Development of simulated and ovine models of extracorporeal life support to improve understanding of circuit–host interactions

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Use of extracorporeal life support (ECLS) in cardiorespiratory failure refractory to maximal medical therapy is now well described, and use in adults is increasing.1,2 Patients requiring ECLS are a critically ill, although otherwise heterogeneous, cohort with varying diagnoses, age, body size and degrees end-organ dysfunction. ECLS itself can induce myriad additional pathophysiological changes. Clinical research in this group of patients is challenging, given the multitude variables that are difficult to control in any given study. Simulated extracorporeal circuits and in-vivo animal models provide valuable means to undertake detailed, systematic research into complex clinical scenarios such as ECLS, and to identify strategies for optimising clinical interventions.

An ideal animal model should mimic the severity of patient illness, reproduce key haemodynamic and immunological aberrations, serve as its own control, mimic histological findings in relevant organs, and provide a means to gain insight into factors contributing to interpatient variability.3 There are many well established ovine models used to study clinically relevant interventions and pathological states, such as cardiopulmonary bypass, myocardial reperfusion, burn injury, sepsis and acute lung injury (ALI).4-16 These

ABSTRACT

Background: Extracorporeal life support (ECLS) is a lifesaving technology that is being increasingly used in patients with severe cardiorespiratory failure. However, ECLS is not without risks. The biosynthetic interface between the patient and the circuit can significantly alter inflammation, coagulation, pharmacokinetics and disposition of trace elements. The relative contributions of the pump, disease and patient in propagating these alterations are difficult to quantify in critically ill patients with multiple organ failure.

Objective: To design a model where the relevance of individual components could be assessed, in isolation and in combination.

Design and subjects: Four ECLS models were developed and tested — an in-vitro simulated ECLS circuit; and ECLS in healthy sheep, sheep with acute lung injury (ALI), and sheep with ALI together with transfusion of old or new blood.

Main outcome measures: Successful design of in-vitro and in-vivo models.

Results: We successfully conducted multiple experiments in the simulated circuits and ECLS runs in healthy and ALI sheep. We obtained preliminary data on inflammation, coagulation, histology, pharmacokinetics and trace element disposition during ECLS.

Conclusions: The establishment of in-vitro and in-vivo models provides a powerful means for enhancing knowledge of the pathophysiology associated with ECLS and identification of key factors likely to influence patient outcomes. A clearer description of the contribution of disease and therapeutic interventions may allow improved design of equipment, membranes, medicines and physiological goals for improved patient care.

models highlight the appropriateness of the use of sheep in the study of human cardiopulmonary disease.

To assess the relative contribution of circuit factors to ECLS ovine model outcomes, we have also established a validated

Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABP</td>
<td>Arterial blood pressure</td>
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<td>ACT</td>
<td>Activated clotting time</td>
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<td>ALI</td>
<td>Acute lung injury</td>
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<td>CCO</td>
<td>Continuous cardiac output</td>
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<td>CVP</td>
<td>Central venous pressure</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<td>ECLS</td>
<td>Extracorporeal life support</td>
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<td>ECMO</td>
<td>Extracorporeal membrane oxygenation</td>
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<tr>
<td>ICE</td>
<td>Intracardiac echocardiography</td>
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<tr>
<td>IJV</td>
<td>Internal jugular vein</td>
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<tr>
<td>IVC</td>
<td>Inferior vena cava</td>
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<tr>
<td>SvO₂</td>
<td>Mixed venous saturation</td>
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in-vitro model of simulated ECLS using contemporary circuitry. Simulated circuits provide conditions that allow control of one variable at a time, which is difficult to achieve in animal and clinical studies. Hence, the aim of this study was to develop and validate more extensive and informative descriptors of ECLS using in-vitro and in-vivo models.

**Methods**

Establishment of a simulated model of extracorporeal life support

We used PLS ECLS circuits (Maquet Cardiopulmonary, Hirrlingen, Germany). This circuit comprises Bioline tubing, a PLS Quadrox D oxygenator and Rotaflow pump head. An R38 reservoir bladder (Medtronic, Minneapolis, Minn, USA) was added to allow fluid sampling from the closed circuit. The circuits were primed with 900 mL Plasma-lyte 148 (Baxter, Sydney, NSW, Australia), then exchanged for 500 mL Albumex 4 (human albumin, 40 g/L; CSL Bioplasma, Melbourne, Vic, Australia). Porcine mucous heparin (5000 U; Pfizer Australia, Sydney, NSW, Australia) was added to the circuits. Fresh whole human blood (mean volume, 420 mL [SD, 52 mL]) was used for the final prime to obtain postoxygenator pressures of 230–250 mmHg. The final circuit configuration is shown in Figure 1.

The final volume of the pressurised circuit was 668 mL (SD, 2 mL) with a mean haemoglobin concentration of 64 g/L (SD, 13 g/L). Activated clotting time (ACT) was maintained between 220 and 250 seconds. A Jostra Rotaflow centrifugal pump (Maquet Cardiopulmonary) was used to maintain a circuit flow rate of 4–5 L/min. Oxygen tension was maintained between 150 and 200 mmHg. Circuit temperature was maintained at 37°C. Carbon dioxide gas or sodium bicarbonate solution was added to the circuit to maintain the pH of the circulating blood between 7.25 and 7.55.

This simulated circuit model system was used to study the effect of circuit factors on drug concentrations, coagulation, inflammation and platelet cell functions under standard physiological conditions. In future, this model will incorporate variables that are of high relevance for a patient on ECLS, such as hypoxia, hyperoxia and hypothermia.

Establishment of an ovine model of extracorporeal life support

Establishment and validation of the ovine model of ECLS was conducted with approval of the Queensland University of Technology Animal Ethics Committee (approval no. 1100000053). An overview of the ECLS ovine model is presented in Figure 2. A summary of the key steps (Figure 3) and rationale is outlined below.

**Theatre set-up**

The theatre was set up to incorporate all equipment required to manage and monitor animal health and wellbeing. The operating table was equipped with a warming blanket to maintain normothermia during chronic instrumentation. A cradle was used to safely maintain the sheep’s sternum in the recumbent position, and a “head box” was used to immobilise the head and neck in a natural position.
Electrocardiograms (ECGs), physiological and pump pressures, saturation and end-tidal carbon dioxide were measured with a Marquette Solar 8000 monitor (GE Healthcare, Little Chalfont, UK) and recorded at 5 s intervals using custom software. Ventilation data were recorded on a breath-by-breath basis using software provided by the manufacturer (Hamilton Medical, Reno, Nev, USA), and ECLS pump data were recorded at 5 s intervals using custom software. Mixed venous saturation (SvO₂) and continuous cardiac output (CCO) were measured with a Vigilance II Monitor (Edwards Lifesciences, Irvine, Calif, USA) and recorded at 5 s intervals using software provided by the manufacturer. Syringe drivers and large- and small-volume infusion pumps were used for anaesthesia maintenance and drug/fluid administration. A fibre-optic bronchoscope was used to obtain bronchoalveolar lavage samples. The operating theatre was set up as shown in Figure 4.

Venous access, induction of anaesthesia and airway management

The sheep were 18-month-old ewes and weighed 40–45 kg. After fasting overnight, the animal was brought into the preparation room in a sling with face, neck and chest shaved. A multilumen central venous catheter and 8 Fr sheath were placed in the left internal jugular vein (IJV) under local anaesthesia. Baseline blood samples were taken. Anaesthesia was induced with intravenous midazolam (0.5 mg/kg) and alfaxalone (3 mg/kg). The animal was intubated with an orotracheal tube, brought into the operating room and positioned in the left lateral position; mechanical ventilation was commenced with expired air capnography. The sheep was ventilated with a Galileo ventilator (Hamilton Medical) using the Acute Respiratory Distress Syndrome Network criteria for lung-protective ventilation. No muscle relaxants were used. Anaesthesia was maintained with infusions of alfaxalone (4–6 mg/kg/h), midazolam (0.25–0.5 mg/kg/h), and ketamine (3–5 mg/kg/h). Doses were titrated to physiological and clinical parameters such as arterial blood pressure (ABP), heart rate, respiratory rate, central venous pressure (CVP), jaw movements, loss of the eyelash reflex and limb withdrawal response to ensure a constant surgical plane and optimal depth of anaesthesia and analgesia. A bolus dose of intravenous buprenorphine 0.01 mg/kg was given for analgesia and subsequently every 6 hours. Three venous sheaths (10 Fr) were introduced in the right IJV. These would later be used for intracardiac echocardiography (ICE) and extracorporeal membrane oxygenation (ECMO) cannulation. A tracheostomy was performed using a size 10 Portex (Smiths Medical, London, UK) tracheostomy tube and the endotracheal tube removed. The length of the sheep’s neck otherwise would prevent adequate bronchoscopy.

Establishment of monitoring

Routine monitoring consisted of pulse oximetry, ECG, ABP, CVP, pulmonary artery pressure and CCO. A pulmonary artery catheter was inserted via the left IJV sheath to measure CCO and SvO₂. A urinary catheter and orogastric tube were inserted. The facial artery was exposed and cannulated under direct vision for ABP monitoring.
**Inducing acute lung injury**

Our validated reproducible smoke-inhalation ALI method was used and has been described in detail elsewhere.\(^{18}\) Briefly, a stainless steel plate is heated to 750\(^\circ\)C and placed on top of 8 g of cotton in a cup. A bellows with transparent walls and a tidal volume of 400 mL is placed on top. The smoke from the combustion then passively fills the bellows. The bellows are then compressed by hand, exhaling the smoke through the base to a one-way valve, acting as one tidal volume breath (10–12 mL/kg) to the sheep. Exhalation is via a one-way valve, thus minimising rebreathing. Twelve breaths are delivered with first load of cotton. After the first cycle, new fuel is placed in the cup, and the process is repeated with eight breaths per cycle. Blood gas analysis provides both oxygenation details and carboxyhaemoglobin content (target 45%–50%), which demonstrates the reproducibility of the smoke “dose” between sheep.

**Extracorporeal life support circuit set-up and cannulation**

The circuits were primed with 900 mL Plasma-lyte 148 (Baxter) and then exchanged for 500 mL Albumex 4 (human albumin, 40 g/L; CSL Bioplasma). The oxygenator was connected to a water heater (Cincinnati Sub-zero, Cincinnati, Ohio, USA) to maintain ovine normothermia (39\(^\circ\)C). A PLS ECMO circuit was used (Maquet Cardiopulmonary AG). This circuit comprised Bioline tubing, a PLS Quadrox D oxygenator and Rotaflow pumphead. The circuit was primed with 900 mL Plasma-lyte 148 (Baxter), then exchanged for 500 mL Albumex 4 (CSL Bioplasma). Porcine mucous heparin (1000 U; Pfizer Australia) was then added to the circuit. The pump driver was a Bio-Medicus 550 Bio-Console pump speed controller (Medtronic) with external drive head. All data from the Bio-Medicus device were captured using the serial data port. Precise mixing of air and oxygen was achieved using a Sechrist air–oxygen mixer (Sechrist Industries, Anaheim, Calif, USA).

Cannulation was performed in the supine position by rewiring the previously placed venous sheaths. A 21 Fr (50 cm) femoral Carmeda BioActive Surface coated venous cannula (Medtronic) was first inserted into the right IJV using the Seldinger technique and positioned using ICE in the proximal inferior vena cava (IVC). After insertion of an access cannula, 30 U/kg of unfractionated porcine heparin (Pfizer Australia) was administered intravenously. A 19 Fr (50 cm) Carmeda BioActive Surface coated femoral venous cannula was used for return blood and also inserted in the right IJV using the Seldinger technique and positioned at the mid right atrium using ICE. Sheep femoral vessels are too small for ECLS cannulation, whereas the jugular veins are much more capacious. Arterial cannulae (25 cm) were too short for this model.

On confirmation of correct cannula positioning, each cannula was locked with unfractionated heparin (10 U/mL) and saline, and anchored around the neck with white endotracheal tube tape. Standard cyanoacrylate adhesive was found to be very effective in fixation of the cannula, but it limited any subsequent manipulation. The sheep was then repositioned from the supine to the sternal recumbent position. The clamped ECLS circuit was connected to the access and return cannulae. The pump speed was dialled to 1000 rpm before releasing the clamps. Pump speed was further increased to target flows at least two-thirds of pre-ECLS cardiac output (or 60–80 mL/kg). Gas flow was set to 80% of pump flows and inspired oxygen concentrations set at 100%. Ventilator settings were then set to “rest” settings (respiratory rate, 6; FiO\(_2\), 0.21; tidal volume, 4–6 mL/kg, positive end-expiratory pressure, 10 cmH\(_2\)O). PaCO\(_2\) was adjusted by altering sweep gas flows with ventilator settings held constant. A schematic model of the sheep ECMO model is shown in Figure 5.

**Intracardiac echocardiography and confirmation of cannula position**

Echocardiography plays a fundamental role in initiating and maintaining ECLS.\(^{19,20}\) Conventionally, transoesophageal echocardiography is used to determine cannula positioning in the clinical setting.\(^{21}\) However, sheep have a particularly capacious oesophagus, preventing good transducer contact and hence poor images are produced. Transthoracic echocardiography is possible in sheep, but there are limited acoustic windows available. ICE has the dual benefits of excellent spatial resolution combined with multiple accessible acoustic windows.\(^{22}\)
An 11 Fr venous access sheath was placed in the right IJV. A 10 Fr ICE catheter was connected to an ACUSON Sequoia CS12 echocardiography machine (Siemens, Munich, Germany) via a Swiftlink catheter connector (Siemens). The ICE catheter was passed down the sheath, through the right heart and into the IVC before cannulation. The guide wire for the access cannula was then passed through the heart and into the IVC under ICE guidance. The access cannula was inserted over this guide wire, and the tip could be visualised on the ICE images as it passed into the IVC and positioned as appropriate. A similar process was followed for the return cannula, but the imaging was performed more proximally. The ICE catheter was withdrawn into the mid right atrium. Appropriate passage of the return guide wire was confirmed with ICE. The return cannula was then passed over this wire and positioned in the mid right atrium (Figure 6).

**Anticoagulation**
The heparin infusion was commenced 4 U/kg/h for a target ACT of 200–300 s. The ACT checks were measured at 1 hour, 2 hours and every 2 hours subsequently.

**Haemodynamic management**
Invasive monitoring allowed for standardised management of haemodynamic variables using crystalloids (0.9% sodium chloride solution), colloids (human albumin, 40 g/L), vasopressor (vasopressin, noradrenaline) and inotrope (adrenaline) infusions as required.

**Fluid and electrolyte management**
Sheep were weighed on the day before and the day of surgery. Volume administration was determined by standard haemodynamic variables including elevated systemic vascular resistance, poor urine output and a low CVP. Maintenance fluids were run at 2 mL/kg/h throughout the study. Additional fluid boluses were allowed to maintain circuit flow and/or for resuscitation. Electrolyte levels were checked via regular blood gas analysis. Serum potassium levels were maintained >3 mmol/L. If required, a potassium chloride infusion was commenced at a rate of 5–20 mmol/h, depending on serum potassium concentrations, urine output, and gastric losses. Any large-volume gastric content lost was returned to normalise acid balance.

**Temperature management**
Temperature control was facilitated by the heater unit connected to the oxygenator. The ambient room temperature could also be adjusted to maintain goal temperatures.

**Euthanasia and postmortem handling**
At completion of experimentation, animals were euthanased with sodium pentobarbitone (295 mg/mL, 0.5 mL/kg). Death was confirmed by loss of cardiac electrical activity, ABP and cardiac output. After euthanasia, organs were retrieved surgically. Histological analysis of organs, including the right lung (right lower lobe), left lung (left lower lobe), heart (left and right ventricles), liver, pancreas, kidneys, spleen, adrenals, stomach, intestine and brain, was performed. The remains of the animals were frozen and stored until disposed of via high temperature incineration.

**Results**
The simulated ECLS circuit experiments (n=4) examining drug disposition in the extracorporeal circuit primed with fresh whole human blood have showed significant alterations in drug behaviour. Preliminary data show significant
sequestration of drugs in the circuit, which may have important clinical implications.

By commencing with a short-term ECLS experiment using healthy sheep, we conducted multiple experiments in the sheep comprising 2-hour ECLS runs in healthy (n=6) and ALI sheep (n=6). We were also successful with 24-hour runs in healthy (n=4) and ALI sheep (n=1). Significant preliminary data were obtained in relation to inflammation, coagulation, histology, pharmacokinetics, biomarkers and trace element disposition.

From a technical point of view, the cannulation, initiation and maintenance of ECLS were uneventful. We captured up to 70% of the cardiac output through our IVC access cannula with minimal recirculation, which is a significant improvement over previously reported ovine models,23 and is the key to maintaining satisfactory oxygenation in animal models of severe ALI. We had no serious issues related to bleeding or clotting, unlike the clinical situation.24 We have now optimised our anticoagulation, haemodynamic management and overall conduct of the experiment.

Discussion
This article describes the use of standard circuitry that includes centrifugal pumps and polymethyl pentene oxygenators to establish simulated circuit and ovine models of ECLS. Previously reported models have used variable technology comprising roller pumps, membrane oxygenators and drainage reservoirs.23,25-28 Some models have used venoarterial ECLS, which is not entirely comparable to the venovenous group. Changes in technology influence circuit–host interactions significantly and having a contemporary model adds to clinical relevance.

A major advantage of the sheep as an animal model of human disease is their large size. With mean body weight between 30 and 90kg, depending on breed,29 the size is comparable to adult humans (67–87 kg).30 Haemodynamic and respiratory measurements can be made using the same clinical techniques and equipment that are used in clinical intensive care units. Also, large volumes of blood, plasma and bronchoalveolar lavage samples can be collected at serial time points during an experiment without compromising the animal’s health. Apart from the size, there are immunological similarities, which are important when studying host–circuit interactions.9,31-34 Previous successful use of other ovine models of human disease, especially ALI,9 as well as closer similarities between sheep and human pulmonary anatomy and physiology, immunology and size, justify the choice of sheep as a relevant in-vivo large animal model of ECLS.

The simulated circuit and ovine models of ECLS described here allow an incremental and systematic approach to characterising the effects of ECLS on host physiology. Knowledge of how each ECLS component contributes to tissue injury will provide novel insight to inform new strategies to control, reduce or eliminate extracorporeal associated tissue injury, which will result in improved ECLS survival rates. The information gathered from this study will also benefit other extracorporeal therapies such as cardiopulmonary bypass. Knowledge gained using this ovine model of ECLS will improve our preparedness for future severe influenza outbreaks. Finally, the establishment and validation of this in-vivo ovine ECLS model provides a means for evaluating interventions (eg, drugs such as serine protease inhibitors) to reduce extracorporeal induced inflammation and tissue injury.

Future work beyond the scope of that described here will use simulated circuits together with healthy and critically ill sheep receiving ECLS to gain insight into the contributions of circuit factors relative to the influence of critical illness on changes in the pharmacokinetics of drugs such as antibiotics, sedatives and analgesics that are used in patients receiving ECLS in the clinical setting. These studies, together with studies in patients receiving ECLS, will provide important data for informing the development of antibiotic dosing guidelines as well as specific sedation protocols for patients receiving ECLS.

Conclusions
We have successfully established reproducible in-vitro and ovine models of ECLS that will be used in future studies to provide novel insight on altered pathophysiology during ECLS. Our on-going work involves collaborative research between major ECLS centres across Australasia and our model systems described herein will enable clinical hypotheses to be tested in a systematic fashion in order to optimise patient outcomes and further refinements in the use of ECLS in the clinical setting.

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Competing interests
The funding sources acknowledged had no influence on study design.
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