Investigation of CytoSorb™ Cytokine and Myoglobin Removal in the Treatment of Trauma and Burn Injury (Cytosorb™)

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FINAL REPORT

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INVESTIGATION OF CYTOSORB™ CYTOKINE AND MYOGLOBIN REMOVAL IN THE TREATMENT OF TRAUMA AND BURN INJURY (CYTOSORB™) HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION IN ACCORDANCE WITH ASSIGNED DISTRIBUTION STATEMENT.

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14. ABSTRACT-

Options for treatment of hyperkalemia in the pre-hospital setting are limited, particularly in the context of natural disaster or combat. Contemporary interventions require extensive resources and technical expertise. Here we utilized a novel and clinically relevant model of ischemia-reperfusion injury inducing acute hyperkalemia to provide evidence for application of extracorporeal hemofiltration technology for serum potassium extraction. We demonstrated that serum potassium can be filtered via hemoperfusion utilizing extracorporeal potassium binding. Hemofiltration to address acute hyperkalemia is feasible with contemporary technology, offering a novel approach to the management of critically ill patients in austere environments. While alternative methods exist to manage hyperkalemia, this technology offers unique advantages to a specific patient base. We believe that continued evolution of extracorporeal hemofiltration alternatives to classic dialysis warrant continued development and evaluation resulting in eventual deployment to serve this unique subset of patients.

15. SUBJECT TERMS-

Extra-corporeal Filtration of Potassium, Hyperkalemia; Bridge Dialysis; Dialysis; Hemofiltration; Trauma

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1.0 EXECUTIVE SUMMARY

Acute kidney injury (AKI) is associated with poor outcomes in both the civilian and the wounded warrior populations. Rhabdomyolysis may play a role in the poor outcomes seen in combat injured personnel specifically. An extracorporeal technique for the removal of myoglobin (the postulated cause of AKI in rhabdomyolysis) could therefore improve outcomes in military casualties. Here, we utilized a novel and clinically relevant model of ischemia-reperfusion injury inducing acute hyperkalemia to provide evidence for application of extracorporeal hemofiltration technology for cytokine and myoglobin extraction. We were able to demonstrate that cytokines and myoglobin can be filtered via hemoperfusion utilizing the device, CytoSorb™. This device allows rapid and minimally invasive filtration of the blood to reduce the effects of mycoplasma in patients who have sustained combat injuries. We believe that continued evolution of extracorporeal hemofiltration alternatives to classic dialysis warrant continued development and evaluation resulting in eventual deployment to serve this unique subset of patients.

2.0 INTRODUCTION

First described in casualties of the London air raids of World War II,[1] rhabdomyolysis is a process by which the cellular contents are released into circulation after damage to skeletal muscle. Their release can result in profound effects on renal function (acute kidney injury – AKI). The major etiologic factors of renal damage are intra-renal vasoconstriction and toxicity of myoglobin.[2] Vasoconstriction is the result of fluid sequestration in the damaged muscle with resultant up-regulation of the sympathetic nervous system, renin-angiotensin-aldosterone system, and arginine vasopressin. Additional factors that promote vasoconstriction, such as F2-isoprostanes, endothelin-1, thromboxane A2, and nitric oxide (NO) deficiency have also been implicated in this process. Tumor necrosis factor α may play an indirect role in this process by decreasing the activity of NO.

Myoglobin is postulated to have deleterious effects in the kidney as a result of oxy-radical production and precipitation with the Tamm-Horsfall protein in the tubule. The traditional therapy for rhabdomyolysis is primarily supportive with aggressive fluid resuscitation.[2] It has also been suggested that urinary alkalization or an osmotic diuretic (i.e., mannitol) could have further benefit. However, no benefit was observed in a 2004 retrospective review of the trauma population[3], and randomized controlled trials are lacking.

Injury to the large arteries in the extremities reduces the blood flow to the limb. If blood flow is not restored rapidly, time-dependent irreversible damage occurs within the muscle, nerve, fat, skin and bone. This damage is not only the result of ischemia (or poor oxygenation) but is paradoxically exacerbated by subsequent (though necessary) re-establishment of blood flow by the release of cell contents and biologically active chemicals. The release of these substances from the ischemic cells can cause life-threatening damage to other organs (such as the kidneys, liver, lungs and heart).

Vascular injury results in muscle damage, with subsequent release of myoglobin. This process is positively correlated with ischemic time. In this model, the mortality rate for animals that received ≥3 hours of ischemia in the context of hemorrhagic shock was 33%. These deaths were attributable to organ failure, consistent with the hypothesis that ischemic injury results in the release of factors (such as myoglobin) that cause organ damage and subsequent mortality.[4]

Hemoperfusion is a method in which blood is removed from the body, put through a filter and then returned to circulation. The hemoperfusion system we tested (Cytosorb™), has been shown to be effective in removing substances (myoglobin and cytokine) that can cause damage to other organs. Using a novel swine model of bilateral hind limb ischemia reperfusion (IR), we examined the effect of this system on the levels of the aforementioned toxic substances and functional outcomes (i.e., survival, hemodynamics, etc.). This study demonstrated a positive outcome when the Cytosorb™ device was used, and it represented the first significant advance in the treatment of this post-trauma disorder and could positively impact the care and outcomes for combat casualties.

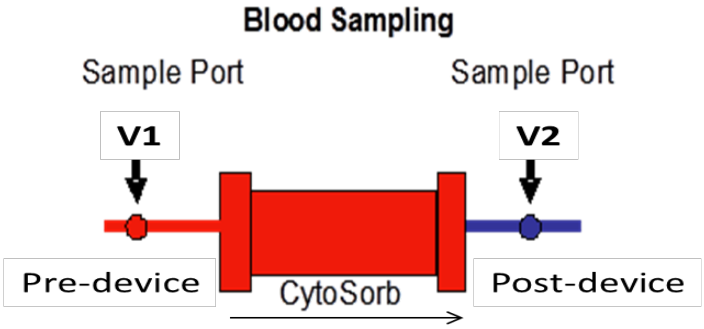

Figure 1. CytoSorb™ Device Filtration

3.0 METHODS, ASSUMPTIONS AND PROCEDURES

3.1 Experiment 1: Cytokine and Myoglobin Filtration

3.1.1 Hypothesis

We hypothesize, the animals given hemoperfusion with the Cytosorb™ filters will have decreased cytokine and myoglobin levels in a model of ischemia/reperfusion as compared to controls and have decreased end organ damage as evidenced by liver function tests, creatinine and histopathology of kidney tissue.

3.1.2 Methods

We used a novel swine model of a bilateral hind limb ischemia reperfusion to examine the effect of a novel hemoperfusion system Cytosorb™ on the levels of toxic cell and biologically active chemicals and functional outcomes. This study occurred in two phases. In the first phase, a novel swine model of bilateral hind limb ischemia reperfusion injury using endovascular balloon

occlusion was developed. The second phase evaluated the efficacy of the Cytosorb™ filter in mitigating the deleterious effects of bilateral hind limb ischemia reperfusion.
Institutional Animal Care and Use Committee Approval for all experiments was obtained prior to commencement of the described study. Experiments were carried out in an Association for the Assessment and Accreditation of Laboratory Animal Care (AALAC) accredited facility at the Wilford Hall Ambulatory Surgical Center, 59th Medical Wing, Joint Base San Antonio-Lackland, Texas. Animals were used in accordance with The Guide for the Care and Use of Laboratory Animals.

Phase I Model Development consisted of 4 groups. **Group 1** consisted of technique refinement for endovascular balloon occlusion of the terminal aorta. For **Group 2,** subjects underwent balloon occlusion of the terminal aorta with 3 hours of ischemia. At the end of three hours, perfusion was restored to the hind limb and animals were recovered and observed for 7 days. **Group 3** consisted of animals that experienced 6 hours of bilateral hind limb ischemia after balloon occlusion. These animals were also recovered and observed for 7 days. **Group 4** subjects underwent bilateral hind limb ischemia for a period of 6 hours as determined by Groups 2 and 3 outcomes. After reperfusion, all animals in all groups were monitored post-operatively for serum biomarkers indicating rhabdomyolysis, Acute Kidney Injury (AKI) and Multisystem Organ Failure (MSOF). During this phase, we identified the ideal bilateral ischemia time required to induce metabolite concentrations required to facilitate acute organ injury, as well as determine the time for creatinine levels to peak and fall.

Phase II Cytosorb™ Efficacy Experiment consisted of two groups, a control group and study group. The Control group consisted of 11 animals that did not receive hemoperfusion with the Cytosorb™ filter; instead, they received sham hemoperfusion with an inactive filter unit. These animals experienced up to 6 hours of bilateral hind limb ischemia induced by endovascular balloon occlusion of the terminal aorta. After reperfusion, the animals were submitted to a sham hemoperfusion session consisting of a blood pump, inactive Cytosorb™ filter cartridge and heparin anticoagulation.

The study group, determined by *in vivo* efficacy of Cytosorb™ in a swine model of ischemia reperfusion injury, consisted of 11 animals that underwent the same bilateral hind limb ischemia reperfusion injury as the control animals. Following reperfusion, these animals received 6 hours of hemoperfusion with an active Cytosorb™ filter and heparin anticoagulation in the manner described below.

Prior to treatment, a blood draw for a CBC was performed in both groups that included Hematocrit (Hct), Platelet count, Red Blood Cell (RBC), Hemoglobin (Hgb), and White Blood Cell (WBC). Study group animals underwent hemoperfusion with a single use CytoSorb™ device for 6 hours, using temporary vascular access (catheters) for a veno-venous system. Prior to hemoperfusion, we flushed and primed the circuit with saline and prevented air from entering into the system. Hemoperfusion was performed at an initial flow rate of 100 cc/min; when blood reached the entire system, the flow rate increased within a couple of minutes of reaching a target of 200-300 cc/min. Animals were systemically heparinized BEFORE all treatment sessions to a Partial Thromboplastin Time (PTT) of 60-80 or an Activated Clotting Time (ACT) of approximately 180% of baseline.

Throughout the treatment session, we monitored the CytoSorb^{TM} device for blood leaks or clots which was an essential data point. Any adverse device events were recorded by indicating the location of leaks or by recording the size of clots present.

Outcome measures recorded for both groups included cytokine levels, myoglobin levels, durable neuromuscular recovery as evidenced by TARLOV scale gait analysis, and any lab and histology evidence of end organ damage.

3.1.3 Data Analysis

Arterial blood samples were taken at baseline, every 30 minutes during the ischemic period, and every 10 minutes for the first 30 minutes following reperfusion. Arterial samples were assayed for blood pH, pO2, pCO2, and HCO3. Venous blood samples were drawn at 3 hours of ischemia and at 6 hours of ischemia (prior to re-perfusion). Venous aspirates were assayed for gross markers of ischemia and reperfusion injury including Blood pH, pO2, pCO2, HCO3, creatine kinase (CPK), lactate, lactate dehydrogenase (LDH), creatinine, AST/ALT, pro and anti-inflammatory cytokines, potassium, phosphate, hematologic parameters, clotting factors, albumin and myoglobin. Physiologic parameters were recorded from cardiac output monitor and physiologic monitors a Q1min intervals for later evaluation. Primary endpoint is blood myoglobin levels.

Statistical Analysis: In prior studies using this model, the mortality rate for animals that received ≥ 3 hours of ischemia in the context of hemorrhagic shock was 33%. Our study utilized animals that receive ≥ 6 hours of ischemia, so we expected a slightly higher proportion of mortality. Power analysis revealed that 22 animals (11 in each group) would be necessary based on an estimated proportion of mortality in the control group of 0.4, 80% power, a difference of 0.6, and a significance level of 0.05 using a two-sided z-test with pooled variance.

3.2 Experiment 2: Potassium Filtration

3.2.1 Hypothesis

Hemofiltration with a potassium-binding substrate would decrease systemic potassium by 1mEq/L during the first hour of treatment as compared to sham controls in a swine model of bilateral hind-limb ischemia reperfusion injury.

3.2.2 Methods

We used a novel model of swine bilateral hind IR injury consisting of 4 phases to investigate the efficacy of extracorporeal filtration of serum potassium as follows: 1) Anesthesia, instrumentation and surgical preparation; 2) ischemic injury; 3) reperfusion; and 4) hemofiltration. Institutional Animal Care and Use Committee (IACUC) approval for all experiments was obtained prior to commencement of the described study. Experiments were carried out in an Association for the Assessment and Accreditation of Laboratory Animal Care

accredited facility at the Wilford Hall Ambulatory Surgical Center, 59th Medical Wing, Joint Base San Antonio-Lackland, Texas. Animals were used in accordance with *The Guide for the Care and Use of Laboratory Animals*.

Twenty female, Yorkshire-Landrace cross swine (*Sus scrofa*, 70-91kg) were randomized into two experimental groups by pairs: 1) animals that underwent hemofiltration with a Cytosorb™ potassium binding filter (Filter) following ischemia-reperfusion injury and functional (*in situ)* nephrectomy; and 2) animals that underwent sham hemofiltration (Control) following the same injury procedure. The study was conducted in four phases: animal preparation, ischemia, reperfusion, and hemofiltration (Figure 2). Research staff were blinded as to which animal received intervention or sham treatment up until hemofiltration was commenced. Blinding for the entire procedure was not logistically feasible due to the support requirements of the hemofiltration circuit.

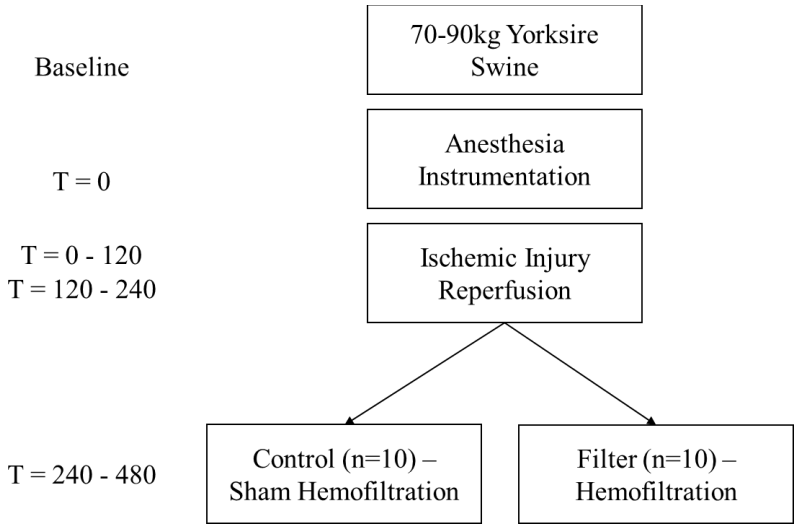

Figure 2. Flow Diagram of Experimental Procedure and Times

In Phase 1, animals were tranquilized with Telazol, intubated, and anesthesia was induced with isofluorane. Instrumentation for measurement of physiologic parameters was placed as follows: (1) Right external jugular cordis for Swan Ganz catheter (cardiac output, mixed venous oxygen saturation, systemic vascular resistance, temperature, end diastolic volume and ejection fraction); (2) right internal jugular Schon XL catheter for hemoperfusion;(3) left external jugular triple lumen catheter for pharmaceutical and fluid infusion; and (4) a micropuncture catheter was inserted into both the left carotid artery and the left femoral artery for invasive measurement of blood pressure. Near-infrared spectroscopy (NIRS) pads were applied to the gracilis and pectoralis muscles. To reduce the requirement for volatile anesthesia, animals received a 3µg/kg fentanyl bolus and maintained on a continuous fentanyl infusion at a rate of 0.02µg/kg/min in normal saline (NS) throughout the entire procedure.

In Phase 2, we accomplished ischemic injury reperfusion by performing a midline laparotomy to enter the left retroperitoneal space. The distal aorta and inferior vena cava were isolated just

proximal to their respective bifurcations and encircled with vessel loops. In the same dissection, the renal arteries were exposed bilaterally, double ligated and divided within the renal hilum. Prior to initiation of bilateral hind limb ischemia, a 100unit/kg loading dose of heparin was administered immediately followed by a 60unit/kg/hr infusion of heparin in normal saline (NS). Further boluses of heparin were administered throughout the procedure to maintain an activated clotting time (ACT) 200% of baseline ACT as measured by i-STAT (Abbott Laboratories, Princeton, NJ) as measured following phase 2.

Ischemia was initiated by securing the vessel loops around the aorta and vena cava in a taut position with vascular clamps. Occlusion of flow distal to the loops was confirmed by invasive arterial pressure telemetry and decreased NIRS tissue oxygenation levels. The abdominal fascia was then closed with running 0-PDS suture and ischemia was maintained for two hours.

In Phase 3, we focused on reperfusion. Following the ischemic period, vascular clamps and vessel loops were removed to restore hind limb flow. Return of flow was confirmed by invasive femoral arterial pressure measure and increased muscle oxygenation (NIRS). One liter of NS was infused upon restoration of flow to mitigate ischemia-reperfusion cardiovascular depression and/or collapse. Animals were observed for two hours after reperfusion to permit IR injury physiologic sequelae to occur. Fifteen minutes prior to the initiation of hemoperfusion and filtration, a 20cm dual-lumen dialysis catheter was placed in the right external jugular vein.

At the onset of Phase 4, hemoperfusion, 1L of NS was administered as a bolus. A second 1L bolus was administered at 2 hours post initiation of hemoperfusion. In filter group animals, two 500cc CytoSorb™ potassium-binding fluidized bed filters (CytoSorbents INC, Monmouth Junction, NJ) were placed in a parallel hemofiltration circuit attached to the dialysis catheter. A Masterflex L/S pump (Cole Parmer, Vernon Hills, IL) was utilized to circulate blood through the filter system at a continuous rate of 300mL/min for 4 hours. Control animals underwent sham hemoperfusion using extracorporeal circuit sans filters. Flow rates were measured using flow probes (Transonic Systems, Ithaca, NY) placed on the extracorporeal circuit tubing just proximal to the filters. Norepinephrine was administered at a rate of 12µg/min in NS if MAP fell below 25mmHg.

3.2.3 Data Analysis

Arterial blood samples were taken at the following time points: pre-surgery (baseline), initiation of ischemia (T=0min), throughout the ischemic period (T=60 & 120min), throughout the reperfusion period (T=150, 180, 210 & 240min), and during the hemoperfusion period (T=225, 270, and Q30min 270-480min). Venous blood samples were drawn from the extracorporeal circuit, proximally and distally to the filters at 255, 300, 360, 420 & 480min. Levels of potassium, lactate, and pH were analyzed from both arterial and venous samples. Physiologic parameters were recorded from cardiac output monitor and physiologic monitors a Q1min intervals for later evaluation. NIRS from the pectoralis and gracilis muscle was recorded Q30sec. Primary endpoint was post-filtration serum potassium. Secondary endpoints were survival, post-filtration platelet count and metrics for physiologic status.

Statistical Analysis: A priori power analysis determined that 18 animals (9 per group) would be required to observe a statistically significant difference (1mEq/L decrease) in serum potassium with a standard deviation (SD) of 0.75mEq, α=0.05 and β=0.80. All continuous variables were reported as mean (SD) and analyzed by t-test. Clinical and laboratory variables measure at multiple time points were analyzed by two-way repeated measures ANOVA with post-hoc pairwise comparisons where appropriate (Holm-Sidak). Statistical software used was GraphPad Prism V6.0f (La Jolla, CA).

4.0 MAJOR EVENTS/MILESTONES/SUCCESS

- IACUC Approval – 1 November 13
- Model Development and Refinement – August 2014
- Full Experimental Matrix – June 2015
- All experimental procedures completed
- Platform presentation at the 2014 Military Health System Research Symposium (MHSRS)
- Poster presentation at the 2015 Association of Surgeons of Great Britain and Ireland (ASGBI)
- Manuscript submitted to Shock on 2 May 2016 (rejected)
- Manuscript submitted to Military Medicine (~April 2017) – conditional acceptance
- Data Analysis – May 2016
- Dissemination of Results – Fall 2017

5.0 RISK ASSESSMENT

5.1 Risk Analysis

Scheduling delays during the project:

- Project start day delayed due to IACUC approval taking four months.
- By July 2014 only 18 experiments were scheduled. Model development completed. IMPACT: Primary resident unable to publish findings as evidence of productive fellowship activities.
- Second Fellow assigned to project July 2014. Only 12 experiments were able to be scheduled between July and December 2014.
- Significant delay in laboratory processing of critical samples
  IMPACT: delayed model development decision making. This delayed project direction decision-making with Army and SBIR partner. Data was not available for abstract submission in December 2014.
- Sponsor planning TTA acquisition strategy for Cytosorb XL Filter technology. SBIR Phase III mechanism identified and planned - desired continued collaboration with AFMS as "Extending Golden Hour" (a top CBA) priority for USAF MAJCOM(s). Delay in SBIR Phase II activities delays TTA and SBIR III.

5.2 Technical Challenges

None.

6.0 TRANSITION PLAN

6.1 Military Relevance

Given the prevalence of extremity injury in the current conflict (as well as subsequent rhabdomyolysis and AKI), if an intervention were shown to improve functional outcomes, it would positively impact morbidity associated with combat injury. The CytoSorb™ device constitutes a major improvement to the military and civilian medical mission by potentially providing emergency medics and surgeons a viable option for improved outcomes in patients who experience sustained injuries with rhabdomyolysis as a complication. It would represent a significant advancement in the treatment of a condition whose management has not significantly changed in the 70 years since its discovery. Additionally, current military studies on acute kidney injury have focused largely on burn patients; however, given what is known about the association of rhabdomyolysis and acute kidney injury and the increased morbidity and mortality associated with AKI, this device would positively impact combat injured military personnel, and eventually civilian patients, who are susceptible to rhabdomyolysis.

AFMS CBA Gap: 2014 CBA: AFMS 72 2015 CBA: RTK AFMS 16 AETC Use of Extracorporeal Life Support Systems Devices in Complex Clinical Scenarios; Research to Knowledge "Advance treatments of hyperkalemia, rhabdomyolysis, and acute kidney injury using an extra-corporeal filtration device (Cytosorb). Transition: Incorporate Cytosorb into ECLS care".

6.2 Transition Strategy

The outcome of this work could lead to advanced treatments of hyperkalemia, rhabdomyolysis, and acute kidney injury using an extra-corporeal Cytosorb™ filtration device. We demonstrated a positive outcome when the CytoSorb™ device is used. Use of this device represents the first significant advancement in the treatment of this post-trauma disorder that can positively impact the care and outcomes for combat casualties. Gained knowledge may be incorporated into extracorporeal life support practices in complex clinical scenarios with results of this study disseminated to the combat casualty care community.

7.0 RESULTS

7.1 Experiment 1: Cytokine and Myoglobin Filtration

The injury created in Experiment 1 was not severe enough to elicit a strong myoglobin and cytokine response. Therefore, no results are available to determine the efficacy of the Cytosorb™ filtration device to remove cytokines and myoglobin from blood.

7.2 Experiment 2: Potassium Filtration

Baseline characteristics: Twenty-two swine were randomly assigned to either the filtration or control arms of the study. Two filtration animals were excluded and replaced due to idiopathic

cardiac arrest prior to initiation of filtration protocol. Twenty animals were included in analysis. Baseline values for both groups were similar in regards to the following: weight, mean arterial pressure (MAP), heart rate (HR), lactate, serum potassium, serum calcium, pH, and platelet count (Table 1.).

Table 1

Baseline Parameters

Parameter	**Filter**	**Control**	**p-value**
Female	10 (100%)	10 (100%)	1.0
Weight (kg)	82.6 ± 5.8	81.1 ± 4.6	0.50
MAP (mmHg)	56.8 ± 10	57.5 ± 11	0.88
HR (beats/min)	74.1 ± 17	80.8 ± 20	0.44
pH	7.47 ± 0.06	7.43 ± 0.04	0.11
Lactate (mmol/L)	1.2 ± 0.3	1.3 ± 0.4	0.34
Potassium (mEq/L)	3.9 ± 0.2	3.8 ± 0.4	0.56
Calcium (mEq/L)	10.2 ± 0.4	10.2 ± 0.4	0.95
Platelets	300 ± 55	278 ± 50	0.35

Physiologic response to injury and filtration: There was minimal effect of extracorporeal filtration on MAP, HR, systemic vascular resistance, cardiac output, or central venous oxygenation. The physiological effects of ischemia-reperfusion resulted in decline of MAP and cardiac output from T = 120 to 480 minutes with a concomitant rise in HR.

Primary Endpoint: Hyperkalemia: Serum potassium was significantly elevated in both groups following ischemia and reperfusion periods (Figure 3, Panel a). At initiation of filtration (T=240), normalized serum potassium was not different between filter and control groups (150% (22) and 149% (12) respectively). During the initial hour of filtration (simulated “golden hour” of pre-hospital care), we observed a significant reduction in normalized potassium values in the filter group which persisted at end of study (480min) (Figure 3, Panel b).

Secondary Endpoints: There was no evidence of significant variation of serum calcium between treatment groups ($p=0.15$). However, beginning at filtration (T =240min), calcium decreased significantly from baseline with a mean decrease of 0.97mEq/L amongst all animals at the end of the experiment ($p<0.0001$). Platelets were significantly lower in the filter arm compared to the control group ($p<0.01$) with a mean difference of 168 $x10^3/\mu L$ at T =480 ($p<0.0001$). On post-hoc multiple comparisons, platelet count began to decline at initiation of filtration and was found to be significantly lower in filter animals as compared to controls by one hour after filtration.

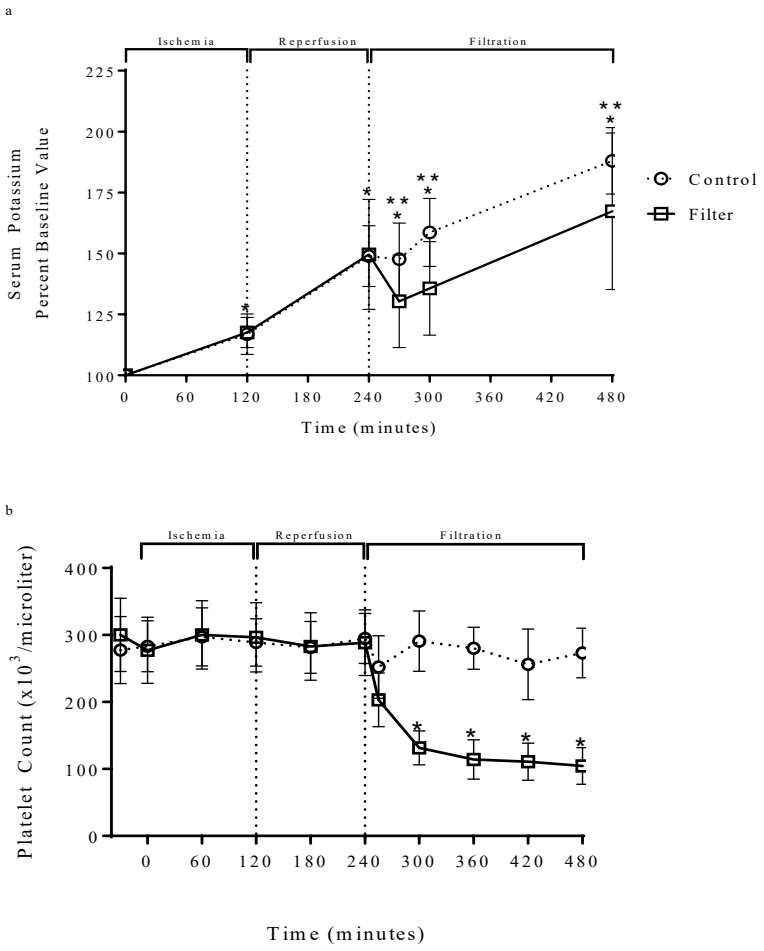

Figure 3. Primary Endpoints

*Note. Panel a. Normalized serum potassium (% baseline) over time. Control group values are represented by open circles and dotted line. Filter group values are open squares with solid line. Values are graphed as mean (SD). * = statistically significant difference from respective baseline value, ** = statistically significant difference from control value at time point. Panel b. Platelet count over time. * = statistically significant difference from control value at time point.*

8.0 CONCLUSION/DISCUSSION

Here we utilized a novel and clinically relevant model of ischemia-reperfusion injury inducing acute hyperkalemia to provide evidence for application of extracorporeal hemofiltration technology for serum potassium extraction. While this is not the first description of hemofiltration technology developed to achieve serum potassium reduction, it is a significant step towards the development of a fieldable intervention for acute hyperkalemia in both the prolonged field care and civilian disaster management scenarios.

Extracorporeal hemofiltration, as we have described herein, may provide a more feasible alternative for the management of casualties where established medical facilities and suitable logistical support are not available. While it may lack the simplicity of temporizing measures like those seen with insulin and glucose administration, the management is simplified to a single

pump that does not require titration or fluid balancing. Additionally, it addresses the underlying condition of hyperkalemia instead of temporarily shifting potassium intracellularly. This "bridge" dialysis is designed to span the gap from point of injury to fixed medical care when transportation options are limited. Current evacuation times in Iraq and Afghanistan average less than 60 minutes from injury to surgery; however, future conflicts in less established battlefields may have substantial delays in patient evacuation. Similarly, this technology offers rapid deployment capability to environmental disasters and humanitarian aid responses.

Although this technology requires continued development, it does offer a potential solution to a critical medical knowledge gap. While alternative methods exist to manage hyperkalemia, this technology offers unique advantages to a specific patient base. We believe that continued evolution of extracorporeal hemofiltration alternatives to classic dialysis warrant continued development and evaluation resulting in eventual deployment to serve this unique subset of patients. More specifically, extracorporeal potassium hemofiltration can be easily applied to critically ill patients in an austere environment with limited access to resources and personnel. For example, our group noted that 5.8% of critically ill service members evacuated from Iraq and Afghanistan achieved a serum potassium greater than 6mEq/L and could have benefitted from this type of technology for improved patient outcomes. In addition to austere environment use, this type of intervention may also be advantageous in humanitarian aid response or under-resourced medical facilities. It is important to note that such technology may be less pertinent to tertiary care centers in the United States.

In conclusion, hemofiltration to address acute hyperkalemia is feasible with contemporary technology, offering a novel approach to the management of critically ill patients in austere environments. Continued development and refinement of the technology will be required in order to achieve longer-term filtration efficiency while reducing risk of thrombocytopenia in hemofiltration circuits utilizing this technology.

9.0 DELIVERABLES

9.1 Publications

9.2 Presentations

1) Platform presentation at the 2014 Military Health System Research Symposium (MHSRS)
2) Poster presentation at the 2015 Association of Surgeons of Great Britain and Ireland (ASGBI)

10.0 COST

This work was funded by Small Business Innovation Research award number W81XWH-12-C-0038. FY13 funding under Project Code Number AC14EM03 of $108,000.00 was received on June 16, 2014 and all funds were expended by September 30, 2015.

11.0 REFERENCES

1. Bywaters, E G, and D Beall. “Crush Injuries with Impairment of Renal Function.” British medical journal 1 (1941): 427–32.

2. Bosch, X, E Poch, and JM Grau. “Rhabdomyolysis and Acute Kidney Injury.” New England Journal of Medicine 361 (2009): 62–72.

3. Brown, Carlos V. R. et al. “Preventing Renal Failure in Patients with Rhabdomyolysis: Do Bicarbonate and Mannitol Make a Difference?” The Journal of Trauma: Injury, Infection, and Critical Care 56.6 (2004): 1191–1196.

4. Hancock, Heather M et al. “Hemorrhagic Shock Worsens Neuromuscular Recovery in a Porcine Model of Hind Limb Vascular Injury and Ischemia-reperfusion.” Journal of vascular surgery 53.4 (2011): 1052–62; discussion 1062.

TABLES AND FIGURES

11.1 Tables

11.2 Figures

12.0 LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

ACT	Activated Clotting Time
AETC	Air Education and Training Command
AFMS	Air Force Medical Service
AFMSA	Air Force Medical Support Agency
AKI	Acute Kidney Injury
ANOVA	Analysis of Variance
ASGBI	Association of Surgeons of Great Britain and Ireland
CBA	Capabilities Based Assessment
CBC	Complete Blood Count
ECLS	Extracorporeal Life Support
Hct	Hematocrit
Hgb	Hemoglobin
HR	Heart Rate
IACUC	Institutional Animal Care and Use Committee
IR	Ischemia Reperfusion
MAP	Mean Arterial Pressure
MHSRS	Military Health System Research Symposium
MSOF	Multisystem Organ Failure
NIRS	Near-Infrared Spectroscopy
NO	Nitric Oxide
NS	Normal Saline
PDS	Polydioxanone Sutures
PTT	Partial Thromboplastin Time
RBC	Red Blood Cells
RTK	Research to Knowledge
SBIR	Small Business Innovation Research
SD	Standard Deviation
TTA	Technology Transfer agreement