ANZSBT project report and synopsis: Alternatives to red cell transfusion to treat anaemia

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Synopsis for ANZSBT web site

After massive bleeding, immediate fluid infusion can restore microvascular blood flow and oxygen delivery to tissues, potentially avoiding or reducing the severity of haemorrhagic shock. Rapid treatment is not always feasible, so when severe haemorrhagic shock occurs, attempts to restore capillary blood flow by aggressively transfusing fluids and blood products may actually worsen microcirculatory dysfunction and reduce delivery of oxygen to tissues. We established a model of severe haemorrhagic shock in sheep to systematically measure the efficacy of novel fluids, including a crystalloid designed for enhanced microvascular perfusion and oxygen delivery (Oxsealife®). The model was designed to invasively measure microvascular blood flow, oxygen saturation and anaerobic metabolism by-products in vital organs, compared to standard monitoring of haemodynamic recovery and non-invasive assessment of tissue oxygen delivery. Protocols were developed and validated for reliable tissue instrumentation, induction of haemorrhagic shock, and clinically-appropriate resuscitation strategies. Treatment outcomes were demonstrated as being relevant for intensive care and emergency resuscitation. A severe haemorrhagic shock model was therefore established, providing functional outcome measures suitable for pre-clinical efficacy assessment of novel resuscitation strategies compared to PRBC transfusion.

Progress report

The aim of this project was to develop sheep models of extreme anaemia (haemorrhagic shock and chronic normovolemic anaemia), with treatment efficacy assessed according to microvascular and tissue oxygenation outcomes. The project provided pilot/feasibility data for a larger study to determine whether novel plasma volume expansion fluids are suitable alternatives to transfusion to treat moderate to severe normovolemic and hypovolemic anaemia. The study was performed at the Medical Engineering Research Facility at The Prince Charles Hospital in Brisbane. The ANZSBT granted $25,000 to this study, with the balance of materials and services funded by a Blood Service R&D project grant. In-kind staff support was provided by the Blood Service and the Critical Care Research Group (based at the Prince Charles Hospital). Experimental work commenced in October 2018. An interim analysis was performed after five experiments to confirm protocols, and the study was completed in April 2019.

The severe haemorrhagic shock model was established using eight female Leicester-cross sheep. We evaluated tissue-specific oxygenation and microvascular function invasively in brain, kidney, liver and skeletal muscle using laser Doppler blood flow, oxygen partial pressure (PtO_2), and micro-dialysis, benchmarked against continuous haemodynamic monitoring. Non-invasive regional tissue oxygen saturation (StO_2) was assessed by near infra-red spectroscopy (NIRS), and sublingual capillary perfusion by incident dark-field imaging. Haemorrhagic shock was induced by withdrawal of at least 40% total blood volume (TBV) over 90min, limited by clinical tolerance and retaining mean arterial pressure (MAP) >30mmHg. More blood was taken if required to induce shock, defined by central venous oxygen saturation ($ScvO_2$) <60% and arterial lactate >4mM. Sheep were randomised to an investigational crystalloid Oxsealife®, allogenic packed red cells (PRBC), or PlasmaLyte®, dosed to the treatment target MAP >65mmHg.

Primary outcomes: All sheep bled to shock targets demonstrated haemodynamic consequences of severe haemorrhage (Figure 1) including critically reduced MAP (38.0±9.2mmHg) and cardiac output (36.0±8.8ml/kg/min), while increased heart rate and/or systemic vascular resistance compensated for low blood pressure. Evidence of tissue hypoxia was consistently achieved during shock-guided haemorrhage, according to reduced $ScvO_2$, whereas only ¼ animals bled to %TBV targets developed shock (Figure1C). Tissue hypoxia measured by NIRS demonstrated conserved cerebral oxygen delivery relative to muscle (16% vs. 76% decrease).

Metabolic evidence of tissue hypoxia was demonstrated by increased arterial lactic acidosis and reduced base excess and pH (Figure 2). Recovery after resuscitation did not depend on haemoglobin. The single transfused sheep remained in metabolic shock, whereas crystalloid treatment resulted in metabolic recovery. Induction of shock and recovery was further demonstrated using invasive microvascular perfusion, tissue oxygen saturation and micro-dialysis probes, and supported by non-invasive sublingual microvascular imaging (Figure 3). Tissue perfusion and PtO2 declined >50% and lactate increased 2-3 fold across all tissues during shock. Although volume replacement improved MAP (70.3±21.7mmHg) and cardiac output (84.7±13.2ml/kg/min) within 30min, sustained recovery from shock, defined by increase to baseline tissue perfusion and oxygenation levels, and decrease in lactate: pyruvate ratios, was achieved independent of reduced haemoglobin after crystalloid treatment. All sheep survived the procedure.

To determine the utility of this model for a study of haemorrhagic shock and resuscitation outcomes, all haemodynamic, metabolic and microvascular parameters associated with haemorrhage and shock and with treatment efficacy were categorised for each animal and presented in a heat map to identify reliable markers of shock and treatment efficacy (Figure 4). Response thresholds were set according to published reference levels for sheep, according to the shock and resuscitation targets, or categories were based arbitrarily on percent change from baseline levels. These data confirmed that the primary markers of shock (cardiac output, $ScvO_2$ and lactate) were present in all shocked animals. Stroke volume, base excess and arterial pH were also consistently associated with shock. Metabolic shock was confirmed in vital organs by an elevated tissue lactate : pyruvate ratio. Resuscitation resulted in moderate to effective resolution of these haemorrhagic shock markers across each treatment group. Although the number of sheep used to develop the model was limited, this model appeared sufficiently robust to detect outcome differences between animals and treatment groups. Haemodynamic outcomes were similar between aggressive vs. MAP-guided resuscitation protocols, but lung injury was increased after aggressive resuscitation. Non-invasive tissue measures showed equivalent responses between the fluid treatment groups. Crystalloid treatment addressed specific parameters according to the formulation; Oxsealife® tended to provide better recovery in $ScvO_2$, cardiac output and stroke volume, while PlasmaLyte® tended to provide better recovery in MAP and acid-base parameters. The single sheep given PRBC experienced some haemodynamic recovery, but failed to achieve sustained microvascular and metabolic recovery.

This pilot study succeeded in developing an ovine model of severe haemorrhagic shock, and provided functional outcome measures based on recovery of microvascular perfusion and tissue oxygenation, benchmarked against standard haemodynamic monitoring and non-invasive tissue oxygenation measures. This model is therefore suitable for pre-clinical efficacy assessment of novel resuscitation strategies compared to PRBC transfusion.

Results from this pilot study were presented as a poster at the 3rd Perioperative PBM Symposium in Brisbane in Feb 2019, and an abstract submitted for presentation at Blood 2019. A manuscript describing the haemorrhagic shock model was submitted to the journal Intensive Care Medicine Experimental. Funding was obtained from the National Blood Authority and a Blood Service R&D project grant to continue the study, with in-kind support from the Blood Service and the Critical Care Research Group.