Extending the "Golden Hour": Prolonged Pre-Hospital Survival and Stabilization of Hemorrhagic Shock That Permits Delayed Whole Blood Resuscitation and Subsequent Survival

Cliff Morgan, PhD
Darren Fryer, BS
Kassandra Ozuna, MBA
Remealle How, MD MAJ MC USAF
Leasha Schaub, MS
Valerie Sams, MD Lt Col MC USAF
Jacob J. Glaser, MD FACS CDR MC USN

FINAL REPORT

25 March 2019

59th Medical Wing
Office of the Chief Scientist
1100 Wilford Hall Loop, BLDG. 4554
JBSA Lackland AFB, TX 78236-7517

DECLARATION OF INTEREST

The views expressed in this article are those of the authors and do not reflect the official views or policy or position of the Department of Defense or its Components. This work was funded by Project Code Number AC15EM02. The experiments reported herein were conducted according to the principles set forth in the National Research Council's Guide for the Care and Use of Laboratory Animals (8th ed.), and the Animal welfare Act of 1966, as amended. Authors are military service members, employees, or contractors of the US Government. This work was prepared as part of their official duties. Title 17 USC §105 provides that 'copyright protection under this title is not available for any work of the US Government.' Title 17 USC §101 defines a US Government work as a work prepared by a military service member, employee, or contractor of the US Government as part of that person's official duties.

NOTICE AND SIGNATURE PAGE

Using Government drawings, specifications, or other data included in this document for any purpose other than Government procurement does not in any way obligate the U.S. Government. The fact that the Government formulated or supplied the drawings, specifications, or other data does not license the holder or any other person or corporation or convey any rights or permission to manufacture, use, or sell any patented invention that may relate to them.

Qualified requestors may obtain copies of this report from the Defense Technical Information Center (DTIC) (http://www.dtic.mil).

Extending the "Golden Hour": Prolonged Pre-Hospital Survival and Stabilization of Hemorrhagic Shock That Permits Delayed Whole Blood Resuscitation and Subsequent Survival

Michele F. Tavish

Michele F. Tavish, DAF
Program Analyst
En route Care Research Program
59MDW Office of the Chief Scientist

Robert T. Gerhardt, MD, MPH, FACEP, FAEMS
Director, Trauma & Clinical Care Research (STT)
59MDW Office of the Chief Scientist

This report is published in the interest of scientific and technical information exchange, and its publication does not constitute the Government's approval or disapproval of its ideas or findings.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
25 March 2019	Final Report	23 June 2016 – 23 June 2018

4. TITLE AND SUBTITLE

Extending the "Golden Hour": Prolonged Pre-Hospital Survival and Stabilization of Hemorrhagic Shock That Permits Delayed Whole Blood Resuscitation and Subsequent Survival

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Cliff Morgan, PhD; Darren Fryer, BS; Kassandra Ozuna, MBA; Remealle How, MD MAJ MC USAF; Leasha Schaub, MS; Valerie Sams, MD Lt Col MC USAF; Jacob J. Glaser, MD FACS CDR MC USN
Email: clifford.morgan.5.ctr@us.af.mil, darren.fryer.1@us.af.mil, Kassandra.m.ozuna.ctr@mail.mil, remealle.a.how.mil@mail.mil, valerie.g.sams.mil@mail.mil, jacob.j.glaser.mil@mail.mil

5d. PROJECT NUMBER
AC16EM02

5e. TASK NUMBER

5f. WORK UNIT NUMBER
G1703

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Naval Medical Research Unit San Antonio
3650 Chambers Pass
Building 3260
JBSA Fort Sam Houston, TX 78234

8. PERFORMING ORGANIZATION REPORT NUMBER

Phone: 210-539-7027

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
59th Medical Wing
Office of the Chief Scientist
1100 Wilford Hall Loop, BLDG. 4554
JBSA Lackland AFB, TX 78236-7517

10. SPONSOR/MONITOR'S ACRONYM(S)
59 MDW

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Distribution A: Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT- Background: Adenosine, lidocaine, and magnesium (ALM) is a cardioplegic agent shown to improve survival by improving cardiac function, tissue perfusion, and coagulopathy in animal models of shock. We hypothesized pre-hospital ALM treatment in hemorrhagic shock would improve survival compared to current Tactical Combat Casualty Care (TCCC) resuscitation beyond the Golden Hour. Methods: Swine were randomized to: 1) TCCC, 2) 2cc/kg vehicle control (VC), 3) 2cc/kg ALM+drip, 4) 4cc/kg ALM+drip, 5) 4cc/kg ALM+delayed drip at 0.5cc/kg/hr, 6) 4cc/kg vehicle control, 7) 4cc/kg ALM for 15 mins + delayed drip at 3cc/kg/hr. Animals underwent pressure controlled hemorrhage to MAP of 30mmHg (S=0). Treatment was administered at T=0. After 120 minutes of simulated pre-hospital care (T=120) blood product resuscitation commenced. Physiologic variables were recorded and labs were drawn at specified time points.Results: TCCC demonstrated superior survival to all other agents. VC and ALM groups had lower mean arterial pressures (MAPs) and systolic blood pressures (SBPs) compared to TCCC. Except for the vehicle control groups, lactate levels remained similar with correction of base deficit after pre-hospital resuscitation in all groups. Kidney function and liver function remained comparable across all groups. Compared to baseline values, TCCC demonstrated significant hypocoagulability. Conclusion: ALM, as administered in this study, is inferior to current Hextend®-based resuscitation for survival from prolonged hemorrhagic shock in this model. In survivors, ALM groups had lower SBPs and MAPs, but provided a protective effect on coagulopathy as compared to TCCC. ALM does not appear to be a suitable low volume replacement to current TCCC resuscitation. The reduced coagulopathy compared to TCCC warrants future studies of ALM, perhaps as a therapeutic adjunct.
Study Type: Translational Animal Model

15. SUBJECT TERMS-
Shock, Hemorrhage, Bleed, Hypovolemia, Ischemia, Reperfusion, Coagulation, Acute Coagulopathy, Coagulopathy, Swine, Pig, Porcine, Sus scrofa domesticus, stabilization, adenosine, lidocaine, magnesium, ALM, Adenocaine, Survival

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			MAJ WILLIAM G GENSHEIMER
U	U	U	UU	23	19b. TELEPHONE NUMBER *(include area code)* (240) 612-1730

TABLE OF CONTENTS

2.0 INTRODUCTION.....Error! Bookmark not defined.

3.0 METHODS, ASSUMPTIONS AND PROCEDURES **3**

4.0 MAJOR EVENTS/MILESTONES/SUCCESS **7**

5.0 RISK ASSESSMENT **7**
5.1 Risk Analysis **7**
5.2 Technical Challenges **8**

6.0 TRANSITION PLAN **8**
6.1 Military Relevance **8**
6.2 Transition Strategy **9**

7.0 RESULTS **9**

8.0 CONCLUSION/DISCUSSION **11**

9.0 DELIVERABLES (Please provide appropriate citations for your work, publications, etc.) **13**
9.1 Publications **13**
How, R., Glaser, J., Schaub, L., Fryer, D., Ozuna, K., Morgan, C., Sams, V., Cardin, S. "Pre-hospital adenosine, lidocaine and magnesium has inferior survival compared to tactical combat casualty care resuscitation in a porcine model of prolonged hemorrhagic shock." JOT: ePUB ahead of print (JT-D-18-08911R1) **13**
9.2 Presentations **13**
How, R. (2018 April 26). Pre-hospital Treatment with Adenosine, Lidocaine, and Magnesium in a Porcine Model of Hemorrhagic Shock: Inferior Compared to Current Tactical Combat Casualty Care Resuscitation. SAUSHEC **13**
How, R. (2019 January 17) Treatment with Adenosine, Lidocaine, and Magnesium (ALM) in the Pre-Hospital Setting Has Inferior Survival Compared to Tactical Combat Casualty Care based Hextend Resuscitation in a Porcine Model of Prolonged Hemorrhagic Shock. EAST **14**
Morgan, C. (2019 February 06) Adenosine, Lidocane, Mg^{2+} as an Adjunct to Whole Blood Resuscitation does not Improve Coagulopathy. ASC **14**

10.0 COST **14**

11.0 REFERENCES **15**

FIGURES AND TABLES **17**

12.0 LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS **23**

EXECUTIVE SUMMARY

In combat casualties, hemorrhagic shock and its sequelae remain the most prevalent cause of potentially survivable mortality, accounting for 97% pre-hospital and 86% in-hospital potentially survivable mortality. To limit the physiologic and metabolic derangements that occur in prolonged hemorrhagic shock, there is an emphasis in pre-hospital trauma care of adhering to the "Golden Hour" concept in which casualties reach hospital care for definitive treatment within one hour of injury. Due to logistical and battlefield constraints, this is not always feasible. Adenosine, lidocaine, and magnesium (ALM) is a cardioplegic agent shown to improve survival by improving cardiac function, tissue perfusion, and coagulopathy in animal models of shock. We hypothesized pre-hospital ALM treatment in hemorrhagic shock would improve survival compared to current Tactical Combat Casualty Care (TCCC) resuscitation beyond the Golden Hour. For prolonged field care studies, swine were randomized to: 1) TCCC, 2) 2cc/kg vehicle control (VC), 3) 2cc/kg ALM+drip, 4) 4cc/kg ALM+drip, 5) 4cc/kg ALM+delayed drip at 0.5cc/kg/hr, 6) 4cc/kg vehicle control, 7) 4cc/kg ALM for 15 mins + delayed drip at 3cc/kg/hr. For whole blood resuscitation adjunct studies, swine were randomized to three treatment groups: (1) Whole blood alone, (2) Whole blood + 30 mL of ALM bolus (A:1mg/kg; L:2 mg/kg; M:0.5 mg/kg) infused at 5 mL/min, or (3) Whole + 30 mL of vehicle control (VC) (0.9% NaCl bolus) infused at 5 mL/min. Animals underwent pressure controlled hemorrhage to MAP of 30mmHg (S=0). For prolonged field care studies, treatment was administered at T=0. After 120 minutes of simulated pre-hospital care (T=120) blood product resuscitation commenced. For coagulopathy studies, ALM was administered at T=60, along with whole blood resuscitation. Physiologic variables were recorded, and labs were drawn at specified time points. TCCC demonstrated superior survival to all other agents. VC and ALM groups had lower mean arterial pressures (MAPs) and systolic blood pressures (SBPs) compared to TCCC. Except for the vehicle control groups, lactate levels remained similar with correction of base deficit after pre-hospital resuscitation in all groups. Kidney function and liver function remained comparable across all groups. Compared to baseline values, TCCC demonstrated significant hypocoagulability. ALM, as administered in this study, is inferior to current Hextend®-based resuscitation for survival from prolonged hemorrhagic shock in this model. In survivors, ALM groups had lower SBPs and MAPs, but avoided the coagulopathy induced by TCCC. ALM does not appear to be a suitable low volume replacement to current TCCC resuscitation. Furthermore, the addition of ALM to whole blood resuscitation at the hospital phase did not improve upon the coagulopathic status of patients.

2.0 INTRODUCTION

In combat casualties, hemorrhagic shock and its sequelae remain the most prevalent cause of potentially survivable mortality, accounting for 97% pre-hospital and 86% in-hospital potentially survivable mortality.[1,2] To limit the physiologic and metabolic derangements that occur in prolonged hemorrhagic shock, there is an emphasis in pre-hospital trauma care of adhering to the "Golden Hour" concept in which casualties reach hospital care for definitive treatment within one hour of injury. Due to logistical and battlefield constraints, this is not always feasible. Because of these limitations, the United States military utilizes a clinical practice guideline for Tactical Combat Casualty Care (TCCC) that outlines resuscitative treatments for medics to use in the battlefield. The guideline emphasizes the use of whole blood as the optimal treatment for resuscitation.[3] If whole blood is not available, the next best preferred treatment is 1:1:1 blood component damage control resuscitation (DCR) followed by dried plasma (DP). Although whole blood is available in Special Operations units, it is not yet

available in all combat units and component blood products, as well as dried plasma, are only available in limited amounts.[4] In these circumstances where blood products are not available in the pre-hospital setting, Hextend® is the preferred treatment over crystalloid; however, Hextend® use has been shown to lead to adverse effects, including increased hypocoagulability and risk of bleeding.[5] Furthermore, the availability of blood or fluids in the battlefield is limited by the amount of gear that medics can carry. Therefore, a low-volume therapeutic that can stabilize a patient in a pre-hospital setting in order to maintain adequate organ perfusion and protection while limiting the metabolic and physiologic derangements would be crucial in improving the potentially survivable mortality associated with hemorrhagic shock. Such a therapeutic may have promise as a suitable treatment modality that can replace Hextend® and avoid the potential adverse effects resulting from its use.

Adenosine, lidocaine, and magnesium (ALM) is a known treatment regimen used in cardiac surgery as a cardioplegic agent in cardiopulmonary bypass. Adenosine hyperpolarizes the myocyte while lidocaine blocks sodium fast channel activation. Magnesium acts as a calcium antagonist and prevents accumulation of intracellular calcium. Magnesium alone has been shown to improve myocardial performance following cardioplegic arrest.[6] Furthermore, hypomagnesemia is associated with higher mortality rates in ICU patients.[7] In open heart surgeries, ALM has been shown to improve metabolic, cardiac, and neurologic outcomes when it is used as a cardioplegic agent.[8-10] Numerous animal models of hemorrhagic shock have demonstrated the therapeutic potential benefits of ALM: in rats, low dose bolus of ALM not only improved cardiovascular function, but also demonstrated an increased survival rate.[11,12] A swine model of hemorrhagic shock demonstrated improved cardiac and renal function, as well as decreased fluid requirements during resuscitation.[13] Other animal studies have shown that ALM may also reverse trauma-induced coagulopathy[14] and prevent secondary immune-mediated complications.[15]

Given these potential therapeutic benefits of ALM, the aim of this study was to determine if 'pre-hospital' treatment with ALM during hemorrhagic shock would: 1) limit metabolic and physiologic derangements; 2) provide end-organ stabilization and protection; and 3) increase overall survival compared to current standardized Hextend®-based Tactical Combat Casualty Care Resuscitation (TCCC) in a prolonged field care scenario.

Primary endpoints:

- Overall survival rate.
- Survival times both prior to, and following, whole blood resuscitation.

Secondary endpoints:

- Cardiovascular function - Assessment to include: continuous invasive arterial blood pressure and waveform monitoring, continuous electrocardiography (lead II ECG).
- Metabolic state - Assessment to include: serum pH, base deficit and lactate levels.
- Coagulation function - Assessment to include: rotational thromboelastometry analyses, as well as, determinations of prothrombin time (PT), activated partial thromboplastin time (aPTT) and international normalized ratios (INR).
- End organ function and protection - Assessment to include: serum chemistries and markers of injury/function, and end organ histology.
- Fluid requirement to maintain permissively hypotensive resuscitation.

3.0 METHODS, ASSUMPTIONS AND PROCEDURES

The initial study proposed the use of one hundred and ten (110) male swine (*Sus scrofa domesticus*), which would divide them randomly into several groups: ten (10) animals for an initial

technical refinement group, ninety (90) animals assigned into five (5) Protocol Studies (i.e. Studies A, B, C, D, and E), and three treatment arms (i.e. Arms 1, 2, and 3) within each Study. There were additionally ten (10) cull animals for possible replacement should animals on the study be lost due to illness or early anesthetic or surgical mortality (***Table 1***).

Studies A, B, C, D, and E would evaluate the use of ALM as a low volume resuscitation fluid in prolonging the 'Golden Hour' to 2, 3, 4, 5, and 6 hours, respectively. However, after the completion of the technical refinement group, and the initial bolus dose in Study A, it was determined that few of the animals were surviving beyond the first hour, as compared to the TCCC standard of care, which had a 90% survival rate at 2 hours.

This prompted communication with the Dobson group, which have been the lead publishers on the use of ALM in hemorrhagic shock models. They provided input on options to modify the dosage and administration of ALM in the experimental models. Initially, ALM was being administered as a rapid bolus, followed immediately by a continuous IV infusion, or 'drip' for the remainder of the first hour. The Dobson group advised delaying the drip until the end of the 1st hour of prolonged field care, as well as administering the bolus over a 15-minute period, rather than all at once. Both of these options were tested and failed to yield better results. The animals utilized in these groups are described in ***Table 2***.

In a final attempt to assess the potential utility of ALM in the modification of TCCC guidelines, it was proposed to utilize it as an adjunct to whole blood resuscitation at the point of hospital care, as previous studies from the Dobson group have demonstrated that ALM can prevent trauma induced coagulopathy. These groups are described in the methods below. Ultimately, these animals did not demonstrate a significant difference in coagulation as compared to control groups. At this point, sixty-one (61) animals had been utilized in the study. Having failed to demonstrate an advantage of ALM as a low volume resuscitation fluid, extend survival beyond the golden hour, or as an adjunct to whole blood resuscitation at the point of hospital care, the study was terminated, and the results disseminated for presentation and publication.

Table 1: Initial Proposed Protocol Arms

Table 1. Protocol Arms		
Technical Refinement		
	Objective	**# of Animals**
	Technical Refinement (surgical, hemorrhage, and resuscitation protocols)	10
Study A - *GOLDEN 2-HOURS*		
	Treatment	**# of Animals**
Arm 1	0.9% NaCl bolus and drip infusion	6
Arm 2	ALM bolus (2.5 mg/kg) + ALM drip infusion (0.7 mg/kg/hr)	6
Arm 3	ALM bolus (2.5 mg/kg) + ALM drip infusion (1.7 mg/kg/hr)	6
	Study A total number of swine	18
Study B - *GOLDEN 3-HOURS*		
	Treatment	**# of Animals**
Arm 1	0.9% NaCl bolus and drip infusion	6
Arm 2	ALM bolus (2.5 mg/kg) + ALM drip infusion (0.7 mg/kg/hr)	6
Arm 3	ALM bolus (2.5 mg/kg) + ALM drip infusion (1.7 mg/kg/hr)	6
	Study B total number of swine	18
Study C - *GOLDEN 4-HOURS*		
	Treatment	**# of Animals**

Arm 1	0.9% NaCl bolus and drip infusion	6
Arm 2	ALM bolus (2.5 mg/kg) + ALM drip infusion (0.7 mg/kg/hr)	6
Arm 3	ALM bolus (2.5 mg/kg) + ALM drip infusion (1.7 mg/kg/hr)	6
	Study C total number of swine	18
Study D - ***GOLDEN 5-HOURS***		
	Treatment	**# of Animals**
Arm 1	0.9% NaCl bolus and drip infusion	6
Arm 2	ALM bolus (2.5 mg/kg) + ALM drip infusion (0.7 mg/kg/hr)	6
Arm 3	ALM bolus (2.5 mg/kg) + ALM drip infusion (1.7 mg/kg/hr)	6
	Study D total number of swine	18
Study E – ***GOLDEN 6-HOURS***		
	Treatment	**# of Animals**
Arm 1	0.9% NaCl bolus and drip infusion	6
Arm 2	ALM bolus (2.5 mg/kg) + ALM drip infusion (0.7 mg/kg/hr)	6
Arm 3	ALM bolus (2.5 mg/kg) + ALM drip infusion (1.7 mg/kg/hr)	6
	Study E total number of swine	18
Project Summary		
	Objective	**# of Animals**
	Technical Refinement	10
Study A	GOLDEN 2-HOURS	18
Study B	GOLDEN 3-HOURS	18
Study C	GOLDEN 4-HOURS	18
Study D	GOLDEN 5-HOURS	18
Study E	GOLDEN 6-HOURS	18
	Project subtotal number of swine	100
	Cull	10
	PROJECT TOTAL NUMBER OF SWINE	**110**

Ethical Approval and Accreditation:

The study protocol was reviewed and approved by the 711th HPW/RHD JBSA-Fort Sam Houston Institutional Animal Care and Use Committee (IACUC) in compliance with all applicable Federal regulations governing the protection of animals in research. All procedures were performed in facilities accredited by the AAALAC international.

Preparation of Test Agent:

ALM solutions were prepared as defined by Dobson et al. Briefly, all bolus doses were comprised of 0.2674g Adenosine (Adenosine, SIGMA A9251), 0.8664g Lidocaine (Lidocaine Hydrochloride Monohydrate, SIGMA L5647), and 0.301g Magnesium ($MgSO_4$)(Magnesium Sulphate, anhydrous SIGMA M7506). This was placed into solution with 1000mL 3% NaCl. For the 'drip' dose, using the same millimole amounts above, Adenosine (0.5g/100mL), Lidocaine (1.0g/100mL), and MgSO4 (0.5g/100mL) were mixed into 100mL of 0.9% NaCl. This allowed for a drip rate of 0.5mL/kg/hour over the two-hour protocol.

Pre-operative Preparation:

Sixty-one male swine (*Sus scrofa domesticus*) weighing 70-90 kilograms were randomized into ten resuscitation groups: 1) Hextend®-based Tactical Combat Casualty Care (TCCC) Resuscitation consisting of up to a maximum of two 500cc boluses of Hextend (1L maximum) and Lactated Ringers infusion to maintain MAPs of 50mmHg; 2) 2 cc/kg vehicle control bolus (3% NS); 3) 2 cc/kg 3% ALM bolus followed by immediate 0.5 cc/kg/hr 0.9% ALM drip; 4) 4 cc/kg 3% ALM bolus followed by immediate 0.5 cc/kg/hr 0.9% ALM drip; 5) 4 cc/kg 3% ALM bolus followed by 60 minute delayed 0.5 cc/kg/hr 0.9% ALM drip (drip delay); 6) 4 cc/kg vehicle (3%

NS) control bolus; or 7) 4 cc/kg 3% ALM bolus with 3 cc/kg/hr delayed 0.9% ALM drip. For ALM as an adjunct to WB resuscitation studies, animals were randomized to one of three groups (n=6): (1) Whole blood alone, (2) Whole blood + 30 mL of ALM bolus (A:1mg/kg; L:2 mg/kg; M:0.5 mg/kg) infused at 5 mL/min, or (3) Whole + 30 mL of vehicle control (VC) (0.9% NaCl bolus) infused at 5 mL/min.

Animals were sedated with Telazol (6.0 mg/kg; Fort Dodge Animal Health, Overland Park, KS, USA), pre-medicated with an analgesic (Buprenex 0.01 mg/kg; Reckitt & Colman Pharmaceuticals Inc., Richmond, VA, USA), and intubated with anesthesia maintained on 1-3% isoflurane. Core body temperature was monitored via a rectal temperature probe and maintained between 36.0-38.0°C.

Four catheters were placed percutaneously or via cut down. Briefly, venous catheters (8Fr; Arrow, Morrisville, NC, USA) were placed for central venous pressure monitoring and fluid infusion in the femoral or jugular veins. Femoral arteries were cannulated with either 8 Fr catheter (Arrow, Morrisville, NC, USA) for hemorrhage or 5 Fr catheter (Cook Medical, Bloomington, IN, USA) for blood pressure monitoring (MAP) and sampling.

Hemorrhagic Shock:

After baseline (BSLN) blood samples were drawn, hemorrhage was initiated by opening the stopcock in line with the right femoral artery allowing for free flow of blood until a mean arterial pressure (MAP) reached 30 mmHg. This marked the beginning (S=0 min) of the shock period, additional blood was withdrawn as needed to maintain a MAP of 30-35 mmHg. The end of shock (T=0 min) was defined when the animal either physiologically decompensated or was unable to maintain a blood pressure, as defined by: 1) 90 min of a MAP 30-35 mmHg; 2) 10 min of a MAP 20-29 mmHg; or 3) 10 sec of a MAP < 20 mmHg. Hemorrhaged blood was collected in a blood donor bag containing anticoagulant citrate phosphate dextrose adenine solution (CPDA) at a 1:10 ratio for subsequent use in resuscitation.

Resuscitation and Euthanasia:

Treatment and study timelines are depicted in ***Figure 1***. Starting at T=0 min, a simulated 'pre-hospital' resuscitation commenced. All ALM products (Adenosine, Lidocaine, and Magnesium) were purchased from Sigma-Aldrich (St. Louis, MO, USA). ALM dosages for each group are described in ***Table 2***.

T=120 min represented a simulated hospital arrival time (120 minutes after injury, to replicate prolonged pre-hospital care by doubling the golden hour), blood samples were collected and resuscitation was initiated with 75% shed blood volume whole blood (WB) infused over 30 min. WB transfusion was preceded with 23% calcium gluconate solution (Vedco, Saint Joseph, MO, USA) at 1 mL/200 mL WB. At end-of-resuscitation (T=150 min) animals received maintenance fluids and were monitored under anesthesia until T=240 min. Swine were humanely euthanized at T=240 min with pentobarbital sodium and phenytoin sodium (Euthasol®, 390 mg/mL, Virbac Corporation, Fort Worth, TX, USA).

For animals in the WB Adjunct arms, simulated hospital arrival occurred at T=60 min, wherein resuscitation was administered as determined by their groups, described above. ALM or VC boluses were administered over the first 5 minutes of this hospital phase. These animals were then monitored under anesthesia until T=300 min, before being humanely euthanized as described previously.

Blood Draws and Laboratory Analysis:

WB was collected as BSLN, S=0, T=0, 60, 120, 180, and 240 min with arterial blood gas being collected every 30 min after T=0 min. Arterial blood gas parameters were assessed utilizing a GEM® Premier 4000 (Instrumentation Laboratory, Bedford, MA, USA). Complete blood counts,

basic metabolic panels, and liver associated enzymes were evaluated on ProCyte Dx Hematology and Catalyst One Chemistry Analyzers (IDEXX Laboratories, Inc., Westbrook, ME, USA).

WB viscoelastic clotting properties were evaluated by rotational thromboelastometry (ROTEM® Delta System, Instrumentation Laboratory, Bedford, MA, USA). ROTEM® analyses included evaluation of extrinsic coagulation pathway function (ExTEM) and ExTEM in the presence of the platelet inhibitor, cytochalasin D (FibTEM) including: clotting time (CT), clot formation time (CFT), alpha angle (α), and maximum clot firmness (MCF).

Concentrations of coagulation factors were evaluated using STAGO® STA Compact® (Diagnostica Stago Inc., Parsippany, NJ, USA). STAGO® analysis included prothrombin time (PT), partial thromboplastin time (PTT), antithrombin (ATIII), von Willebrand Factor (vWF), fibrinogen, and d-dimer.

Platelet aggregation was evaluated by Multiplate® (DiaPharma Group Inc., West Chester OH, USA), using agonist: adenosine diphosphate (ADP) and collagen (COL). Agonist responses are reported as area under the aggregation curve in units (U) over a 6 min measurement period.

Histologic Analysis

Immediately following euthanasia, the following tissue specimens were collected for histopathologic evaluation: heart, lung, liver, and kidney. Tissue specimens were fixed in 10% neutral buffered formalin, paraffin-embedded, cut at 5μm and stained with hematoxylin and eosin (H&E) for histopathologic evaluation. Damage was assessed by pathologic grade from 0 (none) to 4 (severe). The mean pathologic grade for each treatment group was calculated and rounded to the nearest whole number.

Data and Statistical Analysis

Statistical analyses were performed using Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA). Data are presented as mean±SEM. Data with baseline discrepancies were normalized to baseline before statistical analysis. Single time point analyses were performed by one-way ANOVA using post-hoc Tukey and multiple time-point analyses were analyzed by two-way ANOVA using post-hoc Bonferroni correction. P-value <0.05 were considered to be statistically significant.

4.0 MAJOR EVENTS/MILESTONES/SUCCESS

- Kick Off Meeting – 12 June 2017
- IACUC Approval – 15 December 2015
- All experimental procedures completed – 12 April 2018
- Data Analysis – 13 September 2018
- Poster presentation – ASC (Presentation: 06 February 2019), EAST (Presentation: 17 January 2019), SAUSHEC (Poster: 25 April 2018)
- Manuscript submitted to – Journal of Trauma (Accepted: 02 March 2019)

5.0 RISK ASSESSMENT

5.1 Risk Analysis

Risk: Injury model originally outlined in the proposal did not result in a severe physiologic response in the animals.

Mitigation: Our group has extensive experience utilizing a pressure-targeted hemorrhagic shock injury until decompensated shock is achieved. Decompensated shock is defined as a hemorrhagic event where a mean arterial pressure (MAP) of 30-34mmHg is maintained until the animals no longer exhibit cardiovascular compensatory responsiveness to maintain or elevate that MAP. The physiologic insult from this injury yields a reproducible severe model of shock with a significant increase in lactate and alteration to base deficit.

Risk: ALM treatment originally outlined in the proposal did not result in the increased survival.

Mitigation: The animals seem to tolerate the initial bolus administration of the ALM treatment (2-4cc/kg) however during the continued infusion (drip) of the ALM product may not be tolerated. We speculate that during the drip infusion the ALM product builds up intravascularly and prevents an appropriate cardiac response. Instead, this build-up of ALM leads to cardiac arrest occurring roughly 60 minutes following the bolus dose administration. After discussing these interim results with experts there are several other potential explanations, including the timing of the drip as well as the individual dosing concentrations of the ALM components.

Risk: Amended ALM treatments did not result in the increased survival.

Mitigation: Though ALM does not seem to be a feasible pre-hospital resuscitation fluid. One thing we did see, in our secondary endpoints in survivors, was that ALM does seem to have some beneficial effects on coagulation and inflammation. We believe it would be good to look at ALM as an in-hospital adjunct to see if it helps with fighting trauma induced coagulopathy and preserving organs.

Risk: Contracting delays with award for sample analysis kits have pushed the expected completion date to the right.

Mitigation: We are working directly with the contracting office to track the status of these packages and will request delivery of the products upon award. Immediately following receipt of the kits, attention will be turned to executing the laboratory analysis of banked tissues/specimens.

5.2 Technical Challenges

None.

6.0 TRANSITION PLAN

6.1 Military Relevance

Hemorrhage remains the most prevalent cause of PS mortality in our combat casualties, accounting for 97% of pre-hospital and 86% of in-hospital PS mortality (Eastridge, et al., 2012; Eastridge, et al., 2011). Hence, the development of field-deployable, low-volume, permissively hypotensive therapeutic resuscitation strategies to improve survival and outcomes, especially in the context of extended/delayed transport/evacuation times in MODE settings, remains a vital priority. The proposed research, to decrease mortality due to PS hemorrhage, directly addresses

the specific scientific need set forth in the 59th Medical Wing Research Program Announcement (RPA) Priority Area of Extending the Golden Hour. Additionally, the results of the proposed research will provide information regarding Fluid Resuscitation requirements and "best practices" in the context of prolonged hypotensive resuscitation and delayed hospital care in MODE settings. Furthermore, this work has great potential to advance the state of medical science and accelerate the transition of medical knowledge and technologies that can be applied in theater.

6.2 Transition Strategy

While the development of an effective, pre-hospital therapeutics is essential to extend the "Golden Hour," this research effort found that ALM is not an appropriate pre-hospital agent, but may provide protective effects if used as definitive care agent mitigating TIC and organ preservation.

7.0 RESULTS

Demographics, Blood Loss, and Survival:

There were no significant differences between the seven groups for age or total blood loss. Groups 6 and 7 demonstrated significantly lower weights compared to Group 1 (p=0.03) and Group 4 (p=0.01), respectively ***(Table 2)***. Group 1 had a significantly greater percent survival at T=120 min (***Figure 2)*** compared to the other six groups (90.9 vs. 20.0 vs. 40.0 vs. 16.7 vs. 33.3 vs. 20.0 vs. 0.0%). Since Group 1 demonstrated greater than 50% survival compared to Groups 2-7 at T=120 min (the end of pre-hospital phase), further analyses were conducted only out to T=120 min.

Hemodynamics, Heart Rate, and $EtCO_2$:

Systemic hemodynamics were equivalent between groups at baseline ***(Figure 3)***. Regardless of group, MAP and systolic blood pressure (SBP) decreased uniformly in response to hemorrhage, with only Group 1 normalizing pressure by T=60 min. At T=0 min, Groups 1, 2, and 5-7 demonstrated a significant tachycardia response. This tachycardia response remained elevated in Groups 4, 5, and 7 through T=60 min and in Groups 1 and 6 through T=120 min. Compared to Group 1, all other groups demonstrated significantly reduced MAP and SBP at T=60 min ($p<0.001$) with the exception of Group 6.

End-tidal carbon dioxide ($EtCO_2$) was equivalent and significantly declined following hemorrhage (T=0 min) for all groups with the exception of Group 2 **(*Figure 3)***. Across all groups, pre-hospital resuscitation normalized $EtCO_2$ by T=60 min.

MAP was equivalent between groups except at T= 65, when WB+ALM MAP dropped, and at T=75/85 when WB+ALM MAP increased (**Figure 8**). All values were equivalent at T= 300.

Base Excess, Lactate, pH and Bicarbonate:

Base excess was significantly reduced compared to baseline at T=0 min for all groups. Base excess remained significantly decreased in Groups 1 and 4 through T=120 min (1: 9.4±1.7 vs. 3.2±2.1 mmol/L, p=0.02; 4: 12.3±1.8 vs. -1.6 mmol/L, p=0.04). For both vehicle control groups (Groups 2 and 6) base excess significantly lowered further by T=120 min (2: 8.6±0.9 vs. -5.8 mmol/L, p=0.04; 6: 10.2±1.2 vs. -7.3 mmol/L, p=0.008). Lactate levels increased by T=0 mins for all groups, but only the two vehicle control groups (Groups 2 and 6) continued to increase and remained elevated at T=120 mins. The pH values remained equivalent among all groups through T=120 min. Bicarbonate was significantly reduced

at T=0 min for all groups, with Group 1 and 4 remaining reduced through T=120 min (1: 34.1±1.5 vs. 28.4±1.91 mmol/L, p=0.02; 4: 37.0±1.8 vs. 24.3 mmol/L, p=0.04). In the vehicle controls, Groups 2 and 6, bicarbonate significantly lowered further at T=120 min (2: 33.6±0.4 vs. 20.1 mmol/L, p=0.04; 6: 34.5±0.7 vs. 15.8 mmol/L, p=0.008).

Lactate levels were significantly lower in the WB+ALM group compared to the WB+VC groups during shock and resuscitation (T= 0 through T= 120). WB+VC group base deficit was significantly worsened as compared to WB and WB+ALM when treatment began (T= 60).

Organ Function:

Kidney function was assessed by blood urea nitrogen (BUN) and creatinine (CREA). Compared to baseline, BUN was significantly elevated at T=120 min for Group 5. CREA remained elevated through T=120 min for Groups 1-5. Compared to Group 1, CREA was significantly higher at T=120 min for Group 4 and 5 (1.6±0.1 vs. 2.2 vs. 2.0±0.1 mmol/L, $p<0.01$), respectively. Liver function was assessed by albumin (ALB), aspartate transaminase (AST), and alanine transferase (ALT). ALB was significantly reduced in Group 1 and significantly lower in comparison to the other six groups at T=60 min ($p<0.05$). AST and ALT levels were significantly elevated for the lower volume normal saline drip vehicle control group (Group 2) compared to baseline and relative to all the other treatment groups. AST/ALT ratio followed a similar trend for Group 2.

Histopathologic analysis demonstrated no significant difference in organ damage among the treatment groups. Damage ranged from none (Grade=0) to moderate (Grade=2). There was no consistent protective effect conferred by any of the treatment groups.

Complete Blood Count:

Compared to baseline, Group 1 demonstrated significant reduction in hematocrit and hemoglobin. Platelet count was significantly reduced in Group 1 compared to baseline, while levels were significantly reduced at T=120 min for Group 4. Compared to baseline, Group 1 had significantly elevated white blood cell count (WBC) at T=0 min, while Group 7 was significantly elevated at T=120 min. Compared to Group 1, Group 7 demonstrated a significantly elevated WBC at T=60 min (20.4±1.3 vs. 29.0±3.3 K/µL, p=0.02). Neutrophil count was significantly increased following treatment (T=60 min) in all groups with the exception of Groups 2 and 5 and persisted to T=120 min for Groups 1 and 6. At T=120 min Group 5 neutrophil count was significantly lower than Group 1 (6.0±1.1 vs. 12.7±1.0 K/µL, p=0.04).

ROTEM:

Compared to baseline, Group 1 demonstrated significant hypocoagulability with elevated ExTEM clotting time (CT), clot formation time (CFT), and reduced ExTEM alpha angle (α). All ALM and vehicle control groups averted coagulopathy with the exception of Group 5 at T=120 min ***(Figure 4)***. At T=0 min, Group 1 demonstrated significant reduction in plasmatic coagulation factors ATIII and fibrinogen persisting to T=120 min. ExTEM CT was significantly elevated for Group 1 compared to all groups. Group 1 clot strength was significantly diminished via ExTEM maximum clot firmness (MCF), and demonstrated a significant deficiency in fibrin polymerization via FibTEM MCF. FibTEM CT was significantly elevated for Group 1. The data for Group 4's one survivor at T=120 min had an error and was not rerun, and data at that time point was left out of the analysis.

For WB Adjunct groups, after treatment, T= 60, all groups showed comparable viscoelastometry parameters with the exception of ExTEM MCF at T=180 (***Figure 9***).

STAGO:

Compared to baseline, Group 1 demonstrated elevated PT and reduced PTT beginning at T=60 min and persisted through T=120 min ***(Figure 5)***. At T=60 min Group 1 had a significantly elevated PT compared to Group 3 ($p<0.01$) and Group 7 ($p<0.05$), while no significant differences were seen between groups for PTT.

Plasmatic coagulation factors, ATIII and fibrinogen, were significantly reduced in Group 1 compared to baseline values from T=0 through T=120 min, while levels were significantly reduced for Groups 2 and 3 at T=0 min and Group 4 at T=60 min ***(Figure 5)***. Compared to the other six treatment groups, at T=60 min Group 1 had significantly reduced ATIII ($p<0.05$) and fibrinogen ($p<0.05$). Conversely, vWF was significantly elevated at T=60 min in Groups 2, 3, 4, and 6; and remained elevated at T=120 min for Groups 2, 5, and 6. At T=60 min, Groups 2, 3, 4, and 6 demonstrated significantly elevated vWF compared to Group 1 ($p<0.05$). There were no significant differences seen at any time point in d-dimer.

Platelet Aggregation:

Compared to baseline, Group 1 demonstrated significant decrease in platelet aggregation at T=60 min in response to both agonists, ADP and COL, while no significant decreases in platelet aggregation were seen in any of the ALM or vehicle control groups. There were no significant differences between Group 1 and all other groups at any timepoint during the study.

8.0 CONCLUSION/DISCUSSION

In combat trauma, adherence to the Golden Hour paradigm has become difficult due to logistical challenges and constraints in the battlefield. Additionally, treatment of the injured is limited to the amount of gear that military medics can carry in order to administer care. A low volume treatment that can be easily carried and reduce the number of fluids necessary to adequately resuscitate casualties would be beneficial to the combat injured. Based on previous animal studies, ALM has been targeted as a promising candidate to replace current TCCC resuscitation fluids (Hextend®). Our study sought to determine if ALM could serve as a low-volume replacement to TCCC for resuscitation in hemorrhagic shock in the pre-hospital setting. Our results demonstrate that, when compared to TCCC, ALM: 1) did not improve overall survival in hemorrhagic shock; 2) did not improve SBP and MAPs; 3) did not ameliorate metabolic parameters; and 4) did not provide organ protection; but 5) did have protective effects on coagulopathy.

Compared to groups receiving ALM, survival was superior in the TCCC group at a rate of 90.9%. While the ALM treatment groups demonstrated survival rates of less than 50%, the majority of the deaths occurred by T=60 min which is similar to the vehicle control groups. These results suggest that treatment with ALM alone does not improve survival or provide any therapeutic benefit.

Compared to TCCC, ALM treatment did not confer any improvement in blood pressure, with MAPs and SBPs consistently lower than TCCC group. The blood pressure parameters for the ALM treatment groups were comparable to the vehicle control groups. ALM treatment also did not improve heart rate nor $EtCO_2$ compared to TCCC and the vehicle control groups.

Base excess, lactate, pH, and bicarbonate remained comparable across all groups. Although not to a significant value, the vehicle control groups did have lower pH levels, larger

base deficits, and lactate levels compared to the ALM and the TCCC groups. However, these results may be difficult to interpret as only one test subject survived in each of the vehicle control groups. In the 4cc/kg vehicle control group, the subject only survived up to T=120 min.

While the FDA has issued a "black box" warning for hydroxyethyl starch solutions such as Hextend® due to studies demonstrating its association with higher mortality, increased risk of bleeding, and kidney injury and failure,[5] Hextend® has been shown to have lower mortality than Lactated Ringer's solution in near fatal hemorrhagic shock models.[16] In the military setting, while inferior to blood based resuscitation, Hextend® remains a preferred resuscitative fluid over crystalloids (if no blood products are available) due to the smaller volume required for volume expansion seen with Hextend® over crystalloid.[3]

In this particular hemorrhagic shock model, Hextend®-based TCCC resuscitation had comparable effects on kidney function, based on BUN and creatinine levels, to ALM and vehicle control groups. Though not statistically significant over this short-term analysis, the TCCC group's creatinine stabilized by T=120 min, while the ALM and vehicle control groups' levels remained elevated. In our animal model, the known possible detrimental effects to kidney function, following Hextend® administration were not demonstrated in the short-term post-hemorrhagic shock period. Similarly, all treatment groups had comparable effects on liver function. The 2cc/kg vehicle control group demonstrated significantly higher levels of AST and ALT, although only one animal survived in this treatment group after T=60 min, again making interpretation difficult. Histologic analysis further supports the conclusion that ALM and TCCC have no significant differences in their effects on organ function or demonstrate any organ protective effects.

The TCCC group's platelet, WBC, and neutrophil counts all had similar results to the ALM group and the vehicle control group. The hemoglobin and hematocrit levels for TCCC were significantly lower than the ALM and vehicle control groups. The decrease in the hemoglobin did not confer any changes in hemodynamics, given that the MAPs and SBPs in the TCCC group remained consistently higher than the vehicle control and ALM groups, as previously discussed.

The known coagulopathic effects of Hextend® are well demonstrated in this study but were not observed in the ALM or vehicle control groups. When used as an adjunct to WB resuscitation, lactate and BD showed improvement during shock and initial resuscitation with ALM, though these values were equivalent between WB and WB+ALM at all other time points after resuscitation. No differences were noted in coagulopathy. ALM, therefore, may not confer a protective effect from coagulopathy when used as treatment for hemorrhagic shock.

Limitations

One of the major limitations of this study is that 3 out of the 7 low volume resuscitation groups had only one animal in each group survive to T=120 minutes, making the results for these data difficult to interpret beyond this time point. The values of secondary endpoints in the setting of such poor survival is quite variable, making clinical interpretation impossible. However, the primary outcome of survival is demonstrated more profoundly by these low survival rates: the model was sufficiently severe to obtain a high mortality in the test groups, and yet the current TCCC based protocol led to a >90% survival, supporting current fielded practice even over a 120-minute time frame.

Another limitation to the study is the use of a pressure-controlled, pure hemorrhage model. Though certainly a valid animal model for shock, there is no soft tissue, musculoskeletal or major organ trauma in these test subjects. Thus, the inflammatory response present in combat traumas that can exacerbate metabolic and physiologic derangements in hemorrhagic shock may

not be adequately replicated in this particular animal model. Furthermore, in combat traumas, there is uncontrolled bleeding that can lead to rapid decline in blood pressure. While shock in this model was defined physiologically (not a set volume-controlled hemorrhage), it is unlikely to accurately replicate traumatic bleeding seen in combat traumas.

Our study has demonstrated contrary results from other studies of ALM in various animal models of hemorrhagic shock. ALM in our study led to inferior survival and worse outcomes compared to standard resuscitation. However, our model does have differences compared to previous studies. This is the first study to prolong the treatment period of ALM to 120 minutes before definitive treatment (transfusion of shed whole blood) commenced. Prior studies utilized a treatment period that ranged only from thirty to sixty minutes. We also looked at ALM as a complete replacement to TCCC resuscitation; other studies infused fluid to maintain MAPs to around 50mmHg and added ALM as an adjunct. In our study, ALM treatment alone was unable to maintain MAPs and SBPs in the 50mmHg range during the treatment period. It is possible that the significantly decreased blood pressure led to inadequate tissue and organ perfusion that subsequently led to the decreased survival in the ALM treatment groups in this model. Regardless, any benefit that ALM treatment may confer, in these dose ranges, may not have been sufficient to overcome the detrimental effects of inadequate organ perfusion caused by severely low blood pressures in the ALM groups.

Future Directions

While ALM does not appear to be a suitable replacement to TCCC, it may provide beneficial effects as an adjunct, particularly regarding coagulopathy. During the prolonged 120 minutes treatment period, crystalloid infusion, with ALM to augment resuscitation, might be utilized to maintain MAPs to a range required to maintain adequate tissue perfusion. This is currently under investigation in our lab.

Conclusion

In conclusion, ALM demonstrated inferior survival compared to current TCCC resuscitation in this model of hemorrhagic shock and did not improve physiologic parameters or provide organ protection compared to TCCC. Based on this study, it appears that ALM treatment is not a suitable low volume replacement for TCCC. Although, unlike TCCC treatment, ALM did not demonstrate any coagulopathy. However, ALM as an adjunct to WB resuscitation demonstrated no advantage to WB alone. Previous reports of correction of coagulopathy, in similar animal models, were not reproduced here. Further investigation into the interactions of ALM and whole blood may be warranted to clarify ALM's effects on coagulopathy.

9.0 DELIVERABLES

9.1 Publications

How, R., Glaser, J., Schaub, L., Fryer, D., Ozuna, K., Morgan, C., Sams, V., Cardin, S. "Pre-hospital adenosine, lidocaine and magnesium has inferior survival compared to tactical combat casualty care resuscitation in a porcine model of prolonged hemorrhagic shock." JOT: ePUB ahead of print (JT-D-18-08911R1)

9.2 Presentations

How, R. (2018 April 26). Pre-hospital Treatment with Adenosine, Lidocaine, and Magnesium in a Porcine Model of Hemorrhagic Shock: Inferior Compared to Current Tactical

Combat Casualty Care Resuscitation. SAUSHEC

How, R. (2019 January 17) Treatment with Adenosine, Lidocaine, and Magnesium (ALM) in the Pre-Hospital Setting Has Inferior Survival Compared to Tactical Combat Casualty Care based Hextend Resuscitation in a Porcine Model of Prolonged Hemorrhagic Shock. EAST

Morgan, C. (2019 February 06) Adenosine, Lidocane, Mg^{2+} as an Adjunct to Whole Blood Resuscitation does not Improve Coagulopathy. ASC

10.0 COST

This work was funded by the 59th MWD, FAD # 0130-17-04JX-00003. FY17/18 funding under Project Code Number 3080 of $579,000.00 was received on 7 Aug 2017 and the last incremental expenditures occurred on 13 Nov 2018 (7LL1P3) and 13 Feb 2019 (8LL113).

This work was funded by the 59th MWD, FAD # 0130-16-04JX-00070. FY16/17 funding under Project Code Number 3080 of $800,000.00 was received on 9 Feb 2017 and the last incremental expenditures occurred on 6 Sept 2018.
Funds were not received until the 2nd year, so there are no transactions on the FY16 JON series

11.0 REFERENCES

1. Eastridge BJ, Hardin M, Cantrell J, et al. Died of wounds on the battlefield: causation and implications for improving combat casualty care. J Trauma 2011;71:S4-8.

2. Eastridge BJ, Mabry RL, Seguin P, et al. Death on the battlefield (2001-2011): implications for the future of combat casualty care. J Trauma Acute Care Surg 2012;73:S431-7.

3. Butler F, Holcomb J, Schreiber M, et al. Fluid Resuscitation for Hemorrhagic Shock in Tactical Combat Casualty Care: TCCC Guidelines Change 14-01--2 June 2014. J Spec Oper Med 2014;14:13-38.

4. Butler FK, Holcomb J, Shackelford S, et al. Advanced Resuscitative Care in Tactical Combat Casualty Care: TCCC Guidelines Change 18-01:14 October 2018. J Spec Oper Med 2018;18:37-55.

5. Hartog C, Natanson C, Sun J, Klein H, Reinhard K. Concerns over use of hydroxyethyl starch solutions BMJ 2014;349: g5981.

6. Horner SM. Efficacy of Intravenous Magnesium in Acute Myocardial Infarction in Reducing Arrythmias and Mortality: Meta-Analysis of Magnesium in Acute Myocardial Infarction. Circulation 1992;86:774-9.

7. Soliman HM, Mercan D, Lobo SS, Mélot C, Vincent JL. Development of Ionized Hypomagnesemia is Associated with Higher Mortality Rates. Critical Care Medicine 2003;31:1082-87.

8. Dobson G. Organ arrest, protection and preservation: natural hibernation to cardiac surgery: a review. Comp Biochem Physiol B Biochem Mol Biol 2004;139:469–85.

9. Dobson G, Jones M. Adenosine and lidocaine: a new concept in non-depolarizing surgical arrest, protection and preservation. J Thoracic Cardiovasc Surg 2004;127:794–805.

10. Dobson G. Membrane polarity: a target for myocardial protection and reduced inflammation in adult and pediatric cardiothoracic surgery. J Thorac Cardiovasc Surg 2010;140:1213-7.

11. Letson H, Dobson G. Ultra-small intravenous bolus of 7.5% NaCl/Mg2+ with adenosine and lidocaine improves early resuscitation outcome in the rat after severe hemorrhagic shock in vivo. J Trauma 2011;71:708-19.

12. Letson H, Dobson G. Unexpected 100% survival following 60% blood loss using small-volume 7.5% NaCl with adenocaine and Mg2+ in the rat model of extreme hemorrhagic shock. Shock 2011;36:586-94.

13. Granfeldt A, Nielsen T, Solling C, et al. Adenocaine and Mg2+ reduce fluid requirement to maintain hypotensive resuscitation and improve cardiac and renal function in a porcine model of severe hemorrhagic shock. Critical Care Medicine 2012;40:3013-25.

14. Letson H, Pecheniuk N, Mhango L, Dobson G. Reversal of acute coagulopathy during hypotensive resuscitation using small-volume 7.5% NaCL adenocaine and Mg2+ in the rat model of severe hemorrhagic shock. Critical Care Medicine 2012;40:2417-22.

15. Shi W, Jiang R, Dobson GP, Granfeldt A, Vinten-Johansen J. The nondepolarizing, normokalemic cardioplegia formulation adenosine–lidocaine (adenocaine) exerts anti-neutrophil effects by synergistic actions of its components. J Thorac Cardiovasc Surg 2012;143:1167–75.

16. Ferreira E, Terzi RS, WA, de Moraes A. Early colloid replacement therapy in a near-fatal model of hemorrhagic shock. Anesth Analg 2005;101:1785-91.

FIGURES AND TABLES

	Treatment	Bolus Component Concentrations	Drip Component Concentrations	n	Age (months)	Weight (kg)	Blood Loss (%)	Length of Shock (min)	Prehospital Fluids (mL)
Group 1	TCCC (Hextend® + Lactated Ringer's)	---	---	11	3.7±0.1	80.1±1.6	49.6±4.0	57±9	1429±192
Group 2	2cc/kg 3% NaCl bolus + 0.5cc/kg/hr 0.9% NaCl drip	---	---	5	3.6±0.2	75.3±2.6	49.9±4.5	50±13	193±14
Group 3	2cc/kg ALM bolus + 0.5cc/kg/hr ALM drip	Adenosine: 1.0mM Lidocaine: 3.0mM Magnesium: 2.5mM	Adenosine: 18.7mM Lidocaine: 34.6mM Magnesium: 41.5mM	5	4.2±0.2	77.3±2.6	43.8±6.2	81±5	209±14
Group 4	4cc/kg ALM bolus + 0.5cc/kg/hr ALM drip	Adenosine: 1.0mM Lidocaine: 3.0mM Magnesium: 2.5mM	Adenosine: 18.7mM Lidocaine: 34.6mM Magnesium: 41.5mM	6	4.0±0.0	82.0±1.7	52.3±4.2	67±12	367±15
Group 5	4cc/kg ALM bolus + 60min delayed 0.5cc/kg/hr ALM drip	Adenosine: 1.0mM Lidocaine: 3.0mM Magnesium: 2.5mM	Adenosine: 18.7mM Lidocaine: 34.6mM Magnesium: 41.5mM	6	4.0±0.0	76.9±1.2	56.8±3.4	72±7	326±7
Group 6	4cc/kg/15 min 3% NaCl bolus + 60min delayed 3cc/kg/hr 0.9% NaCl drip	---	---	5	4.4±0.2	71.5±1.1	58.7±4.0	40±11	304±29
Group 7	4cc/kg/15 min ALM bolus + 60min delayed 3cc/kg/hr ALM drip	Adenosine: 0.5mM Lidocaine: 1.5mM Magnesium: 1.2mM	Adenosine: 14.6mM Lidocaine: 27.0mM Magnesium: 36.5mM	5	4.4±0.2	71.5±1.1	59.6±3.5	77±6	348±26

Table 2. Treatment and Demographics

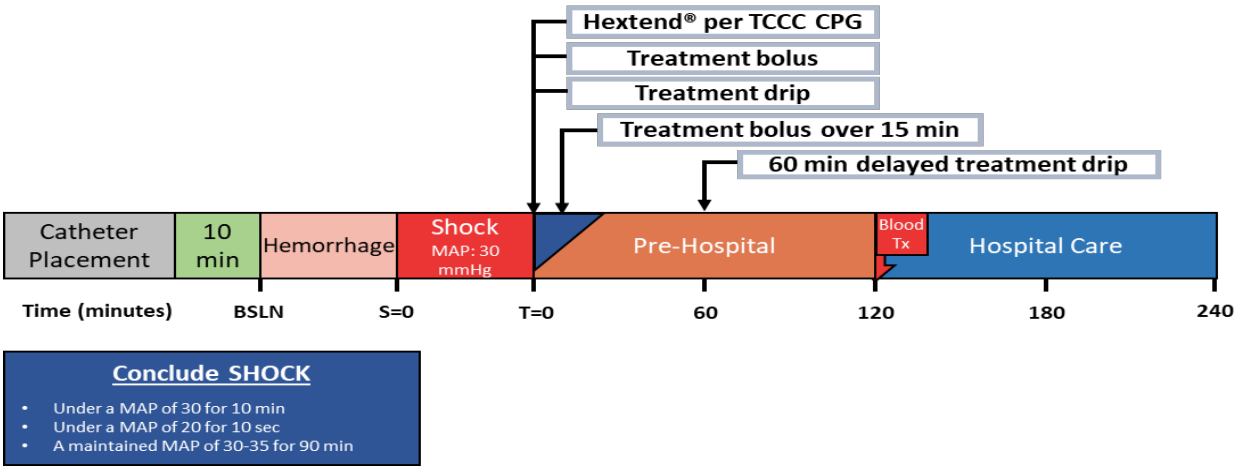

Figure 1. Timeline

Schematic depicting experimental design including time points of hemorrhage, pre-hospital and hospital care. Whole blood was drawn for labs (arterial blood gas, complete blood count, serum chemistries, ROTEM, STAGO, and Multiplate) at baseline (BSLN), start of shock (S=0 min), end of shock (T=0 min), T=60 min, 120 min, 180 min, and 240 min.

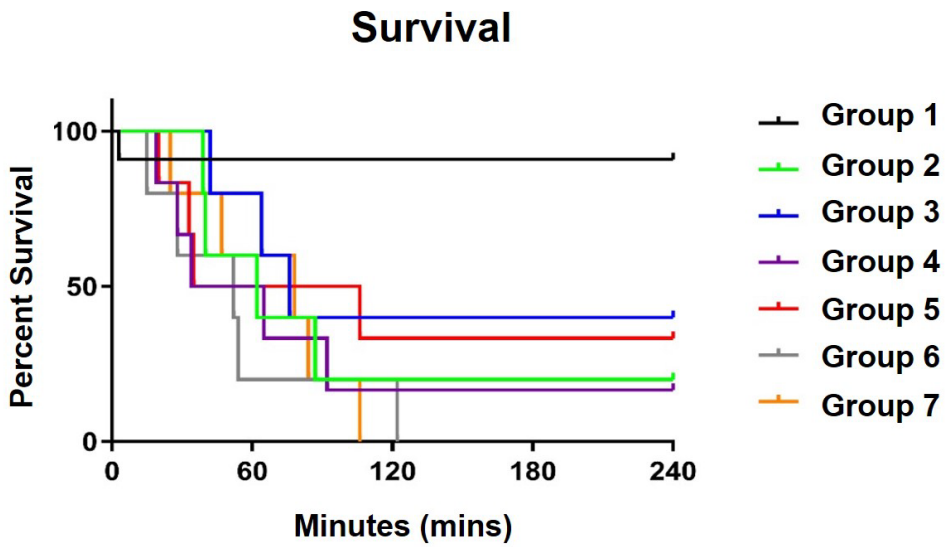

Figure 2. Kaplan-Meier Curve

Kaplan-Meier Curve analysis of percent survival.

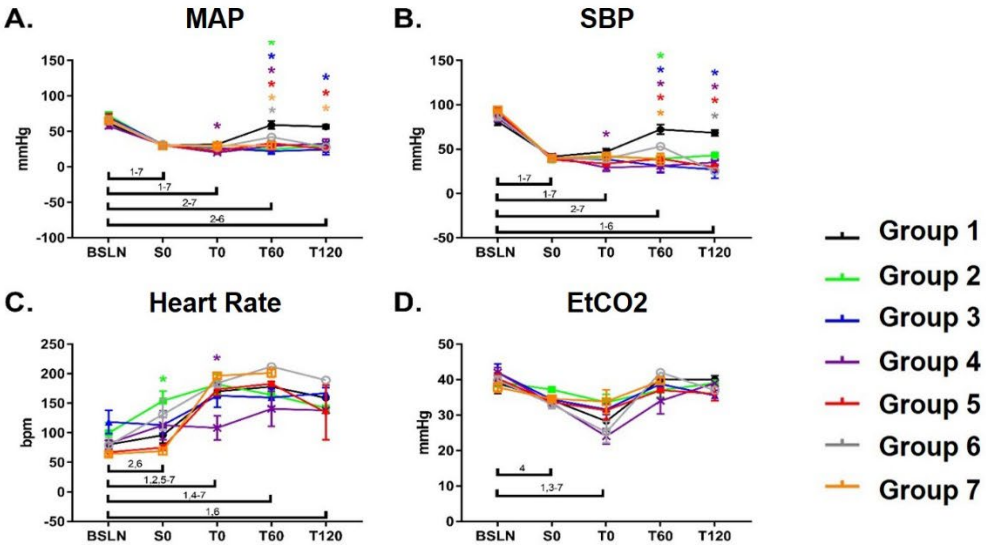

Figure 3. Vital Signs

Vitals were continuously monitored throughout the investigation. Depicted are the mean±SEM for A) mean arterial pressure (MAP), B) systolic blood pressure (SBP), C) heart rate, and D) end-tidal carbon dioxide ($EtCO_2$). Two-way ANOVA with post-hoc Bonferroni correction was performed with $p<0.05$ considered to be a statistically significant difference for groups from Group 1 (*, relative to color). Significant differences relative to baseline are indicated by horizontal bars.

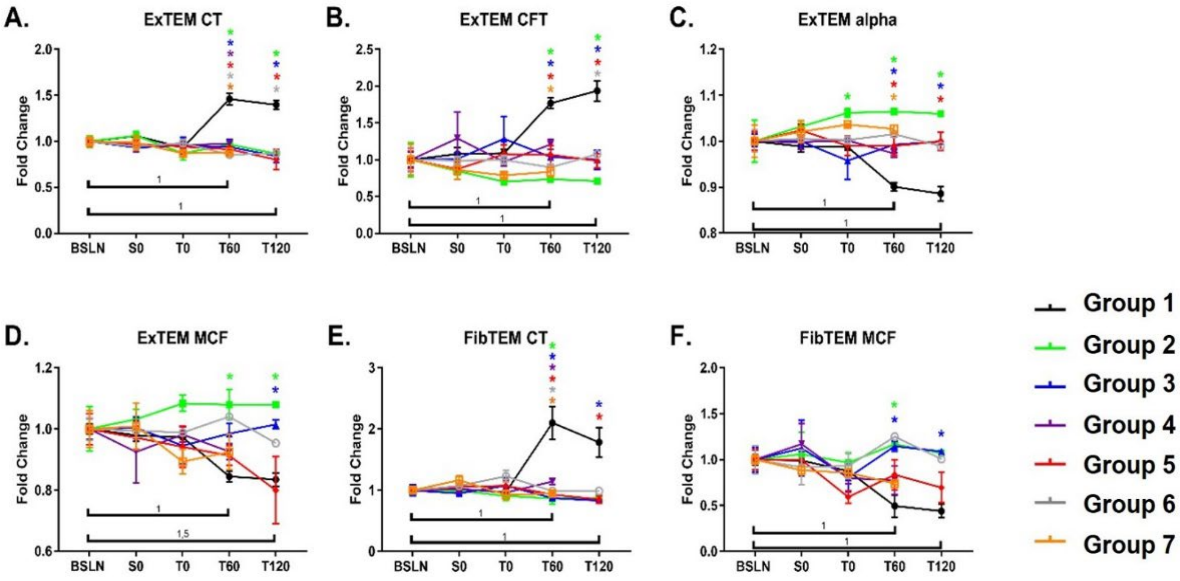

Figure 4. ROTEM

Viscoelastic clotting properties, ExTEM and FibTEM, were measured for treatment groups at baseline (BSLN), beginning of shock (S0), end of shock (T0), T60, and end of pre-hospital (T120). Data are the mean±SEM for A) ExTEM clotting time (CT); B) ExTEM clot formation time (CFT); C) ExTEM alpha angle (α); D) ExTEM maximum clot strength (MCF); E) FibTEM CT; and F) FibTEM MCF. Two-way ANOVA with post-hoc Bonferroni correction was performed with $p<0.05$ considered to be a statistically significant difference for groups from Group 1 (*, relative to color). Significant differences relative to baseline are indicated by horizontal bars.

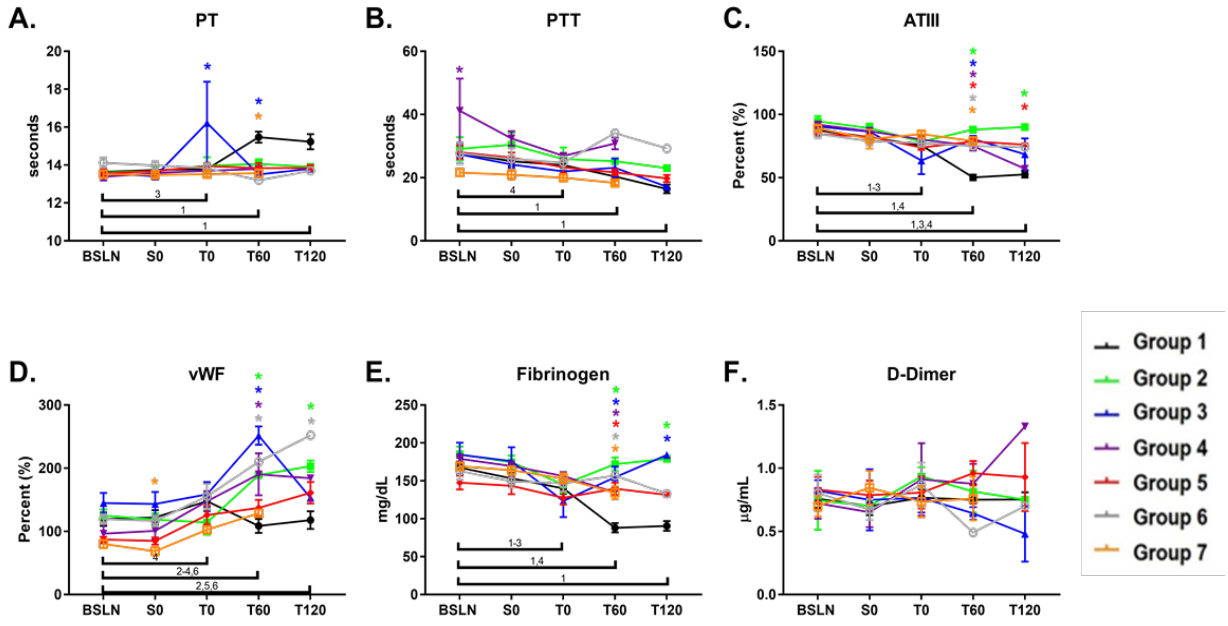

Figure 5. STAGO

Concentrations of coagulation factors were measured for treatment groups at baseline (BSLN), beginning of shock (S0), end of shock (T0), T60, and end of pre-hospital (T120). Data are the mean±SEM for A) prothrombin time (PT), B) partial thromboplastin time (PTT), C) antithrombin III, D) von Willebrand Factor (vWF), E) fibrinogen, and F) d-dimer. Two-way ANOVA with post-hoc Bonferroni correction was performed with $p<0.05$ considered to be a statistically significant difference for groups from Group 1 (*, relative to color). Significant differences relative to baseline are indicated by horizontal bars.

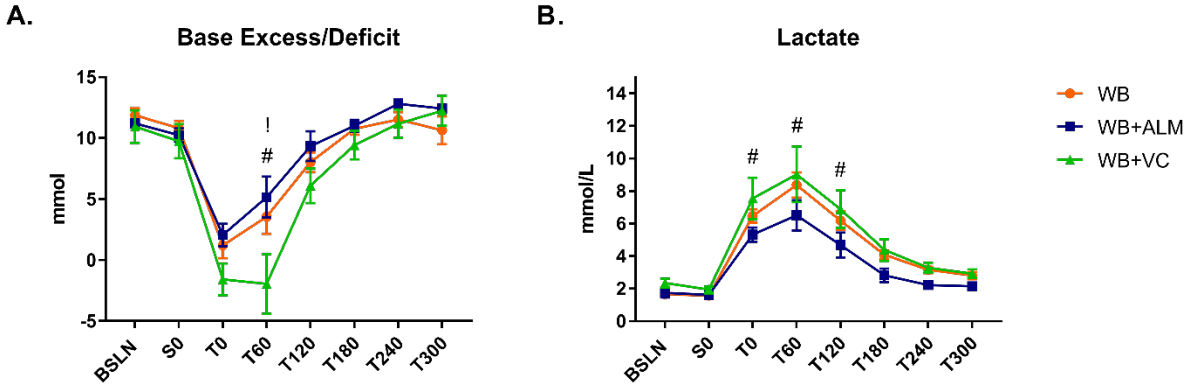

Figure 6. Arterial Blood Gas

Arterial blood gases were measured at baseline (BSLN), beginning of shock (S0), beginning of TCCC (T0), treatment administration (T60), and every 60 min until T300. Data are the mean ± SEM for (A) Base Excess/Deficit and (B) Lactate. Two-way ANOVA with post-hoc Bonferroni correction was performed and $p<0.05$ was considered to be a statistically significant difference between groups (WB vs WB+VC !, WB+ALM vs WB+VC #).

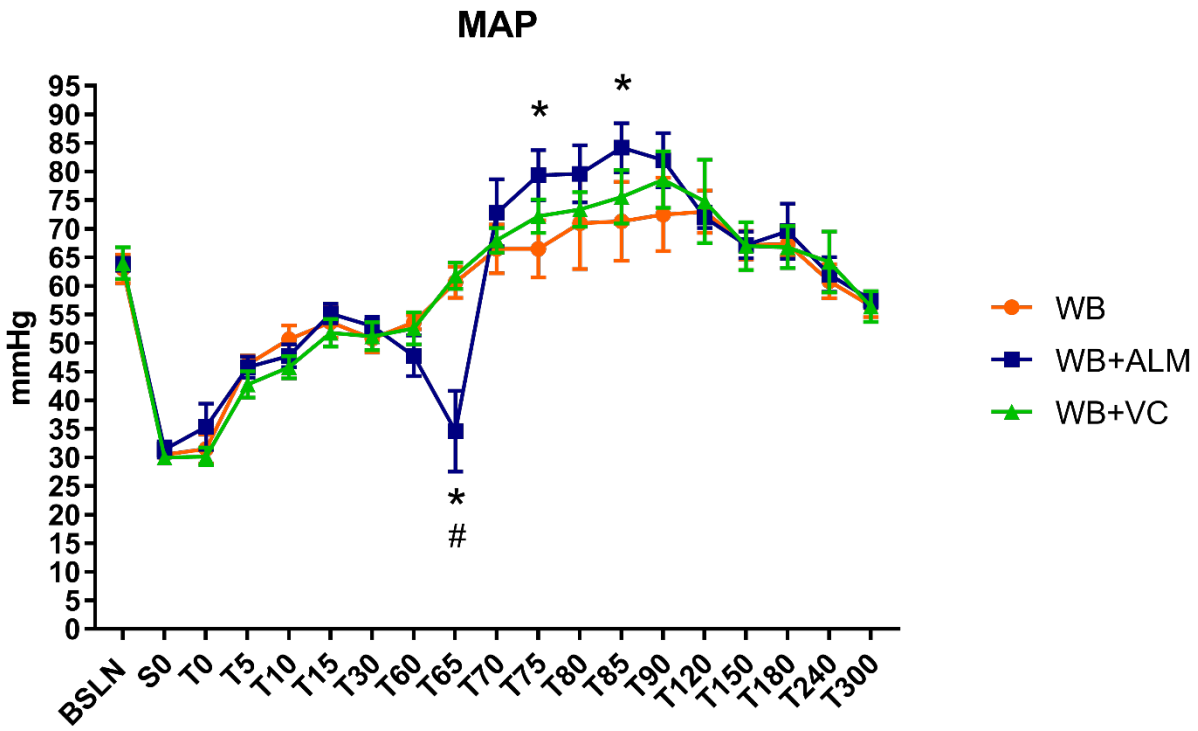

Figure 7. MAP

MAP was continuously monitored throughout the entire investigation. Data are presented as mean ± SEM. Two-way ANOVA with post-hoc Bonferroni correction was performed and $p<0.05$ was considered to be a statistically significant difference between groups (WB vs WB+ALM *, WB+ALM vs WB+VC #).

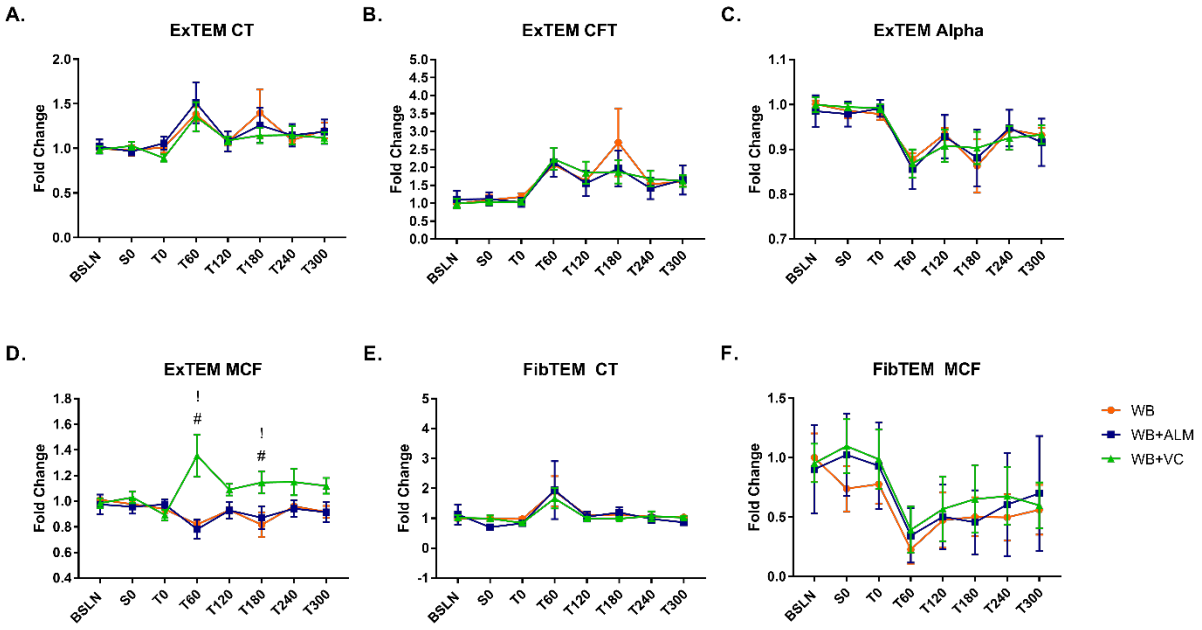

Figure 8. Viscoelastometry

ExTEM and FibTEM were measured at baseline (BSLN), beginning of shock (S0), beginning of TCCC (T0), treatment administration (T60), and every 60 min until T300. Data are the mean ± SEM for (A) ExTEM CT, ExTEM CFT, ExTEM alpha, (D) ExTEM MCF, (E) FibTEM CT, (F) FibTEM MCF. Two-way ANOVA with post-hoc Bonferroni correction was performed and $p<0.05$ was considered to be a statistically significant difference between groups (WB vs WB+VC !, WB+ALM vs WB+VC #).

12.0 LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

®	Rights Reserved
α	Alpha Angle
µL	Microliter
ADP	Adenosine Diphosphate
ALB	Albumin
ALM	Adenosine, lidocaine, and magnesium
ALT	Alanine Transferase
aPTT	Activated Partial Thromboplastin Time
ASC	Academic Surgical Congress
AST	Aspartate Transaminase
ATIII	Anti-thrombin III
BSLN	Baseline
cc	Cubic Centimeter
CT	Clot Time
CFT	Clot Formation Time
COL	Collagen
DC	Damage Control
DP	Dried Plasma
EAST	the Eastern Association for the Surgery of Trauma
ECG	Electrocardiography
$EtCO_2$	End Tidal Carbon Dioxide
Fr	French
g	Gram
hr	Hour
IACUC	Institutional Animal Care and Use Committee
INR	International Normalized Ratios
IV	Intravenous
K	($x10^3$) Thousand
kg	Kilogram
L	Liter
MAP	Mean Arterial Pressure
MCF	Maximum Clot Firmness (strength)
Mg^{2+}	Magnesium
$MgSO_4$	Magnesium Sulfate
Min	Minute
mmol	Millimole
NaCl	Sodium Chloride – Normal Saline
SBP	Systolic Blood Pressure
PS	Potentially Survivable
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
RPA	Research Program Announcement
SAUSHEC	San Antonio Uniformed Services Health Education Consortium
SEM	Standard Error of the Mean
T	Time
TCCC	Tactical Combat Casualty Care
TIC	Trauma Induced Coagulopathy
U	Unit
VC	Vehicle Control
vWF	von Willebrand Factor
WB	Whole Blood