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Efficacy of Tranexamic Acid and Prothrombin Complex Concentrate for Traumatic Coagulopathy in Acidosis and Shock States, with Additional Evaluation of Resuscitative Endovascular Balloon Occlusion of the Aorta



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TABLE OF CONTENTS

	Page
LIST OF FIGURES	iii
LIST OF TABLES	iii
1.0 EXECUTIVE SUMMARY	1
2.0 INTRODUCTION	2
2.1 Background	2
2.2 Relevance to Identified Military Research Gaps	3
2.3 Specific Aims and Hypotheses	4
2.4 Revised and Additional Aims and Hypotheses.....	4
2.5 Added Study on Resuscitative Endovascular Balloon Occlusion of the Aorta (REBOA)	5
3.0 METHODS, ASSUMPTIONS, AND PROCEDURES.....	6
3.1 In Vitro Analysis of the Efficacy of TXA and PCC in the Setting of Severe Metabolic Acidosis	6
3.2 <i>In Vivo</i> Analysis of the Efficacy of TXA in the Setting of Severe Metabolic Acidosis	7
3.3 Comparison of the Safety and Efficacy of 3-Factor vs. 4-Factor PCC Products in the Setting of Severe Traumatic Coagulopathy	8
3.4 Comparison of Pharmacodynamics and Efficacy of TXA Administered via the IO vs. IV Routes.....	10
3.5 Evaluation of Plasma Co-Administration vs. TXA and PCC Administration Alone or in Combination in Severe Traumatic Coagulopathy.....	11
3.6 Evaluation of the Efficacy of REBOA in the Setting of Isolated Major Abdominal Venous Injury.....	12
4.0 RESULTS AND DISCUSSION	13
4.1 <i>In Vitro</i> Analysis of the Efficacy of TXA and PCC in the Setting of Severe Metabolic Acidosis	14
4.1.1 <i>In Vitro</i> TXA and PCC Study Results	14
4.1.2 <i>In Vitro</i> TXA and PCC Study Discussion.....	16
4.2 <i>In Vivo</i> Analysis of the Efficacy of TXA in the Setting of Severe Metabolic Acidosis	17
4.2.1 <i>In Vivo</i> TXA Results	17
4.2.2 <i>In Vivo</i> TXA Discussion	20

TABLE OF CONTENTS (concluded)

	Page
4.3 Comparison of the Safety and Efficacy of 3-Factor vs. 4-Factor PCC Products in the Setting of Severe Traumatic Coagulopathy	21
4.3.1 Comparison of 3-Factor vs. 4-Factor PCC Results.....	21
4.3.2 Comparison of 3-Factor vs. 4-Factor PCC Discussion.....	24
4.4 Comparison of Pharmacodynamics and Efficacy of TXA Administered via the IO vs. IV Routes.....	26
4.4.1 IO vs. IV TXA Study Results	26
4.4.2 IO vs. IV TXA Study Discussion	28
4.5 Evaluation of Plasma Co-Administration vs. TXA and PCC Administration Alone or in Combination in Severe Traumatic Coagulopathy.....	30
4.5.1 Plasma Co-Administration vs. TXA/PCC Alone Results.....	30
4.5.2 Plasma Co-Administration vs. TXA/PCC Alone Discussion	32
4.6 Evaluation of the Efficacy of REBOA in the Setting of Isolated Major Abdominal Venous Injury.....	34
4.6.1 REBOA for Abdominal Venous Injury Results.....	34
4.6.2 REBOA for Abdominal Venous Injury Discussion.....	36
5.0 CONCLUSIONS.....	37
6.0 REFERENCES	38
LIST OF ABBREVIATIONS AND ACRONYMS	42

LIST OF FIGURES

	Page
Figure 1. ROTEM analysis of TXA	14
Figure 2. TXA impact on MCF at baseline vs. with severe metabolic acidosis	15
Figure 3. Prothrombin time correction with PCC alone vs. FFP	15
Figure 4. ROTEM analysis from animals in TXA group at baseline, during maximum fibrinolysis, and after TXA administration.....	17
Figure 5. ROTEM results for CFT, MCF, and ML	19
Figure 6. Effect of TXA on cytokine levels	20
Figure 7. Development of CC by fibrinogen level	24
Figure 8. ROTEM demonstrating hyperfibrinolysis after TPA given in both groups	27
Figure 9. Plasma TXA concentration curves for IV and IO TXA groups.....	27
Figure 10. Change in ROTEM with TXA administration in IV and IO groups.....	28
Figure 11. ROTEM values with significant differences between study groups.....	31
Figure 12. Dynamic changes in fibrinogen levels by group.....	32
Figure 13. Kaplan-Meier survival curves for REBOA vs. control groups.....	35

LIST OF TABLES

	Page
Table 1. ROTEM Results for TXA and Control Animals at 45 Minutes	18
Table 2. Comparison of Laboratory Data of Resuscitation and Coagulation with Mean $\pm$ SD Between Animals With or Without Development of Consumptive Coagulopathy	23
Table 3. Baseline Physiologic and Hematologic Data by Study Group	26
Table 4. Lab and ROTEM Values at 4 Hours.....	31
Table 5. Baseline and Post-Intervention Physiologic and Hemodynamic Data	34

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1.0 EXECUTIVE SUMMARY

Hemorrhage remains the number one cause of preventable death after trauma. One of the primary thrusts of military medical research and development has been the treatment of post-traumatic hemorrhage and the systemic effects of hemorrhagic shock. This has particularly come to the forefront over the past decade of combat operations in the Global War on Terrorism. The development of acute coagulopathy of trauma shock is a relatively frequent event that causes significant morbidity and mortality among injured service members.

The primary goal of this study is to investigate in a porcine model the efficacy of two novel agents that have been proposed for the treatment of acute coagulopathy of trauma shock: tranexamic acid (TXA) and prothrombin complex concentrate (PCC). Both have previously been shown to help prevent or reverse coagulopathy. However, critical gaps remain in the understanding of how to use these agents in the severely injured patient, including data regarding the efficacy, dosing, timing, and impact of associated metabolic alterations (such as severe acidosis) on the use of TXA and PCC in the battlefield setting.

Our original research plan proposed linked *in vitro* and *in vivo* studies exploring the efficacy of TXA and PCC in our previously established large animal model of hemorrhage, ischemia, and acidosis that reproduces the metabolic and physiologic changes seen in severely injured service members. In the interim between the original grant application and the awarding and distribution of funds, several other valid questions regarding both TXA and PCC arose that we felt we were ideally positioned to answer with our research. These included the efficacy of TXA when administered via the intraosseous vs. intravenous routes and a comparison of the safety and efficacy of a newer PCC product (4-factor PCC) compared to the older standard 3-factor PCC product.

In addition to the research aims and goals outlined above from the initial and revised grant application and study plan, we identified an opportunity to perform an add-on study, which would incur no additional costs and could utilize animals from an existing arm of the primary study. This involved the study of a newly deployed technique for early control of non-compressible truncal hemorrhage via resuscitative endovascular balloon occlusion of the aorta (REBOA). One significant unanswered question related to REBOA was whether this intervention would be effective in the setting of an isolated major abdominal venous injury, such as an injury to the iliac veins or the vena cava. We therefore designed and executed a study evaluating the efficacy of REBOA in the setting of an isolated major iliac vein injury that utilized animals assigned to one of the study arms listed above from the primary grant.

This project produced a series of experiments involving multiple arms and study questions that have provided new data and insights into critical questions surrounding the use of these therapies for the treatment of battlefield hemorrhage and coagulopathy. This also resulted in a series of multiple presentations at both military and civilian scientific meetings and a total of six peer-reviewed publications as of the writing of this technical report that are outlined and referenced in this report. In addition, data from this series of experiments have been circulated among the military trauma community and utilized in the revision of existing battlefield clinical practice guidelines and in the creation of new clinical practice guidelines and forward combat care policies.

2.0 INTRODUCTION

2.1 Background

One in every four trauma patients develops acute coagulopathy of trauma shock (ACoTS), which is associated with a fourfold increase in mortality [1,2]. ACoTS is caused by the synergistic interaction of tissue injury, hemodilution, hypothermia, metabolic acidosis, inflammation, and shock. A number of therapies targeting reversal of specific components of ACoTS have been proposed for use in both civilian and military trauma patients. However, dosing regimens and anticipated efficacy are often extrapolated from results in healthy animal models or from human non-trauma populations with normal or near-normal physiology. The severe physiologic alterations seen in major trauma patients, especially battlefield casualties, would be expected to have significant impact on the volume, distribution, metabolism, and activity of many of these agents. We have developed a highly reliable and reproducible porcine model that mimics the severe sustained systemic metabolic acidosis, coagulopathy, and shock state seen in humans with major hemorrhagic shock and tissue injury. The primary goal of this study is to investigate in a porcine model the efficacy of two novel agents that have been proposed for the treatment of ACoTS: tranexamic acid (TXA) and prothrombin complex concentrate (PCC). Both have previously been shown to help prevent or reverse coagulopathy. However, critical gaps remain in the understanding of how to use these agents in the severely injured patient, including data regarding the efficacy, dosing, timing, and impact of associated metabolic alterations (such as severe acidosis) on the use of TXA and PCC in the battlefield setting.

TXA is an inexpensive anti-fibrinolytic agent that inhibits both plasminogen activation and plasmin activity, preventing clot breakdown. A recent large prospective randomized trial (Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage-2, CRASH-2) found that TXA may result in reduced mortality following civilian trauma [3]. The subsequent Military Application of Tranexamic Acid in Trauma Emergency Resuscitation Study (MATTERS), a retrospective examination of coalition experience with TXA, found that this simple intervention may be of great value in theaters of modern combat, particularly among combat casualties requiring >10 units of blood products after injury [4]. Recent studies have also demonstrated that TXA may have significant anti-inflammatory properties. PCC is purified, freeze-dried, concentrated coagulation factors and now comes in both 3-factor and 4-factor formulations [5-7]. Both formulations have previously been used in hemophiliacs. This laboratory and others have shown promising protective effects with regard to administration of TXA and PCC at the onset of trauma. We recently have shown *in vitro* that TXA and PCC are able to significantly improve severe coagulopathy and hyperfibrinolysis and that there is no significant decline in their efficacy even when administered in the setting of severe metabolic acidosis.

Severe traumatic injuries result in a massive cytokine release with a resultant massive inflammatory response. It is well known that massive inflammation is associated with higher morbidity and mortality. Recent studies have suggested that TXA has anti-inflammatory properties in non-trauma surgical patients. It is not known if PCC has any effect on the inflammation cascade. The use of TXA and PCC on the battlefield may not only prevent severe coagulopathy, but may minimize the associated massive inflammation cascade and improve overall morbidity and mortality in these patients. This study will also assess the impact of these agents on inflammatory cytokine profiles by utilizing a multiplex immunoassay platform.

This research project performed linked *in vitro* and *in vivo* studies exploring the efficacy of TXA and PCC in our previously established large animal model of hemorrhage, ischemia, and acidosis that reproduces the metabolic and physiologic changes seen in severely injured service members. The data obtained from this line of research and downstream investigations will provide critically needed information that could lead to changes in the way that TXA is administered to injured service members or to changes in the dosing, timing, and administration of other adjuncts that could result in improved efficacy with resultant decreased morbidity or mortality. The data obtained from this research related to PCC and TXA may lead to the more widespread adoption of these agents for use by forward-deployed medical assets as another adjunct in the treatment of wounded service members who are suffering from or at risk for traumatic coagulopathy and hemorrhagic shock and also clarify indications for their use.

2.2 Relevance to Identified Military Research Gaps

Hemorrhage remains the number one cause of preventable death on the battlefield [8,9]. One of the primary thrusts of military medical research and development has been the treatment of post-traumatic hemorrhage and the systemic effects of hemorrhagic shock. This has particularly come to the forefront over the past decade of combat operations in the Global War on Terrorism. The development of major coagulopathy (ACoTS) is a relatively frequent event that causes significant morbidity and mortality among injured service members [2].

Various strategies and agents have been proposed to treat battlefield casualties and to prevent or reverse ACoTS. In addition to obtaining immediate hemorrhage control, the U.S. military has widely adopted a strategy of resuscitating battlefield casualties with a balance of blood products known as “damage control resuscitation.” Although effective, this is highly resource intensive and not available at the most forward or austere levels of care. There has been great interest in pharmacologic adjuncts that could treat traumatic coagulopathy. One such agent, TXA, was adopted for use by several U.S. allies and more recently has begun to be used by U.S. military medical units to treat injured patients with major hemorrhage. Another agent, PCC, has begun to be used in many civilian trauma centers and may be of benefit in battlefield trauma. However, there are critical gaps in the available knowledge and understanding of how to use these agents in the severely injured patient. This includes data regarding the efficacy, dosing, timing, and impact of associated metabolic alterations (such as severe acidosis) on the use of TXA and PCC in the battlefield setting.

The data obtained from this initial proposed line of research and downstream investigations will provide critically needed information that could lead to changes in the way that TXA is administered to injured service members or to changes in the dosing, timing, and administration of other adjuncts that could result in improved efficacy with resultant decreased morbidity or mortality. The data obtained from this research related to PCC may lead to the adoption of this agent for use by forward-deployed medical assets as another adjunct in the treatment of wounded service members who are suffering from or at risk for traumatic coagulopathy and hemorrhagic shock. The data from this research study will be made immediately available to the leadership of the Joint Theater Trauma System (JTTS) and any other interested parties to be considered in decisions regarding alterations to the current JTTS clinical practice guidelines (CPGs) for the use of TXA and the possible development of a new CPG for the use of PCC in the combat setting.

2.3 Specific Aims and Hypotheses

Our original research plan proposed linked *in vitro* and *in vivo* studies exploring the efficacy of TXA and PCC in our previously established large animal model of hemorrhage, ischemia, and acidosis that reproduces the metabolic and physiologic changes seen in severely injured service members.

Specific Aims:

1. Determine if the administration of TXA and PCC following severe injury improves coagulopathy and if these agents are significantly impacted by acidosis.
2. Evaluate the impact of TXA and PCC on the inflammatory cascade *in vivo*.
3. Evaluate the efficacy and any adverse effects of administering these agents alone or in combination with each other and with plasma during resuscitation.

Original Hypotheses:

1. PCC and TXA will significantly improve coagulopathy as measured by rotational thromboelastometry (ROTEM), prothrombin time (PT), activated partial thromboplastin time (PTT), fibrinogen, and international normalized ratio (INR) when administered in a severely acidotic environment.
2. TXA and PCC may significantly impact the levels of post-traumatic inflammatory cytokines.

2.4 Revised and Additional Aims and Hypotheses

In the interim between the original submission of this grant and the final awarding, there were multiple developments involving this line of inquiry, both in the medical literature and through our own preliminary work on this topic. We completed all *in vitro* data collection and analysis, which supported the validity of our hypotheses and study design regarding the efficacy of TXA and PCC in serum from acidotic animals in hemorrhagic shock. We also completed data collection and analyses on control animals (adult swine) that confirmed the presence of a significant dilutional coagulopathy and evidence of shock. However, even with this degree of hemorrhage and shock, adult swine have a high resistance to developing major fibrinolysis. We overcame this problem with the administration of recombinant tissue plasminogen activator (TPA) to induce massive fibrinolysis. Although there was some concern that this was an artificial means of inducing hyperfibrinolysis, subsequent preliminary work by researchers from the Denver Health System has actually demonstrated that TPA release is the primary mechanism of hyperfibrinolysis that is seen in ACoTS (unpublished data). This lends additional confirmation that our model is a valid reproduction of ACoTS.

In addition to the question of improving the model of inducing and sustaining ACoTS in adult swine, several other valid questions regarding both TXA and PCC have arisen that we felt we were ideally positioned to answer with our research. These included:

1. Is TXA absorption, systemic levels, and efficacy equivalent when administered via an intraosseous (IO) route as compared to the standard intravenous (IV) route? This is particularly relevant to battlefield medicine, as many patients have IO lines placed due to the lack of peripheral access. The existing JTTS CPG at that time recommended against

administration of TXA via an IO line due to the lack of data on the safety and efficacy of administration via this route.

2. The standard PCC product used in the United States until 2014 was a “3-factor” product that did not contain factor VII. In 2014 a “4-factor” product was approved by the Food and Drug Administration for use in the United States. There is currently no literature on the relative efficacy of the 3-factor vs. the 4-factor product when used in the setting of severe injury and coagulopathy.

Thus, we added on several additional aims and hypotheses to the previous list to address these clinically relevant issues and questions.

Additional Specific Aims:

4. Determine the safety and efficacy of administration of TXA via an IO route compared to the standard IV route.
5. Compare the efficacy of 3-factor vs. 4-factor PCC products for the correction of coagulopathy in a severe traumatic coagulopathy model.

Additional Hypotheses:

3. TXA will be equally effective at reversing hyperfibrinolysis when given via an IO route vs. the IV route.
4. Both 3-factor and 4-factor PCC products will have equivalent efficacy in correcting the coagulopathy of trauma.

2.5 Added Study on Resuscitative Endovascular Balloon Occlusion of the Aorta (REBOA)

In addition to the research aims and goals outlined above from the initial and revised grant application and study plan, we identified an opportunity to perform an add-on study that would incur no additional costs and could utilize animals from an existing arm of the primary study. Our lab has developed an interest in REBOA, a new technique for rapid control of non-compressible truncal hemorrhage by inserting a balloon-tipped catheter into the aorta via percutaneous femoral artery access and temporarily occluding aortic blood flow [10]. This has been shown to provide immediate hemorrhage control for exsanguinating abdominal vascular and solid organ injuries and has recently been widely fielded in both Iraq/Syria and Afghanistan [11]. However, there are multiple remaining questions and ill-defined aspects regarding patient selection, injury types, and efficacy of REBOA with various specific injuries.

One significant unanswered question related to REBOA was whether this intervention would be effective in the setting of an isolated major abdominal venous injury, such as an injury to the iliac veins or the vena cava. All prior animal models of REBOA had utilized either an isolated vascular injury or a major solid organ injury, and there were no existing data for how REBOA would affect bleeding and survival in a patient with hemorrhage from a major abdominal venous injury. We therefore designed and executed a study evaluating the efficacy of REBOA in the setting of an isolated major iliac vein injury that utilized animals assigned to one of the study arms listed above from the primary grant. Once all data completion related to the primary TXA/PCC study was complete, animals were then utilized for this add-on REBOA

study. The specific aims of this study were to evaluate the efficacy of REBOA in this setting, using the primary endpoints of survival time, hemodynamics, and blood loss.

3.0 METHODS, ASSUMPTIONS, AND PROCEDURES

This study involved multiple arms organized around various specific phases and focused research questions and resulted in a series of peer-reviewed publications. We have therefore grouped the reporting of the methods into subheadings primarily organized by individual study phases and the subsequent related journal publication. This also roughly follows the temporal order of the study arms, beginning with the preliminary *in vitro* data, proceeding through the *in vivo* experiments, and concluding with the added-on REBOA study outlined in the Introduction.

The study protocol was reviewed and approved by the Madigan Army Medical Center Institutional Animal Care and Use Committee (IACUC). Animals were handled and studies were conducted under a program of animal care accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and in accordance with the National Research Council's 2011 *Guide for the Care and Use of Laboratory Animals* (in compliance with Department of Defense Instruction 3216.1).

3.1 In Vitro Analysis of the Efficacy of TXA and PCC in the Setting of Severe Metabolic Acidosis [12]

Genetically similar Yorkshire-mix swine between 35 and 45 kg were obtained from a local breeder. Swine were sedated and intubated and vascular access (both jugular and carotid) was obtained through a limited neck dissection. This allowed pulmonary artery wedge pressures (PAWPs) through a Swann-Ganz catheter and dynamic blood pressure monitoring through the carotid arterial line. Blood samples were drawn at this time point to represent baseline. One of these samples was spun down and the plasma withdrawn to be later administered as a simulation of fresh frozen plasma (FFP). This was placed on ice and served as the FFP control when looking at the effects of PCC and FFP on dilutional coagulopathy.

One of the major limitations of porcine trauma models is the presence of baseline hypercoagulation as well as a highly effective buffering system. As a result, hemorrhagic-only models have had to rely on infusing exogenous acids. Our lab developed a porcine model that combines hemorrhage with a severe truncal ischemia-reperfusion injury. This model creates a uniform and highly reproducible native metabolic acidosis and shock state that can be maintained with resuscitation over a period of 4-8 hours and allows for the study of resuscitation or interventions. All animals undergo midline laparotomy to allow direct bladder catheterization, placement of a 6 French (Fr) catheter in the inferior vena cava (IVC), and supra-celiac aortic control. A controlled hemorrhage of 35% calculated blood volume (based on weight) was then performed over 20 minutes by withdrawal through the IVC line to simulate the initial traumatic hemorrhage. After this was completed, 5000 units of heparin was administered intravenously and the supra-celiac aorta was cross-clamped for 50 minutes. The clamp was then slowly removed over a 5-minute period with crystalloid fluids and epinephrine supplementation to keep the mean arterial pressures (MAPs) above 40 mmHg. This phase simulates the in-hospital operative hemorrhage control and restoration of adequate perfusion with resuscitation.

A simple algorithm was used to guide resuscitation, and MAPs were maintained between 40 and 49 mmHg. If the MAP dropped below 40 mmHg, IV fluids, or epinephrine, or both were increased. [If PAWP was <150% of baseline, IV crystalloid fluids were increased/bloused; if PAWP was >150%, epinephrine was titrated up.] If MAP rose above 50 mmHg, resuscitative measures were likewise reduced. The swine were resuscitated for 6 hours after the injury to simulate the postoperative resuscitation phase. To avoid the confounding effects of the administration of blood products, no red blood cell or whole blood products were administered during resuscitation. Using both a hemorrhagic and an ischemic component, we were able to create a severe native lactic acidosis without the use of exogenous acid.

Three blood samples were drawn at baseline. The first sample served as the control. The second sample received the *in vitro* equivalent of 100 mg TPA to create a severe fibrinolysis. The final sample received the same TPA dose, but was then given the equivalent of 1000 mg TXA. This entire process was then repeated after hemorrhage, cross-clamp removal, and 6 hours of resuscitation (in the acidotic pH). In addition to standard labs (PTT, PT/INR), each sample was analyzed using ROTEM® (TEM International, Munich, Germany). For our study, we used the EXTEM assay and looked specifically at the maximum clot firmness (MCF). MCF values less than 45 mm represent fibrinolysis and a bleeding risk.

To study PCC, three blood samples were drawn after hemorrhage, cross-clamp, and 6 hours of resuscitation (to allow time for a native dilutional coagulopathy). One served as the control, one received a 45-IU/kg equivalent dose of PCC, and the final tube received the equivalent dose of 4 U autologous plasma (FFP). Standard coagulation parameters were obtained at each time point, with the primary measure of interest being PT.

All data were collected and stored on a secure electronic network. Descriptive statistics as well as Student's t-test, paired t-test, and analysis of variance (ANOVA) were performed using PASW 18.0 (SPSS, Armonk, NY).

3.2 *In Vivo* Analysis of the Efficacy of TXA in the Setting of Severe Metabolic Acidosis [13]

The protocol for our trauma hemorrhagic ischemic porcine model has been previously described above. Briefly, genetically similar Yorkshire adult swine weighing between 35-45 kg were obtained from a local breeder. Sedation, intubation, and general anesthesia were provided and invasive hemodynamic monitoring (PAWPs and dynamic blood pressure) was achieved through limited neck dissection of the carotid artery and jugular vein. Blood samples were drawn for baseline routine labs (hemoglobin, hematocrit, arterial blood gas, alanine transferase, creatinine, lactate, fibrinogen, and coagulation panel) and repeated throughout the experiment at the following time points: 30 minutes and hours 1 and 4 of the resuscitative phase. To achieve a hemorrhage ischemic-reperfusion injury, all study animals underwent a midline laparotomy with a controlled 35% weight-based hemorrhage after cannulation of the IVC followed by clamping of the supra-celiac aorta for 50 minutes to create a severe truncal ischemia, which allowed us to create a severe native lactic acidosis with the use of exogenous acid. The clamp was gradually removed over a 5-minute period with crystalloid fluids and epinephrine infusions to keep MAPs above 40 mmHg simulating a reperfusion injury. The clamp was then removed over 5 minutes to simulate in-hospital operative hemorrhage control and restoration of adequate perfusion. A standard resuscitation phase was carried out according to our protocol. The swine were resuscitated for 4 hours after the injury to simulate the postoperative resuscitation phase. No

blood products were administered during resuscitation to prevent a confounding effect and to examine the drug effect in isolation.

To achieve a significant hyperfibrinolysis in addition to the dilutional coagulopathy, all animals received a 100-mg bolus of recombinant TPA (Alteplase, Genentech, Inc., San Francisco, CA) at 30 minutes into the resuscitation phase. This dosing is identical to human TPA dosing for treatment of pulmonary embolism and was previously validated in an *in vivo* porcine thrombotic model. Experimental animals (TXA group) were then treated with a 1-gram bolus of TXA (Cyklokapron, Pfizer, Inc., New York, NY) at 35 minutes into the resuscitation phase. Control animals received only isovolemic saline vehicle.

In addition to standard labs (PTT, PT/INR), ROTEM was utilized to measure response to TXA. Blood samples for ROTEM were taken at the following time points: baseline, 30 and 45 minutes, and 4 hours into the resuscitation phase. For our study, we used the EXTEM assay analyzing the clotting time (CT), MCF, maximum lysis (ML), and clot formation time (CFT). CT values two times the normal range (38-79 seconds) represent a bleeding risk. MCF values less than 45 mm represent fibrinolysis and a bleeding risk. ML values greater than 15% within 1 hour represent a pathologic hyperfibrinolytic state and were the primary value of interest for examining *in vivo* TXA effects. CFT values two times the normal range (34-159 seconds) represent a bleeding risk from lack of factors or factor activity in clot generation.

The levels of six pro- and anti-inflammatory cytokines were simultaneously measured in plasma samples from experimental swine using a multiplex immunoassay system (Meso Scale Discovery Systems [MSD], Gaithersburg, MD). Cytokines were measured in all study animals at baseline and at 1, 2, 3, and 4 hours of the resuscitation phase. A minimum of three swine from each condition was used. Data were normalized to baseline and expressed as the average maximum fold change over baseline during the 4-hour reperfusion period after cross-clamp removal. Capture antibodies for porcine interleukin (IL)-6, IL-8, IL-10, IL-4, tumor necrosis factor-alpha, and IL-1 beta (R and D Systems, Minneapolis, MN) were printed on an MSD Multi-Spot plate. MSD technology uses electrochemical stimulation of a SULFO-TAG label conjugated to the detection species to generate a light signal that is detected by a camera built into a Sector Imager. The technology enables highly sensitive detection of multiple analytes simultaneously with broad linear dynamic range.

All data were collected and stored on a secure electronic network. Descriptive statistics, one-way and repeated measures ANOVA, analysis of covariance, and independent t-test were performed using PASW 18.0 (SPSS, Armonk, NY). Statistical significance was set at an alpha of 0.05.

3.3 Comparison of the Safety and Efficacy of 3-Factor vs. 4-Factor PCC Products in the Setting of Severe Traumatic Coagulopathy [14]

Genetically similar research-grade adult Yorkshire swine weighing between 35 and 55 kg were sedated, intubated, and maintained on general anesthesia. A limited neck dissection was performed to obtain central vascular access and dynamic physiologic monitoring by placement of a carotid arterial line, an internal jugular 10 Fr introducer, and a pulmonary artery catheter. A midline laparotomy was performed with placement of an IVC 10 Fr introducer and a suprapubic bladder catheter with reapproximation of the abdominal wall with penetrating towel clips.

To replicate traumatic blood loss, acute anemia was then generated with a controlled venous hemorrhage utilizing the IVC catheter to exsanguinate 26 mL/kg, or 35% of the

circulating blood volume. To simulate ischemia and reperfusion, truncal ischemia was created with occlusive clamping of the supra-celiac aorta for 50 minutes, with graduated release over the final 5 minutes to avoid hemodynamic collapse from rapid reperfusion. Post-operative resuscitation and hemodilution were recreated by following a standardized algorithm utilizing a combination of crystalloid IV fluid and epinephrine to maintain a MAP above 40 mmHg with stepwise weaning guided by pulmonary artery values and continued for 6 hours from the removal of the aortic cross-clamp.

Physiologic measures were recorded at baseline, following hemorrhage completion, after cross-clamp removal, at 30 minutes into the resuscitation phase, and then on every hour until 6 hours of resuscitation were completed. Routine laboratory studies (arterial blood gas, arterial lactate, complete blood count, alanine aminotransferase, blood urea nitrogen, serum creatinine, PT, PTT, and fibrinogen) were drawn at baseline, following hemorrhage, after cross-clamp removal, and during the resuscitation phase at hours 1, 4, 5, and 6. Blood for viscoelastic clotting assessment using ROTEM was drawn at baseline and again at resuscitation hours 1, 4, 5 and 6. Serum concentrations of vitamin-K dependent coagulation factors (II, VII, IX, and X) were drawn immediately prior to PCC administration at 4 hours of resuscitation and again at six hours. All animals were euthanized following completion of 6 hours of resuscitation or if pre-determined criteria signifying futility were met.

To compare 3-factor PCC (3PCC) and 4-factor PCC (4PCC), animals received either an infusion of the 3PCC (Profilnine® SD, Grifols Biologicals Inc., Los Angeles, CA) or 4PCC (Kcentra™, CSL Behring LLC, Kankakee, IL) products. A weight-based, 45-IU/kg dose was chosen based on previous *in vitro* work performed by our laboratory. Given the number of available PCC products, these particular formulations of PCC were chosen due to our institutional pharmacy formulary, prepared by research pharmacists, and administered according to manufacturer guidelines. Previous *in vitro* work with acidotic porcine blood from our model comparing autologous plasma and 3PCC had demonstrated a limited correction of coagulopathy after 3PCC administration. However, given the well-publicized clinical experience with PCC in reversing supra-therapeutic warfarin effects, we hypothesized that delivery of PCC in the setting of a profound coagulopathy and metabolic acidosis could potentially improve or reverse this highly lethal coagulation defect. Our previous *in vitro* studies had utilized blood from the first hour of the resuscitation phase prior to the development of the worst coagulopathy and acidosis; as such, the timing of PCC delivery for this *in vivo* study was changed from hour 1 of the resuscitation phase to hour 4 of the resuscitation phase.

To further characterize the response to PCC in the setting of trauma, our IACUC authorized an additional eight animals to receive the superior PCC product (as determined by interim analysis) following administration of two unit equivalents of autologous plasma during the initial resuscitation phase. The intent of this arm of the study was to increase the available fibrinogen and coagulation factor substrate prior to PCC administration, while avoiding simultaneous administration. This autologous plasma was prepared using the weight-based hemorrhaged blood, which had been collected in citrated blood collection bags (Teruflex® Blood Bags, Terumo Corporation, Tokyo, Japan) and aseptically centrifuged into platelet-poor plasma. The plasma was then infused over 20-30 minutes via venous central catheter after the initial stabilization, 45 minutes following removal of the supra-celiac aortic cross-clamp. Following interim analysis, 4PCC was determined as the superior agent for further analysis due to lower rate of consumptive coagulopathy (CC) and improved effect in the absence of complications. Descriptive statistics, one-way and repeated-measures ANOVA with adjustments for multiple

comparisons, and Student's t-test for independent measures were performed using SPSS® Statistics 22 (IBM Corp., Armonk, NY). Statistical significance was set at a p-value of <0.05.

3.4 Comparison of Pharmacodynamics and Efficacy of TXA Administered via the IO vs. IV Routes [15]

Eighteen adult swine (35-55 kg, *Sus scrofa*) underwent a hemorrhage and ischemia reperfusion injury protocol that had been previously validated to produce systemic acidosis, coagulopathy, and shock physiology with marked hyperfibrinolysis. They were then randomized to either an IV dose of TXA (n=9) or a tibial IO dose (n=9).

After the induction of general anesthesia, all animals underwent placement of invasive monitoring devices. A neck dissection was performed and the right carotid artery and right internal jugular veins were identified. An arterial line was placed in the carotid artery to obtain continuous blood pressure monitoring. A central venous introducer sheath was placed in the right internal jugular vein and a pulmonary artery catheter was floated from this site to give continuous hemodynamic monitoring. A laparotomy was then performed and a large-bore infusion catheter was placed in the IVC. The aorta was then identified and controlled with a vessel loop via an incision in the diaphragm. The abdomen was then temporarily closed using penetrating towel clamps.

After the setup phase, each animal underwent a controlled, 35% blood volume hemorrhage (26 cc/kg). This hemorrhage was done as tolerated until the animal's MAP dropped below 40 mmHg. If the MAP dropped below 40 mmHg, the rate of hemorrhage was slowed. The maximum allowed time for hemorrhage was 30 minutes. If the hemorrhage could not be completed in this time, it was stopped. All animals in the study completed the entire hemorrhage phase. After the hemorrhage had been performed, a supra-celiac aortic cross-clamp was placed. This was kept in place for 45 minutes. When the aortic occlusion time reached 45 minutes, the cross-clamp was slowly released over 5 minutes. An epinephrine infusion was initiated at the start of cross-clamp release and was turned down as tolerated by the animal, ensuring that the MAP stayed above 40 mmHg.

After the release from of the supra-celiac aortic cross-clamp, the subjects underwent a 4-hour resuscitation and monitoring phase where they were given IV fluids and vasopressors (epinephrine) as needed to keep their MAP above 40 mmHg. After release of the cross-clamp, the animals were stabilized for 30 minutes. At 30 minutes post cross-clamp release, we induced a state of hyperfibrinolysis via an injection of 100 mg of TPA through the internal jugular catheter. This induces an immediate and profound hyperfibrinolytic state as validated previously by our lab and mechanistically appears similar to human post-traumatic hyperfibrinolytic states. After the injection was completed, a blood sample was drawn and ROTEM was performed to confirm the existence of a hyperfibrinolytic state. Five minutes after the injection of the TPA, the animals were given a 1-gram dose of TXA over 10 minutes. This was the only dose of TXA given and was not followed by a continuous infusion as we wished to assess the response to the single bolus dose only. In the animals that were randomized to the IV group, the TXA was given through the right internal jugular catheter. The animals randomized to the IO group received TXA through an IO catheter placed in the tibial plateau.

Hemodynamics and labs values (MAP, pH, lactate, INR) were monitored to assess the animals' physiologic state at the removal of the cross-clamp, immediately before TPA delivery, 1 hour after cross-clamp removal, and 4 hours after cross-clamp removal. ROTEM analysis was

performed immediately after the TPA dose was given, at 1 hour after cross-clamp release (15 minutes after completion of TXA dose), and 4 hours after cross-clamp release. To assess plasma concentrations of TXA, we used a custom-made and validated high-performance liquid chromatography assay for TXA levels developed by the University of Washington. To test for serum drug concentrations and establish a pharmacokinetic response curve, we drew samples at 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 minutes from the start of the TXA infusion in both study groups. All statistical analyses were performed with standard statistical software. To determine significance, a t-test analysis was used; $p < 0.05$ was considered significant.

3.5 Evaluation of Plasma Co-Administration vs. TXA and PCC Administration Alone or in Combination in Severe Traumatic Coagulopathy [16]

General anesthesia was induced and maintained in genetically similar research-grade adult Yorkshire swine weighing between 35 and 55 kg. Central venous and arterial access was gained through a midline neck dissection with placement of a carotid arterial line and a jugular 10 Fr introducer used as a conduit for a Swan-Ganz pulmonary artery catheter. A 10 Fr infrarenal IVC catheter was placed following midline celiotomy as well as an intra-abdominal Foley catheter. The midline incision was re-approximated after setup was completed. Animals underwent the combined hemorrhage and ischemia-reperfusion injury protocol as described for the previous study arms.

Animals were assigned to one of eight groups: control (n=12), a single agent group (TXA, n=10; PCC, n=8; or FFP, n=6), or combination groups (TXA+FFP, n=10; PCC+FFP, n=8; TXA+PCC, n=6). The sample size was based on power analysis for each treatment, and each group had its own controls, which ultimately resulted in a larger than typical control group size. Controls had induction of the injury without hemostatic adjuncts administered. Given the resistance to coagulopathy in the porcine model, we pharmacologically induced hyperfibrinolysis with TPA. Alteplase (Activase, Genentec, Inc., San Francisco, CA) was administered intravenously via the internal jugular central line at a dose of 100 mg. It was administered as a bolus 30 minutes after cross-clamp removal. The dosing and administration of the experimental arms were as follows.

PCC (Kcentra™, CSL Behring LLC, Kankakee, IL) was given in a weight-based fashion at 45 IU/kg, based on previous *in vitro* work performed by our laboratory. PCC was prepared by research pharmacists and administered according to manufacturer recommendations. The timing of PCC delivery for this *in vivo* study was during hour 4 of the resuscitation phase, as this correlated to the peak of coagulopathy seen in this model.

FFP for swine was generated in our laboratory using autologous plasma from the controlled hemorrhage collected in citrated blood collection bags (Teruflex® Blood Bags, Terumo Corporation, Tokyo, Japan) and aseptically centrifuged into platelet-poor plasma (approximately 25-26 mL/kg). The plasma was then infused over 20-30 minutes via venous central catheter after the initial stabilization, 45 minutes following removal of the supra-celiac aortic cross-clamp.

TXA (Cyklokapron, Pfizer, Inc., New York, NY) was dosed in the standard fashion in accordance with the CRASH-2 trial. TXA 1 gram was administered via internal jugular central line, 5 minutes after induction of hyperfibrinolysis using TPA (35 minutes after removal of cross-clamp). The above drugs were also given in three groups of two drug combinations: TXA+PCC, TXA+FFP, and FFP+PCC. Dosage and timing of the drugs were not changed when

the intervention was in combination. The TXA alone, TXA+PCC, and TXA+FFP groups all had TPA administered as described above to induce coagulopathy and thus had TPA controls for standard comparison.

Data points were collected and stored electronically on a secure network. Descriptive statistics, one-way ANOVA with adjustments for multiple comparisons, post hoc analysis, and Student's t-test for independent measures were performed using IBM® SPSS® Statistics 22 (IBM Corp., Armonk, NY). Statistical significance was set at $p < 0.05$. Groups were compared using values collected at baseline, 1 hour, and 4 hours for hemodynamic parameters and laboratory and ROTEM values using one-way ANOVA and multivariate linear regression for repeated measures, with Tukey post hoc to delineate significance between groups. Variances of mean values at baseline were corrected by calculating the delta change between time points. Subgroup analysis was completed comparing mean values between FFP containing (FFPc) groups and non-FFP containing (nFFP) groups using Student's t-tests.

3.6 Evaluation of the Efficacy of REBOA in the Setting of Isolated Major Abdominal Venous Injury [17]

Prior to starting the control and experimental arms of the experiment, five adult swine were used in a model development phase to create a reliably reproducible model of massive intra-abdominal venous bleeding. Ultimately this was done by identifying and dissecting the right common iliac vein. Using a ruler to confirm distance, the spot for injury was identified on the anterior aspect of the vessel, 1-2 cm distal to the confluence of the iliac veins with the IVC. A side-biting clamp was then applied to the anterior aspect of the vessel, and after measuring a 4- to 5-mm section, Metzenbaum scissors were used to remove this section of the anterior portion of the vein. This was able to be reproduced in all experimental arms.

Once we had determined our method for creating a venous injury that would lead to massive blood loss, we randomized 10 adult swine (35-55 kg, *Sus scrofa*) to either a standard resuscitation only arm (n=5) or a treatment (REBOA) arm (n=5). All animals underwent a blood loss and ischemia reperfusion injury protocol to produce shock physiology prior to entering the venous injury and REBOA phase of the experiment. After the induction of general anesthesia, all animals underwent placement of invasive monitoring devices. A neck dissection was performed and the right carotid artery and right internal jugular veins were identified. An arterial line was placed in the carotid artery to obtain continuous blood pressure monitoring. A Cordis was placed in the right internal jugular vein and a pulmonary artery catheter was floated from this site to give continuous hemodynamic monitoring. This access site was also used to deliver fluids and vasopressors as needed. A laparotomy was then performed and a Cordis infusion catheter was placed in the IVC. The aorta was then identified and controlled with a vessel loop via an incision in the diaphragm. The abdomen was then temporarily closed using penetrating towel clamps.

After the setup phase, each animal underwent a controlled, 35% blood volume hemorrhage (26 cc/kg). This hemorrhage was performed by drawing blood from the IVC Cordis as rapidly as possible until the animals' MAP dropped below 40 mmHg. If the MAP dropped below 40 mmHg, the rate of hemorrhage was slowed until the animal was able to compensate. The maximum allowed time for hemorrhage was 30 minutes. If the hemorrhage could not be completed in this time, it was stopped. All animals in the study completed the entire hemorrhage.

After the hemorrhage had been performed, a supra-celiac aortic cross-clamp was placed. This was kept in place for 45 minutes. When the aortic occlusion time reached 45 minutes, the

cross-clamp was slowly released over 5 minutes. An epinephrine infusion was initiated at the start of cross-clamp release and was turned down as tolerated by the animal, ensuring that the MAP stayed above 40 mmHg. After the release from the supra-celiac aortic cross-clamp, the subjects underwent a 4-hour resuscitation and monitoring phase where they were given IV fluids and vasopressors (epinephrine) as needed to keep their MAP above 40 mmHg. Lab values were drawn at predetermined intervals for this period and recorded.

After the 4-hour resuscitation from their previous controlled 35% blood loss, the animals entered the REBOA protocol. During this time their fluids and vasopressor requirements were not adjusted or titrated. In animals randomized to the control arm (no intervention), a 4- to 5-mm section of the anterior portion of the right common iliac vein was removed approximately 1-2 cm distal to the iliac confluence with the IVC. A vascular clamp was left in place over the injury until a temporary abdominal closure with towel clamps could be performed. The vascular clamp was then released allowing for free bleeding to occur. Vital signs and hemodynamics were recorded every 5 minutes until the animal demonstrated a sustained MAP of 20 mmHg or less for at least 10 seconds. Blood loss was collected with suction and into an empty canister. Hematoma was weighed using a standard balance.

In the experimental arm, a 14 Fr REBOA catheter was prepositioned in zone I (supra-celiac aorta) via direct cut-down of the left common femoral artery. The artery was cannulated with a standard arterial line kit and upsized to a 7 Fr Cordis. A 0.35J-Wire was then used to gain access to the aorta. A 14 Fr sheath was used and the REBOA was placed with the balloon down. After preplacement of the device, our injury was created in the right common iliac vein as previously described and the abdomen was closed. The vascular clamp was released and free-bleeding was allowed for 60 seconds. After 60 seconds, the balloon was inflated until there was a loss of distal pulses and hemodynamic changes were seen on the monitor. The animals were then continued on their current rate of fluid and vasopressor without titration until a sustained MAP of less than 20 mmHg (sustained defined as longer than 10 seconds) or 1 hour, whichever happened first. Blood loss was calculated in a similar fashion.

All animals were euthanized in accordance with IACUC policy at the completion of the experiment. After euthanasia was achieved, placement of the balloon was confirmed by opening the thoracic aorta and confirming location. All statistical analyses were performed with standard statistical software. To determine significance, a t-test analysis was used; $p < 0.05$ was considered significant. A power analysis was conducted by our local biostatistician, and it was determined that a minimum of five animals in a control and experimental arm would be required to detect a difference.

4.0 RESULTS AND DISCUSSION

This study involved multiple arms organized around various specific phases and focused research questions and resulted in a series of peer-reviewed publications. We have therefore grouped the reporting of the results and discussion into subheadings primarily organized by individual study phases and the subsequent related journal publication that correspond to the same subheading numbers in the Methods section above. This also roughly follows the temporal order of the study arms, beginning with the preliminary *in vitro* data, proceeding through the *in vivo* experiments, and concluding with the added-on REBOA study outlined in the Introduction section.

4.1 *In Vitro* Analysis of the Efficacy of TXA and PCC in the Setting of Severe Metabolic Acidosis

4.1.1 *In Vitro* TXA and PCC Study Results. Thirty-one swine were used in the study. The mean values at baseline were as follows: hematocrit (Hct) 24.28% (± 2.3), pH 7.56 (± 0.09), INR 1 (± 0.05), and lactate 1.47 mmol/L (± 0.48). Following hemorrhage, aortic cross-clamp, and 6 hours of resuscitation, the mean values were Hct 15.93% (± 2.97), pH 7.11 (± 0.13), INR 1.72 (± 0.55), and lactate 10.26 mmol/L (± 2.75).

TXA was studied by looking at the MCF. The mean MCF at baseline was 71.71 mm (± 3.09). After a dose of TPA, the mean MCF was 0.29 mm (± 0.53), representing a severe fibrinolysis. However, with one dose of TXA, the mean MCF immediately returned to a normal value, 69.06 mm (± 3.14). All these changes were statistically significant ($p < 0.001$). After hemorrhage, cross-clamp, and 6 hours of resuscitation, the mean MCF was 59.03 mm (± 11.95). TPA dosing again created a profound fibrinolysis, with a mean MCF of 1.06 mm (± 2.72); again, this was fully reversed with one dose of TXA, bringing the mean MCF back to 57.81 mm (± 10.39). These changes were also statistically significant ($p < 0.001$). However, 57.81 mm was not statistically different than 59.03 mm ($p = 0.476$), meaning that TXA was able to fully reverse the severe fibrinolysis, even in a severely acidotic environment with mean pH 7.1 (Figure 1).

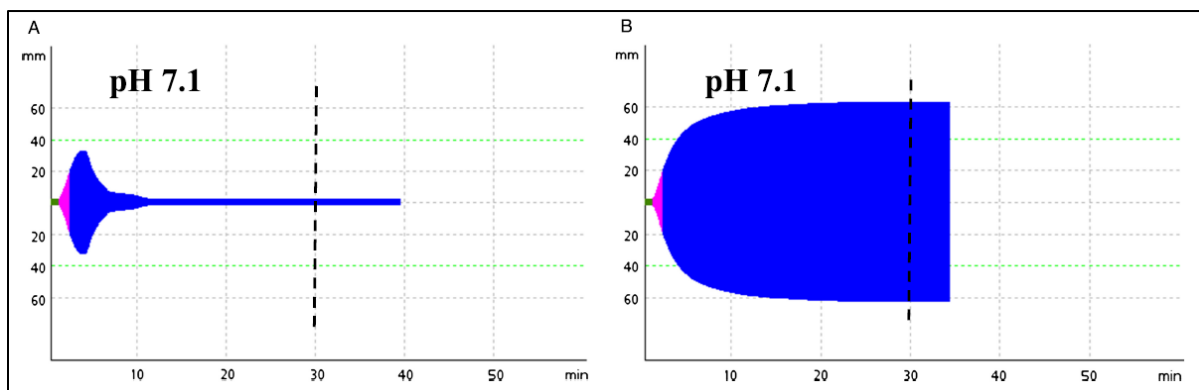


Figure 1. ROTEM analysis of TXA. MCF was measured at 30 min (dashed line). A: Severe fibrinolysis in the absence of TXA. B: Complete reversal after a single dose of TXA.

To look more closely at TXA's function in acidosis, we compared the ability of TXA to correct the MCF in acidosis vs. at normal pH. At baseline (mean pH 7.56), TXA was able to bring the MCF back to within 2.65 mm of the starting (pre-TPA) value. In the setting of severe acidosis (mean pH 7.11), TXA was able to bring the MCF to within 1.23 mm (2.65 mm vs. 1.23 mm; $p = 0.4$). Therefore, TXA did not function differently in the setting of metabolic acidosis and maintained baseline activity (Figure 2). Both TPA and TXA had a small but statistically significant effect on PT and PTT. At baseline pH (before hemorrhage and ischemia), the mean PT was 12.94 seconds (± 0.53 ; INR 0.99) and PTT was 31.83 seconds (± 5.99). One dose of TPA slightly raised PT (13.26 seconds; INR 1.03; $p = 0.003$) and PTT (34.89 seconds; $p < 0.001$), but a subsequent dose of TXA brought these elevated values back to control values (PT = 12.8 seconds, INR 0.98, PTT 32.22 seconds; $p = 0.052$ and 0.661, respectively).

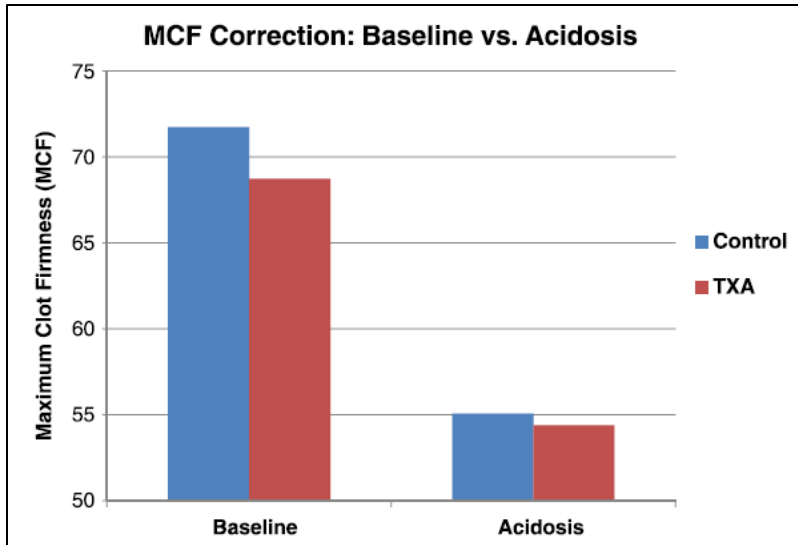


Figure 2. TXA impact on MCF at baseline vs. with severe metabolic acidosis.

The effects of FFP and PCC are best studied using PT. Mean baseline PT was 13 seconds (± 0.49). After hemorrhage, ischemia, and several hours of resuscitation, mean PT rose to 23.03 seconds (± 10.08). An *in vitro* dose equivalent to 4U FFP brought the PT down to 17.44 seconds (± 2.66 ; $p=0.001$), whereas the equivalent of 45 IU/kg of PCC reduced the PT to 20 seconds (± 7.52 ; $p=0.001$) (Figure 3). The ability of the drug to reverse the coagulopathy and “normalize” the PT is best seen in the mean PT correction. The mean PT correction seen with FFP was 5.58 seconds (± 7.84) and with PCC was 3.03 seconds (± 2.96), which was statistically significant ($p=0.013$). Fibrinogen levels were drawn at random from five swine after trauma and resuscitation and all showed severe hypofibrinogenemia (mean 76.8 ± 15.77).

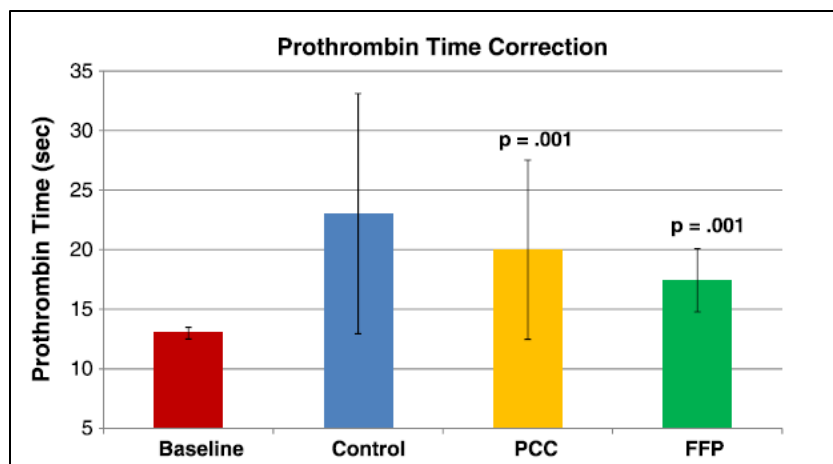


Figure 3. Prothrombin time correction with PCC alone vs. FFP.

4.1.2 In Vitro TXA and PCC Study Discussion. TXA clearly has the ability to completely reverse a severe fibrinolysis. Using real-time ROTEM analysis, we could easily quantify the level of fibrinolysis as well as confirm reversal and return to normal. We used a full dose of TPA to augment the fibrinolysis, and TXA was still able to fully reverse this coagulopathy. Additionally, using our acidotic trauma model, we were able to directly compare TXA's function in the normal pH (7.57) vs. an acidotic pH (7.11). TXA showed no loss of function and could reliably reverse a severe fibrinolysis at both pH levels.

Fibrinolysis is rapidly and easily assessed objectively with ROTEM (or thromboelastography) but not with standard labs (PT/PTT). Relying solely on standard lab analysis during evaluation of a trauma patient could lead to overlooking hyperfibrinolysis, a prevalent process with significant morbidity and mortality [18,19]. We ran PT (INR) and PTT on all of our blood samples. Although several of our findings reached statistical significance, the severe fibrinolysis did not cause any abnormal PT/PTT changes. A full dose of TPA or TXA only changed the INR by 0.05, emphasizing the inability of standard labs to diagnose fibrinolysis. In addition to being highly effective, TXA has several additional attractive features. A full dose of TXA is contained within a 10-mL ampule that is stable at room temperature and costs less than \$40. TXA is cost effective and highly portable, which makes it perfectly suited for military usage or in the civilian pre-hospital setting [20].

Head-to-head, in our study, FFP outperformed PCC, which contradicts previously published hemorrhagic models [5,21,22]. This may be due to the severe acidosis component of our model, which has been absent in previous hemorrhagic-only models. Additionally, some studies have used PCC in combination dosing with fibrinogen, a glycoprotein that is notably present in FFP yet absent in PCC. An additional major issue that may impact the comparison of studies using PCC products is that both 3-factor and 4-factor (includes factor VII) preparations are available. The 4-factor preparations were not available for use in the United States at the time of this study; thus, the 3-factor preparation was used. Although effective in reducing PT, higher doses of the 3-factor preparation, or the co-administration of other agents such as factor VII or FFP, may be required to produce equivalent results to the 4-factor product and FFP. Further studies directly comparing the 3 and 4-factor products are indicated and were subsequently performed as part of this research grant as outlined in sections 3.4 and 4.4.

Noteworthy is that PCC is immediately effective and available without cross-matching or thawing. PCC also performed more consistently than FFP with a much narrower standard deviation (SD) after treatment. Moreover, a large dose of PCC is approximately one-tenth the volume of the equivalent FFP, making it a more stable, portable, reliable, and potent alternative to FFP. At this time, optimal therapy may include PCC initially with FFP and vitamin K dosing to maintain PT/INR goals over the subsequent hours to days. In conclusion, our trauma model produced a severe anemia and a consistent native metabolic acidosis. TXA seems best studied with thromboelastometry and was able to fully reverse a severe fibrinolysis at a normal and acidotic pH with hemodilution. PCC at a dose of 45 IU/kg contains the necessary clotting factors to significantly reduce the PT/INR but was outperformed by 4U FFP in our model. TXA and PCC offer exciting new treatment options that are portable, stable, reliable, and effective, with applications in both the civilian and military setting.

4.2 In Vivo Analysis of the Efficacy of TXA in the Setting of Severe Metabolic Acidosis

4.2.1 In Vivo TXA Results. Ten swine were included in our study, and all survived to study completion. Post hoc power analysis was performed and power was >80%. Baseline vital signs were similar between the two groups with the exception of central venous pressure (CVP, 4.2 mmHg control vs. 2.2 mmHg TXA, $p=0.20$). Vital signs at 1 and 4 hours were similar between the two groups. Urine output, IV fluids, and epinephrine administration were similar between groups. Mean laboratory values at baseline were similar: Hct 27.6% (± 7.8), pH 7.51 (± 0.064), INR 1.04 (± 0.04), fibrinogen 218 mg/dL (± 21), and lactate 1.49 mmol/L (± 0.61). At 30 minutes, 1 hour, and 4 hours of resuscitation, mean values were similar between the groups. At 30 minutes of resuscitation, the mean values were pH 7.2 (± 0.06), base excess (BE) -7.4 mEq/L (± 0.67), and lactate 9.4 mmol/L (± 1.5), demonstrating a severe lactic acidosis.

To study the efficacy of TXA, we performed repeated measures ANOVA on ROTEM values CFT, MCF, and ML at points baseline, 30 minutes resuscitation (time of TPA administration), 45 minutes resuscitation (10 minutes from time of TXA administration), and 4 hours resuscitation (end time) (Figure 4). At baseline, mean CFT was 41.4 seconds in controls vs. 43 seconds in the TXA group. After hemorrhage and cross-clamp removal, but prior to TXA administration, mean CFT was 59.4 seconds in controls vs. 69.2 seconds in the TXA group. Following TXA administration, mean CFT was 53.4 seconds in the TXA group vs. 59.2 seconds in controls. There was no significant difference between the two groups at any time point ($p=0.110$).

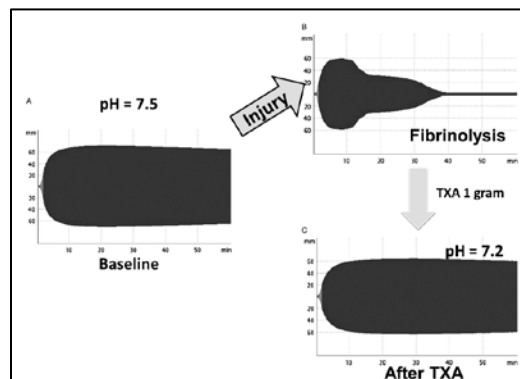


Figure 4. ROTEM analysis from animals in TXA group at baseline, during maximum fibrinolysis, and after TXA administration.

At baseline, mean MCF was 74.8 mm in controls vs. 47.8 mm in the TXA group. After hemorrhage and cross-clamp removal, but prior to TXA administration, mean MCF was 36.8 mm for controls and 32.6 mm in the TXA group, demonstrating a severe fibrinolysis. Following TXA administration, MCF increased to 60.4 mm in the TXA group and 64.4 mm in the control group. There was no difference between the study groups ($p=0.214$). The baseline ML for controls was 8.6% vs. 9.2% in the TXA group. After hemorrhage and cross-clamp removal, but prior to TXA administration, mean ML was 100% for controls and 99.4% in the TXA group, demonstrating a severe fibrinolysis (as shown in Figure 4). Following TXA administration, ML decreased to 9% in the TXA group, which is a complete reversal of severe fibrinolysis, but remained elevated in the control group at 91.6% (Table 1). There was a

significant difference between the study groups noted for the ROTEM parameter of ML ($p < 0.001$) (Figure 5).

Table 1. ROTEM Results for TXA and Control Animals at 45 Minutes

ROTEM Value	Control, Mean (SD)	TXA, Mean (SD)	95% Confidence Interval	p
CT, s				
Baseline	42.4 (3.04)	47.8 (4.55)	42.3-47.9	0.214
30 min	52.4 (6.84)	48.8 (6.14)	45.9-55.3	
45 min	44.2 (6.42)	44.6 (5.03)	40.2-48.6	
4 h	43.8 (6.26)	53.6 (5.6)	44.3-53.0	
CFT, s				
Baseline	4.14 (3.21)	43.0 (5.89)	38.7-45.7	0.11
30 min	59.4 (10.16)	69.2 (4.55)	58.6-70.04	
45 min	53.4 (7.96)	59.2 (8.29)	50.4-62.2	
4 h	61.8 (9.09)	72.4 (9.24)	60.4-73.8	
MCF, mm				
Baseline	74.8 (3.05)	47.8 (4.54)	72.6-75.4	0.516
30 min	36.8 (5.45)	32.6 (4.62)	31.0-38.3	
45 min	60.4 (6.8)	64.4 (1.67)	58.8 -66.0	
4 h	68.8 (3.70)	65.4 (3.51)	64.5-69.7	
ML, %				
Baseline	8.6 (4.16)	9.2 (1.92)	6.5-11.3	<0.001
30 min	100.0 (0)	99.4 (1.34)	99.0-100.4	
45 min	91.6 (18.78)	9.0 (2.45)	40.5-60.1	
4 h	1.8 (3.03)	2.8 (1.30)	0.6-4.0	

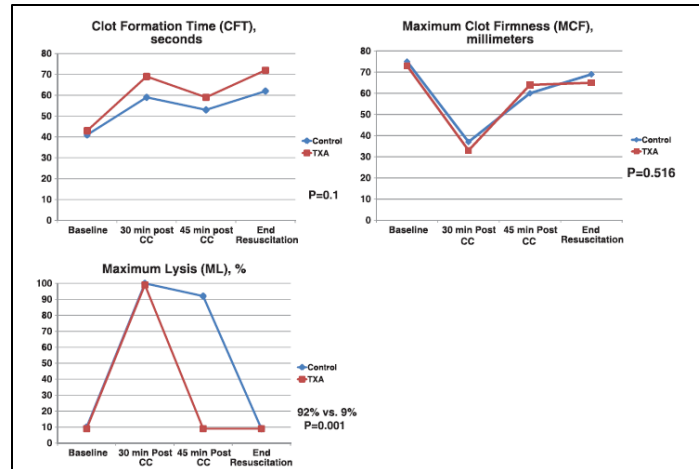


Figure 5. ROTEM results for CFT, MCF, and ML.

A one-way ANOVA was then performed to further evaluate TXA efficacy. Baseline CVP, serum bicarbonate, alanine aminotransferase and blood urea nitrogen, and lactate at 4 hours were significant variables ($p < 0.05$). An independent t-test was performed for the unadjusted significant independent variables for those ROTEM values that were significant in the repeated measures (CT at 4 hours and ML at 45 minutes, $p < 0.05$). To adjust for significant independent variables, an analysis of covariance was performed with ML at 45 minutes and CT 4 hours as the dependent variables. There were no significant differences between the two groups for CT at 4 hours (43.8 ± 6.26 seconds control vs. 53.6 ± 5.6 seconds, $p = 0.479$). ML at 45 minutes was significantly different between the two study groups ($91.6 \pm 18.78\%$ control vs. $9 \pm 2.45\%$, $p = 0.007$). This indicates that pH did not have an effect on TXA's ability to reverse severe fibrinolysis in an acidotic environment (mean pH 7.2).

Cytokine levels were analyzed in both groups. The maximum fold-change in the IL-4 level over baseline was significantly higher in the TXA group vs. controls. In control animals, the maximum IL-4 level during the reperfusion period was approximately 57% of baseline, whereas the maximum IL-4 level in TXA-treated animals was approximately 121% of baseline (Figure 6). IL-6, IL-10, tumor necrosis factor-alpha, and IL-1b showed no significant difference between study groups at baseline and reperfusion (data not shown). IL-8 levels were below the lower limit of detection in all samples.

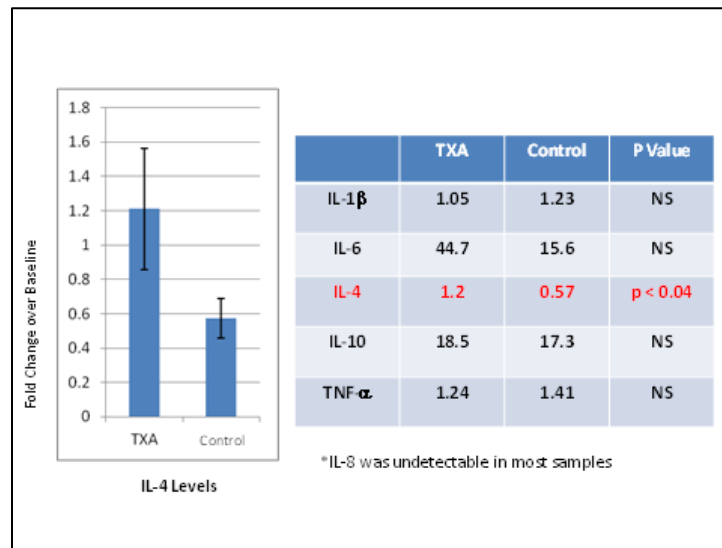


Figure 6. Effect of TXA on cytokine levels.

4.2.2 In Vivo TXA Discussion. The role of severe hyperfibrinolysis seen in acute traumatic coagulopathy has been further elucidated and characterized by improved technologies such as ROTEM. This allows both researchers and clinicians to identify and treat patients previously not identified with traditional labs such as PT/PTT/INR and fibrinogen. TXA, an anti-fibrinolytic, has shown promise in clinical studies such as the CRASH-2 and MATTERS trial, but little data are available regarding basic science, animal data in the underlying mechanism, pharmacokinetics and efficacy, and anti-inflammatory effects of TXA in the treatment of severely injured trauma patients [4,23,24]. Using our model, we are able to demonstrate that the administration of TXA rapidly reverses a severe fibrinolysis even in an acidotic environment (mean pH of 7.1 to 7.2). This confirms the previous findings in our *in vitro* study outlined in sections 3.1 and 4.1. We also analyzed the effect of TXA on both anti- and pro-inflammatory cytokine levels. To our knowledge, this is the first study on the anti-inflammatory effects of TXA in a large animal model. Levels of IL-4, an anti-inflammatory cytokine, were decreased only in the control animals, suggesting an anti-inflammatory effect from TXA. This is consistent with human studies that showed a decrease in the pro-inflammatory cytokine IL-6 upon TXA administration [25,26]. Further studies are needed to confirm this finding and further elucidate the effect of TXA on inflammatory mediators.

In 2012, the Department of Defense completed a review on the knowledge gaps and research requirements regarding the use of TXA in traumatic hemorrhage [27]. They identified a number of priorities, with drug safety, mechanism of action, efficacy, mechanism of action, timing of administration, and patient selection being among the most important. Of particular interest are the efficacy and safety in the prehospital setting. Additionally, the MATTERS II trial recently reported improved mortality associated with TXA alone and a combination of TXA and cryoprecipitate on severely injured military trauma casualties, with the most benefit noted in the combination therapy [4]. The wide availability, cost (\$40 per vial), and stability at room temperature of TXA make it an attractive tool in the armament of trauma care in both military and civilian settings. Future research should be directed at further *in vivo* studies evaluating different TXA dosing regimens, the timing of TXA administration as a pre-hospital intervention,

TXA in combination with PCC, cryoprecipitate, or other agents, and further mechanistic studies of TXA beyond its anti-fibrinolytic activity. In conclusion, TXA produced a significant and rapid reversal of a severe fibrinolysis by ROTEM parameters even in the setting of a severe native metabolic acidosis. TXA also may have a role in modulating activated inflammatory pathways in severely injured trauma patients, and our results suggest that this may be via increased expression of anti-inflammatory cytokines such as IL-4. Future research should be directed at further *in vivo* studies evaluating different TXA dosing regimens, the timing of TXA administration as a pre-hospital intervention, TXA in combination with PCC, cryoprecipitate, or other agents, and further mechanistic studies of TXA beyond its anti-fibrinolytic activity.

4.3 Comparison of the Safety and Efficacy of 3-Factor vs. 4-Factor PCC Products in the Setting of Severe Traumatic Coagulopathy

4.3.1 Comparison of 3-Factor vs. 4-Factor PCC Results. Twenty animals underwent the described protocol. Seventeen animals survived to randomization, with nine animals receiving 3PCC and eight receiving 4PCC. One animal receiving 3PCC did not survive to the sixth hour of the resuscitation phase, but received the study drug with near-complete data collection and was included in this study. The remaining 16 animals survived to completion of the sixth hour of resuscitation. Following interim analysis, an additional eight animals received 4PCC and two unit-equivalents of autologous plasma transfusion as previously described. Five animals in a control group underwent hemorrhage, ischemia, reperfusion, and algorithmic resuscitation without PCC administration.

There were no clinically significant differences in baseline physiologic measures. The model created significant physiologic derangement replicating trauma-induced coagulopathy. At the fourth hour of resuscitation (immediately prior to PCC administration), the animals demonstrated significant acidosis, coagulopathy, and shock physiology with mean pH 7.21 ± 0.12 , BE -8.8 ± 4.98 mEq/L, lactate 9.60 ± 3.10 mmol/L, Hct $16.4 \pm 3.17\%$, PT 15.5 ± 1.97 seconds, PTT 20.3 ± 5.77 seconds, fibrinogen 111.8 ± 34.49 mg/dL, and platelets $306.1 \pm 76.99 \times 10^3/\mu\text{L}$.

At the first hour of resuscitation following model creation, control animals demonstrated a clinically and statistically significant consumption of fibrinogen 118.4 ± 32.3 vs. 195.8 ± 41.6 mg/dL ($p=0.011$) and platelets 340.6 ± 49.7 vs. $461.6 \pm 98.9 \times 10^3/\mu\text{L}$ ($p=0.04$). From the fourth hour of resuscitation to the sixth hour of resuscitation, the five control animals demonstrated a non-significant worsening of their measures of coagulopathy. This was manifest by PT 15.9 ± 1.34 vs. 15.1 ± 1.3 seconds, PTT 23.4 ± 3.44 vs. 26.4 ± 5.8 seconds, fibrinogen 117.8 ± 30.0 vs. 122.6 ± 30.7 mg/dL, and platelets 334.6 ± 39.8 vs. $360.6 \pm 50.5 \times 10^3/\mu\text{L}$ (all $p>0.5$).

Model creation resulted in clinically and statistically significant coagulopathy and consumption with PT 15.7 ± 3.1 vs. 13.0 ± 0.5 seconds, fibrinogen 94.1 ± 25.6 vs. 173.2 ± 12.0 mg/dL, and platelets 285.6 ± 111.2 vs. $366.9 \pm 124.3 \times 10^3/\mu\text{L}$ (all $p<0.05$). Six of nine animals (66.7%) receiving 3PCC developed a laboratory profile consistent with a severe CC following 3PCC administration. Coagulation studies at the 5-hour mark (1 hour after 3PCC administration) when compared with their 4-hour values revealed a worsening coagulopathy with significant worsening of PT 40.3 ± 33.4 vs. 16.1 ± 3.0 seconds, fibrinogen 66.6 ± 30.5 vs. 92.3 ± 25.6 mg/dL, and circulating platelet volume 170.0 ± 109.6 vs. $301.7 \pm 106.4 \times 10^3/\mu\text{L}$ (all $p<0.05$). This coagulopathy continued to worsen through study completion, with 6-hour labs also

demonstrating significantly deranged PTT 148.6±87.8 vs. 51.7±58.2 seconds (p=0.019). The 6-hour coagulation studies with PT, PTT, fibrinogen, and platelet count were all significantly worse than controls both clinically and statistically.

For the 4PCC arm, model creation generated clinically and statistically significant coagulopathy and consumption with PT 14.9±1.3 vs. 13.3±0.5 seconds and fibrinogen 111.9±34.3 vs. 187.5±54.1 mg/dL (both p<0.05). Laboratory findings consistent with CC developed in two of eight animals (25%) following 4PCC administration. In these animals, 4PCC therapy resulted in no improvement of coagulopathy at 5 hours. At completion of the sixth hour of resuscitation, when compared with control animals, they demonstrated significant depletion of coagulation substrates with fibrinogen 70.1±30.5 vs. 117.8±30.0 mg/dL and platelets 198.8±75.9 vs. 334.6±39.8 x 10³/μL (both p<0.05). Overall their 6-hour coagulation parameters were superior to those of 3PCC: PT 21.9±14.6 vs. 63.4±43.6 seconds, PTT 21.8±7.2 vs. 148.6±87.8 seconds, and platelets 198.8±75.9 vs. 108.0±83.7 x 10³/μL (all p<0.05).

We then examined the combination of 4PCC + two units of autologous plasma. The model generated clinically and statistically significant coagulopathy and consumption of substrate with PT 15.0±0.9 vs. 13.9±0.3 seconds and fibrinogen 127.6±22.6 vs. 201.6±33.5 mg/dL (both p<0.05). The transfusion of two units of autologous plasma prior to 4PCC administration appeared to stabilize or provide transient improvement in the coagulopathy. Two of the eight animals receiving 4PCC and plasma developed laboratory parameters consistent with CC. Administration of 4PCC resulted in no improvement of coagulopathy at 5 hours, and at completion of the sixth hour of resuscitation, when compared with control animals, they demonstrated significant depletion of circulating platelet volume 186.8±102.3 vs. 334.6±39.8 x 10³/μL (p=0.011).

Lacking consensus criteria for CC in swine, the development of this condition was determined by adapting human laboratory criteria that would equate with disseminated intravascular coagulation (DIC) even with unknown fibrin split product levels: a fibrinogen ≤60 mg/dL, a ≥50% decrease in platelet count, and a PT ≥25 seconds (corresponding with an INR of ≥2.5) [25]. In this study, the administration of PCC resulted in 67% of 3PCC animals and 25% of 4PCC animals developing CC profile consistent with DIC. Despite the addition of autologous plasma transfusion, 25% of these animals receiving 4PCC developed DIC as well. When compared with those animals without CC, the animals developing this syndrome had demonstrated physiologic and laboratory parameters consistent with the classic “lethal triad” prior to receiving PCC. This was manifested with significantly lower temperatures (36.3 vs. 37.8°C, p=0.007), greater degrees of acidosis (pH 7.14 vs. 7.27, p=0.006; BE -12.1 vs. -6.5 mEq/L, p=0.004), and coagulopathy (PT 17.1 vs. 14.6 seconds, p=0.001; fibrinogen 87.9 vs. 124.1 mg/dL, p=0.009) (Table 2). In the animals that did not develop CC, the administration of both 3PCC and 4PCC improved levels of individual clotting factors and transiently improved PT at the 5-hour time point. However, despite the improvement in individual factors, there was a significant depletion of fibrinogen and platelets without a lasting improvement of coagulopathy by the end of the 6-hour resuscitative phase (Table 2).

Table 2. Comparison of Laboratory Data of Resuscitation and Coagulation with Mean $\pm$ SD Between Animals With or Without Development of Consumptive Coagulopathy

Variable	Control	Non-CC	CC
Baseline			
Temperature (°C)	37.8 $\pm$ 0.9	37.4 $\pm$ 0.8	37.6 $\pm$ 0.9
pH	7.51 $\pm$ 0.04	7.55 $\pm$ 0.09 ^a	7.45 $\pm$ 0.09 ^b
BE (mEq/L)	9.6 $\pm$ 1.14	10.7 $\pm$ 4.87	7.3 $\pm$ 5.06
Lactate (mmol/L)	1.68 $\pm$ 0.34	1.91 $\pm$ 0.67 ^a	3.45 $\pm$ 2.19 ^b
PT (s)	13.6 $\pm$ 0.63	13.3 $\pm$ 0.56	13.5 $\pm$ 0.60
EXTEM MCF (mm)	75.2 $\pm$ 3.96	74.1 $\pm$ 3.48	73.5 $\pm$ 2.51
PTT (s)	31.4 $\pm$ 6.11	23.4 $\pm$ 5.97 ^c	25.0 $\pm$ 10.30
Fibrinogen (mg/dL)	195.8 $\pm$ 41.55	191.3 $\pm$ 42.68	180.2 $\pm$ 27.22
FIBTEM MCF (mm)	46.6 $\pm$ 9.84	47.2 $\pm$ 7.46	57.2 $\pm$ 12.46 ^b
Platelets (10 ³ /μL)	461.6 $\pm$ 98.92	348.1 $\pm$ 83.55 ^c	380.7 $\pm$ 114.93
4 Hours Resuscitation			
Temperature (°C)	37.6 $\pm$ 0.4	37.8 $\pm$ 0.8 ^a	36.3 $\pm$ 1.6 ^b
pH	7.20 $\pm$ 0.12	7.27 $\pm$ 0.13 ^a	7.14 $\pm$ 0.05 ^b
BE (mEq/L)	-9.0 $\pm$ 5.24	-6.5 $\pm$ 5.18 ^a	-12.1 $\pm$ 2.28 ^b
Lactate (mmol/L)	9.31 $\pm$ 3.01	8.79 $\pm$ 3.21	10.97 $\pm$ 2.75
PT (s)	15.1 $\pm$ 1.27	14.6 $\pm$ 0.91 ^a	17.1 $\pm$ 2.49 ^b
EXTEM MCF (mm)	70.8 $\pm$ 3.90	71.5 $\pm$ 3.91	66.4 $\pm$ 5.17 ^b
PTT (s)	26.4 $\pm$ 5.81	19.1 $\pm$ 4.91 ^c	19.1 $\pm$ 5.47 ^c
Fibrinogen (mg/dL)	122.6 $\pm$ 30.71	124.1 $\pm$ 32.25 ^a	87.9 $\pm$ 22.98 ^{b,c}
FIBTEM MCF (mm)	30.2 $\pm$ 12.68	35.4 $\pm$ 8.12	28.7 $\pm$ 21.67

^ap<0.05 vs. DIC.

^bp<0.05 vs. non-DIC.

^cp<0.05 vs. control.

In analyzing the effect of fibrinogen at the time of PCC delivery, a normal range of fibrinogen levels was determined (using the baseline serum fibrinogen levels of 66 genetically similar Yorkshire swine) to create stepwise cut-offs corresponding to those utilized in previous retrospective human studies [26]. In the “critical” group (<77 mg/dL), 100% (3 of 3) of the animals developed laboratory parameters consistent with CC following administration of PCC. In the “abnormal” group (77-139 mg/dL), 41.2% (7 of 17) of the animals developed CC. No animals (0 of 5) developed a CC in the “normal” group with pre-administration fibrinogen levels >139 mg/dL (Figure 7).

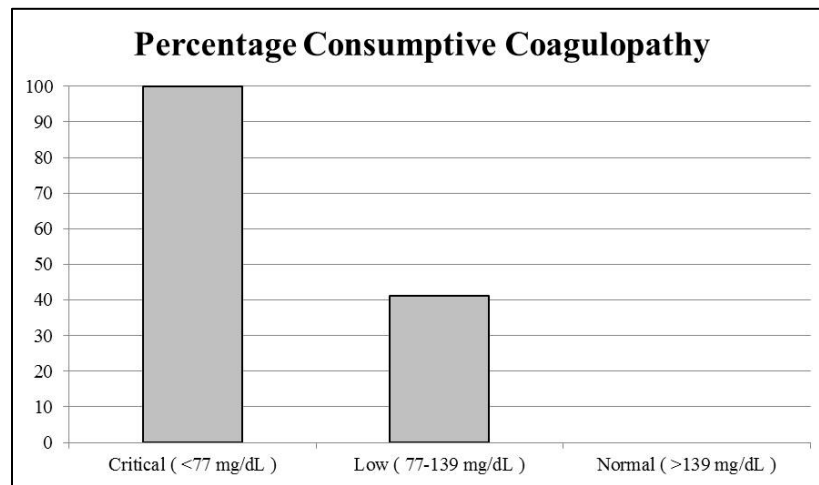


Figure 7. Development of CC by fibrinogen level.

4.3.2 Comparison of 3-Factor vs. 4-Factor PCC Discussion. While PCC products have demonstrated promising efficacy and acceptable safety in the correction of inborn coagulopathy and reversal of oral anticoagulants, *in vitro* studies have revealed poor improvement of coagulopathy in the setting of shock with severe systemic acidosis [12]. Given this finding and the lack of previous safety and efficacy data, we sought to test the effects of PCC under conditions replicating a hemorrhagic shock-associated coagulopathy with the presence of severe metabolic acidosis. This constellation of acidosis, hypothermia, and coagulopathy is a feared syndrome in the fields of surgery and critical care and dictates that as patients become colder and more acidotic, their probability of developing coagulopathy and their risk of mortality increase. Moreover, it recognizes that not only does acidosis or hypothermia worsen clot formation individually, but their effects are also additive [3]. In addition, we aimed to compare and contrast the effects of administration of the 3PCC product compared with the newer 4PCC product.

Although the exact compositions of coagulation factors and inhibitors vary by manufacturer, PCCs are standardized by their concentration of factor IX and can be broadly grouped into 3-factor or 4-factor formulations: 3PCC contains significant concentrations of factors II, IX, and X as well as low levels of factor VII, and 4PCC contains significant concentrations of all vitamin K-dependent clotting factors (II, VII, IX, and X). Data comparing 3PCC and 4PCC are limited, yet retrospective studies reveal that 4PCC is also more effective than 3PCC at reversing the effects of warfarin, specifically when the INR >4 [28,29]. Additionally, 4PCC has been found to be more cost effective than 3PCC in reversing oral anticoagulant effects in bleeding patients [30].

Given the effectiveness and acceptable safety profile of PCC in correcting inborn and iatrogenic coagulopathies – as well as shelf-stable packaging, low volume, and easy preparation for administration – PCCs have been used in Europe in laboratory-guided “factor-based” resuscitations and are now proposed as a possible substitute to FFP in the treatment of trauma-associated coagulopathy [19,21]. Beyond potential logistical benefits of a “factor-based” approach, PCCs have been shown to be superior to recombinant activated factor VII for the reversal of coagulopathy in porcine shock models, possibly due to PCC containing all of the factors in the extrinsic pathway [31]. However, several concerns in the use of PCCs have arisen,

including reported cases of fatal thrombosis, CCs, and disseminated intravascular coagulation in both laboratory models and in clinical use. Factor II, or prothrombin, is currently believed to be the driver of consumptive or thrombotic complications.

Although used in the setting of traumatic injury to reverse anticoagulation, PCCs have not been trialed in acidosis, which is a major component of traumatic-induced coagulopathy. The acidosis associated with this coagulopathy is known to affect endogenous and exogenously administered coagulation factors, and this has been identified as an area for potential use of PCC. *In vivo* evaluations of 3PCC and 4PCC have not been performed in the setting of acidosis, nor have they been compared in this setting. Due to the vitamin K-dependent factor's ultimate dependence upon fibrinogen, PCC is only administered in severely injured patients with the combined delivery of FFP or a fibrinogen concentrate or following determination of normal fibrinogen levels. As a result, data are lacking for the use of PCC in isolation to reverse trauma-induced coagulopathy, especially in the setting of acidosis and hypofibrinogenemia. As the building block of fibrin and final step of the coagulation cascade that all resuscitative strategies are attempting to improve, the relationship of fibrinogen and trauma-induced coagulopathy has been described. Retrospective clinical evaluations of fibrinogen's role in massive transfusions following trauma describe a steep, stepwise increase in mortality as fibrinogen levels decrease [32]. The groups were stratified based on admission fibrinogen concentration with cut-offs of "normal" (>180 mg/dL), "abnormal" (101-180 mg/dL), and "critical" (<100 mg/dL).

In this study, a CC developed in two-thirds of the animals receiving 3PCC and one-quarter of the animals receiving 4PCC (with or without autologous plasma transfusion). This is especially notable because none of the control animals developed a CC, despite equivalent mean physiologic derangements at the fourth hour of resuscitation. The development of CC in the 4PCC animals that received autologous plasma further suggests that there may be thresholds (of increasing levels of physiologic derangements) to the development of this condition and that previous administration of plasma may not be protective. The addition of a profound metabolic acidosis utilizing a single dosing strategy in our model suggests that this physiologic derangement may be associated with the increased risk of CC, or DIC had we assessed for and demonstrated microthrombi or elevated fibrin split products, when PCC is administered in these conditions. Therefore, caution should be used when attempting to extrapolate previous studies documenting the safe use of PCC in traumatically injured, but physiologically healthy, patients.

We found that significantly more animals receiving 3PCC developed a CC compared to 4PCC (67% vs. 25%, $p < 0.05$). As such, while careful consideration must be given to the use of 4PCC in the setting of acidosis and trauma-induced coagulopathy, the results suggest the use of a 3PCC should especially be cautioned. This difference may highlight the importance of factor VII in the treatment of trauma-associated coagulopathy, as the 3PCC solutions lack significant concentrations of factor VII. Other authors have reported improved coagulopathy reversal when recombinant factor VII is co-administered with 3PCC vs. 4PCC alone for warfarin-induced coagulopathy in the traumatically injured patient.

While the clinical relevance of PCC remains undisputed in the reversal of medication-induced coagulopathy and possibly in the early post-injury setting, prior to the development of severe physiologic derangement, this study suggests the need for caution and further investigation of the potentially detrimental effects of PCC when administered in a state of severe acidosis. Our findings suggest that while these agents may offer transient improvements in the levels of individual clotting factors, without further treatment these effects are limited, and often progressively worsen. The development of a CC when PCC is administered in the setting of

severe acidosis is highly concerning and may suggest that PCC contributes uniquely to this process. Our results also highlight the importance of adequate thrombogenic substrate, namely, a baseline fibrinogen level that is not significantly depleted when administering PCC in the setting of acidosis and coagulopathy. Finally, these results raise significant concerns about the administration of the 3PCC product in this setting, which appears to carry a higher risk of inducing a pathologic CC compared to the 4PCC product.

4.4 Comparison of Pharmacodynamics and Efficacy of TXA Administered via the IO vs. IV Routes

4.4.1 IO vs. IV TXA Study Results. Eighteen animals were used to conduct this study and were randomized to one of two groups: IV (n=9) or IO (n=9). Baseline hemodynamic (MAP, heart rate, cardiac index) and laboratory (pH, lactate, BE, INR) parameters prior to drug delivery were similar between the two groups (Table 3). After giving TPA, all animals became hyperfibrinolytic (Table 3 and Figure 8).

Table 3. Baseline Physiologic and Hematologic Data by Study Group

Variable	IV TXA (n=8)	IO TXA (n=8)	p-Value
<i>Physiologic Parameters</i>			
MAP, mmHg	48±4.4	49.5±4.7	0.16
Heart Rate, bpm	144±22.1	140±21.6	0.34
Cardiac Index	4.87±0.81	4.77±1.06	0.42
<i>Hematologic Data</i>			
pH	7.20±0.05	7.22±0.05	0.14
Lactate, mmol/L	11.1±1.33	10.8±0.96	0.10
BE, mEq/L	-9.1±2.67	-8.3±2.17	0.27
INR	1.6±0.12	1.6±0.18	0.37
<i>ROTEM Data after TPA</i>			
EXTEM Lysis Index 30 min	0.89±1.36	0.22±0.44	0.11
INTEM Lysis Index 30 min	0.44±0.72	0.11±0.33	0.14

At the time of randomization to either the IV or IO arms of the experiments, all animals demonstrated shock physiology and there were no differences in the measured baseline characteristics of MAP, pH, lactate, BE, or INR. The mean baseline MAP was 48 mmHg in the IV arm and 49.5 mmHg for the IV arm (p=0.16). The starting pH for the IV group was 7.20 and 7.22 for the IO group (p=0.14). There were also no differences in the baseline lactate, BE, or INR of IV and IO arms (lactate 11.3 vs. 10.8 mmol/L, p=0.10; BE -9.1 vs. -8.3 mEq/L, p=0.37; and INR 1.6 vs. 1.6, p=0.37).

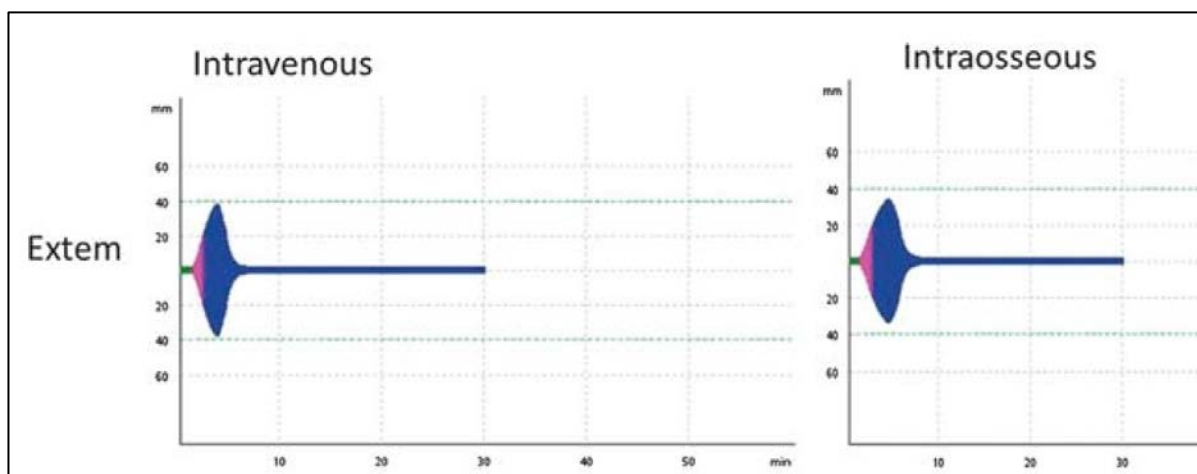


Figure 8. ROTEM demonstrating hyperfibrinolysis after TPA given in both groups.

Serum TXA concentrations were measured immediately before infusion of the drug and then up to 240 minutes after the completion of the infusion. In both the IV and the IO infusion groups, there was an initial spike in serum concentrations that peaked at the 10-minute post TXA infusion lab draw and then slowly tapered off throughout the 4-hour monitoring phase of the experiment. The mean peak concentration in the IV group was 160.9 $\mu\text{g/mL}$ and 132.57 $\mu\text{g/mL}$ in the IO group ($p=0.053$). At the end of the 4-hour monitoring phase, the serum concentrations of TXA were 20.59 $\mu\text{g/mL}$ and 23.53 $\mu\text{g/mL}$ in the IV and IO groups, respectively. There were no statistically significant differences in serum drug concentrations between IV and IO infusions at any of the measured time points (Figure 9).

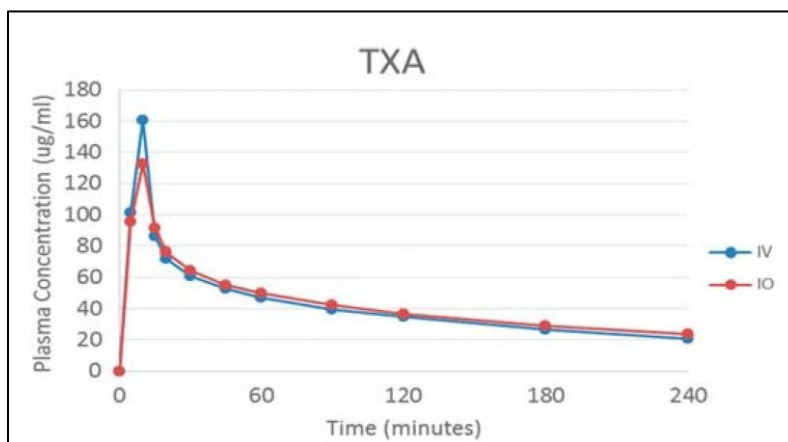


Figure 9. Plasma TXA concentration curves for IV and IO TXA groups.

As previously discussed, all animals became hyperfibrinolytic after being given the 100-mg bolus of TPA with EXTEM lysis index at 30 minutes of 0.88 and 0.22 in the IV and IO groups ($p=0.11$); the INTEM lysis index at 30 minutes in the IV and IO groups was 0.44 and 0.11 ($p=0.14$). In both the IV and the IO groups, the EXTEM and INTEM lysis indices at 30 minutes corrected with the administration of TXA. In the EXTEM analysis, the lysis index at 30 minutes in the IV group corrected to 99.8 from 0.88 and to 100 from 0.22 in the IO group (Figure 10). Similar results were seen when analyzing the INTEM. The lysis index at 30 minutes corrected from a mean of 0.44 to a mean of 98.4 in the IV group and from a mean of 0.11 to a mean of 98.3 in the IO group ($p=0.44$).

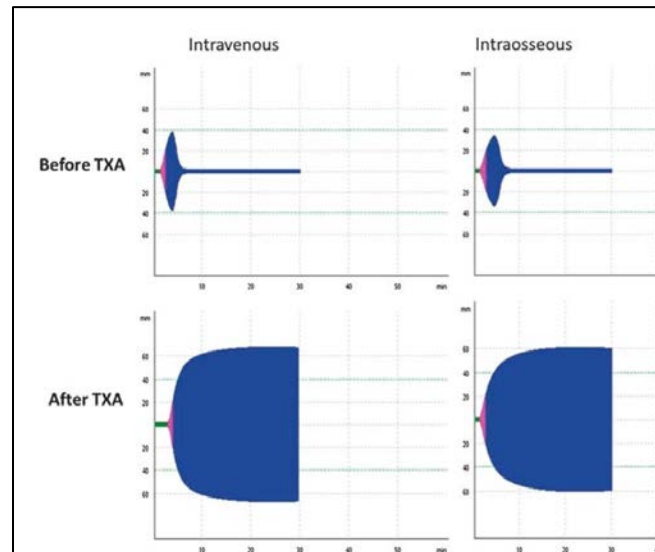


Figure 10. Change in ROTEM with TXA administration in IV and IO groups.

4.4.2 IO vs. IV TXA Study Discussion. Both U.S. and international military forces have successfully used IO access for infusions in combat zones, and the techniques are used in both adult and pediatric civilian trauma patients. IO access and infusion have become particularly important in the modern military setting due to the change in the predominant mechanism of wounding from standard projectiles to improvised explosive devices. The resultant massive and often multi-system blast injury pattern typically includes multiple mangled or amputated extremities, which makes standard peripheral IV access much more difficult or even impossible. The worst of these types of injuries, now commonly referred to as the “dismounted complex blast injury,” carries an exceedingly high risk of massive hemorrhage and massive transfusion [33]. The current tactical combat casualty care doctrine emphasizing early hemorrhage control with tourniquets and hemostatic dressings and liberal use of IO access (typically sternal or humeral) has resulted in a significant improvement in battlefield survival, but also a heavier reliance on IO infusion [34,35]. Therefore, it is critically important to have both clinical and animal data validating the safety and efficacy of fluids or therapeutics administered via this route.

The updated JTTS CPG on massive transfusion codified TXA administration as a theater-wide best practice for both Iraq and Afghanistan. Among patients who do not have immediate IV access, TXA has anecdotally been delivered via an IO route, but without any existing data validating a similar systemic absorption, duration of action, or efficacy for reversing

hyperfibrinolysis. In recognition of the lack of data validating the pharmacodynamics and therapeutic efficacy of IO TXA infusion compared to the IV route, a 2013 report of a Department of Defense scientific panel identified this as one of the key “gaps” in research for TXA [27]. In response to this well-identified gap and our lab’s interest in TXA, we designed this study, which is the first to our knowledge that examines the efficacy and pharmacokinetics of TXA when administered via IO infusion in a hemorrhagic shock model. By studying the profile of TXA administered through the IO route, these data provide much-needed scientific evidence to support its safety and efficacy and allay concerns of a marked alteration of the well-described profile seen with IV administration. In addition, these data provide support (from a pharmacologic standpoint) for the use of TXA in various civilian and military out-of-hospital settings where the need for IO infusion would likely be much greater.

When looking at our data, the peak serum concentration of the IV group trended toward statistical significance; however, it did not reach it. There are likely two mechanisms that contribute toward this finding. One is intrinsic to the protocol and likely, in part, due to the infusion sites chosen. We compared a central venous (right internal jugular) injection with the most peripheral IO site (tibial plateau) accessible in our model. It is likely that had we compared serum concentrations of TXA after infusion through a tibial IO with that of a peripheral IV this trend toward significance would decrease. It is also possible that if the IO injection site were more centrally located (humeral or sternal), this gap would decrease even more. The second reason that would potentially be applicable to a hemorrhaging trauma patient is that in a hypovolemic shock state the blood flow to the bone decreases, potentially allowing less of the drug to be absorbed. While the peak concentration trended toward significance, from a functional standpoint it does not seem to matter. When given through an IO, the serum concentration of the drug is high enough to reverse hyperfibrinolysis, even when in a hypovolemic shock state.

While the potential battlefield applications of the IO administration of TXA, particularly among blast-injured or multiple-amputation patients, are clear, we believe this also has significant civilian trauma applications. There are multiple scenarios where IV access, particularly early in the evaluation and resuscitation process, may be difficult or significantly delayed and an IO is required in the meantime [36,37]. Known factors such as obesity, advanced age, and prior IV drug use may complicate attempts at IV access, and in many protocols the next option for immediate access is placement of an IO line. Another obvious civilian application comes in the setting of pediatric trauma, where IO infusion is more commonly required due to the difficulties of IV access in smaller children or babies.

In conclusion, we feel that the IO route of infusion is a reliable way to reach effective plasma levels of TXA administered via a single bolus dose. There are clear implications of this finding to tactical combat casualty care where it could be utilized immediately by advanced providers and combat medics who find themselves caring for casualties in austere environments. We also feel that there is an obvious application to the civilian trauma setting, particularly in any setting where IV access can be exceptionally difficult, or in rural/austere settings where transport to a major trauma center and establishment of reliable IV access may significantly delay the administration of TXA beyond the currently accepted 3-hour window of early administration.

4.5 Evaluation of Plasma Co-Administration vs. TXA and PCC Administration Alone or in Combination in Severe Traumatic Coagulopathy

4.5.1 Plasma Co-Administration vs. TXA/PCC Alone Results. Baseline values for hemodynamics, laboratory values, and ROTEM measures were relatively similar between study groups, and the majority fell within normal range. There was variation noted on ANOVA for the following baseline variables: PAWP ($p=0.006$), Hct ($p=0.009$), creatinine ($p<0.001$), INR ($p<0.001$), activated PTT ($p<0.001$), fibrinogen ($p=0.036$), platelets ($p=0.019$), and ML ($p<0.001$). To statistically correct for baseline differences, the remaining comparisons were completed using multivariate linear regression analysis and mean differences between time points. Hemorrhagic shock physiology was induced in all 60 animals. This is evidenced by mean values measured at 1 hour after cross-clamp release showing hypotension (MAP 44 mmHg), tachycardia (heart rate 145 bpm), acidosis (pH 7.18, lactate 10.6 mmol/L), anemia (Hct 16.6%), and coagulopathy (fibrinogen 106.5 mg/dL). All animals survived to the 4-hour time point.

On analysis of the lab values at the 1-hour time point, notable differences between treatment groups were seen for MAP ($p=0.02$), PAWP ($p=0.03$), lactate ($p=0.008$), creatinine ($p=0.006$), PTT (0.001), fibrinogen ($p=0.02$), and ML ($p<0.0001$). Post hoc analysis found that MAP was lower in TXA+PCC (41 mmHg) when compared to 49 mmHg with TXA+FFP ($p=0.03$), which tightly correlated to and accounted for significant differences in PAWP between the same two groups. Lactate was higher in PCC (11.8 mmol/L) vs. FFP+PCC (10.1 mmol/L, $p=0.03$). Creatinine was found to be significantly higher with FFP+PCC (1.9 mg/dL) when compared to PCC (1.4 mg/dL), TXA (1.5 mg/dL), and TXA+FFP (1.6 mg/dL), with p -values of 0.005, 0.02, and 0.01, respectively. Fibrinogen was higher in FFP+PCC at 128 mg/dL compared to TXA at 89 mg/dL (0.01 mg/dL).

Mean values are shown in Table 3 with correlating significance seen based on regression analysis. Values reviewed on post hoc included MAP ($p=0.003$), PAWP ($p=0.004$), PTT ($p=0.0001$), fibrinogen ($p=0.009$), platelets ($p=0.03$), ML ($p<0.0001$), CT ($p=0.03$), and MCF ($p=0.02$). The only significant difference found related to resuscitation fluids was the PCC alone group required over 11 liters of crystalloid on average, which was significantly higher than all other groups on post hoc analysis ($p<0.01$).

Post hoc analysis revealed significant differences between TXA+FFP for MAP (60 mmHg) compared to PCC and PCC+FFP at 46 mmHg and 47 mmHg, respectively ($p=0.01$, 0.02). PTT was significantly elevated in the TXA+FFP group with a value of 33 seconds compared to PCC (18 seconds), TXA (24 seconds), and PCC+FFP (22 seconds), all with p -values of <0.001 , and TXA+PCC at 24 seconds ($p=0.006$). PTT for the FFP group was higher than when PCC was given alone ($p=0.01$). Worse fibrinolysis was seen with TXA alone, with fibrinogen levels of 89 mg/dL compared to PCC+FFP at 134 mg/dL ($p=0.02$). ROTEM values showed longer CT with PCC compared to TXA+FFP at 72 seconds vs. 54 seconds ($p=0.007$). MCF was larger with TXA+PCC at 75 mm than TXA alone at 68 mm ($p=0.02$) (see Figure 11).

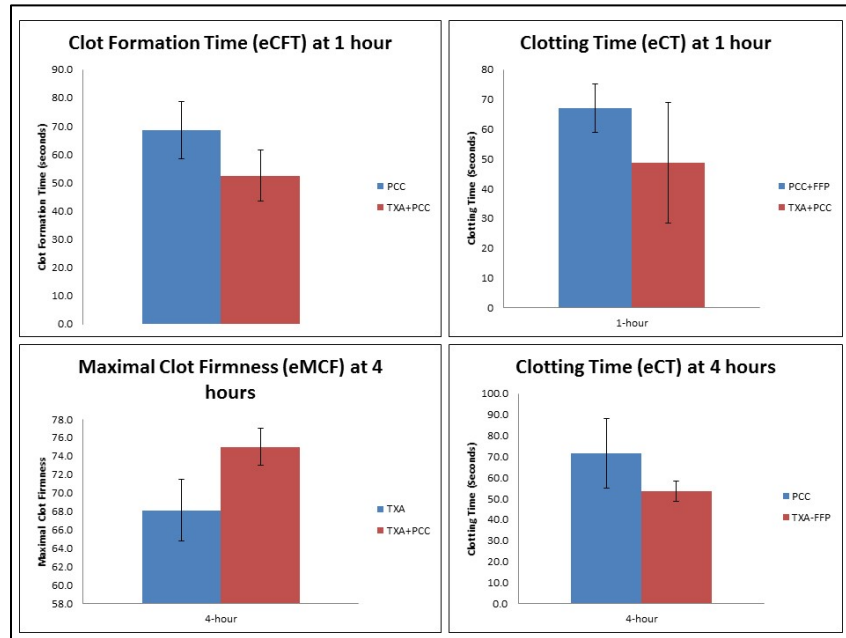


Figure 11. ROTEM values with significant differences between study groups.

ROTEM values associated with hyperfibrinolysis were evaluated independently. The change in ML was significantly raised in the PCC and TXA groups at both 1 and 4 hours. This was significant when comparing to all other treatment groups (Table 4). PCC showed an ML of 6.4% and 6.1% at 1 and 4 hours, respectively, and post hoc found p-values all <0.0001 when compared to all groups except TXA alone. TXA also was significantly higher with ML of 4.6% and 3.9% at 1 and 4 hours, respectively, and this was significantly higher when compared to all treatment groups at all p-values <0.02.

Table 4. Lab and ROTEM Values at 4 Hours

Variable	Control	PCC	TXA	FFP	TXA+FFP	PCC+FFP	TXA+PCC	p-Value
Labs								
pH	7.20±0.12	7.23±0.16	7.25±0.17	7.28±0.16	7.34±0.09	7.24±0.13	7.26±0.09	0.61
BE, mEq/L	-9.0±0.52	-8.9±5.6	-5.2±5.5	-4.0±6.0	-1.5±4.4	-7.1±5.9	-5.8±4.8	0.13
Lactate, mmol/L	9.3±3.0	10.1±3.1	7.8±2.9	6.5±2.1	5.6±2.8	7.6±2.6	6.9±3.4	0.13
Hct, %	18.4±4.4	15.3±2.6	16.2±3.6	17.2±2.6	18.0±2.7	16.8±1.2	17.2±2.7	0.35
Creatinine, mg/dL	2.0±0.3	1.7±0.3	1.8±0.4	1.8±0.4	1.7±0.2	2.2±0.4	1.8±0.6	0.15
INR	1.2±0.2	1.3±0.2	1.3±0.2	1.4±0.2	1.2±0.1	1.3±0.2	1.3±0.1	0.45
PTT, s	26.3±5.2	17.5±4.1	24.2±4.6	26.3±4.0	33.1±6.1	22.4±3.8	24.0±4.2	0.0001
Fibrinogen, mg/dL	122.6±30.7	104.6±42.9	89.8±18.1	113.8±17.3	125.0±16.5	134.1±24.5	83.7±40.6	0.009
Platelet	360.6±50.5	287.4±49.0	265.0±46.4	312.6±36.2	267.9±27.9	295.9±71.4	280.4±62.3	0.03
TEG								
CT, s	67.8±10.2	71.6±16.6	61.9±10.3	63.8±6.7	53.5±4.7	63.6±10.5	63.0±3.6	0.03
CFT, s	56.8±9.2	70.1±19.5	66.0±11.1	51.8±6.7	56.8±6.5	57.5±8.8	58.0±12.1	0.10
MCF, mm	72.3±5.1	69.6±4.4	68.1±3.3	73.3±2.8	72.1±2.4	70.8±4.1	75.0±2.0	0.03
ML, %	0.0±0.0	6.1±4.3	3.9±2.1	0.0±0.0	0.2±0.4	0.5±1.1	0.2±0.4	0.0001

Percent change between 1 and 4 hours demonstrated that PCC had an increased INR by 17% vs. a correcting INR for TXA+FFP (1.28 to 1.17, 10%) and FFP (1.3 to 1.25, 10%; p=0.001). Fibrinogen levels significantly increased from 1 to 4 hours for PCC+FFP (128 to 134 mg/dL, 4%) over TXA, which remained unchanged (0%; p=0.02).

Since FFP containing (FFPc) groups globally displayed better metrics, we investigated comparisons between FFPc groups (FFP alone, FFP+PCC, FFP+TXA) and compared these to non-FFP containing groups (nFFP) (TXA alone, PCC alone, TXA+PCC) in aggregate. FFPc had lower lactate levels than nFFP at 1 hour (10.2 vs. 11.3 mmol/L, $p=0.004$) and 4 hours (6.6 vs. 8.3 mmol/L, $p=0.039$). Both FFPc and nFFP groups had fibrinogen levels of 176 mg/dL at baseline. At 1 hour, both FFPc and nFFP fibrinogen levels dropped to 110.8 and 100.1 mg/dL ($p=0.116$), respectively. However, by the 4-hour time point, the fibrinogen levels of FFPc rebounded while nFFP continued to decrease (126.3 vs. 94.8 mg/dL, $p<0.001$) as shown in Figure 12.

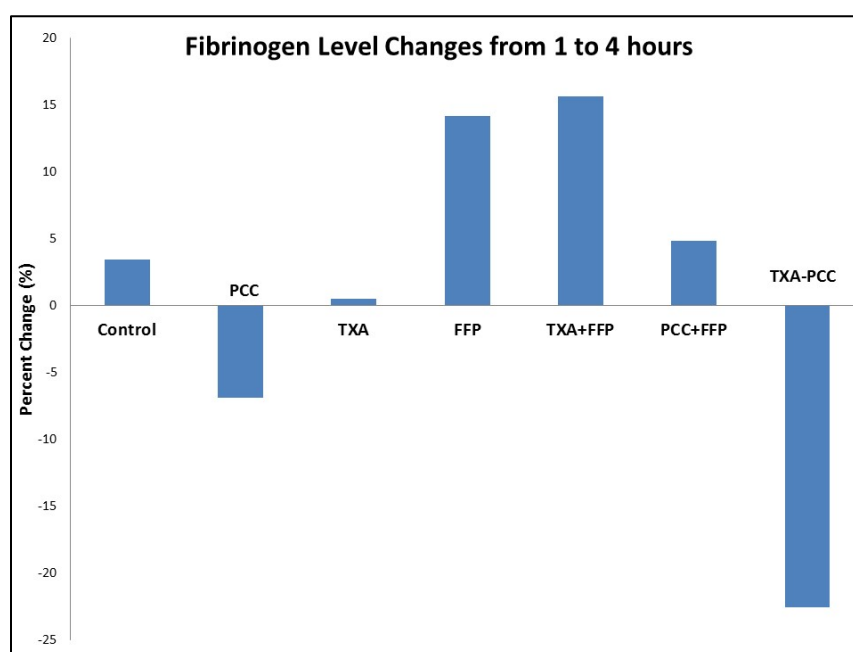


Figure 12. Dynamic changes in fibrinogen levels by group.

4.5.2 Plasma Co-Administration vs. TXA/PCC Alone Discussion. There has been significant efforts in both the military and civilian trauma communities to develop strategies and products that can be pushed further forward, even to the point of injury, to attempt to prolong survival and prevent or lessen ACoTS. This has included programs to carry blood products on medical evacuation transport platforms, to develop more portable and field-ready component products such as freeze-dried plasma, and to utilize pharmacologic products such as TXA and PCC as earlier resuscitation components [38]. Of most significance to this study, and of particular concern to the authors, are the efforts to push these pharmacologic adjuncts or factor concentrate products further forward into settings where they would likely be administered alone or in combination to patients in hemorrhagic shock, but without immediate co-administration of either whole blood or adequate component therapy. These patients may be delayed for several hours or even 12-24 hours in austere military environments, during which time they would likely receive only crystalloid fluid resuscitation and any agent (such as TXA) that is available. This study was designed to address the lack of any controlled animal or human data on the use of these products, and particularly with PCC, in this type of scenario and to evaluate efficacy of this approach.

Various resuscitation regimens were evaluated using pharmaceutical and/or plasma-based methods either alone or in combination with each other using a standardized and validated hemorrhage-induced coagulopathy model. To summarize, hemodynamic profiles of all the animals demonstrated shock over the 4-hour time period. Elements of the “lethal triad” including acidosis and signs of end organ failure were also universally demonstrated in the groups. These parameters were slightly improved in terms of lactic acidosis at 1 hour in groups containing FFP. However, the most profound findings were in relation to coagulopathy. FFPc groups had improved INR and PTT with less severe changes in these values over the 4 hours. Fibrinogen levels were able to correct with plasma containing groups, while fibrinolysis continued to worsen in animals in the arms with the pharmaceutical hemostatic agents administered alone. *In vivo* hemostatic dynamics based on ROTEM values demonstrated that plasma-based regimens were associated with significantly firmer clot strength and more rapid clot formation while preventing significant fibrinolysis. Although not statistically significant, CT was quicker in the TXA and TXA+PCC groups at 1 hour. This, however, was reversed and CTs were actually slower at the 4-hour time period suggesting that the factors made available by FFP ultimately aided in quicker CTs.

PCC has been highly beneficial in the rapid reversal of factor-based, medically induced coagulopathy patients in trauma [22,39]. The vast majority of the literature reviewing the use of PCC is in massively bleeding patients who are known to be on a vitamin K antagonist. Excitement for its use in factor-based resuscitation for ACoTS has been borne out of success and superiority seen when combined with fibrinogen compared to FFP alone. A recent prospective randomized trial out of Austria had to terminate its study early due to increased treatment failure and the need for rescue therapy in patients treated with FFP alone when compared to those treated with a factor-based resuscitation including fibrinogen, PCC, and factor XIII [21]. PCC did show to improve MCF at 4 hours when combined with TXA, suggesting a synergistic effect of PCC when fibrin is available. However, PCC alone does not seem to provide the same collective benefit. Our lab has previously shown that PCC administration results in a CC in ACoTS. We found PCC alone to be inadequate in correcting coagulopathy again in the study. However, when used alone, a worsening fibrinolysis was seen with PCC—a leading cause of ACoTS.

Vascular endothelium and the associated glycocalyx layer have been increasingly recognized in their role in ACoTS [40,41]. This matrix is composed of molecules that ultimately affect inflammation, leukocyte extravasation, and vascular permeability. In ACoTS, these molecules are effectively shed from the endothelium, resulting in increased vascular permeability, which potentiates the effects of coagulopathy and shock physiology. Benefits of FFP likely can be partially attributed to its effect on the endothelium, as it has been shown in rat models to restore the glycocalyx integrity after injury due to hemorrhagic shock. Decreased plasma colloid osmotic pressures are early indicators of traumatic shock and have been shown to be effectively corrected with the administration of FFP [42]. Syndecan-1 is one specific molecule found in the endothelium glycocalyx that displays decreased shedding in response to plasma administration in ACoTS. Research in this arena is ongoing, but it seems clear that we do not fully understand the value of plasma in resuscitation of patients with traumatic shock and that the use of plasma will continue to be a critical component of successful regimens.

Marked enthusiasm exists surrounding the potential for factor-based pharmaceutical regimens to aid in the correction of traumatic coagulopathy. We were unable to demonstrate any benefit of PCC or TXA as single agents or in combination therapy for resuscitation of hemorrhagic shock and correction of ACoTS in our swine model. However, plasma co-administration with these agents appears to provide the best outcomes in the treatment of hemorrhagic shock by improving hypotension, decreasing lactic acidosis, improving coagulopathy, and improving clot formation and strength. Further study, and particularly safety and efficacy data, is warranted before widely deploying any of these pharmacologic or factor concentrate products to more remote settings without immediate access to standard blood products.

4.6 Evaluation of the Efficacy of REBOA in the Setting of Isolated Major Abdominal Venous Injury

4.6.1 REBOA for Abdominal Venous Injury Results. Ten animals were used to conduct this study and were randomized to one of two groups: control (n=5) and REBOA (n=5). Baseline hemodynamic, laboratory, and/or pressure parameters prior to hemorrhage were similar between the two groups (Table 5).

Table 5. Baseline and Post-Intervention Physiologic and Hemodynamic Data

Variable	Control (N=5)	REBOA (N=5)	p-Value
Baseline Physiologic Parameters			
MAP, mmHg	42±5.5	50±6.2	0.106
Heart Rate, bpm	142±35.4	162.8±11.8	0.185
Cardiac Index	4.04±0.83	4.66±0.55	0.213
Mean PAP, mmHg	13.2±3.11	15.8±3.3	0.060
Mean CVP, mmHg	2.8±1.6	1.8±1.6	0.176
Mean PAWP, mmHg	4.6±1.3	4.6±1.1	0.500
Hematologic Data			
pH	7.29±0.08	7.26±0.10	0.233
Lactate, mmol/L	6.19±3.6	6.26±2.23	0.491
BE, mEq/L	-3.2±3.84	-4.6±4.96	0.231
INR	1.2±0.08	1.3±0.24	0.249
MAP after Injury			
T+5, mmHg	19.2±15.36	97.2±37.76	0.040
T+10, mmHg	N/A	65.4±25.32	
T+45, mmHg	N/A	42.0±20.00	
After Balloon Occlusion			
Mean CVP			
T+5, mmHg		2.8±1.30	0.206
T+10, mmHg		2.4±2.30	0.361
T+45, mmHg		2.8±1.50	0.302
Mean PAWP			
T+5, mmHg		4.0±1.87	0.152
T+10, mmHg		3.6±2.19	0.095
T+45, mmHg		3.0±1.41	0.040

PAP = pulmonary artery pressure.

At the time of randomization to either the control or REBOA arms of the experiments, all animals demonstrated shock physiology and there were no differences in the measured baseline characteristics of MAP, mean pulmonary artery pressure, CVP, PAWP, pH, lactate, or INR. The mean baseline MAP was 42 mmHg in the control arm and 50 mmHg for the REBOA arm ($p=0.11$). In regard to baseline measurements taken from the pulmonary artery catheter, mean measurements in the control and REBOA arms were PAP 13.2 and 15.8 mmHg ($p=0.06$), mean CVP 2.8 and 1.8 mmHg ($p=0.18$), and mean PAWP 4.6 and 4.6 mmHg ($p=0.5$). The starting pH for the control group was 7.29 and for the REBOA arm was 7.26 ($p=0.23$). There were also no differences in the baseline lactate or INR of control and experimental arms (lactate 6.19 vs. 6.26 mmol/L, $p=0.49$ and INR 1.2 vs. 1.3, $p=0.25$).

The use of the REBOA in the experimental arm conferred a significant survival advantage (Figure 13). Survival time was defined as time of injury to time of sustained MAP of 20 mmHg or less. Mean survival time in the REBOA arm was 40.1 minutes as compared to the control arm with a mean survival time of 4.1 minutes ($p=0.0008$). The range of survival times in the control arm was from 1.88 minutes to 7.83 minutes.

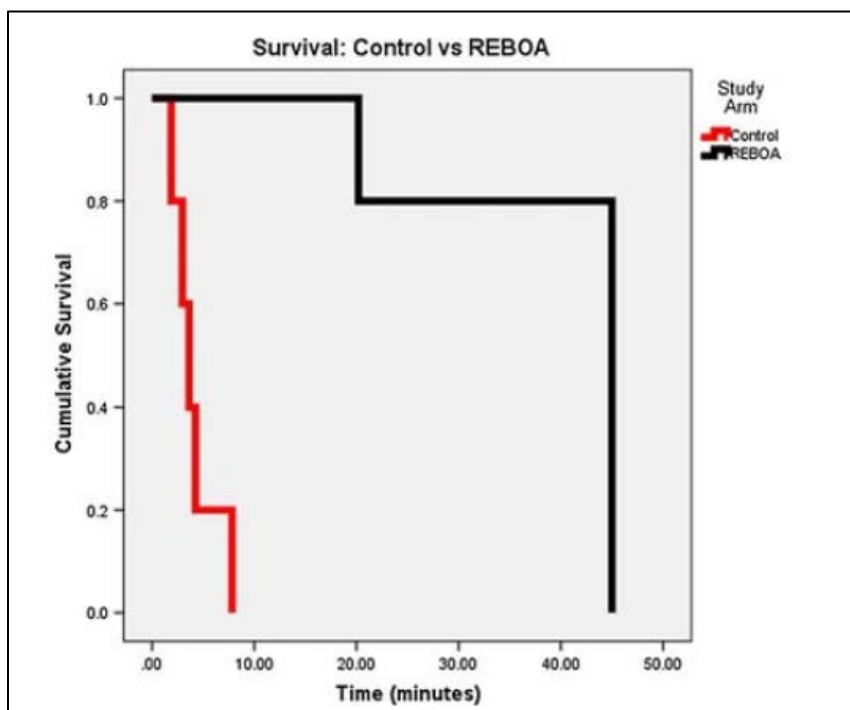


Figure 13. Kaplan-Meier survival curves for REBOA vs. control groups.

After injury, all animals in the control arm became increasingly hypotensive until they reached the experiment endpoint of MAP less than 20 mmHg. In the REBOA arm, the animals had a decrease in their mean MAP from 50.6 mmHg to 45.4 mmHg with the 60-second free bleeding ($p=0.08$). However, with the inflation of the balloon catheter, the animals' MAPs increased to a mean of 97.2 mmHg ($p=0.04$). All of the REBOA animals survived to the 45-minute endpoint except one, where all of the control animals reached a MAP of 20 mmHg well before the 45-minute study period (Figure 13).

There was no difference in baseline mean PAP, CVP, or PAWP between the control and REBOA arms (Table 5). After balloon occlusion was performed, these values were compared to the baseline values in the experimental group. At 5, 10, and 45 minutes after balloon occlusion, the mean PAP in the REBOA arm was 14.6, 12.0, and 14.0 mmHg ($p=0.29, 0.089, 0.29$). At the same time points, the mean CVP was 2.8, 2.4, and 2.75 mmHg ($p=0.21, 0.36, 0.30$). The mean PAWPs were 4.0, 3.6, and 3.0 mmHg ($p=0.15, 0.09, 0.04$).

The blood loss for each group was equal. The mean blood loss in the control group was 630 cc, which was the same for the REBOA group. To quantify this amount over time, we calculated a bleeding rate, which was defined as amount of blood loss divided by the time the animal was able to sustain a MAP over 20 mmHg. In the control group, the bleeding rate was found to be 197.85 cc/min. The bleeding rate for the REBOA animals was significantly lower at 14.02 cc/min ($p=0.02$).

4.6.2 REBOA for Abdominal Venous Injury Discussion. While REBOA has been used in the setting of both blunt and penetrating trauma, the current literature has primarily evaluated it as a means of controlling arterial hemorrhage or solid organ injuries. However, the applicability of these studies to the patient with a major abdominal venous injury with hemorrhage is highly limited and prompted our investigation in this type of injury model.

One concern that has been raised about the use of REBOA in the setting of venous injuries is that occluding aortic outflow may lead to increased pulmonary and CVPs that could potentially lead to increased bleeding [43,44]. While it is likely that the animals in the experimental arm had some continued bleeding from their iliac vein injury, their pulmonary pressures and CVPs were not significantly different from their values at baseline before balloon occlusion. Based on these results, we do not believe that REBOA leads to increased venous bleeding as compared to no intervention. This statement is also supported by the overall blood loss being equivalent between the REBOA and control arm. If the use of REBOA in the setting of a large volume abdominal venous hemorrhage were to lead to increased back bleeding, we would expect a larger volume of blood loss as compared to the control arm, especially in the setting of increased survival time. This is particularly important to appreciate for several reasons. Most importantly is the fact that the exact injury type and source of hemorrhage are almost never known prior to utilizing REBOA in the trauma setting. It is therefore critical that as much pre-clinical data as possible be obtained regarding the impact and efficacy of REBOA in the setting of a wide variety of isolated major hemorrhagic injuries, as well as combined injuries. With the lack of any available literature on the performance of REBOA in the setting of an isolated major abdominal venous injury, such as an iliac vein or vena cava injury, we felt this study was indicated to fill this gap.

Although we theorized that REBOA would prove beneficial even when used in the setting of major abdominal venous injury and hemorrhage, we also considered the possibility that it could be largely ineffective due to lack of direct proximal inflow occlusion, from ongoing non-pulsatile antegrade and retrograde hemorrhage from the vein injury, and from possible elevation of the CVP during zone 1 aortic occlusion. Based on our invasive monitoring, we believe that this fear is likely theoretical, as there was no significant increase in CVPs with balloon occlusion of the aorta; however, we do acknowledge that these results may be slightly skewed by the lack of active resuscitation. A future direction would be to reevaluate pulmonary and CVPs in a similar setting but with the addition of active resuscitation to more closely mimic clinical practice. Our results demonstrated a very clear advantage of zone 1 REBOA deployment in this

injury model in terms of central hemodynamics, survival time, and overall survival to the study endpoint.

An additional important consideration in the utility and in the overall risk:benefit ratio of REBOA is the location of placement and balloon deployment. The current widely accepted practice is to attempt to place and inflate the balloon in the descending thoracic aorta (zone 1) for abdominal hemorrhage of unknown source or for a known source above the level of the pelvis and to place in the distal abdominal aorta (zone 3) for cases of proven or suspected isolated pelvic hemorrhage [45]. This distinction is important primarily because the adverse effects and morbidity due to truncal ischemia and reperfusion injury are significantly greater for zone 1 placement vs. zone 3. In our study, we chose to place the balloon in zone 1 of the aorta, even in the setting of a known proximal iliac vein injury. Data do suggest that zone 3 placement is able to offer a period of stabilization for transfer to definitive management in the setting of pelvic hemorrhage; however, we chose to operate under the assumption that this technology would be used in the setting of intra-abdominal hemorrhage from an unknown source and therefore would better reflect clinical reality where the balloon would likely be positioned in zone 1.

This study demonstrates the efficacy of REBOA in the setting of a large, truncal venous injury and shock. We found that REBOA was effective at preventing cardiovascular collapse and providing a prolonged window of survival in the setting of massive intra-abdominal venous bleeding that was highly fatal with standard management and resuscitation. Similarly to its use in the setting of arterial and solid organ injuries, REBOA leads to an increase in systolic and mean arterial blood pressures, preservation of cardiac output, and markedly prolonged survival times. The amount of bleeding does not seem to be decreased; however, this is confounded by the fact that these animals did live longer and likely bled at a much slower rate. We conclude that even in the setting of massive central venous hemorrhage, REBOA has an application in aiding resuscitation efforts and in providing a bridge to definitive operative or endovascular hemorrhage control.

5.0 CONCLUSIONS

The series of experiments outlined above included a set of sequenced experiments examining the safety and efficacy of TXA, PCC, and plasma products for resuscitation in the setting of severe hemorrhagic shock and metabolic acidosis. In addition to these primary experiments and main study objectives, we were able to perform two additional nested sub-projects that examined critical areas of uncertainty in the clinical use of these products in the battlefield setting. This included whether TXA can be effectively administered via an IO route and a head-to-head comparison of the two currently available formulations of PCC products (3-factor vs. 4-factor). Finally, we were able to incorporate a separate add-on study utilizing animals from select arms of these experiments to assess the efficacy of REBOA for hemorrhage control in the setting of an isolated major abdominal venous injury.

This work has led to a number of novel insights and findings related to these interventions, including several that have had immediate clinical impact or influence on battlefield trauma care and guidelines. The following list is a brief summary of the key findings from each arm or phase of the research performed under this grant award:

1. *In vitro* analysis demonstrated that TXA maintains full efficacy even in the setting of severe metabolic acidosis. There is a slight degradation of PCC efficacy in the setting of metabolic acidosis.
2. *In vivo* data demonstrate that TXA maintains full efficacy for the reversal of hyperfibrinolysis when administered in the setting of shock and severe metabolic acidosis.
3. The administration of both 3-factor and 4-factor PCC products had limited efficacy as stand-alone therapeutics in the setting of severe dilutional/consumptive coagulopathy. In animals with critically low fibrinogen, there was a marked risk for PCC inducing disseminated intravascular coagulopathy, and this risk appears to be significantly higher with the 3-factor PCC products.
4. TXA demonstrates equivalent pharmacodynamics and serum drug levels, and full efficacy for reversing hyperfibrinolysis, when administered via the IO route vs. the standard IV route.
5. Plasma co-administration at the time of PCC administration showed superior outcomes compared to administration of both PCC and TXA alone or in combination.
6. REBOA demonstrated clear efficacy in terms of hemorrhage control and prolonging survival in an animal model of isolated major abdominal venous injury.

6.0 REFERENCES

1. Davenport RA, Brohi K. Cause of trauma-induced coagulopathy. *Curr Opin Anaesthesiol*. 2016; 29(2):212-219.
2. Brohi K, Singh J, Heron M, Coats T. Acute traumatic coagulopathy. *J Trauma*. 2003; 54(6):1127-1130.
3. Roberts I, Shakur H, Coats T, Hunt B, Balogun E, et al. The CRASH-2 trial: a randomised controlled trial and economic evaluation of the effects of tranexamic acid on death, vascular occlusive events and transfusion requirement in bleeding trauma patients. *Health Technol Assess*. 2013; 17(10):1-79.
4. Morrison JJ, Dubose JJ, Rasmussen TE, Midwinter MJ. Military Application of Tranexamic Acid in Trauma Emergency Resuscitation (MATTERs) Study. *Arch Surg*. 2012; 147(2):113-119.
5. Franchini M, Lippi G. Prothrombin complex concentrates: an update. *Blood Transfus*. 2010; 8(3):149-154.
6. McSwain N Jr, Barbeau J. Potential use of prothrombin complex concentrate in trauma resuscitation. *J Trauma*. 2011; 70(5 Suppl):S53-S56.
7. Schöchl H, Schlimp CJ, Maegele M. Tranexamic acid, fibrinogen concentrate, and prothrombin complex concentrate: data to support prehospital use? *Shock*. 2014; 41 Suppl 1:44-46.
8. Eastridge BJ, Mabry RL, Seguin P, Cantrell J, Tops T, et al. Death on the battlefield (2001-2011): implications for the future of combat casualty care. *J Trauma Acute Care Surg*. 2012; 73(6 Suppl 5):S431-S437.
9. Martin M, Oh J, Currier H, Tai N, Beekley A, et al. An analysis of in-hospital deaths at a modern combat support hospital. *J Trauma*. 2009; 66(4 Suppl):S51-S60; discussion S60-S61.

10. Rasmussen TE, Eliason JL. Military-civilian partnership in device innovation: development, commercialization and application of resuscitative endovascular balloon occlusion of the aorta. *J Trauma Acute Care Surg.* 2017; 83(4):732-735.
11. Manley JD, Mitchell BJ, DuBose JJ, Rasmussen TE. A modern case series of resuscitative endovascular balloon occlusion of the aorta (REBOA) in an out-of-hospital, combat casualty care setting. *J Spec Oper Med.* 2017; 17(1):1-8.
12. Porta CR, Nelson D, McVay D, Salgar S, Eckert M, et al. The effects of tranexamic acid and prothrombin complex concentrate on the coagulopathy of trauma: an in vitro analysis of the impact of severe acidosis. *J Trauma Acute Care Surg.* 2013; 75(6):954-960.
13. DeBarros M, Hatch Q, Porta CR, Salgar S, Izenberg S, et al. Tranexamic acid corrects fibrinolysis in the presence of acidemia in a swine model of severe ischemic reperfusion. *J Trauma Acute Care Surg.* 2014; 76(3):625-632; discussion 632-633.
14. Moe DM, Lallemand MS, McClellan JM, Smith PJ, Marko ST, et al. Three- versus four-factor prothrombin complex concentrates for “factor-based” resuscitation in a porcine hemorrhagic shock model. *J Trauma Acute Care Surg.* 2017; 83(6):1114-1123.
15. Lallemand MS, Moe DM, McClellan JM, Loughren M, Marko S, et al. No intravenous access, no problem: intraosseous administration of tranexamic acid is as effective as intravenous in a porcine hemorrhage model. *J Trauma Acute Care Surg.* 2018; 84(2):379-385.
16. Kuckelman J, Barron M, Moe D, Lallemand M, McClellan J, et al. Plasma co-administration improves resuscitation with tranexamic acid or prothrombin complex in a porcine hemorrhagic shock model. *J Trauma Acute Care Surg.* 2018 [Epub ahead of print]. doi:10.1097/TA.0000000000001942.
17. Lallemand MS, Moe DM, McClellan JM, Smith JP, Daab L, et al. Resuscitative endovascular balloon occlusion of the aorta for major abdominal venous injury in a porcine hemorrhagic shock model. *J Trauma Acute Care Surg.* 2017; 83(2):230-236.
18. Holcomb JB, Jenkins D, Rhee P, Johannigman J, Mahoney P, et al. Damage control resuscitation: directly addressing the early coagulopathy of trauma. *J Trauma.* 2007; 62(2):307-310.
19. Klages M, Zacharowski K, Weber CF. Coagulation management in trauma-associated coagulopathy: allogenic blood products versus coagulation factor concentrates in trauma care. *Curr Opin Anaesthesiol.* 2016; 29(2):245-249.
20. Ausset S, Glassberg E, Nadler R, Sunde G, Cap AP, et al. Tranexamic acid as part of remote damage-control resuscitation in the prehospital setting: a critical appraisal of the medical literature and available alternatives. *J Trauma Acute Care Surg.* 2015; 78(6 Suppl 1):S70-S75.
21. Innerhofer P, Fries D, Mittermayr M, Innerhofer N, von Langen D, et al. Reversal of trauma-induced coagulopathy using first-line coagulation factor concentrates or fresh frozen plasma (RETIC): a single-centre, parallel-group, open-label, randomised trial. *Lancet Haematol.* 2017; 4(6):e258-e271.
22. Matsushima K, Benjamin E, Demetriades D. Prothrombin complex concentrate in trauma patients. *Am J Surg.* 2015; 209(2):413-417.
23. CRASH-2 Trial Collaborators, Shakur H, Roberts I, Bautista R, Caballero J, et al. Effects of tranexamic acid on death, vascular occlusive events, and blood transfusion in trauma patients with significant haemorrhage (CRASH-2): a randomised, placebo-controlled trial. *Lancet.* 2010; 376(9734):23-32.

24. Morrison JJ, Ross JD, Dubose JJ, Jansen JO, Midwinter MJ, Rasmussen TE. Association of cryoprecipitate and tranexamic acid with improved survival following wartime injury: findings from the MATTERs II Study. *JAMA Surg.* 2013; 148(3):218-225.
25. Graham EM, Atz AM, Gillis J, Desantis SM, Haney AL, et al. Differential effects of aprotinin and tranexamic acid on outcomes and cytokine profiles in neonates undergoing cardiac surgery. *J Thorac Cardiovasc Surg.* 2012; 143(5):1069-1076.
26. Jiménez JJ, Iribarren JL, Brouard M, Hernández D, Palmero S, et al. Safety and effectiveness of two treatment regimes with tranexamic acid to minimize inflammatory response in elective cardiopulmonary bypass patients: a randomized double-blind, dose-dependent, phase IV clinical trial. *J Cardiothorac Surg.* 2011; 6:138.
27. Pusateri AE, Weiskopf RB, Bebart V, Butler F, Cestero RF, et al. Tranexamic acid and trauma: current status and knowledge gaps with recommended research priorities. *Shock.* 2013; 39(2):121-126.
28. Jehan F, Aziz H, O'Keeffe T, Khan M, Zakaria ER, et al. The role of 4-factor prothrombin complex concentrate (4-PCC) in coagulopathy of trauma: a propensity matched analysis. *J Trauma Acute Care Surg.* 2018 Apr 16. [Epub ahead of print].
29. Young H, Holzmacher JL, Amdur R, Gondek S, Sarani B, Schroeder ME. Use of four-factor prothrombin complex concentrate in the reversal of warfarin-induced and nonvitamin K antagonist-related coagulopathy. *Blood Coagul Fibrinolysis.* 2017; 28(7):564-569.
30. Yanamadala V, Walcott BP, Fecci PE, Rozman P, Kumar JI, et al. Reversal of warfarin associated coagulopathy with 4-factor prothrombin complex concentrate in traumatic brain injury and intracranial hemorrhage. *J Clin Neurosci.* 2014; 21(11):1881-1884.
31. Dickneite G, Dorr B, Kaspereit F, Tanaka KA. Prothrombin complex concentrate versus recombinant factor VIIa for reversal of hemodilutional coagulopathy in a porcine trauma model. *J Trauma.* 2010; 68(5):1151-1157.
32. Inaba K, Karamanos E, Lustenberger T, Schöchl H, Shulman I, et al. Impact of fibrinogen levels on outcomes after acute injury in patients requiring a massive transfusion. *J Am Coll Surg.* 2013; 216(2):290-297.
33. Andersen RC, Fleming M, Forsberg JA, Gordon WT, Nanos GP, et al. Dismounted complex blast injury. *J Surg Orthop Adv.* 2012; 21(1):2-7.
34. Butler FK. Two decades of saving lives on the battlefield: tactical combat casualty care turns 20. *Mil Med.* 2017; 182(3):e1563-e1568.
35. Anonymous A. TCCC updates: Committee on Tactical Combat Casualty Care meeting minutes. Davis Conference Center, MacDill AFB, Fl 4-5 February 2014. *J Spec Oper Med.* 2014; 14(2):113-120.
36. Lewis P, Wright C. Saving the critically injured trauma patient: a retrospective analysis of 1000 uses of intraosseous access. *Emerg Med J.* 2015; 32(6):463-467.
37. Day MW. Intraosseous devices for intravascular access in adult trauma patients. *Crit Care Nurse.* 2011; 31(2):76-89; quiz 90.
38. Bennett BL, Butler FK Jr, Wedmore IS. Tactical combat casualty care: transitioning battlefield lessons learned to other austere environments. *Wilderness Environ Med.* 2017; 28(2S):S3-S4.
39. Patanwala AE, Acquisto NM, Erstad BL. Prothrombin complex concentrate for critical bleeding. *Ann Pharmacother.* 2011; 45(7-8):990-999.
40. Kozar RA, Peng Z, Zhang R, Holcomb JB, Pati S, et al. Plasma restoration of endothelial glycocalyx in a rodent model of hemorrhagic shock. *Anesth Analg.* 2011; 112(6):1289-1295.

41. Rahbar E, Cardenas JC, Baimukanova G, Usadi B, Bruhn R, et al. Endothelial glycocalyx shedding and vascular permeability in severely injured trauma patients. *J Transl Med.* 2015; 13:117.
42. Peng Z, Pati S, Potter D, Brown R, Holcomb JB, et al. Fresh frozen plasma lessens pulmonary endothelial inflammation and hyperpermeability after hemorrhagic shock and is associated with loss of syndecan 1. *Shock.* 2013; 40(3):195-202.
43. Davidson AJ, Russo RM, Reva VA, Brenner ML, Moore LJ, et al. The pitfalls of resuscitative endovascular balloon occlusion of the aorta: risk factors and mitigation strategies. *J Trauma Acute Care Surg.* 2018; 84(1):192-202.
44. Napolitano LM. Resuscitative endovascular balloon occlusion of the aorta: indications, outcomes, and training. *Crit Care Clin.* 2017; 33(1):55-70.
45. Morrison JJ, Galgon RE, Jansen JO, Cannon JW, Rasmussen TE, Eliason JL. A systematic review of the use of resuscitative endovascular balloon occlusion of the aorta in the management of hemorrhagic shock. *J Trauma Acute Care Surg.* 2016; 80(2):324-334.

LIST OF ABBREVIATIONS AND ACRONYMS

3PCC	3-factor prothrombin complex concentrate
4PCC	4-factor prothrombin complex concentrate
ACoTS	acute coagulopathy of traumatic shock
ANOVA	analysis of variance
BE	base excess
CC	consumptive coagulopathy
CFT	clot formation time
CPG	clinical practice guidelines
CRASH-2	Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage 2 Study
CT	clotting time
CVP	central venous pressure
DIC	disseminated intravascular coagulopathy
FFP	fresh frozen plasma
FFPc	fresh frozen plasma containing
Fr	French
Hct	hematocrit
IACUC	Institutional Animal Care and Use Committee
INR	international normalized ratio
IL	interleukin
IO	intraosseous
IV	intravenous
IVC	inferior vena cava
JTTS	Joint Theater Trauma System
MAP	mean arterial pressure
MATTERS	Military Application of Tranexamic Acid in Trauma Emergency Resuscitation Study
MCF	maximum clot firmness
ML	maximum lysis
MSD	Mesoscale Discovery Systems

nFFP	non-fresh frozen plasma containing
PAWP	pulmonary artery wedge pressure
PCC	prothrombin complex concentrate
PT	prothrombin time
PTT	partial thromboplastin time
REBOA	resuscitative endovascular balloon occlusion of the aorta
ROTEM	rotational thromboelastometry
SD	standard deviation
TPA	tissue plasminogen activator
TXA	tranexamic acid