70	11
HH 291	F	58	114.7	CVA	92	100/50	12
HH 295	M	34	68.0	Cardiac arrest	92	130/75	-
HH 308	F	36	53.0	CVA, previously transplanted	87	97/71	10
Average		53.2	80.7		89.0	110/65	12
Standard Dev.		18.4	31		11.6	14/10	2.2

BP=blood pressure; HR=heart rate; CVA= cerebrovascular accident; CVP=central venous pressure

Table 3-2: Hemodynamic performance of each reanimated heart/lung bloc specimen after hemodynamic stabilization post-defibrillation

Swine Specimens							
Specimen	HR (bpm)	LVSP (mm Hg)	LVEDP (mm Hg)	+dLVP/dt (mm Hg/s)	-dLVP/dt (mm Hg/s)	Tau	Lung
1	95.8	91.2	12.4	982.8	-903.0	31.2	Right
2	91.0	25.0	-2.0	430.8	-343.8	36.2	Right
3	100.0	77.0	-4.0	961.0	-462.0	30.0	Right
4	81.7	73.5	2.3	772.3	-354.5	37.7	Right
5	91.8	85.7	1.3	927.0	-509.8	33.2	Right
6	99.5	62.8	11.3	574.0	-435.0	30.2	Right
7	55.8	82.0	5.7	600.2	-358.2	63.0	Right
8	90.3	79.3	-4.3	842.5	-618.0	33.5	Left

9	92.7	75.7	12.0	623.3	-771.7	32.5	Left
10	102.5	67.5	1.2	637.8	-513.2	29.7	Right
11	76.7	101.7	10.7	786.5	-501.5	39.7	Right
12	123.3	76.3	1.3	729.0	-624.5	26.0	Right
13	124.8	58.7	2.7	762.5	-532.7	24.0	Right
14	84.5	87.2	0.7	888.8	-646.0	54.0	Right
15	114.0	91.7	-2.7	922.3	-808.0	27.5	Right
16	105.0	70.8	0.0	607.7	-446.3	28.8	Right
17	101.3	56.5	3.8	529.8	-317.7	29.8	Right
18	91.2	75.7	7.0	618.3	-810.2	33.0	Right
Average	95.7	74.3	3.3	733.2	-553.1	34.4	
Standard Dev.	16.3	17	5.4	163.6	177.6	9.7	
Human Specimens							
HH 277	85.8	65.7	-7.3	624.5	-475.5	37.2	Right
HH 284	81.2	79.5	1.7	848.2	-377.7	37.5	Right
HH 291	70.3	53.3	8.0	341.3	-273.7	45.3	Both
HH 295	81.3	72.5	4.2	415.7	-343.0	37.7	Right
HH 308	57.2	73.0	0.0	963.0	-469.8	56.2	Right
Average	75.2	68.8	1.3	638.5	-387.9	42.8	
Standard Dev.	11.6	9.9	5.7	268.1	86	8.2	

HR=heart rate; LVSP=left ventricular systolic pressure; LVEDP=left ventricular end-diastolic pressure; +dLVP/dt= maximal positive derivative of left ventricular pressure with respect to time; -dLVP/dt= maximal negative derivative of left ventricular pressure with respect to time.

It should be noted that one of the early reanimated swine heart-lung specimens (#2) elicited poor hemodynamic performance from the beginning of reanimation. We suspect that injury occurred during isolation and/or that emboli caused poor coronary perfusion. Additionally, recorded data from several hearts elicited negative values for end-diastolic pressures; we suspect that this is due to drift in the sensors or a vacuum or syphoning effect, potentially occurring in the current system modification to incorporate the lungs.

Interestingly, compared to our long-term experience with lone heart reanimation using endoscopes, a large degree of remaining particulate and blood within the lung complicated our initial imaging during certain studies. Therefore, more frequent buffer changes were required to obtain clear, high-fidelity images and video.

As previously mentioned, the main benefit of this model is the maintenance of proper pulmonary ostia and vessel anatomies. A selected anatomical image series of a videoscope being retracted from either the pulmonary arteries or veins is shown in Figure 3-2; a corresponding video can be accessed via supplemental materials (Supplemental Video) or online at http://www.vhlab.umn.edu/atlas. The study of cryoballoon ablation procedures motivated much of the development of this model, and a series of cryoballoon procedures have been performed, as viewed from within the vein as displayed in Figure 3-3, panel C. The Supplemental Video of the functioning pulmonary arteries and veins gives the reader an appreciation of the truly dynamic nature of these vessels, which are usually thought to be relatively passive structures.

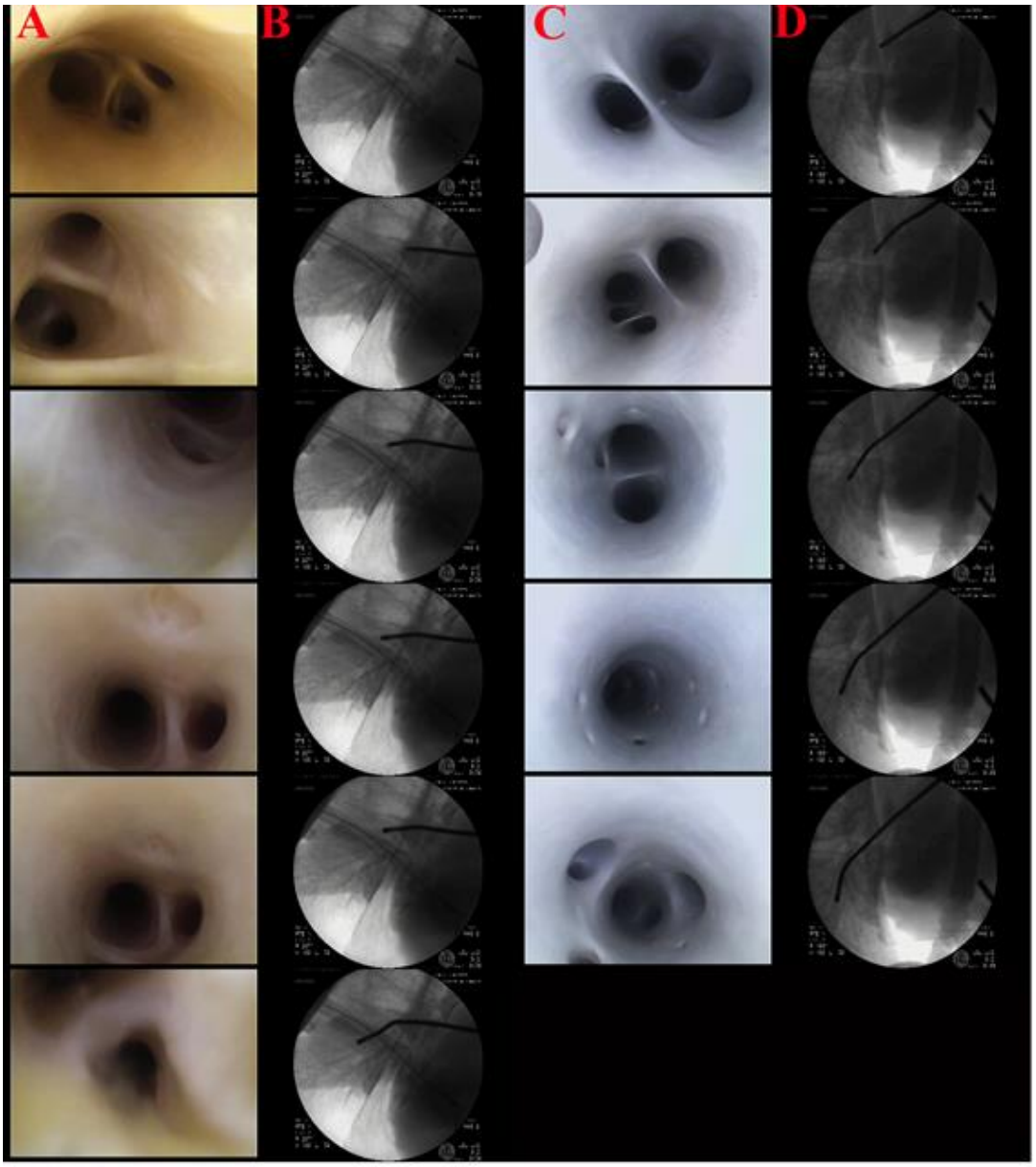

Figure 3-2: Image series obtained from reanimated human heart-lung bloc #284 (A,B) and #277 (C,D). Series shows the path through the distal pulmonary arteries and veins, respectively. The corresponding fluoroscopic images (B,D) in each case show the relative locations of the videoscopes (A,C). A video of the journey through the vasculature can be viewed as well (see online Supplementary Video).

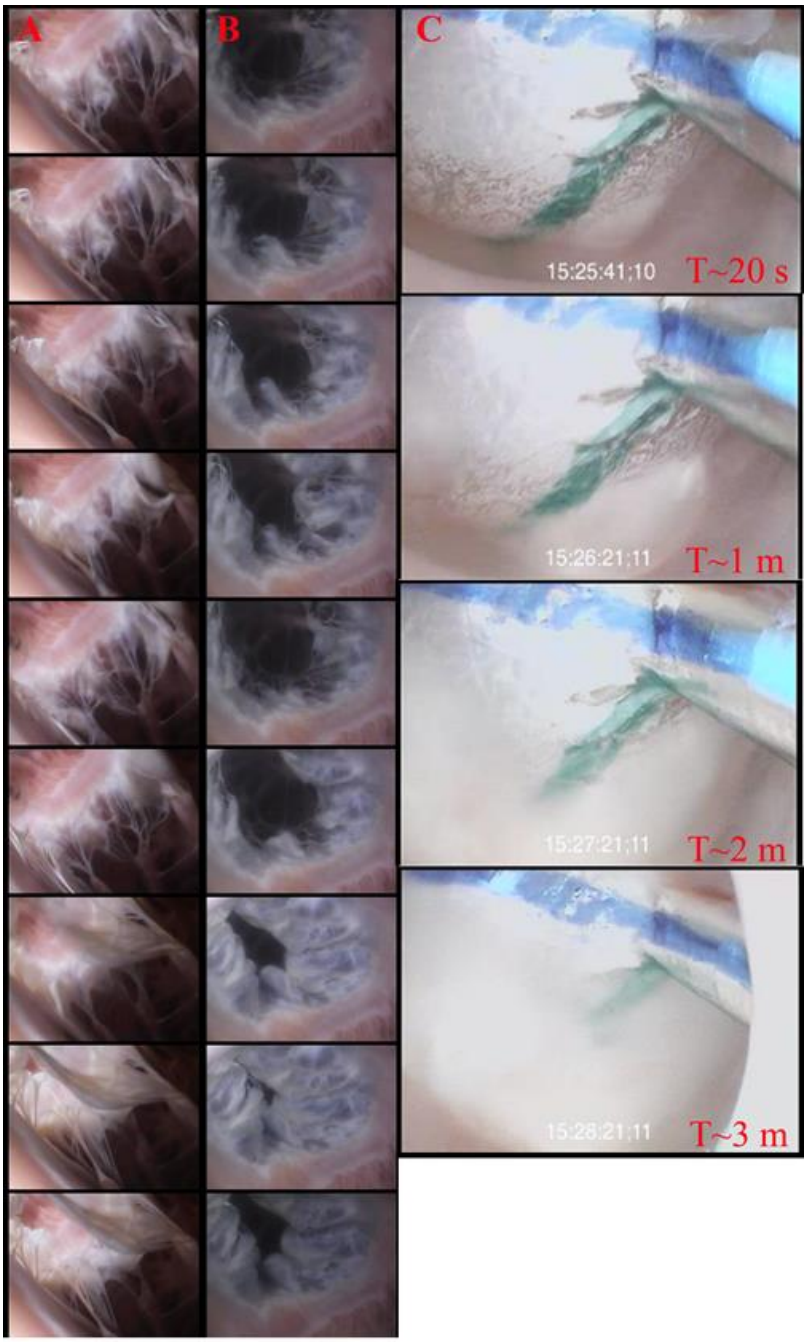

Figure 3-3: Time series of images from human heart #277. Series shows tricuspid valve closure from the right ventricle (A) and right atria (B). Images are displayed 1/15th per second apart in time. Panel C displays ice formation on the distal portion of a cryoballoon ablation catheter (Artic Front, Medtronic, Inc., Minneapolis, MN) as seen from within the pulmonary vein. The images are spaced post-ablation 30 seconds, 1, 2, and 3 minutes apart.

Conclusions

To the authors' knowledge, this report has provided first time dynamic images of the pulmonary vasculature during normal cardiac function in both reanimated human and swine heart-lung blocs. This model provides a unique in vitro approach for the study of novel medical therapeutics from both human and large mammalian heart-lung specimens.

In a similar embodiment (i.e., without the lungs), this reanimated heart model has been utilized in numerous cardiac studies. In the electrophysiology area, these studies have included the use of endocardial noncontact mapping, pacemakers, defibrillators, leads, and catheters [16]. The Visible Heart® model has also been employed to study the dynamic nature of valves and transcatheter valve deployment [17]. Importantly these methodological approaches also allow for use of echocardiography and fluoroscopy to guide procedures, such as for comparative imaging [18]. Most recently, this approach has proven to be quite valuable for the study of novel cardiac treatments, such as leadless pacing devices [19]. Nevertheless, the novel addition of a lung(s) to this paradigm still allows for any of the prior studies to be conducted, but may in turn reduce the number of hemostasis valve access points that were previously available.

Our continued use and enhancement of Visible Heart® methods have also facilitated the creation of an open-access educational website, The Atlas of Human Cardiac Anatomy

(http://www.vhlab.umn.edu/atlas) [20]. The anatomical images and videos on this website are free to download and use for presentations and teaching, however we request that proper citations be used. In other words, the novel images and/or comparative imaging of functional cardiac anatomies are of high value in teaching the nuances of cardiac anatomy, especially of active, complex structures such as valves. It should be noted that this website also provides instructional tutorials on cardiac anatomy and physiology, as well as full cadaveric thoracic cavity dissections. Finally, a cardiac device tutorial is also available, which has been well noted as being beneficial in explaining therapies to patients.

The model described here is not without limitations, as is true with all in vitro systems. Despite supersaturating the buffer with oxygen, there remains a significant difference in the oxygen content of the buffer compared to blood. For this reason, the function of the heart slowly declines over time from the initial reanimation. Yet reasonable physiologic function to perform the aforementioned investigations is generally elicited for up to 4 to 8 hours, based on our previous experiences. It is possible that the addition of the lungs may extend the functionality, a theory which we tend to believe at this point but need more data to substantiate. Most recently, we are employing a full anesthesia suite ventilator to more closely control the ventilation parameters (e.g., provide positive end-expiratory pressure). In such experimentation, one also needs to consider that although it is also known that hypothermal transport of organs is protective, global ischemic injury still occurs to some degree. Further, our current system is designed to replicate physiologic

pre- and after-loads, but there potentially are important physiological effects that are not completely replicated, such as vessel compliance. Likewise, in our studies the heart-lung blocs are cradled on soft foam sponges which may focally alter perfusion compared to the natural state. Finally, due to the nature of acquiring non-viable donor human heart-lung specimens, there are numerous differences between inherent statuses of the hearts that cannot be controlled. Such differences in status include, but are not limited to: method of cardiac arrest, transport time before arriving in the laboratory, inotropic support, potential air or other emboli in the coronary vasculature, and prior pathologies. Despite being unable to control for these parameters, the described system produced comparable pressures to other isolated human heart alone preparations [2, 21]. As reviewed in Table 3-2, despite these variances the hemodynamic data are fairly comparable from specimen to specimen. It should be specifically noted that we are extremely grateful and privileged to obtain these donated human heart-lung preparations as gifts for research.

To conclude, this extension of Visible Heart® methods has enabled novel, functional anatomical heart-lung anatomical visualization. Further, unique abilities to image the device-tissue interactions using this approach are unparalleled. Therefore, we consider that these obtained images are of high value to the medical device designer, educator, and clinician for both training and educational purposes. The lab will continue to reanimate hearts and heart-lung blocs using these methods and thereby enable the study of dynamic anatomies, insights into the device-tissue interface, and creation of new materials for the free-access Atlas of Human Cardiac Anatomy website.

Acknowledgements

The authors would like thank the generous individuals and families that donated hearts for research and LifeSource for facilitation of this research. A special thank you to Gary Williams for his computer assistance and Monica Mahre for preparation of this manuscript.

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Chapter 4 : OCS Lung System Baseline/Whole Blood

Preface

The University of Minnesota's cardiothoracic transplant team began performing lung transplants using a normothermic temperature preservation device (Organ Care System™ (OCS), Transmedics MA) in 2014. These first transplants were part of the INSPIRE clinical trial for the new Lung OCS™ a 1 to 1 comparison against standard procurement of lungs on ice. An additional OCS™ unit was procured for the purposes of basic science research to be performed in collaboration with the Visible Heart® Laboratory with myself as the primary operator of the system.

As a first steps for utilizing this as an experimental research tool, we: 1) assessed the device as utilizing procured swine lungs utilizing standardize clinical protocols, that is utilizing blood processed through a cell saver (control studies); and next 2) investigated a modification to the approach as a means to extend the viabilities of the preparation out to beyond 24 hours (therapeutic groups). This modification followed from my work in integrating lungs with the Visible Heart® preparation (Chapter 3), which mirrored the failure modes that are observed here in the clinical model from 9-12 hours with severe edema.

The use of whole blood for sustaining lungs in the OCS™ was our first therapeutic group and provided for both a large reduction in overall edema over time as well as a consequently longer viability reaching out to over 24 hours. In other words,

otherwise healthy swine lungs sustained on the OCS™ system with standard clinical methods would no longer be considered viable for transplant after 9 hours, but if whole blood was utilized specimens were still viable beyond 24 hours of perfusion/ventilation.

The implications of an organ's window of viability being pushed beyond 24 hours on a mobile platform are immense. For example, organ procurement organizations would then have a much better chance to place an available organ with an appropriate recipient even if they are in a remote geography. Additionally this would allow for a longer time period to stabilize and prepare a very sick recipient, while at the same time allowing for the potential of reconditioning the donated organ. An organ that is compromised for example may have an opportunity to be recovered by the techniques that are being developed on this very system as it is employed in the laboratory for translational research.

My contributions include writing, personally collecting/analyzing the majority of data, and preparing the figures below with the exception of the diagram in Figure 4-1.

Prolonged Swine Lung Preservation Using Donor Whole-Blood Perfusion in the Organ Care System (OCS™)

Running title: Prolonged ex vivo lung preservation

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This study was funded by grants from United Therapeutics Corporation (Silver Spring, MD) and from the Lillehei Heart Institute, the Department of Surgery, and the Institute for Engineering in Medicine, all of the University of Minnesota (Minneapolis, MN). Additional equipment was provided by TransMedics, Inc. (Andover, MA).

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At-a-glance commentary:
Scientific knowledge: Ex vivo lung perfusion (EVLP) has the potential to improve donor lung function and prolong viability pre-transplant. Portable devices such as the Organ Care System (OCS^{TM}) are designed to transport lungs in their physiologic state while monitoring their progress. But EVLP is still in its infancy and has, thus far, been limited to short-term preservation.
What this study adds to the field: This is the first study to evaluate the effect of the blood's composition on prolonged donor lung preservation. Prolonged preservation is ideal for marginal lungs requiring time to recover, outside the eventual recipient.

All of the individuals listed as authors made substantial contributions to the design of the work and to the acquisition and interpretation of data. All were involved in drafting the work and providing final approval. Each author agrees to be accountable for all aspects of the work and its presentation.

Summary

Background: Acellular preservation solutions with or without isolated red blood cells (RBCs) have been used clinically for short term ex vivo lung perfusion (EVLP). We sought to establish the ideal blood perfusate for prolonged swine lung preservation.

Methods: All swine lungs (n = 8) were retrieved in the standard fashion followed by instrumentation and implantation into the Organ Care System (OCS^{TM}) perfusion chamber. For the perfusate in our control group (n = 4), we used a 1:2 ratio of isolated RBCs and OCS^{TM} solution; in our whole-blood group (n = 4), a 2:1 ratio of leukocyte-depleted autologous whole donor blood and OCS^{TM} solution.

Results: Both groups met clinical standards for transplantation at 8 hours. At 8 hours, the whole-blood perfusate was superior (vs. the control group's RBCs) for maintaining stability of all monitored parameters, as indicated by the following means: pulmonary artery pressure (PAP), 6.8 (vs. 9.0) mmHg; pulmonary vascular resistance (PVR), 333 (vs. 1158) dyn.s/cm^5; reservoir volume replacement (RVR), 85 (vs. 1607) ml; PaO_2:FiO_2 (P:F) ratio, 541 (vs. 56); and hematocrit (HCT), 16.8% (vs. 23.3%). At 24 hours, lungs in the control group had gross and bronchoscopic evidence of profound edema, as compared with the whole-blood group, whose parameters stayed similar to baseline.

Conclusion: Swine lungs exposed to prolonged perfusion with autologous whole donor blood—but not with isolated RBCs—were viable for transplantation at 24 hours. Further research is needed to verify these findings in human lungs and to determine the utility of protective agents that might match our whole-blood results.

Introduction

Today, despite a steady increase in the number of patients listed for lung transplantation and an increase in organ donation rates, only a small fraction of lung offers are considered for use (< 20%). Ex vivo lung perfusion (EVLP) devices offer the potential to increase donor yield through their ability to monitor and even recondition lungs that are initially deemed marginal.[1-3]

The 2 EVLP devices that are currently available can be used to preserve human lungs outside of the body for up to 4 to 8 hours: —the XVIVO Perfusion System (XPS™, XVIVO Perfusion AB, Göteborg, Sweden) or the Organ Care System (OCS™, TransMedics, Inc., Andover, MA, USA).[4,5] Unpublished clinical data have shown viable perfusion of human lungs on the OCS™ for up to 12 hours. In general, lungs are deemed transplantable if the pulmonary vascular resistance (PVR), PaO_2:FiO_2 (P:F) ratio, and mean pulmonary artery pressure (PAP) all remain stable or improve during preservation.

Several additional approaches are evolving that also aim at lung reconditioning on ex vivo platforms, including genetic and stem cell therapy.[6,7] Such approaches are promising for recoverable lungs suffering from trauma, edema, transfusions, infections, or other donor-related issues. However, effective genetic alterations and/or protein expressions will require longer time periods. To date, the relative effects of prolonged lung perfusion with isolated red blood cells (RBCs) are unknown.

In our study, using a swine model, we compared the effects of EVLP—specifically, the OCS™—with either isolated RBCs (control group) or autologous whole

donor blood (whole-blood group). We assessed various parameters at 8 hours (early) and at 24 hours (prolonged preservation).

Methods

Animals

For our study, we used lungs from castrated male Yorkshire swine. Each pig underwent general anesthesia and the same surgical preparation. Baseline arterial blood gases were measured. The lungs were ventilated with a positive end-expiratory pressure (PEEP) of 5 mmHg and 8 cc/kg tidal volume. The Institutional Animal Care and Use Committee of the University of Minnesota approved our study.

Lung Procurement

After performing a median sternotomy, we placed an aortic root cannula to prepare the heart for administration of cardioplegia. After exploring the lungs for any abnormalities, we administered 30,000 units of heparin and allowed it to circulate for 3 minutes. Next, we collected 1.5 L of blood. To achieve cardiac arrest, we crossclamped the aorta, delivering cold cardioplegia through the aortic root and venting through the left atrial appendage.

We ventilated the lungs at low tidal volumes. Then, through the pulmonary artery, we administered 2 L of cold OCS^{TM} lung solution. (Note that 1000 ml contains 50 g of dextran 40, 2 g of glucose monohydrate, 0.201 g of magnesium sulfate heptahydrate, 0.4 g of potassium chloride, 8 g of sodium chloride, 0.058 g of sodium phosphate dibasic dihydrate, and 0.63 g of monopotassium phosphate.). The first bag of OCS^{TM} solution

contained 50 mg of nitroglycerin. Around the heart and lungs, we placed ice slush. After excising the heart, we passed it from the operative field, leaving the bilateral lung block and left atrial cuff. We dissected the posterior attachments off the esophagus, and then stapled the trachea near the cricoid cartilage.

Blood collection

To infuse RBCs in the swine lungs in our control group, we drained blood through the inferior vena cava via a cannula routed to an autoLog® Autotransfusion System (Medtronic, Minneapolis, MN, USA). We rinsed the blood with normal saline, thereby separating the isolated RBCs from other components (such as platelets, leukocytes, lysed cells, and other factors).[8]

For autologous whole blood collection in our whole-blood group, we routed blood from the inferior vena cava cannula directly to the OCS™ blood collection reservoir. It was then passed through a leukocyte reduction filter, before being infused into the OCS™ device.

Perfusion

Through the pulmonary vein ostia, we administered a cold retrograde flush of 1,000 ml of OCS™ solution. The lungs were instrumented with appropriate-size tracheal and pulmonary artery cannulas. Before loading the lungs onto the perfusion chamber, we primed the control group with 900 ml of isolated RBCs and 1500 ml of OCS™ solution; the whole-blood group, with 1600 ml of whole blood and 700 ml of OCS™ solution. In both groups, we also infused standard additives: 1 g of cefazolin, 200 mg of

ciprofloxacin, 200 mg of voriconazole, 500 mg of methylprednisolone, 1 vial of multivitamins, 20 IU of regular insulin, 4 mg of milrinone (Primacor®), 20 mEq of $NaHCO_3$, 50 mg of nitroglycerin, and a 50% dextrose solution.

Next, we placed the lungs onto the OCS™ perfusion chamber (Figure 4-1Figure 1A). For our initial 30-minute assessment period, we slowly increased flows to a goal of 2 to 2.5 L/min, rewarming lungs to 37 degrees Celsius and ventilating them at 6 cc/kg with a PEEP of 7 mmHg (Figure 2A); we chose the 30- min mark, corresponding with the time point of "0 hours," to begin measuring arterial blood gases. Thus, we measured arterial blood gases at 0, 2, 4, 6, 8, and 24 hours, as permitted, and calculated the P:F ratio using a GEM® 3000 (Instrumentation Laboratory, Bedford, MA, USA).

Throughout our 24-hour experiments, we continuously tracked PAP, PVR, oxygen saturation, temperature, peak airway pressure (PAWP), and hematocrit (HCT). Initial rises in vascular PVR were treated with 10-mg infusions of nitroglycerin, as needed to maintain the mean PAP at < 20 mmHg and to allow increases in flow.

We placed oxygen saturation probes on both the inflow tubing (from the oxygenator toward the pulmonary artery) and the outflow tubing (from the drain toward the oxygenator). Unless we were assessing the lungs, we kept them in preservation mode. During preservation, a 12% O_2 tank delivered oxygen to the oxygenator and to the ventilator (Figure 4-1B). During assessment, a high-nitrogen, low-O_2 tank flushed the oxygen from the oxygenator, so that deoxygenated blood reached the lungs, which were then ventilated with 21% oxygen (room air) (Figure 4-1C). Doing so allowed us to assess the oxygenating capacity of the lungs.

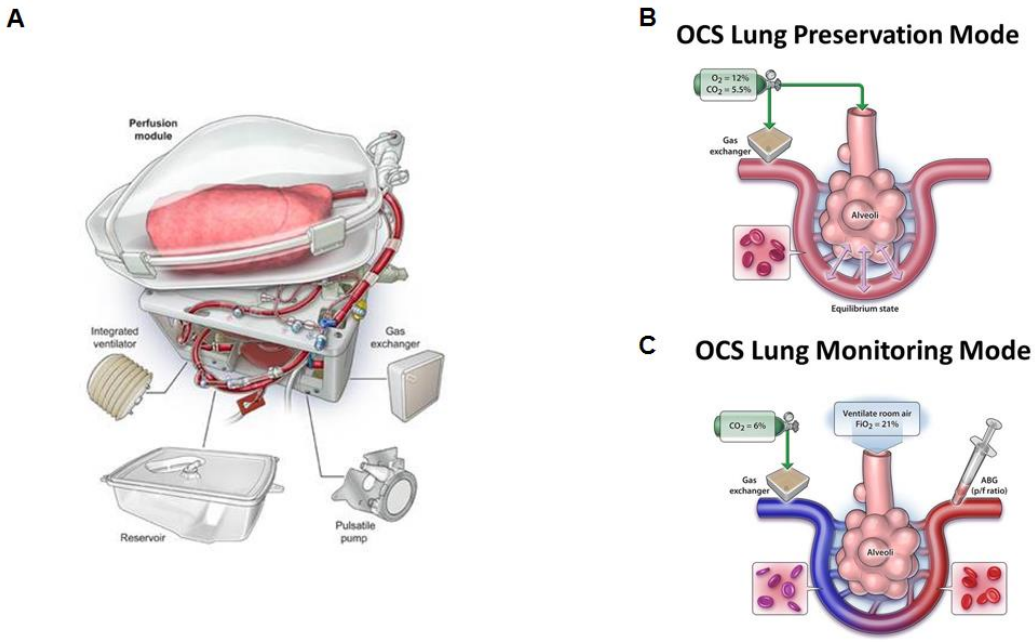

Figure 4-1 (A) The Organ Care System (OCS^{TM}) device is an integrated platform that includes 5 main parts. (B) During lung preservation mode, a 12% O_2 tank delivers oxygen to the oxygenator and ventilator. (C) During lung monitoring mode, a mixture with low oxygen and high nitrogen flushes the oxygenator while the lungs are ventilated with room air. *ABG = arterial blood gases; P:F = PaO_2:FiO_2.*

Periodically, we checked the reservoir volumes, adding volume, as needed, to maintain at least 500 ml. At the completion of each experiment, we obtained images of the lungs, flushed them with 1 L of OCS^{TM} solution, and procured specimens for histologic evaluation. To check for evidence of gross contamination, we used Gram staining. Throughout OCS^{TM} perfusion, we administered bicarbonate and dextrose, as needed, to keep bicarbonate levels > 20 meq/L and glucose levels > 70 mg/dL.

To track pulmonary edema (often associated in EVLP with pulmonary dysfunction and disruption of physiologic cellular barriers), we assessed weight, HCT,

and reservoir volume replacement (RVR), as well as the evidence from gross inspection and from flexible bronchoscopy.

Statistical analysis

For our numeric findings, we calculated the mean +/- standard deviation. For our statistical comparisons, we used the standard *t* test, with a *P* value < 0.05 considered statistically significant. To determine differences in continuous measurements, we used analysis of variance (ANOVA).

Results

A total of 8 OCS^{TM} trials were included in our comparative study: 4 control and 4 whole-blood experiments. All runs were technically successful, with a mean time from procurement to OCS^{TM} perfusion of 20 minutes. At baseline, all lungs appeared healthy, with equal bilateral ventilation, compliance, and recoil. During the procurement process, we successfully treated any minor blebs or lung abrasions with a surgical stapler and sealant, as needed (1 in each group).

Of note, throughout our 24-hour experiments, we observed no technical failures of the OCS^{TM} device. In all experiments, we achieved flow rates of 1.5 to 1.75 L/min for preservation and of 2 to 2.5 L/min for continuous assessment of arterial blood gases. Over the 24 hours, our administration of bicarbonate and dextrose was volumetrically similar in the 2 groups (77+/- 23 ml in the control group vs. 85 +/- 33 ml in the whole-blood group)

Over the initial 12 hours, lactic acid production steadily increased in both groups, but with a higher maximum in the whole-blood group consistent with the greater cellular constituents. More dextrose was required in the whole-blood group (3.3 +/- 1.9 g in the control group vs. 9.2 +/- 1.7 g in the whole-blood group), suggesting higher overall energy consumption and metabolism in the whole-blood group. In both groups, pH was kept stable through the addition of bicarbonate (Figure 4-2B).

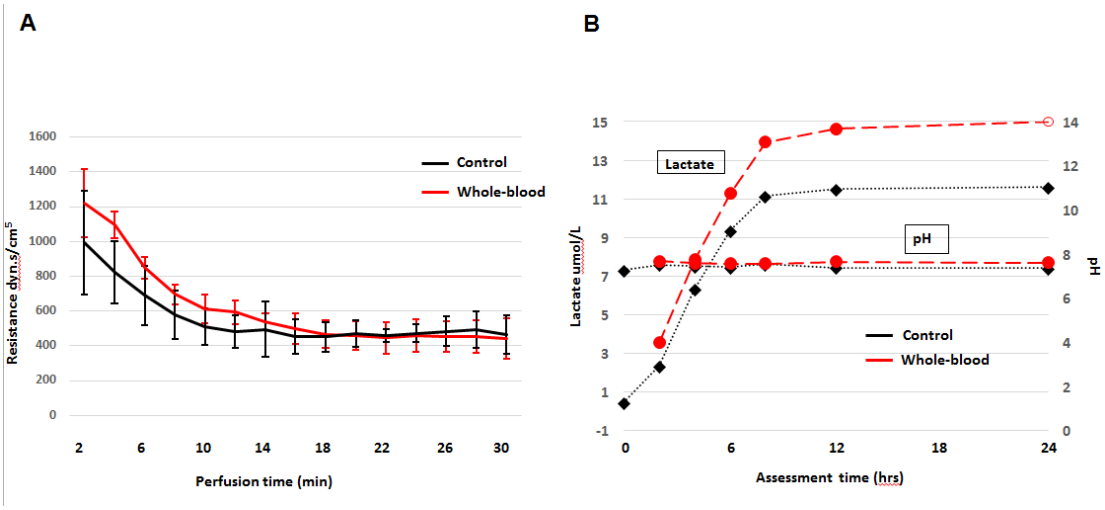

Figure 4-2 (A) During startup of the Organ Care System (OCS™) device, the resistance of the lungs in the control group improved faster than in the whole-blood group, as the lungs recovered from cold ischemia and gradually warmed to 37 degrees Celsius. Note the onset of ventilation at about 10 minutes and our initial assessment starting at 30 minutes. (B) Over the initial 12 hours, lactic acid production steadily increased in both groups, but with a higher maximum in the whole-blood group. In both groups, pH was kept stable through the addition of bicarbonate.

Pulmonary physiology

At the beginning of perfusion, PVR was universally elevated, corresponding to recovery from cold ischemia. But recovery was faster in the control group (Figure 4-2A). In the whole-blood group, PVR was initially elevated and variable, gradually recovering

over the 24 hours (Figure 4-3). In contrast, in the control group, PVR was initially stable and superior (lower) at 8 hours, then sharply increased.

At 8 hours, PAWP was higher in the whole-blood group, then remained stable; however, in the control group, it continued to rise (Figure 4-3C).

Initially, the mean PAP was superior in the control group, but deteriorated (increased) during the prolonged preservation period (Figure 4-3D).

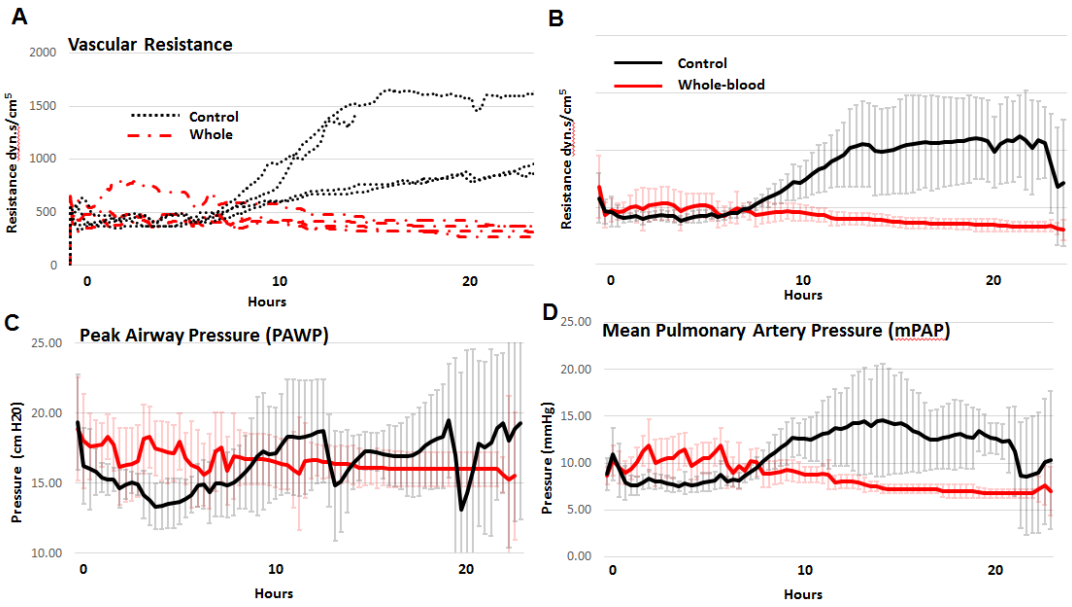

Figure 4-3 We analyzed pulmonary vascular resistance (PVR) in the control group (n = 4) and whole-blood group (n = 4) as (A) individual trials and (B) combined analysis. We also analyzed (C) peak airway pressure (PAWP) and (D) mean pulmonary artery pressure (mPAP). Note: major jogs correspond to individual experiments receiving bolus contributions to buoy the reservoir volumes.

Oxygenation

As time on the OCS™ device progressed, oxygen levels were stable, and even showed a trend toward improvement, in the whole-blood group (effectively collinear in the first several hours) (Figure 4-4A,B). We found that the P:F ratio was an important

indicator of general lung function and quality; currently, in clinical transplant models, a P:F ratio of 300 is considered standard for acceptance. In our study, both groups had a similar P:F ratio up to 8 hours, indicating viability for transplantation (Figure 4-4C). However, beyond 9 hours, most of the P:F ratios were not measurable in the control group; in fact, at 24 hours, we were able to assess only 1 set of lungs in the control group for purposes of the P:F ratio, and even in that set, assessment required an escalation in flow rates that those lungs were not able to tolerate. In contrast, in the whole-blood group, the overall mean P:F ratio improved by 24 hours.

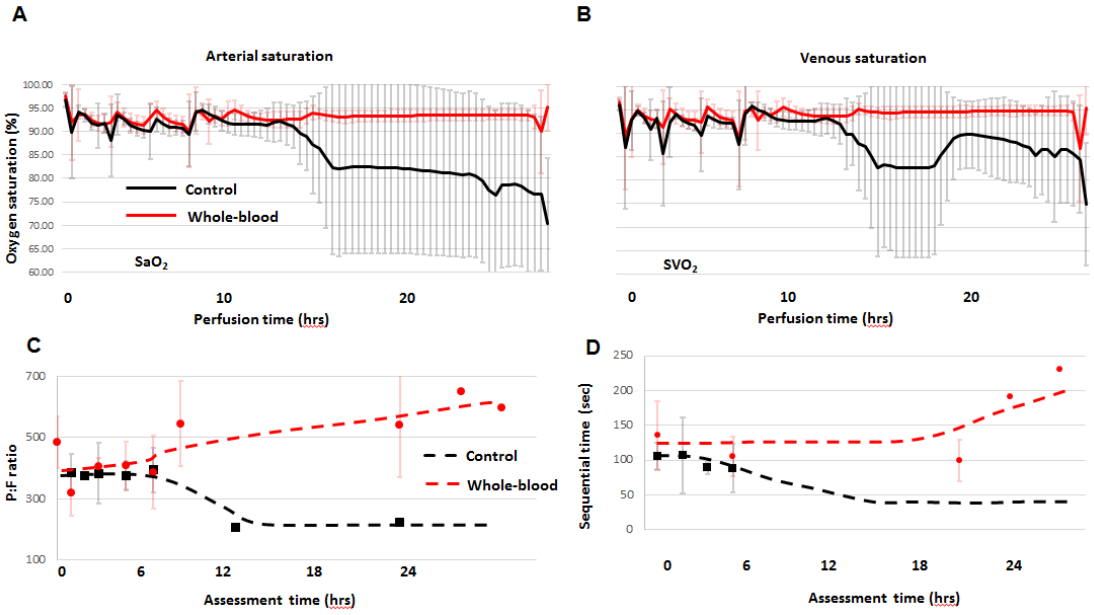

Figure 4-4Figure 4. We analyzed (A) arterial O_2 saturation (SaO_2), (B) venous O_2 saturation (SvO_2), and (C) the P:F ratio at various time points with trends depicted by dashed lines. (D) In addition, sequential mode measurements reveal the time it takes to reoxygenate the blood in seconds. Trends are depicted by dashed lines. Sequential measurements could not be attained in the controls beyond 12 hours due to prohibitively high resistance values.

For our sequential measurements of arterial blood gases (to test the rates at which the lungs could fully oxygenate RBCs), we calculated results in seconds: shorter times

suggested better oxygenating potential. In the control group, sequential measurements improved from an initial 106 +/- 20 sec, to 88 +/- 35 sec at 6 hours; however, if the lungs were functional enough to even attempt a measurement at 24 hours, we aborted all attempts after 220 sec. In contrast, in the whole-blood group, sequential measurements did not improve as dramatically: from an initial 135 +/- 48 sec to, at 6 hours, 105 +/- 28 sec—and strikingly to, at 24 hours, 100 +/- 30 sec.

Edema

The mean weight of the swine lungs at the start of each experiment was 610 ± 68 g in the control group and 615 ± 38 g in the whole-blood group (P = 0.908). At 24 hours, the mean weight was 2951 ± 43 g in the control group and 836 ± 147 g in the whole-blood group (P < 0.0001) (Figure 4-5A).

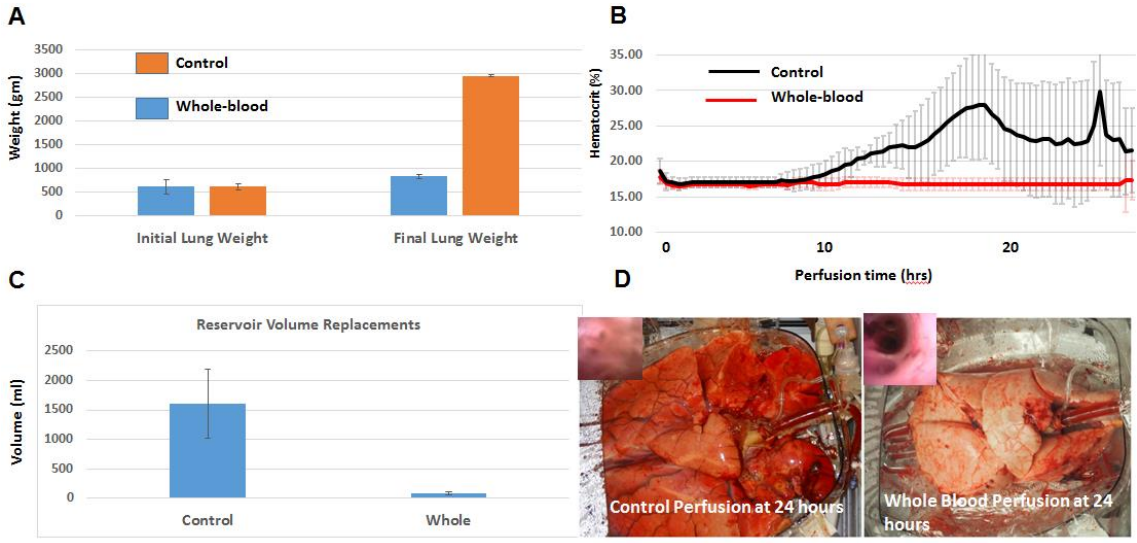

Figure 4-5 To track edema, we measured (A) lung weights differences at baseline (initial) and at the end of prolonged perfusion (final), (B) hematocrit, and (C) reservoir volume replacement (RVR). The 2 photos (D) depict gross edema in a control group lung (left)

and a whole-blood group lung (right); note the dimensions of the lung chamber as compared with the lung volume at 24 hours. The 2 insets show representative bronchoscopic images of the distal airway; note the lack of lumen because of extensive edema and froth in the control group lung (left, top corner).

Beyond 9 hours, HCT became concentrated in the perfusate in the control group, but remained stable in the whole-blood blood group (Figure 4-5B).

Throughout the 24 hours of our experiments, we found that RVR (which steadily decreases with time if there is any leakage into the lung parenchyma) was a sensitive predictor of pulmonary edema. During the initial 8 hours, RVR in the control group decreased consistently; over the prolonged preservation period, repeated fluid boluses or continuous drips of OCS^{TM} solution were required to maintain any volume in the reservoir. (The residual volume trigger for volume administration was 700 ml.) Thus, in the control group, volume requirements were significantly higher (Figure 4-5C). In contrast, in the whole-blood group, the reservoir was stable, with no volume requirements above what we administered for dextrose and pH.

Global edema primarily occurred in the control group; it was associated with compromises of other parameters that we continuously measured. By 12 hours, edema was appreciable on gross inspection; by 24 hours, it became impressive (Figure 4-5D). In contrast, in the whole-blood group, gross evidence of edema was minimal at 24 hours. These findings were confirmed on flexible bronchoscopy as well.

Thus, in general, we noted a consistent trend in lung deterioration in the control group in numerous parameters. The first to become abnormal were PAWP, PVR, RVR, and HCT, followed by the mean PAP, oxygen saturation, the P:F ratio, and tidal volume.

Clearly, changes in compliance, vascular permeability, and internal resistance were early indicators of lung failure; poor exchange of arterial blood gases and poor ventilation were late findings.

Discussion

The goal of portable EVLP using OCS™ is to maintain the lung in its warm and physiologic environment until just before implantation.[5] In addition, system metrics allow exclusion of marginal lungs that fail to recover.[4] Thus far, EVLP has focused on early preservation, with protocols and devices optimized to achieve the best results within an initial window of 6 to 8 hours. The OCS™ device is the only portable EVLP platform and it uses isolated RBCs to limit viscosity, maximize availability, and/or reduce interference with other transplant teams at the donor hospital.

In our study, we showed that isolated RBC perfusion in the OCS™ device initially resulted in better performance relative to whole blood. Thus, continuing that practice has value for anticipated short runs in standard donor lungs. However, beyond 8 hours, we found that whole-blood perfusion parameters improved, while perfused RBC lungs steadily deteriorated.

Several explanations are possible for the effects of packed RBCs on pulmonary outcomes. Blood cells damaged by age or trauma can harbor increased free hemoglobin, iron, and blood cell fragments.[9,10] Iron and free heme produce oxidant-induced cellular damage.[11-15] Qing et al. showed that lung endothelial cells incubated with packed RBCs undergo necrotic cell death, perhaps mediated by the release of high-mobility group box

1 protein from the endothelial cells.[16] Thus, structural and cellular signaling factors may be responsible for RBC-induced pulmonary injury.

Moreover, during cardiac surgery, packed RBC transfusions have historically been linked to poorer survival and pulmonary outcomes.[17-22] In critically ill patients, lungs appear to be even more susceptible to packed RBC-induced injury.[23,24]

Our study's finding that isolated RBC-induced injury was nullified with the use of whole-blood perfusion is striking and important. Whole blood maintains clotting factors and platelets, thereby helping to prevent propagation of injury. Further, isolated cell perfusates may lack the matrix necessary for optimal oxygen delivery over time, perhaps leading to stress from energy deprivation. The content in whole blood is clearly protective to the circulating RBCs, preventing both RBC damage and RBC-mediated cellular injury while maintaining oxygen delivery.

It is important to emphasize that, as recommended by the manufacturer, OCS™ blood perfusion is not intended to go beyond 8 to 12 hours. In addition, human lungs are more resilient than swine lungs. Isolated RBCs are logistically a good choice for early perfusion. Packed RBCs are readily available and easy to transport. In contrast, whole blood requires an additional collection system at the donor hospital; moreover, 1.5 L of donor blood is required, an amount that will undoubtedly result in hypotension and could compromise the recovery of other organs from that same donor. Nonetheless, with good communication and the establishment of expectations ahead of time, such obstacles could be prevented and overcome. For early preservation and transportation of standard donor

lungs, isolated RBC perfusion in the OCS™ device is an acceptable, and probably ideal, perfusion method.

However, for marginal lungs, and even for standard lungs that require prolonged preservation for logistical reasons, whole blood is currently the best option. The use of whole blood in this context will require foresight and communication. Scenarios in which prolonged lung preservation is valuable will need to be clarified. For example, edematous lungs, infected lungs, contused lungs, and lungs with borderline levels of arterial blood gases might benefit from a longer stay on the OCS™ device. The use of mesenchymal stem cells in attempts to rehabilitate marginal donor lungs would likely require longer periods of perfusion for adherence and cellular protection.[6]

Conclusion

Swine lungs exposed to prolonged perfusion in the OCS™ device with autologous whole donor blood—but not with isolated RBCs—were viable for transplantation at 24 hours. Thus, the OCS™ device proved to be a reliable preservation platform for early and prolonged EVLP. Clinically, whole blood should be included in the standard donor consent form and anticipated in the procurement process as a valuable resource for transplantation using EVLP, although packed RBCs are acceptable for standard donor lungs and for shorter preservation times.

Further research is needed to verify our study's findings in human lungs and to determine the utility of protective agents that might match our whole-blood results. Future studies must clarify the mechanisms of RBC-induced pulmonary injury. Mitigating such mechanisms would be valuable and would help to expand the application of EVLP. Post conditioning agents, such as delta opioids or fatty acids, could bolster pulmonary cells' protective mechanisms and protect RBCs' integrity.[25-29]

Chapter 5 : OCS Thermal Monitoring of Lungs

Preface

Isolating the lungs has several other advantages to research beyond what has been discussed in previous chapters. In particular the isolated lungs are an excellent opportunity to better understand everything from basic lung physiology to characterizing the injuries and mechanisms which affect lung function. Lungs have been imaged by others, but the imaging of functional lungs has been restricted to the external skin surface or complex MRI/CT routines tracking helium isotopes. Being able to look at the lungs directly by themselves is a simple but powerful thing that has yet to be properly elucidated and is only now possible. Considered here is a novel way of directly imaging the lungs for the purpose of assessing physiological function or response to various therapeutics and describing manners in which those images may be interpreted.

Our experimental results show measureable and reproducible thermal behaviors in responses to: the onset of edema, the recovery of lung function, and/or the acute lung injury. Utilizing this approach we have gained a better understanding of the circulation dynamics which currently dictate important aspects of the clinical preservation methods.

My contribution includes acquisition of the images, concurrent with experiments described in other chapters, manuscript preparation, data analysis and correlation to functional metrics.

This paper is currently awaiting submission for publication.

Thermal Assessments of Isolated Lungs and Lung Injury on the Organ Care System (OCS™)

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Summary

An isolated lung model on the Organ Care System (OCS™) is assessed by different means of thermal monitoring in the case of healthy, damaged, and progressively compromised lungs using a large mammalian (porcine). In conjunction with the integrated system tracking the temperature of the perfusing fluid we use surface temperature probes along with handheld and video thermal imaging of exposed lung anatomy to investigate the perfusing characteristics of the lungs and how those modalities might assist in clinical assessment of organ viability. We determine the presence of local edema or atelectasis can exhibit a decrease in the local cooling coefficient and compromises to the perfusion are predictable associated with an inability for portions of anatomy to achieve/maintain normal temperatures. Direct thermal imaging of the normothermic lungs, shows the effectiveness of the perfusion fluid in restoring and maintaining temperature in the bulk of the lung tissue while the distal lobe tips lag. The fluid temperature is seen to maintain a much tighter control on the fluid temperature than the anatomy as monitored by direct thermal imaging or additional surface temperature probes which were effective in demonstrating the change in thermal properties in progressively injured tissue as well as the improvement and recovery of atelectasis over 24 hours of isolated perfusion time on the OCS™.

Keywords:
Thermal Imaging, Injury, Edema, Atelectasis, Organ Care System, OCS

Introduction/background

Lungs can be looked at a variety ways for many different reasons. With particular attention to conditions including cancers and cystic fibrosis, lung imaging has made advances in X-Ray/CT[1,4,and 6], MRI [2,3], Endoscopy [7] and more [5]. But lungs are a unique case for thermal regulation; it is an intricate internal organ which is exposed continuously to the ambient air conditions on the inside while protected behind a thick layer of membranes, bones, and tissue on the outside it is perfused with warm pulsatile blood directly from the heart. The lungs are an amalgamation of very fine yet pliable structure that is at the mercy of the diaphragm and various thoracic muscle groups to perform their most basic function in the exchange of gases. Combined with emerging isolated lung systems for transplantation, we are afforded a unique test bed for exploring the thermal behavior of healthy and ailing lungs alongside assessing the efficacy and true utility these platforms now offer.

Despite the complexity and apparent frailty, it is none the less a necessarily resilient organ that can withstand being removed, placed in near freezing conditions and successfully transplanted into a waiting recipient. The current standard of treatment in transplantation with ice storage of organs is being challenged by emerging technologies in favor of more normo-thermic. Transmedics' Lung Organ Care System (OCS™) is one such technology which preserves the organ from donor to recipient in a warm perfusion and ventilated state. As a laboratory instrument this device can be employed to explore the physiology and corresponding preservation methods associated with the thermal properties of the lungs. It is a first opportunity to assess thermal monitoring technologies,

what they are capable of telling us, how that may relate to tissue damage and clinical viability, and how they might help make decisions for patients to help improve outcomes.

We hypothesized that the soft, spongy lung tissue may be particularly susceptible to exhibiting changes in local thermal properties that may be effectively monitored in the isolated organ system(s) via a variety of thermal monitoring techniques. The lung tissue is acutely prone to damage in the form of edema or atelectasis which provides a denser and locally larger thermal mass effectively changing how the tissue's temperature responds to warming and cooling. Also of consideration is the compromise or absence of warm perfusion that is key to preservation of tissues which can result from surgical damage or other non-physiological conditions; all of which may result in modifications to the lungs' thermal profile.

The thermal behavior and monitoring techniques of the isolated lungs are tested under nominal and progressive damage models as monitored by the OCS™ device and multiple temperature sensing modalities.

Methods

The porcine lungs were procured under a variety of conditions in conjunction with ongoing experiments to elucidate and optimize the operation of the OCS lung preservation, replicating both injured and normal/healthy lung preservation methods over the course of at least 24 hours. Once procured from a donor the lungs are cooled with surgeon administered antigrade and retrograde flushes consistent with normal clinical practice. The thermal tracking was done on porcine lungs procured in accordance with

current clinical practice using packed red blood cells (pRBCs) (N=4), with a substitution of leukocyte filtered whole blood for the pRBCs (N=4), and more extensively in the separate investigation of an injury model for DCD using both perfusion solutions. Lungs are situated in the OCS™ as though in a supine patient and perfused with a combination of drugs and a fluid base of Transmedic's OCS™ solution. Lungs were weighed before being placed on the OCS and again after being removed.

Three methods of thermal tracking were employed during the course of the experiments. Initial investigation used a hand held thermal camera (FLIR IRC30(?)) to take global time-lapsed images of the lungs. A thermal video camera (FLIR A300) was used to monitor the detailed time dependent thermal behavior over the exposed surface of the lungs. A third method was employed using a video-monitored, direct contact surface temperature probe at a select location on the lungs and tracking the temperature at 5 second increments.

Following periodic assessments of the lung function, the lungs were uncovered for a direct line of sight measurement of the exposed surface temperatures by means of the optical infrared devices. The ambient exposure of the lungs was additionally limited to an approximately two minute time window to provide adequate time for observable cooling trends as well as not to exceed what may be done in clinical practice as for a practical intervention. This made tracking of the cooling trends very efficient via the optical means but the static surface temperature thermocouple was additionally useful in demonstrating the re-warming characteristics of the lungs when they were re-wrapped and enclosed within the OCS device. The system was maintained in a temperature

controlled lab and the lungs were constantly perfused at a consistent rate of 1.5 L/min during thermal assessment. During the warm up period, the lungs were laid open to the air while the circulating fluid was brought up to temperature and ventilation began.

Phases of the lung deployment

The porcine lungs are subject to a conformational change between initial hookup to the system and the measurements tracking subsequent time points. This means that in general, different portions of the lungs are exposed during the initial warming phase as compared with our intermittent assessments.

Following procurement and cannulation the lungs are removed from the temporary ice bath and placed into the primed OCS chamber, and connected to perfusion. While the perfusion begins, the lungs are effectively splayed open, predominantly exposing the interior surfaces of the lobes during this initial perfusion. Once the lungs have risen to the appropriate temperature as measured by the OCS monitoring of the perfusing fluid and ventilation has started, the lungs are wrapped in plastic and closed within the hard plastic container.

When wrapped, the distal tips of the lungs are pulled together in an approximation of their natural inter-thoracic positioning. At subsequent measurements, the porcine lungs tend to maintain their position as wrapped making the exterior surfaces of the lungs exposed (Figure 5-1). The human lungs for which the system was designed may not suffer from the same sort of topological change from the wrapping process but it is a confounding issue that we must address in this animal model.

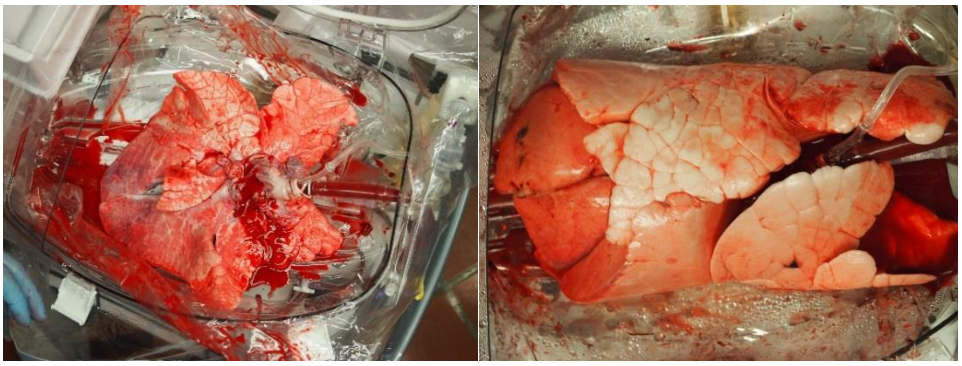

Figure 5-1: Relative positioning of the lungs during startup (A) and as unwrapped for thermal imaging following periodic assessments.

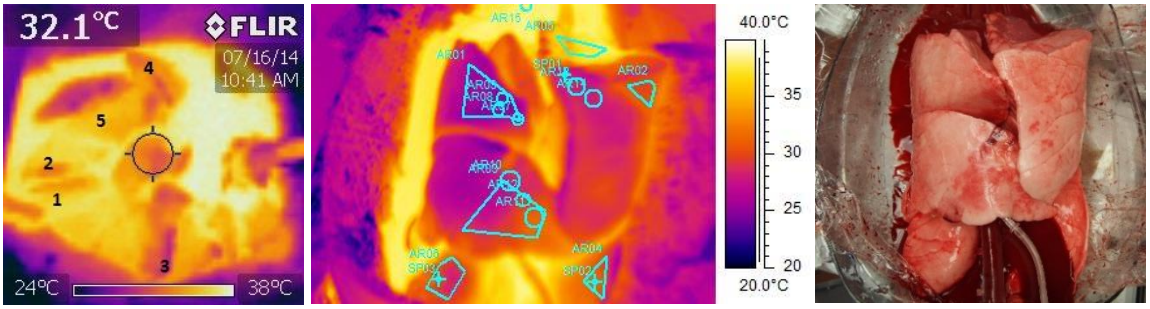

Figure 5-2: The thermal video imaging can continuously monitor/calculate the average area temperature or pointwise approximation while the lung surface is exposed. Multiple lobe areas were were defined for investigative/comparative purposes.

The anterior distal tip of the medial right lobe was selected as the primary site for a surface thermocouple as it is the furthest removed from the warm and constant drainage. The drainage from the lungs exits the pulmonary veins and is collected on the inferior side, effectively bathing the distal tips on the superior/inferior distal tips of the left and right superior and inferior lobes. This is warm external flow serves to raise and maintain the temperature of this anatomy regardless of the local internal perfusion.

Results

Initial Warming

The porcine lungs are briefly held in an ice bath between procurement and placement on the OCS during which it is cannulated to be hooked up to the system. In the bath, external temperatures range from about 5-15°C.

Once placed on the OCS the lungs are observed to warm rapidly through the major vessels (Figure 5-3). Perfusion through the lungs generally only act to acutely drop the perfusion fluid's temperature by a degree or two as monitored by the system. The anterior tips of the lobes are observed to warm at a much slower rate.

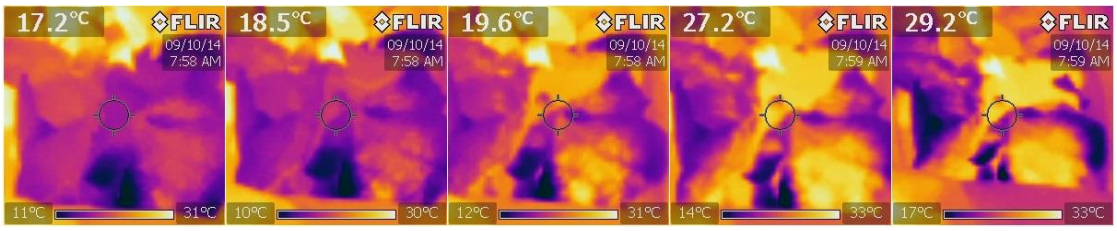

Figure 5-3: Time lapse of the first minute following reperfusion of the lungs on the OCS. The core temperature rises quickly and spreads to areas that are less generously vascularized. Note that the relative temperature scale also shifts between images.

The thermal camera imaging is capable of tracking the global perfusion behavior but it is currently limited to the short terms where the lungs are exposed to the ambient room temperature (here maintained 21.0+/-0.5°C). This allows the tracking of the initial warming behavior but for the purposes of thermal trends, the time-lapse and video is subsequently relegated to studying cooling trends in the lungs as periodically exposed.

Conversely the static thermocouple is capable of tracking the temperature even following the complete isolation of the lungs (Figure 5-4).

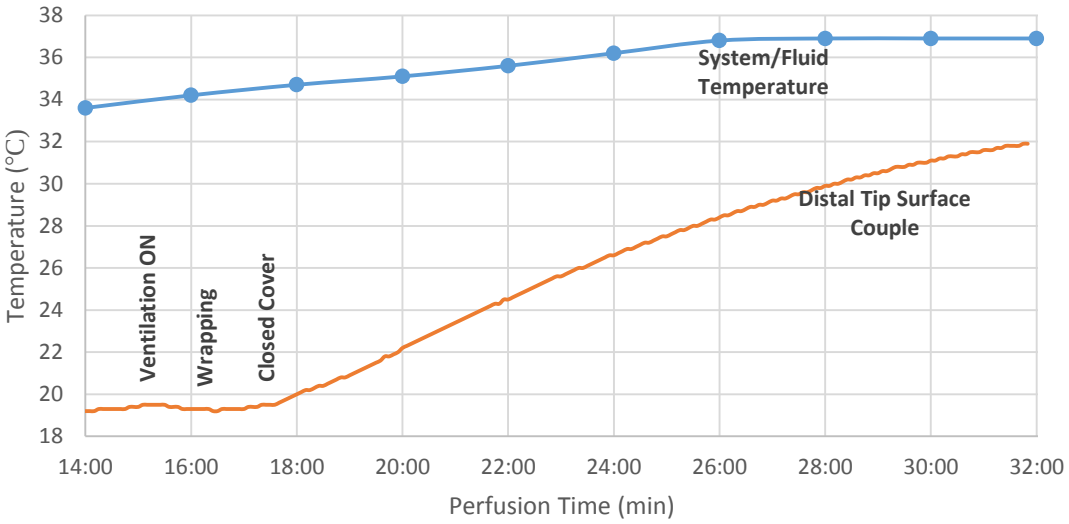

Figure 5-4: Thermal profile taken from a surface probe on the well ventilated distal tip of the right medial lobe during the warming phase.

The static thermocouple trend pictured (Figure 5-4) tracks the temperature change of the right medial lobe's anterior tip during the temperature rise. Note first that there is a significant difference between the surface probe's measures of the lung temperature as compared with that from the system's measurement of the fluid's temperature. Ventilation appears slightly competitive with the temperature rise; additionally confirmed with optical measurements.

At the onset of ventilation and wrapping, the fluid temperature has risen to maintain at least 34°C but there clearly maintains a discrepancy between the distal portions of anatomy and that system-tracked temperature. That system temperature currently dictates much of the warm-up process in the OCS™ system.

What is still unclear from this trend is whether the following temperature rise is brought about by the physical act of wrapping the lungs to approximate the correct position in the body that allows for better perfusion, or if the thermal isolation and corresponding reduction of heat loss. The thermal isolation appears to be the main culprit, but the perfusion component is as of yet an open issue.

Static Surface Probe Placement

At the start of ventilation, the perfusion fluid is maintaining at least 34 °C but the temperature of the distal tip of the right medial lung where this measurement is taken demonstrates a relatively slow rise (also visible in Figure 5-3 time-lapse) as compared with that of the lung once it is wrapped and closed. This suggests a relatively small amount of the perfusing fluid is traveling through the distal tip. It must also be noted that the tip corresponds to a feature of the anatomy which makes it the most responsive to ambient temperatures changes and heat dissipation and its placement here was intended to encompass that activity.

Periodic Temperature Characteristics and Recovery/Injury

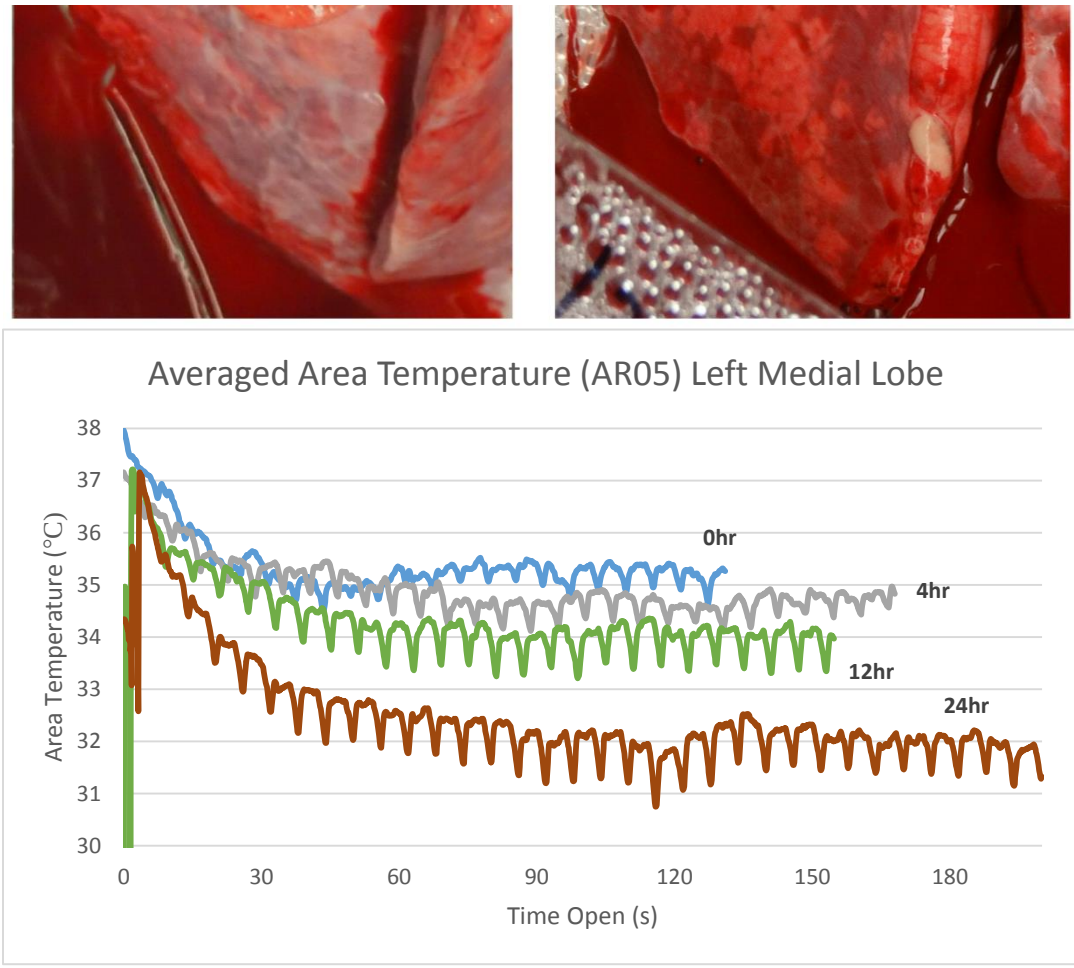

Figure 5-5: Atelectasis on the lower right lobe (Left) as tracked by the progressive thermal video (below) shows a much quicker cooling associated with improved ventilation of the lower lobe over the course of 24 hours of recovery (Right). Additionally a lower steady state temperature is reached as the injury recovers as the lobe is inflated away from the warm blood drainage in the OCS™ device.

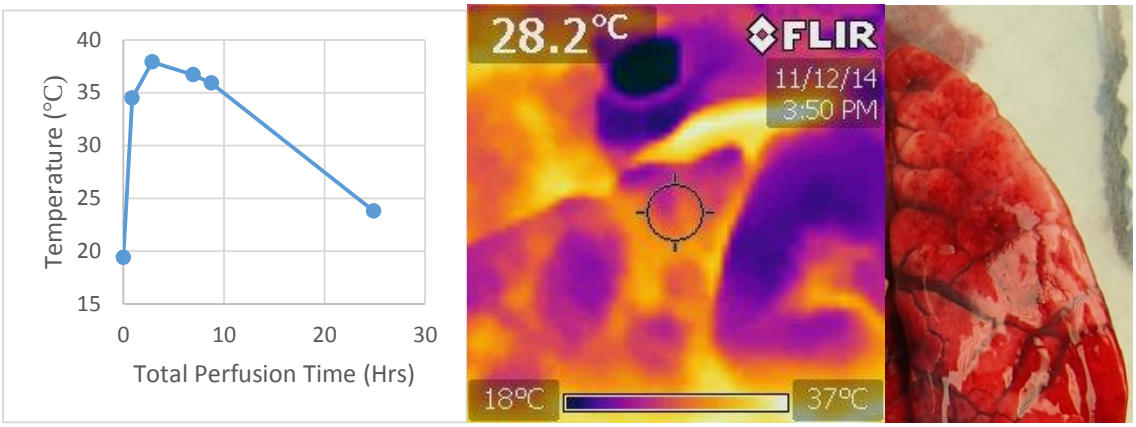

Figure 5-6: Tracking the initial temperature (left) in the left medial lobe (right) via a handheld IRC30 thermal camera shows a correlation with the rapid growth of edema in the lobe observed after ~10hrs of perfusion in an injury model.

It should be noted that the temperature variation does not itself appear to correlate with any adverse preservation characteristics at this time, and inherently the lungs are an organ well equipped to bridge the thermal gap as one of few internal organs to directly deal with the constant influx of external temperature with the ambient air flow.

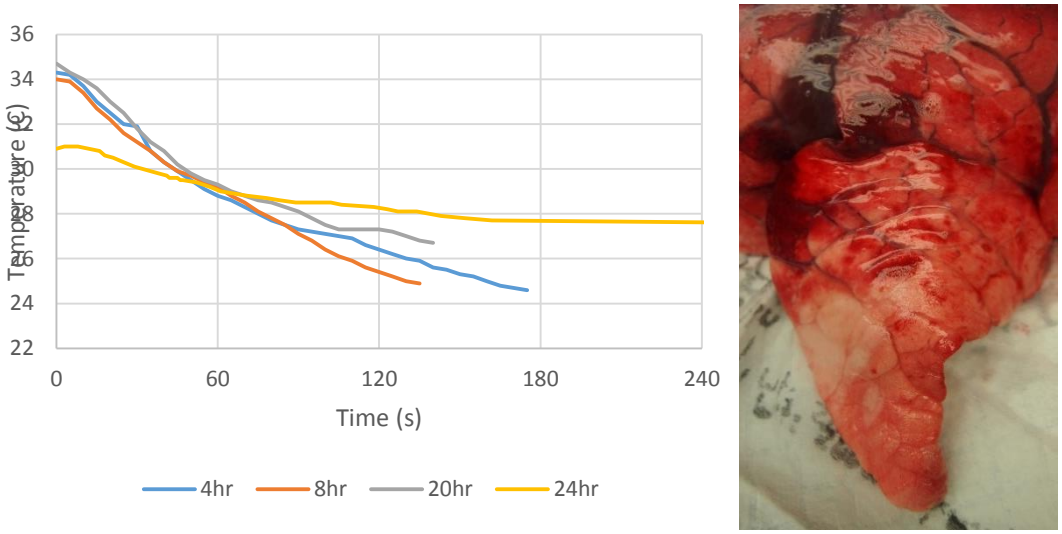

Figure 5-7: A thermal surface couple on the distal anterior surface of the right medial lobe tracks the cooling characteristics in the lobe as it becomes increasingly edematous over 24 hours of preservation. This lobe is often the last to succumb to global edema, maintaining visibly appreciable ventilation until the end.

At the 24 hour time point, the cooling rate is clearly reduced and it is also accompanied by a lower initial temperature which has not recovered since the cooling trial at 20 hours, indicating the . Difference between the 20 and 24 hour starting temperatures may be indicative of the reduced temperature recovery between the time points.

Discussion

The main utility of these various methods is that injured or otherwise compromised lung tissue exhibit measureable changes in their dynamic thermal profiles that are observable by multiple temperature tracking modalities. Our analysis demonstrates: 1) Damage (local and global) to lungs appears strongly correlated with a

decrease in the cooling rate, 2) (Conversely) Improvement of lung function and local damage correlates well with an increase in the cooling rate where lungs are better aerated and maintained at high initial temperature with proper circulation, 3) In short time frames, cooling rates change most drastically in comparison to the total temperature difference over time which 4) correlates well with damage or improvement.

As a high level assessment, this swath of investigations provides opportunities to assess and apply lessons in both anatomical behavior and practical applications to emerging clinical medicine. Anatomically, the distal portions of the lungs are most susceptible to exhibiting these changes and localization of damage can be assessed. For practical application, contact thermocouples are most easily recorded, consistent, and easiest to distill information from both in cooling and warming situations; thermal imaging on the other hand currently requires a direct line of sight, a steady hand for consistency, with less accuracy and limitation to cooling profiles, but it does not require the affixing of thermal probes. It is clear from the cooling characteristics that the lungs are on the whole maintained at a more globally normal temperature by the wrapping and containment process. The different anatomy across the lung however still appears to maintain an inherent temperature gradient (+/- about 2 °C) even across the surface of the lungs.

In principle these thermal behaviors make a great deal of sense when consideration to edema/inflammation and other mechanisms of injury and may be *very* applicable to other organ preservation systems such as for the heart, liver, kidneys, etc.

The exposure of organs and isolated investigations offers a lot of new questions and possibilities for answers as well. For example, in the case of the lungs investigated here, we have yet to determine if the conformational change of the physiology responsible for an increase in the perfusion/temperature in the distal lobes following the wrap, or if the rise in the temperature is due to conductive heat transfer from the better perfused base portions of the lungs? Future investigations also look to address if the lungs in situ demonstrate the same surface temperature gradient across the surface, or is it more uniform as might be expected from constant contact, not with a piece of plastic and air, but with intrinsically body-temperature tissue? Or can we assess the thermal impact of surgical techniques which preserve the bronchial arteries for better perfusion ex vivo?

Chapter 6 : Recovery of DCD Lungs on OCS

Preface

A further research investigation utilizing the Lung OCS™ (Chapter 4, Chapter 5) was undertaken with the direct goal of extending the available donor pool; specifically targeting an underutilized donor population categorized as Donation after Cardiac Death (DCD). That means that the cardiovascular system (heart and lungs) are allowed to come to a complete stop in the patient before procurement of donor organs can start, the problem with this being an extended state of warm ischemia which can be very damaging to lungs. This is different from a case where a donor may have suffered brain death though the heart continues to sustain the body. Typically a lung procurement team will only wait 15-20 minutes following withdrawal of life support before the damage to the lungs are considered too grievous to risk being utilized for transplantation. ***Consequently, only a handful of lungs from over one thousand DCD donors annually are utilized.***. With the OCS lung model however we have the opportunity to both directly assess the injury incurred and also attempt recovery of that organ with prolonged preservation methods which includes novel treatment strategies.

The work in this section of my thesis: 1) demonstrated that the equivalent of a 1 hour warm ischemia can still produce stable lungs that meet criteria for transplantation which is three to four times longer than current accepted clinical practice, 2) showed that the likely limits of this current technology, even utilizing whole blood is after maintaining lungs recovered in their near worst case clinical scenario: i.e., using lungs subjected to a double pneumothorax with 2 hour of warm ischemia or uncontrolled DCD

more similar to acute cases with unknown cardiac down time, and 3) it shows how extending the viable timeframe for an organ can result in recovery of isolated lung function *happening beyond 8 hours of isolation* That means a set of lungs out of an otherwise sick patient, while possibly damaged or otherwise compromised in assessment within a patient, has the potential to perform just as well as lungs assessed to be immediately transplantable.

Using the preferred whole blood group from (Chapter 4) as a control group, the results of this experiment demonstrated the recovery of injured lungs and established a set of injury models from which have become a basis to further improve clinical outcomes with various experimental pre- and/or post-treatments as well as improvements to recovery methods or utilization of the OCS.

Demonstrating currently unacceptably sick and injured lungs can be recovered to be transplantable, and sustained over 24 hours, means that there can be more lungs available for transplant that could travel further (anywhere in the world) for better matches and improved patient outcomes and opportunities. My contribution to this paper was complete from the ground up from method development to data collection, writing, and preparation of figures.

Extended Functional Assessment and Recovery of Porcine Lungs for Donation after Cardiac Death (DCD) Using Portable Ex Vivo Lung Perfusion (EVLP)

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Summary

Purpose: Donation after cardiac death (DCD) has the potential to increase the donor lung pool. But currently, such lungs are not commonly used for clinical transplantation in the United States, primarily because of concern regarding their variable warm ischemic times. We developed a porcine DCD model to investigate a portable ex vivo lung perfusion (EVLP) system for monitoring and conditioning lungs subjected to varying hours of warm ischemia.

Methods: In our porcine study, 12 healthy adult male swine (70 to 90 kg) were fully ventilated, anesthetized, and given a bolus dose of heparin (30,000 units). We divided them into 3 lung procurement groups. In the control group, cardiac arrest was induced, and the lungs were *not* subjected to ischemia (n = 4). In the 1H DCD group, swine were extubated and experienced a 15-minute preagonal phase; the lungs were subjected to *1 hour* of warm ischemia *with* continued ventilation (n = 4). In the 2H DCD group, swine were extubated and experienced a 15-minute preagonal phase; the lungs were subjected to *2 hours* of warm ischemia *without* ventilation (n = 4). The 1H DCD group and 2H DCD group each represented a potential spectrum of clinical DCD scenarios. All sets of procured lungs were flushed and placed in the Organ Care System (OCS^{TM}) portable EVLP platform; then, lungs were perfused and ventilated for ≥ 24 hours, using standard additives with autologous donor whole blood.

Results: The mean peak airway pressures, at baseline and 24 hours, were 18 and 16 mm Hg in the control group, 18 and 14 mm Hg in the 1H DCD group, and 23 and 18 mm Hg in the 2H DCD group. The mean tidal volumes delivered, at baseline and 24 hours, were

400 and 400 ml in the control group, 350 and 362 ml in the 1H DCD group, and 197 and 412 ml in the 2H DCD group. Initially, at baseline, all 3 groups had similar vascular resistances (range, 400 to 500 dyn-sec/cm^5) and similar mean pulmonary artery pressures (10 to 11 mm Hg); at 24 hours, both values improved in all 3 groups (vascular resistances: range, 300 to 390 dyn-sec/cm^5; mean pulmonary artery pressures: 7 to 8 mm Hg). In the 2H DCD group, oxygen saturation (SaO_2) was considered to be compromised in the first 8 hours (76% to 84%), but recovered to a maximum value of 90% at 24 hours. The ratios of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PO_2:FIO_2) at baseline and 24 hours, were 317 and 590 in the control group, 498 and 404 in the 1H DCD group, and 286 and 222 in the 2H DCD group.

Conclusion: In our porcine study, we showed that DCD lungs, if placed on an EVLP system, could recover. We found reduced oxygenation, however, in swine lungs that were exposed to 2 hours of warm ischemia. EVLP should be standard of care for evaluating donor lungs with protracted warm ischemic intervals.

Introduction

Donation after cardiac death (DCD) could substantially expand the donor lung pool for transplantation. Currently, in the United States, the waitlist mortality remains 15.1 per 100 waitlist years; since 2008, DCD lungs have accounted for only 0.8% to 1.9% of lung transplants each year.[1]

A partial solution could be what is known as uncontrolled DCD, which has begun to be practiced in Europe, but not in the United States. Uncontrolled DCD is defined as recovery of lungs from a donor who has died either (1) outside of the hospital (Maastricht category I) or (2) in the hospital after attempted resuscitation (Maastricht category II).[2] Each year in the United States, an estimated 80,000 deaths are attributed to out-of-hospital cardiac arrest, and another 80,000 to in-hospital cardiac arrest; as many as 40,000 of those deceased patients' lungs could be suitable for transplantation, per conservative estimates[3]

The primary reason that DCD lungs are not widely used for clinical transplantation in the United States is concern regarding their variable warm ischemic times. Warm ischemia is known to injure the organ, leading to increased rates of bronchiolitis obliterans syndrome (BOS), primary graft dysfunction (PGD), and even airway complications.[4-7] Yet some groups have reported excellent outcomes with DCD lungs; improved monitoring capabilities pre-transplant would likely clarify which DCD lungs are transplantable.[4,8-12] Unfortunately, the dry run rate is now as high as 30% to 45% of attempted procurements.[10,13]

Ex vivo lung perfusion (EVLP) provides a mechanism for monitoring DCD lungs outside the donor pre-transplant. According to clinical studies, lungs that remain stable on EVLP do better post transplant.[14-16] In addition, lungs that have any form of acute injury—whether from cardiac arrest, resuscitation efforts, or other mechanisms—can be reconditioned on EVLP to a normal state.[17-21] Acute injury to DCD lungs is likely to occur at the time of procurement. Yet most EVLP platforms require some period of ice time for transportation prior to placement onto the perfusion circuit; it is unclear whether that period of cold ischemia adds insult to injury. In other words, the use of a portable EVLP system might decrease the cold ischemic insult and expedite recovery.

In our porcine study, we used a portable EVLP system—namely, the Organ Care System (OCS™, TransMedics, Andover, MA, USA)—for monitoring and conditioning DCD swine lungs exposed to variable warm ischemic times. Our 2 main goals were to assess such lungs and to help them recover, if possible. Previous work from our group showed that autologous donor whole-blood perfusion was beneficial for use in EVLP, so we again used it in an attempt to extend the preservation window. We continuously assessed the physiology and morphology of DCD lungs over a 24-hour period.

Methods

Our research protocol was approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

A total of 12 healthy adult male swine (York X breed), weighing 70 to 90 kg were anesthetized and placed supine on the surgical table. They were intubated and then

ventilated with 8 cc/kg of air, with a positive end-expiratory pressure (PEEP) of 5 mm Hg and about a 1.5 minimal alveolar concentration (MAC) of isoflurane. To expose the thoracic cavity, we performed a median sternotomy. We administered a bolus dose of 30,000 units of intravenous (IV) heparin and allowed it to circulate for at least 3 minutes. We divided the 12 swine into 3 lung procurement groups. In the control group, cardiac arrest was induced, and the lungs were *not* subjected to ischemia (n = 4). In the 1H DCD group, swine were extubated and experienced a 15-minute preagonal phase; the lungs were subjected to *1 hour* of warm ischemia *with* continued ventilation (n = 4). In the 2H DCD group, swine were extubated and experienced a 15-minute preagonal phase; the lungs were subjected to *2 hours* of warm ischemia *without* ventilation (n = 4). Control group

In the control group, cardiac arrest was induced by cross-clamping the ascending aorta and delivering 1 liter of chilled Krebs cardioplegic solution. During this time, the lungs were flushed antegrade with 2 liters of cold OCS^{TM} solution. Note that 1,000 ml of the OCS^{TM} solution contains 50 g of dextran 40, 2 g of glucose monohydrate, 0.201 g of magnesium sulfate heptahydrate, 0.4 g of potassium chloride, 8 g of sodium chloride, 0.058 g of sodium phosphate dibasic dihydrate, and 0.63 g of monopotassium phosphate; we added 50 mg of nitroglycerin to the first liter of flush. Then, we applied topical ice to the lungs, which were ventilated with 6 cc/kg of air, with a PEEP of 5 mm Hg during the flush.

Immediately after the cardiac arrest, we collected autologous donor whole blood via a cannula inserted into the inferior vena cava. The blood was then passed through a leukocyte reduction filter, before being infused into the OCS™.

Per our routine, we first explanted the heart, followed by the lungs. Next, we delivered 1 liter of OCS™ retrograde flush, without nitroglycerin, via the pulmonary veins. We placed the lungs onto the perfusion circuit of the portable EVLP system, the OCS™, in accordance with the manufacturer's specifications[16].

DCD groups

The 1H DCD group and 2H DCD group each represented a potential spectrum of clinical DCD scenarios (Figure 6-1Fig. 1).

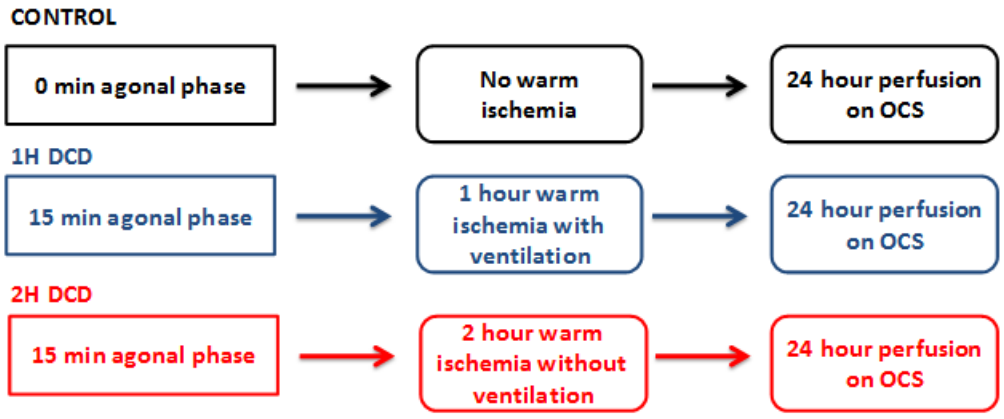

Figure 6-1: In the control group, cardiac arrest was induced, and the lungs were *not* subjected to ischemia (n = 4). In the 1H DCD group, swine were extubated and experienced a 15-minute preagonal phase; the lungs were subjected to *1 hour* of warm ischemia *with* continued ventilation (n = 4). In the 2H DCD group, swine were extubated and experienced a 15-minute preagonal phase; the lungs were subjected to *2 hours* of warm ischemia *without* ventilation (n = 4).

In the 1H DCD group (again, a15-minute preagonal phase followed by 1 hour of warm ischemia with ventilation, swine were heparinized during the preagonal phase as well as disconnected from the ventilator.

In the 2H DCD group, swine also underwent a 15-minute preagonal phase, but their lungs were subjected to 2 hours (not 1) of warm ischemia, and without (rather than with) any ventilation.

In both the 1H DCD group and 2H DCD group, we collected blood only after the cardiac arrest. In general, it took 8 to 12 minutes to reach pulseless electrical activity. We still waited the full 15 minutes before excising the heart and closing the chest with the lungs intact: during these periods, internal thoracic temperatures were between 37 and 38° C.

Perfusion

All sets of procured lungs, from all 3 groups, were flushed and placed in the OCS™; then, lungs were perfused and ventilated for ≥ 24 hours, using standard additives with autologous donor whole blood. The OCS™ perfusion chamber was primed with 1600 ml of whole blood and 700 ml of OCS™ solution. We also infused standard recommended additives: 1 g of cefazolin, 200 mg of ciprofloxacin, 200 mg of voriconazole, 500 mg of methylprednisolone, 1 vial of multivitamins, 20 IU of regular insulin, 4 mg of milrinone (Primacor®), 20 mEq of $NaHCO_3$, 50 mg of nitroglycerin, and a 50% dextrose solution. We slowly increased flows to a goal of 2 to 2.5 L/min while rewarming lungs to 37° C.

We did not begin ventilation until the lungs were at 34° C. Ventilations were set to 6 cc/kg with a PEEP of 5 mm Hg. In between assessments, flows were kept at 1.5 L/min; we used 12% oxygen to oxygenate both the bloodstream oxygenator and the ventilator.

We measured arterial blood gases and blood chemistry parameters at 0, 2, 4, 6, 8, and 24 hours. To calculate the relative PO_2:FIO_2, we used a Radiometer ABL90 (Radiometer America, Inc., Brea, CA, USA). During assessment, a high-nitrogen, low-O_2 tank (6% CO_2, balanced N_2) flushed the oxygen from the oxygenator, so that deoxygenated blood reached the lungs, which were then ventilated with 21% oxygen (room air). We continuously tracked pulmonary artery pressure (PAP), pulmonary vascular resistance (PVR), oxygen saturation (SaO_2), temperature, peak airway pressure (PAWP), and hematocrit (HCT). Initial rises in PVR were treated with 10-mg infusions of nitroglycerin, as needed, to maintain the mean PAP < 20 mm Hg and to allow increases in flow.

To continuously capture SaO_2 in the pulmonary artery and venous return, we used saturation probes. To track PO_2:FIO_2 in the venous return, we measured arterial blood gases. SaO_2 reflected the amount of blood that drained from the pulmonary veins before passing through the oxygenator. The oxygenator was connected to an oxygen tank (12% O_2, 5% CO_2, balanced nitrogen) during preservation (i.e., rest mode) or to a deoxygenated tank (0% O_2, 5% CO_2, balanced nitrogen) during assessment. During preservation, the lungs were ventilated with 12% O_2, 5% CO_2, balanced nitrogen. During

assessment, the lungs were ventilated with room air (21% O_2). Thus, SaO_2 reflected the oxygen contribution of the oxygenator and lungs combined, during preservation; the lungs alone, during assessment.

Periodically, we checked the reservoir volumes, adding volume, as needed, to maintain at least 500 ml. We obtained serial images of the lungs. At the end of the 24 hour experiment, we flushed them with 1 L of OCS^{TM} solution, and procured specimens for BAL and histologic evaluation. Throughout OCS^{TM} perfusion, we administered bicarbonate and dextrose, as needed, to maintain bicarbonate levels > 20 meq/L and glucose levels > 70 mg/dL.

To track pulmonary edema, we assessed weight, HCT, reservoir volume replacement (RVR), as well as serum sodium and osmolality. To determine the extent of edema, we also used gross inspection and flexible bronchoscopy.

Histologic and cytokine analysis

We obtained serum samples from the swine (time point -1) before cardiac arrest and procurement, then from the lungs at baseline (time point 0) and at additional follow-up times on the OCS^{TM}. We obtained bronchial alveolar lavage (BAL) samples at the end of each experiment. To analyze interleukin-6 (IL-6) and 8 (IL-8) levels in serum and BAL, we used a Luminex assay using bead sets from Millipore. At 24 hours, we retrieved portions of lung tissue, froze them at -20° C with liquid nitrogen, and then processed them for hematoxylin and eosin (H&E) staining.

Statistical analysis

All values presented here are expressed as the means +/- standard deviation (SD). For our statistical comparisons, we used the standard *t* test, with a *P* value < 0.05 considered statistically significant. To determine differences in continuous measurements, we used analysis of variance (ANOVA).

Results

We completed a total of 12 lung isolation experiments, divided equally among our 3 groups: the control group (n = 4), the 1H DCD group (n = 4), and the 2H DCD group (n = 4). The mean weight of the 4 post-procurement lung specimens in the control group was 619 g; in the 1H DCD group, 656 g; and in the 2H DCD group, 817 g. All 12 experiments were technically successful; the mean time from lung explantation to perfusion on the OCS^{TM} was 20 minutes. Before the swine's cardiac arrest, all lungs demonstrated normal recoil and gross morphology. Lung abrasions from procurement and blebs were rare, and were successfully treated with a surgical stapler and/or sealant without altering ventilation.

Perfusion

In all 12 experiments, we achieved mean flow rates of 1.5 to 1.75 L/min during preservation. Our pH measurements suggested relative alkalosis in all 3 groups; the 2H DCD group was the least alkalotic in the initial 6 hours (Figure 6-2a). At 24 hours, the

final pH was 7.69 in the control group, 7.52 in the 1H DCD group, and 7.56 in the 2H DCD group. Initially, bicarbonate levels were higher in the control group than in the other 2 groups, but then became nearly identical, throughout the experiments, in all groups (range, 22 to 25 meq/L) (Figure 6-2b). Lactic acid production was lowest in the control group, but rose steadily in all 3 groups: the final values were 12.9 mmol/L in the control group, 20 mmol/L in the 1H DCD group, and 22 mmol/L in the 2H DCD group (Figure 6-2c). Note that bicarbonate is administered at each assessment to keep levels above 20 meq/L.

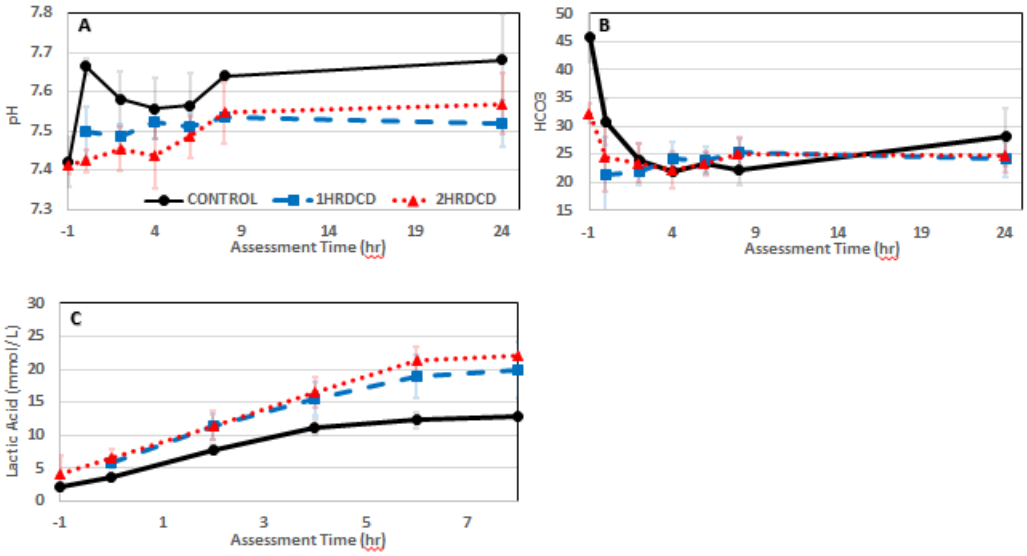

Figure 6-2: Perfusion characteristics on the OCS™ in the 3 groups: a. The 2H DCD group was the most acidotic in the initial 6 hours. b. Bicarbonate (HCO_3) levels were higher in the control group than in the other 2 groups, but then became nearly identical, throughout the experiments, in all groups. c. Lactic acid production was lowest in the control group, but rose steadily in all 3 groups. Time point negative 1 (-1) denotes the lung sample obtained from the swine before cardiac arrest and procurement; time point zero (0) denotes the lung sample, at baseline, on the OCS™.

Potassium levels steadily rose in all 3 groups. The mean potassium levels, at baseline and 24 hours, were 4.4 and 6.1 meq/L in the control group, 5.5 and 7.6 meq/L in the 1H DCD group, and 5.5 and 7.6 meq/L in the 2H DCD group. Those levels potentially reflected both cell death and the inability to clear electrolytes in the system. Similarly, the mean calcium levels, at baseline and 24 hours, were 0.8 and 1.5 meq/L in the control group, 2.9 and 2.3 meq/L in the 1H DCD group, and 2.9 and 2.3 meq/L in the 2H DCD group. Glucose levels were elevated at baseline in both DCD groups but gradually decreased to a mean, at 24 hours, of 135 mg/dL in the control group, 135 mg/dL in the 1H DCD group, and 183 mg/dL in the 2H DCD group.

Pulmonary function

Tidal volume delivery in the control group was fairly constant at 400 ml; in contrast, in the initial 6 hours, it was markedly reduced in the 2H DCD group, but normalized thereafter (Figure 6-3a). In the 1H DCD group, we maintained lungs at a maximum ventilation of 350 ml, in order to limit PAP. We were unable to ventilate the lungs in the 2H DCD group in the first 4 to 6 hours, but saw gradual improvement until ventilation became nearly identical, at 12 hours, to the control group.

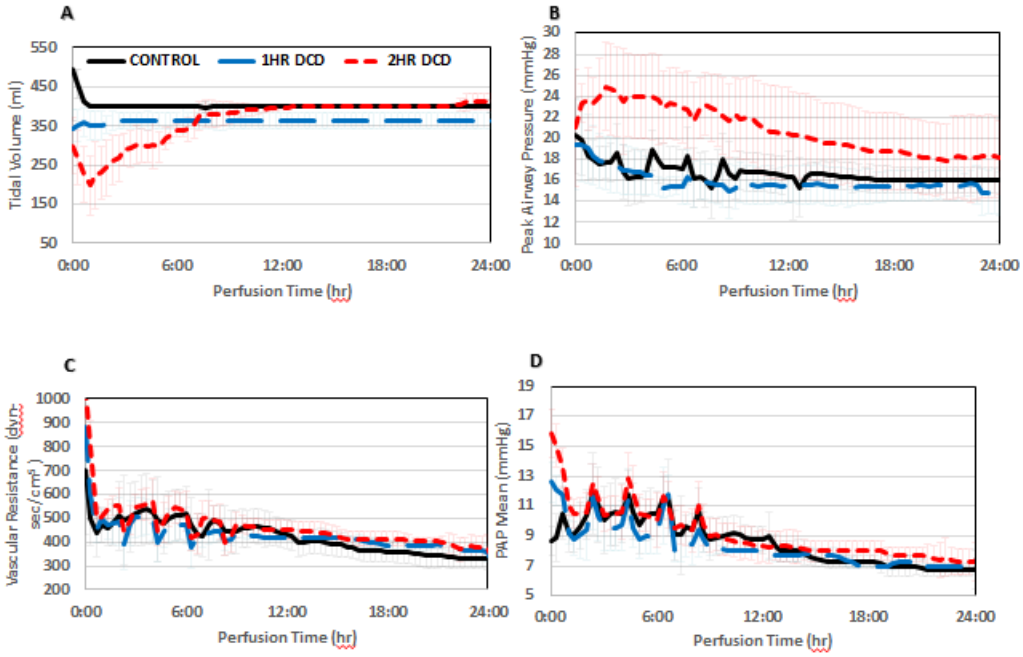

Figure 6-3: Pulmonary function on the OCS™ in the 3 groups: a. Tidal volume delivery in the control group was fairly constant at 400 ml; in contrast, in the initial 6 hours, it was markedly reduced in the 2H DCD group. b. Peak airway pressure (PAWP) was highest in the 2H DCD group. c. In all 3 groups, pulmonary vascular resistance (PVR) gradually improved. d. In all 3 groups, the mean pulmonary artery pressure (PAP) also gradually improved.

PAWP mirrored our PAP findings. In the 2H DCD group, the lungs experienced high PAWP, which prohibited early tidal volume delivery (Fig. 3b). In all 3 groups, PVR and PAP (used to document stability or improvement of pulmonary parameters on the OCS™) were initially elevated, but gradually improved over time (Figure 6-3c and d).

Oxygenation

SaO_2 ranged from 90% to 100% in both the control group and in the 1H DCD group throughout preservation. In the 2H DCD group, SaO_2 indicated severely impaired

gas exchange in the first 6 hours of assessment (Figure 6-4a). In the control group, the mean PO_2 ranged from 60 to 124 mm Hg and showed steady improvement throughout the experiments. In the 1H DCD group, PO_2 gradually decreased over the first 6 hours, reaching a mean of 85 mm Hg at 24 hours (Figure 6-4b). In the 2H DCD group, PO_2 substantially decreased throughout the initial 6 hours, reaching a mean of 46.8 at 24 hours. The mean PO_2:FIO_2 at 24 hours was 590 mm Hg in the control group, 404 mm Hg in the 1H DCD group, and 222 mm Hg in the 2H DCD group (Figure 6-4c).

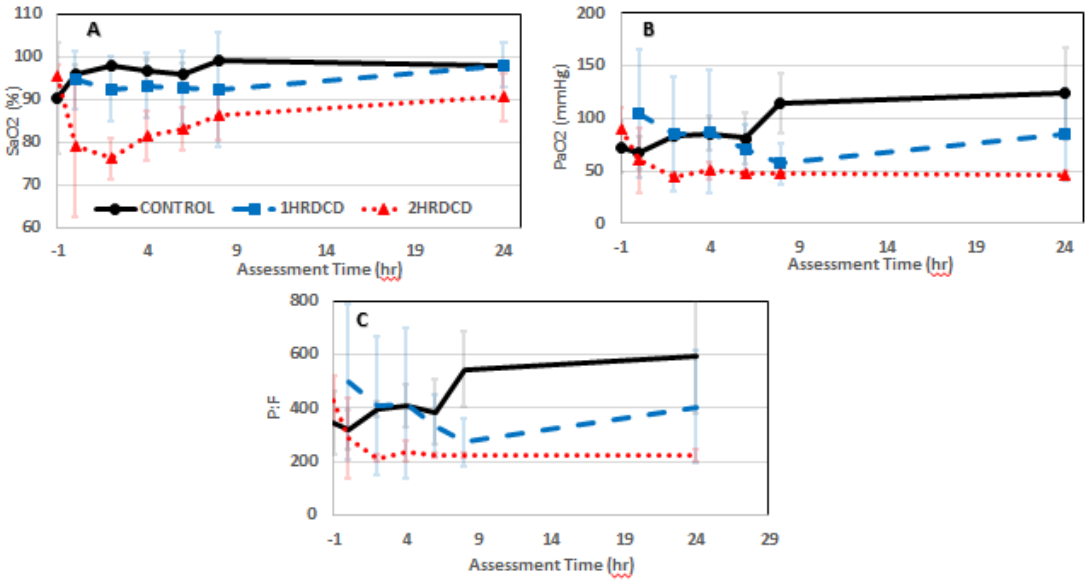

Figure 6-4: Oxygenation on the OCS™ in the 3 groups: a. Oxygen saturation (SaO_2) was lowest in the 2H DCD group, but slowly recovered to a mean of 90% b. In both DCD groups, PO_2 from the venous return decreased. c. The mean ratios of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PO_2:FIO_2) at 24 hours showed transplantable values (i.e., > 300 mm Hg) in the control group and in the 1H DCD group, but not in the 2H DCD group ($P<0.05$ by ANOVA). Time point negative 1 (-1) denotes the lung sample obtained from the swine before cardiac arrest and procurement; time point zero (0) denotes the lung sample, at baseline, on the OCS™.

Pulmonary edema

Since the OCS™ is a closed system, elevations in serum osmolality, sodium levels, and HCT likely suggest fluid leak into the lungs. In all 3 groups throughout our experiments, osmolality steadily increased; the highest elevation was in the 2H DCD group (Figure 6-5a). Sodium levels gradually increased in all 3 groups to a mean, at 24 hours, of 155 meq/L in the control group, 164 meq/L in the 1H DCD group, and 171 meq/L in the 2H DCD group (Figure 6-5b). HCT was more concentrated in both DCD groups compared with controls, despite identical autologous donor whole-blood composition and volumes (Figure 6-5c). The mean weight differences in all 3 groups suggested a modest increase in edema throughout OCS™ perfusion. However, the greatest initial weights were in the 2H DCD group, reflecting those swine's greater initial injuries and leaks (Figure 6-5d).

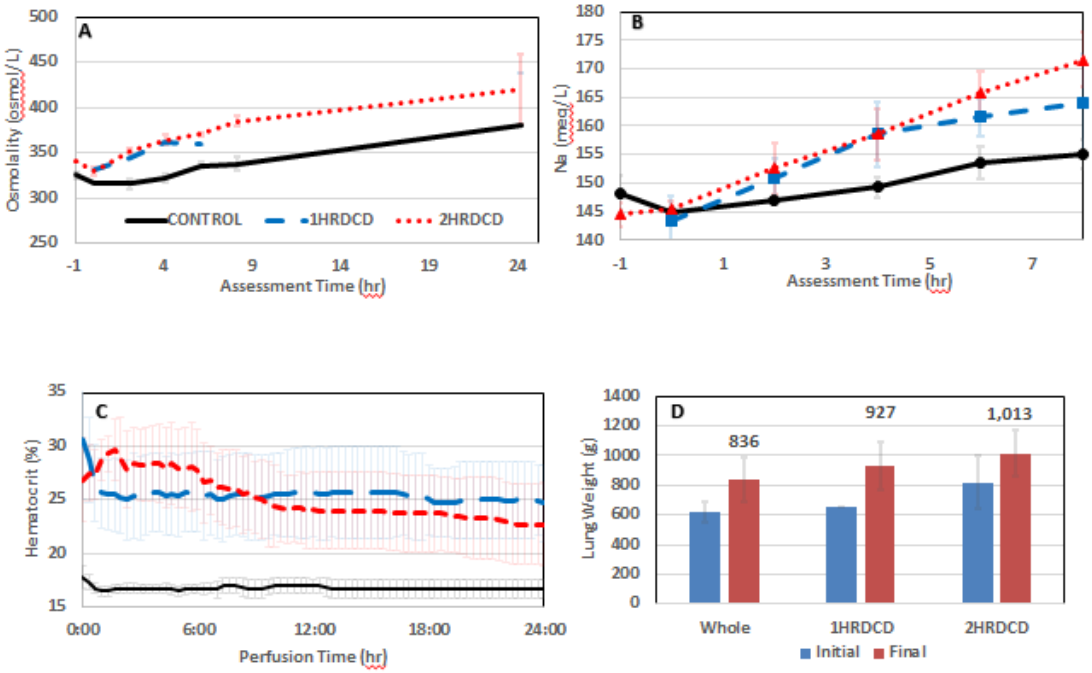

Figure 6-5: Pulmonary edema in the OCS™ chamber, as indirectly tracked through perfusate concentrations and lung weights in the 3 groups: a. In all 3 groups, osmolality steadily increased; the highest elevation was in the 2H DCD group. b. Likewise, sodium levels were highest in the 2H DCD group at 24 hours, but increased in all 3 groups. c. Hematocrit (HCT) was more concentrated in both DCD groups. d. The greatest initial weights were in the 2H DCD group. All 3 groups had a modest increase in lung edema at 24 hours. Time point negative 1 (-1) denotes the lung sample obtained from the swine before cardiac arrest and procurement; time point zero (0) denotes the lung sample, at baseline, on the OCS™.

Histologic and cytokine analysis

Between experiments, IL-8 production in the serum varied markedly in terms of the amount produced and the timing of production (Figure 6-6). In general, the greatest and most consistent elevations were seen in the 2H DCD group, but both DCD groups showed increased production. IL-8 production began at roughly the same time that the lungs began to show improvement (6 hours). The BAL specimens at 24 hours showed IL-8 counts of 9,635 in the 1H DCD group, as compared with 8,513 in the 2H DCD groups

(P = 0.88). For unclear reasons, one of the four 1H DCD experiments (#10) showed significantly higher BAL and serum IL-8 levels than the others. If one were to mask that experiment (#10), BAL and serum IL-8 levels were markedly higher in the 2H DCD group than 1H DCD.

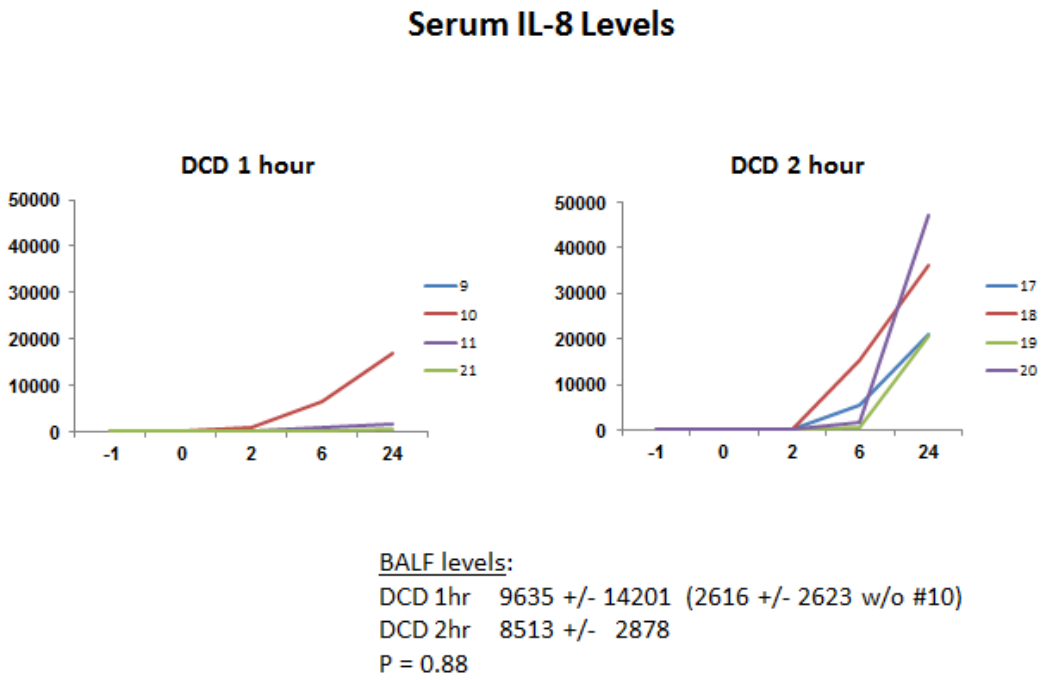

Figure 6-6: Interleukin-8 (IL-8) production in 8 representative experiments. At 24 hours, IL-8 production in the serum varied markedly in the amount produced and in the timing of production in both DCD groups. Greater increases were noted in the 2H DCD group. One experiment (#10) in the 1H DCD accounted for the increased production noted in that group's serum and BAL IL-8. Time point negative 1 (-1) denotes the lung sample obtained from the swine before cardiac arrest and procurement; time point zero (0) denotes the lung sample, at baseline, on the OCS^{TM}.

The complete lungs in the control group and the 1H DCD group appeared grossly and histologically normal throughout the experiment and at 24 hours (Figure 6-7).

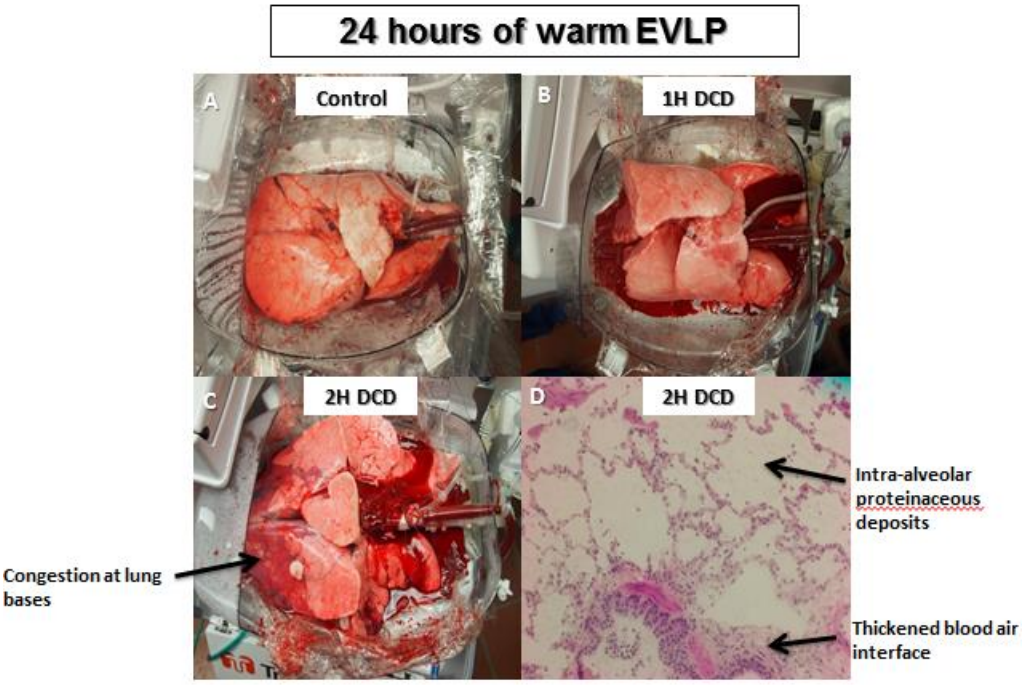

Figure 6-7: Gross morphologic assessment of swine lungs after 24 hours of OCS^{TM} perfusion: a. in the control group. b. in the 1H DCD group. c. in the 2H DCD group. d). Histologic assessment of normal-appearing segment of swine lungs after 24 hours of OCS^{TM} perfusion in the 2H DCD group.

Gross assessment of the lungs in the 2H DCD group showed hyperemic lungs at 2 hours, with development of blebs and air leaks. The lungs gradually became normal in external appearance. At 24 hours, residual areas of necrosis remained at the tips of the lingula and the lower lobes, with a congested appearance in the dependent posterior aspect of the lower lobes. These regions accounted for roughly 30% of the lung; the rest of the lung appeared normal Figure 6-7).

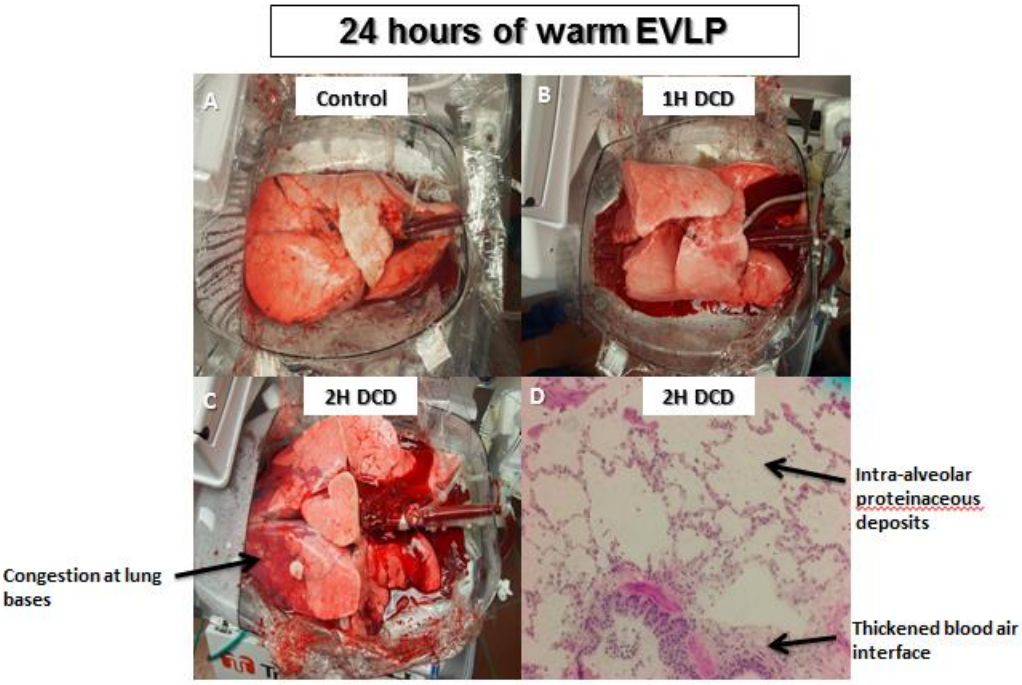

Figure 6-7c/d). Histologic sectioning of a normal portion of the 2H DCD lung showed dense proteinaceous material in the alveoli and thickening of the air blood interface Figure 6-7).

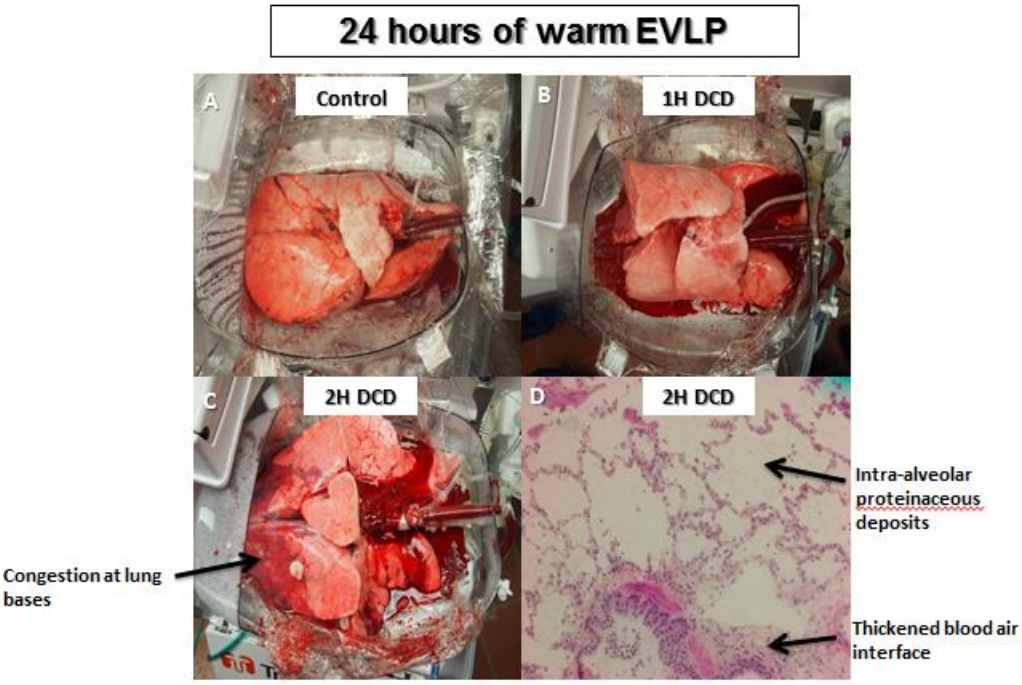

Figure 6-7.

Discussion

The use of DCD lungs could significantly increase the donor pool for transplantation. Yet today, such lungs account for less than 2% of lung transplants in the United States, mostly because of concern regarding their variable warm ischemic times and reperfusion injury.

Furthermore, uncontrolled DCD is not practiced in the United States, but has the potential to provide an additional 40,000 donor lungs, per conservative estimates[3] (given the 80,000 deaths from out-of-hospital cardiac arrest and another 80,000 deaths from in-hospital cardiac arrest). As opposed to controlled DCD (where the procurement team is

on hand as the prospective donor goes into cardiac arrest), uncontrolled DCD requires that the team must be mobilized, and family consent is obtained after the prospective donor goes into cardiac arrest. Uncontrolled DCD can expose the lungs to significantly greater periods of warm ischemia.

In the current porcine study, we characterized the effects of a graded stepwise increase in warm ischemic insults on potential donor lungs. We observed that 1 hour of warm ventilated ischemia had only a modest effect on pulmonary compliance, acidosis, PVR, and PAP, but did cause a significant reduction in initial abilities for oxygenation, which recovered after 6 to 8 hours of warm reperfusion on the OCS™. In contrast, 2 hours of non-ventilated warm ischemia resulted in a severe early impairment in pulmonary compliance, which improved, and in significant impairments in oxygenation, which did not recover fully. In both of our DCD groups (1 hour and 2 hours), IL-8 production was delayed but present; the greatest production was seen in the 2H DCD group.

Clinical studies have also employed EVLP as a means to evaluate the effects of warm ischemia on DCD lungs pre-transplant. Bozso et al. performed 3 successful transplants with no early recipient deaths after controlled DCD and EVLP using the OCS™.[22] Machuca et al. demonstrated less primary graft dysfunction, a shorter hospital length of stay, and shorter ventilator times after DCD and EVLP: they screened out 20% of prospective DCD donors as not eligible.[23] Suzuki et al. used uncontrolled DCD and EVLP for 3 hours for a transplant in the United States; the recipient was discharged home

after 9 days.[24] Snell et al. found acceptable lung function after EVLP in DCD lungs exposed to 60 minutes of warm ischemia, with or without topical cooling.[25]

In addition, EVLP is considered to be a very useful platform to assess experimental approaches for aiding lung recovery. In one such study, Machuca et al. showed that lungs with elevated nitric oxide metabolites and endothelin-1 levels on EVLP were more likely to have impaired oxygenation and greater primary graft dysfunction post-transplant.[26] Additionally, Kaneda et al. found a strong correlation between donor lung function and relative IL-8, IL-6, and IL-10 levels: IL-10 was protective.[27] Furthermore, Fisher et al. showed that elevated IL-8 levels were associated with poor graft function in lung transplant recipients.[28] Yet it is still unclear what the actual role of cytokine expression is in EVLP. In our porcine study, IL-8 was released after pulmonary compliance and oxygenation were already impaired, so it might be a marker, rather than a mediator, of lung injury in EVLP.

EVLP also provides a highly useful platform to investigate therapeutic interventions for reconditioning DCD-injured lungs. In our porcine study, we were not able to produce a transplantable organ after EVLP in any lung subjected to 2 hours of warm ischemia, even after 24 hours on the OCS™. Vascular function and pulmonary compliance improved, but not oxygenation. While we do not know the implication of transplanting a DCD lung with sub-standard PF ratios after 24 hours of EVLP, it is unlikely that many recipients or surgeons would be willing to experiment with this clinically anytime soon.

Our study also suggests that, after 2 hours of warm ischemia, placement on the OCS™ will result in a reperfusion injury, mostly affecting the interstitial cells and the alveolar epithelial cells. Pulmonary compliance and oxygenation were the most affected after reperfusion, while vascular flow steadily improved over time. Yet it remains unclear whether or not this injury is reversible. During EVLP, the lungs might have limited ability to regenerate damaged cells, although in the lung transplant recipient, regeneration might be more feasible. If the cells are simply dysfunctional rather than dead, post conditioning pharmaceutical interventions might be successful. For example, if the reperfusion injury is mostly mediated by oxidant damage, then perhaps it can be attenuated by adding antioxidants at reperfusion.[29,30]

Several investigators have explored novel protective methods along with EVLP. In a study in rats, Dong et al. showed a reduction of ischemia reperfusion injury in lungs subjected to carbon monoxide ventilation in an EVLP mini-circuit.[31] Similarly, in a porcine model, Inci et al. showed that surfactant inhalation before reperfusion improved PVR and gas exchange after aspiration injury.[19] Inci et al. also showed successful reconditioning of donor lungs treated with surfactant on an EVLP circuit.[20] Finally, hydrogen gas ventilation has been shown to reduce inflammation and improve lung function on EVLP.[18]

Our study has several limitations. First, we relied on the use of autologous donor whole blood but doing so might be logistically challenging in human DCD donors. Still, our method of evaluating EVLP of DCD lungs was unique: using autologous donor whole blood (instead of an acellular or isolated red blood cell perfusate, as is common in

EVLP) allowed for an extended period of perfusion. The use of isolated red blood cells is logistically easier for standard donor lungs, but our research suggests that time on EVLP is an important factor in the recovery of marginal lungs: enough time can only be afforded by the use of autologous donor whole blood in our model.

Second, in our study, the swine were all heparinized before induction of the agonal phase. In a true uncontrolled DCD scenario, heparinization is unlikely to be allowed until about 30 minutes after death at the earliest. Nevertheless, previous work has demonstrated successful recovery of DCD lungs without heparin.[32]. Also, in some cases of uncontrolled DCD, much longer intervals of downtime will likely be required, even more than our 2 hours, since it takes a considerable amount of time and effort to mobilize procurement teams and allocate the organs. Interestingly, Nakajima et al. evaluated longer ischemic intervals—up to 4 hours—and then placed lungs on an EVLP system using acellular perfusate. They did show impaired oxygenation and PVR; however, EVLP reduced the number of microthrombi, reduced the number of inflammatory markers, and improved adenosine triphosphate levels.[33] And in our study, we showed that ventilation before procurement reduced lung injury after reperfusion: clinically, this is potentially feasible in uncontrolled DCD. Once the decision to donate is made, the prospective DCD donor can easily be heparinized and ventilated until the procurement team arrives. This would also be true in a "expedited controlled DCD model" whereby logistics dictate that the donor is extubated before the team is able to arrive. Additionally, the use of topical cooling may be a helpful adjunct.[34]

In conclusion, we developed and tested a unique porcine model of DCD and EVLP, using prolonged normothermic perfusion on the OCS^{TM}. Our approach allowed for critical physiologic assessment, after perfusion, of lungs subjected to a graded stepwise increase in ischemic insults. We found significant injury patterns relative to the severity of ischemia, with the worst injury after 2 hours of warm downtime. In controlled DCD, EVLP should be the standard of care, in order to reduce the risk of exposing transplant recipients to lungs injured by warm ischemia while proceeding to take advantage of uninjured lungs. To clinically apply uncontrolled DCD, much work is first needed, in order to better assess and optimize recovery logistics. We showed that 1 hour of warm downtime with ventilation and heparin consistently yielded transplantable lungs. Longer warm ischemic intervals may require additional novel pharmaceutical interventions on EVLP and/or other strategies in the DCD donor before procurement, in order to attenuate reperfusion injury. We believe that an ideal clinical scenario for uncontrolled DCD would involve immediate heparinization and reintubation of a prospective lung donor who suffers cardiac arrest and cannot be resuscitated, with the intent to transplant the lungs after monitoring them on a portable EVLP system.

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Thesis Summary

The culmination of my thesis projects was to establish advanced and stable isolated organ investigation systems which then could be utilized to have impact on translation research. This has included identifying novel pharmacological therapies, means for enhanced and previously unexplored monitoring of isolated organs, characterization of basic physiology, and is being utilized extensively now for the design and testing of novel cardiothoracic devices. My work makes the isolated heart and lungs function better individually *and* together. I have demonstrated here both new assessment tools and a body of evidence that demonstrates means for improving utilization of current donor lungs and expanding the available populations of donor organs that has the potential to save lives in addition to furthering scientific understanding.

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