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TITLE: A SOF Damage Control Resuscitation Cocktail.

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14. ABSTRACT

Objective: The goal of this project is to develop a new damage control resuscitation (DCR) cocktail made of fibrinogen, Hextend, Vasopressin, and tranexamic acid for use by SOF's that is capable of improving survival from polytrauma in austere settings. These components were tested in a swine polytrauma model of hemorrhagic shock with traumatic brain injury; free internal bleeding from an aortic tear, and femur fracture treated using tactical combat casualty care (TCCC) over 6 hours. Results: Fibrinogen concentrate decreased internal blood loss and enabled 3 animals to survive to 6 hours while also enhancing brain and vital organ blood flow. There was no effect of TXA in this model. In conclusion, our cocktail consisting of Hextend, Vasopressin, and fibrinogen concentrate decreased blood loss and enabled survival to 6 hours in an otherwise lethal swine polytrauma model.

15. SUBJECT TERMS

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1. INTRODUCTION:

The goal of this project is to develop a new damage control resuscitation (DCR) cocktail for use by SOF's that is capable of improving survival from polytrauma in austere settings. The cocktail components include Hextend for volume resuscitation and tissue perfusion, fibrinogen concentrate for hemostasis, and tranexamic acid (TXA) for hemostasis. These components are tested in a combat-relevant swine polytrauma model of hemorrhagic shock with traumatic brain injury.

2. KEYWORDS:

Damage Control Resuscitation; Traumatic Brain Injury; Hemorrhage; Polytrauma; Delayed Evacuation; Austere Medicine; Tactical Combat Casualty Care; Fibrinogen; Vasopressin

3. ACCOMPLISHMENTS:

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

1) **Project Preparation: (Year 1: Months 1-3):** Goal- Use approvals and preparation for experiments. Complete on time.
2) **Objective 1 Experiments (Year 1: Months 4-12)** Goal – Determine baseline model performance and determine optimal fibrinogen concentration. Result: Completion delayed due to model change by need to add vasopressin to induce more bleeding resulting in 2 of 3 fibrinogen concentrations tested. 6 month delay, but completed in year 2.
3) **Objective 2 Experiments (Year 2: Months 13-15)** Goal- Perform albumin control experiments to determine the specific effect of fibrinogen aside from any general protein effect. Completed successfully in year 2.
4) **Objective 3 Experiments (Year 2: Months 16-22)** Determine effect of adding tranexamic acid (15mg/kg). Completed successfully in year 2.
5) **Study Close (Year 2: Months 23-24)** Completed on time

What was accomplished under these goals?

Objective 1 Experiments: Goal: Determine baseline model performance and determine optimal fibrinogen concentration. *Result: Vasopressin increases vital organ blood flow and increases blood loss. Fibrinogen at 100mg/kg total dose is optimal to reduce blood loss and extend survival*
Objective 2 Experiments (Year 2: Months 13-15) Goal- Perform albumin control experiments to determine the specific effect of fibrinogen aside from any general protein effect. *Result: No effect of albumin on bleeding or survival compared to control. Hemostatic effects are fibrinogen-specific.*
Objective 3 Experiments (Year 2: Months 16-22) Determine effect of adding tranexamic acid (15mg/kg). Completed successfully in year 2. *Result: No effect of TXA on bleeding due to lack of fibrinolysis.*
Study Close (Year 2: Months 23-24)*: Overall, a cocktail consisting of 7ml/kg Hextend, 0.4U/kg Vasopressin, and 50mg/kg Fibrinogen concentrate given as two boluses per TCCC guidelines performed best in this swine model. Animals receiving fibrinogen bled less, had improved vital organ blood flow and were more likely to survive to 6 hours (Kaplan Meier p=0.05)*

What opportunities for training and professional development has the project provided?

All team members significantly improved knowledge of hemorrhagic shock, physiological responses to polytrauma with hemorrhagic shock, and TCCC guidelines. All team members significantly improved their technical skills regarding large animal polytrauma research.

How were the results disseminated to communities of interest?

Select medical students, bioengineering undergraduate, and graduate students were given the opportunity to observe or participate in experiments, thus increasing their awareness and interest in military-relevant research. Inerim results were presented at the Western Society for Academic Emergency Medicine, April 2, 2016. Abstracts are accepted for presentation at the annual Shock Society Meeting June 2016. Manuscripts are in preparation for peer reviewed scientific publications.

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to Report

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

There are two major impactful insights gained from this study:

1. Early vasopressor support may be required to improve cerebral and vital organ perfusion during damage control resuscitation of polytrauma.
2. Fibrinogen concentrate is a viable hemostatic agent that reduces noncompressible internal bleeding and improves vital organ blood flow during damage-control resuscitation of polytrauma with traumatic brain injury and hemorrhagic shock.

What was the impact on other disciplines?

These results have potential impact on resuscitation from civilian polytrauma, hemorrhagic shock, and traumatic brain injury.

What was the impact on technology transfer?

A viable new resuscitation cocktail has been developed with potential for technology transfer.

What was the impact on society beyond science and technology?

Nothing to Report

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

The project met with one primary scientific hurdle which was the lack of bleeding and lack of blood pressure response to a single dose of Hextend alone after injury. This hurdle was overcome by combining Vasopressin with Hextend to encourage more bleeding. In doing so, we also found that Vasopressin paradoxically exerts favorable effects on vital organ blood flow despite increasing internal blood loss.

Actual or anticipated problems or delays and actions or plans to resolve them

The project met with one primary scientific hurdle which was the lack of bleeding and lack of blood pressure response to a single dose of Hextend alone after injury. This hurdle was overcome by combining Vasopressin with Hextend to encourage more bleeding. In doing so, we also found that Vasopressin paradoxically exerts favorable effects on vital organ blood flow despite increasing internal blood loss.

Changes that had a significant impact on expenditures

No significant changes to expenditures were required.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Addition of Vasopressin to the experimental protocol.

Approved by UW IACUC on: 12-4-2014
Approved by ACURO on: 1-13-2015

Significant changes in use of biohazards and/or select agents

Addition of Vasopressin.

Approved by UW IACUC on: 12-4-2014
Approved by ACURO on: 1-13-2015

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications.

Nothing to Report, manusctripts in preparation

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

1. Shock Society Meeting, accepted for presentation, June 2016
2. Western Society for Academic Emergency Medicine, April 2, 2016

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

1. Creation of a viable swine polytrauma model with traumatic brain injury and internal bleeding suitable for testing of new therapies for damage-control resuscitation.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	***Nathan White MD, MS***
Project Role:	*PI*
Nearest person month worked:	*2.4*
Contribution to Project:	*Primary project manager, assisted with experiments, primary data analysis and manuscript writing.*
Name:	***Susan A Stern MD***
Project Role:	*Co-I*
Nearest person month worked:	*1.2*
Contribution to Project:	*Assisted with project design, review of data and outcomes.*
Name:	***Xu Wang MD***
Project Role:	*Research Scientist*
Nearest person month worked:	*9*
Contribution to Project:	*Technical lead. Performed experiments and collected data. Lab manager*
Name:	***Esther Lim BS***
Project Role:	*Assistant Research Scientist*
Nearest person month worked:	*9*
Contribution to Project:	*Assisted with experiments, data collection, and daily lab management.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

- *Other*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Appenidix A. Accepted Abstracts for Presentation

Shock Society Annual Meeting, June 2016

Effects of vasopressin on early resuscitation of polytrauma with traumatic brain injury and free internal bleeding.

Alex E. St. John MD MS, Xu Wang MD, Esther B. Lim BS, Diana Chien BS, Matt L. Statz BA, Susan A. Stern MD, Nathan J. White MD MS

Emergency Medicine Research Laboratory, Division of Emergency Medicine, University of Washington, Seattle, WA, USA

Background: After traumatic brain injury (TBI), hypotension is detrimental in a dose- and time-dependent fashion. However, fluid resuscitation directed at preventing hypotension is often of limited value because of blunted cardiovascular responses stemming from TBI. TBI is also often accompanied by internal vascular injuries that are prone to bleeding during aggressive fluid resuscitation. Administration of a low-dose vasopressor may be beneficial after TBI with freely-bleeding injuries by restoring cardiovascular reflexes and maintaining cerebral perfusion without increasing blood loss.

Objective: Determine the effects of vasopressin on vital organ perfusion and blood loss in a swine model of polytrauma with traumatic brain injury and free internal bleeding.

Methods: Fifteen immature female swine underwent fluid percussion TBI, femur fracture, catheter hemorrhage to induce hemorrhagic shock, and a 4 mm aortic tear as a source of internal bleeding. Animals were randomized to receive one of three resuscitation strategies: no fluid resuscitation (NR, n=5), 14 ml/kg hydroxyethyl starch solution (Hextend) (H, n=5), or Hextend with 0.4 U/kg vasopressin (HV, n=5) divided into two boluses given 30 minutes apart. Hemodynamics, lactate concentration, and organ-specific blood flow by colored microspheres were compared at 60 minutes, and 6-hour hemorrhage volume was measured. Results were compared between groups by one-way ANOVA with Tukey adjustment. Survival was compared by Kaplan-Meier log rank test.

Results: After 60 minutes of resuscitation, MAP was not different for NR (mean ± SD: 31.6±16.0 mmHg) vs. H (41.0±20.3) (p=0.67) or for H vs. HV (49.8±13.5) (p=0.76). Lactate concentration was similar for NR (3.7±1.1 mmol/L) vs. H (4.8±1.8) (p=0.58) and for H vs. HV (5.2±1.9) (p=0.92). Blood flow was not different in the injured cerebral cortex (NR: 0.04±0.03 mL/min/g vs. H: 0.14±0.24, p=0.87; H vs. HV: 0.32±0.42, p=0.60) or heart (NR: 0.32±0.14 mL/min/g vs. H: 1.03±0.64, p=0.46; and H vs. HV: 1.50±1.37, p=0.70). Hemorrhage volume per minute of survival time was similar for NR (0.041±0.022 mL/kg/min) compared to H (0.094±0.126) (p=0.97) and for H compared to HV (0.465±0.663 mL/kg) (p=0.32). Survival was similar for all groups (60-minute survival: 5/5 for NR, 5/5 for H, and 4/5 for HV, p=0.29).

Conclusion: There were non-significant improvements in MAP and vital organ blood flow when vasopressin was added to Hextend in this model, even though there was a trend toward increased blood loss. Low-dose vasopressin may support resuscitation of polytrauma with TBI if the associated increase in blood loss can be mitigated.

Shock Society Annual Meeting, June 2016

Effects of fibrinogen on early resuscitation of polytrauma with traumatic brain injury and free internal bleeding.

Alex E. St. John MD MS, Xu Wang MD, Esther B. Lim BS, Diana Chien BS, Matt L. Statz BA, Susan A. Stern MD, Nathan J. White MD MS

Emergency Medicine Research Laboratory, Division of Emergency Medicine, University of Washington, Seattle, WA, USA

Background: The goals of resuscitation for hemorrhagic shock (HS) and traumatic brain injury (TBI) are seemingly at odds. HS is best managed with limited volume resuscitation, while TBI is sensitive to even transient episodes of hypotension. Furthermore, polytrauma is often complicated by the development of trauma-induced coagulopathy (TIC), which is worsened by both fluid administration and shock and is characterized by an early critical decrease in fibrinogen concentration. We have recently shown that low-dose vasopressin may improve resuscitation after polytrauma but at the cost of increased post-injury bleeding. The co-administration of fibrinogen could promote early hemostasis, allowing both improved vital organ perfusion and decreased blood loss.

Objective: Determine the effects of fibrinogen on survival and blood loss in a swine model of polytrauma with TBI and free internal bleeding when co-administered with vasopressin.

Methods: Twenty-one immature female swine underwent fluid percussion TBI, femur fracture, catheter hemorrhage to induce hemorrhagic shock, and a 4 mm aortic tear as a source of internal bleeding. Animals were randomized to receive one of three resuscitation strategies: 14 ml/kg hydroxyethyl starch solution (Hextend™) with 0.4 U/kg vasopressin control (HV, n=5); Hextend, vasopressin, and low-dose fibrinogen concentrate (100 mg/kg) (LF, n=8); or Hextend, vasopressin, and high-dose fibrinogen concentrate (200 mg/kg) (HF, n=8) divided into two boluses given 30 minutes apart. Hemodynamics and lactate concentration were compared at 60 minutes, and 6-hour hemorrhage volume was measured. Results were compared between groups by one-way ANOVA with Tukey adjustment. Survival was compared by Kaplan-Meier log rank test.

Results: Survival was not different between HV (60-minute survival: 4/5) and LF (7/8) (p=0.37) or between HV and HF (5/8) (p=0.86). Hemorrhage volume per minute of survival was not significantly different between HV (mean±SD: 0.47±0.66 ml/kg/min) and LF (0.13±0.14) (p=0.37) or between HV and HF (0.34±0.43) (p=0.86). After 60 minutes of resuscitation, lactate concentration was not different between HV (4.8±3.6 mmol/L) and LF (4.3±2.9) (p=1.00) or between HV and HF (2.8±1.6) (p=0.77).

Conclusion: There were non-significant improvements in survival and hemorrhage volume when fibrinogen was added to Hextend and low-dose vasopressin in this model. These effects were especially true for low-dose fibrinogen, possibly owing to higher oncotic pressure of the higher-dose fibrinogen without added hemostatic benefit. When used in combination with Hextend and vasopressin, low-dose fibrinogen may support an optimized resuscitation strategy by improving vital organ perfusion and limiting hemorrhage.

Walking the Line: Achieving balance between resuscitation and bleeding after hemorrhagic shock with traumatic brain injury.

Nathan J White, Alex St. John, Xu Wang, Esther Lim, Susan A. Stern.
Division of Emergency Medicine, University of Washington, Seattle, WA, USA

Background: The combination of hemorrhagic shock (HS) with traumatic brain injury (TBI) presents a difficult treatment challenge because damage control resuscitation (DCR) can reduce bleeding, but may also intensify secondary brain injury.

Objectives: We hypothesize that early vasopressor support can restore brain perfusion during resuscitation of HS with TBI and free internal bleeding.
Methods: Simultaneous TBI and HS were induced in immature swine using fluid wave brain percussion and catheter hemorrhage with free internal bleeding from an aortic tear. Animals were then given DCR as 2 hydroxyethyl starch solution (Hextend) 7mg/kg boluses separated by 30 minutes with and without 4U/kg of vasopressin. Vital signs, blood loss, cerebral blood flow by injected colored microspheres, and survival time were recorded to 360 minutes and compared between groups.
Results: N=5 animals were given Hextend and N=5 animals were given Hextend with Vasopressin. Vasopressin increased mean arterial pressure by 5.8 mmHg, 95%CI of the difference = [-3.8, 15.3] and arterial lactate concentration by 0.6mmol/L [-0.9, 2.0] during the first 90 minutes of fluid resuscitation. Vasopressin also increased peri-injury brain blood flow by 0.19 ml/min/g [-0.55, 0.92] at 60 minutes of resuscitation. Survival time was, mean (Std. Error), 208 (83) minutes after Hextend and 146 (94) minutes after Hextend with Vasopressin. Mean intraperitoneal blood loss was 13.5 (4.7) ml/kg after Hextend and 25.8 (6.1) ml/kg after Hextend with Vasopressin.
Conclusion: In this model, DCR of HS with vasopressor support did not significantly improve blood pressure, metabolic shock, or significantly increase blood flow to the injured cerebral cortex after TBI. This result was likely due to increased blood loss, suggesting a critical role for early hemostatic support to stop bleeding during fluid resuscitation of hemorrhagic shock with traumatic brain injury.

Appendix B. Detailed Report of Project Outcomes.

INTRODUCTION

Executive Summary: The goal of this project is to develop a new damage control resuscitation (DCR) cocktail for use by SOF's that is capable of improving survival from polytrauma in austere settings. The cocktail components include Hextend for volume resuscitation and tissue perfusion, fibrinogen concentrate for hemostasis, and tranexamic acid (TXA) for hemostasis. These components are tested in a combat-relevant swine polytrauma model of hemorrhagic shock with traumatic brain injury; free internal bleeding from an aortic tear, and femur fracture treated using tactical combat casualty care (TCCC). Model development and validation, and objective 1 was completed in year 1 with an official project start date of April 15, 2014. Objectives 2 and 3 were completed in year two (Start Date: April 15, 2015). The project has met with one primary scientific hurdle which has been overcome by combining Vasopressin with Hextend to encourage more bleeding. In doing so, we have found that Vasopressin paradoxically exerts favorable effects on vital organ blood flow despite increasing internal blood loss. With the addition of fibrinogen concentrate at 100mg/kg decreased internal blood loss and enabled 3 animals to survive to 6 hours, whereas this severe polytrauma model was universally lethal without fibrinogen. Surprisingly, fibrinogen also increased brain and vital organ blood flow, and in many cases, fully restored brain blood flow in the injured brain cortex to pre-injury levels despite systemic hypotension. This effect was due specifically to fibrinogen, and there was no effect of adding TXA, since no fibrinolysis was detectable in control animals. *In conclusion, we have successfully selected components of a DCR cocktail that is suitable for SOF use which includes Hextend, Vasopressin, and fibrinogen concentrate.* This cocktail may provide vital organ support, reduce secondary brain injury, and reduce blood loss during prolonged field care of the polytrauma casualty in austere settings. Further optimization is required in terms of how the cocktail is administered, its performance in the setting of overt coagulopathy, and its performance in combination with whole blood transfusion.

BODY

Stopping blood loss is paramount for the survival of military casualties and the hemostatic protein fibrinogen is a logical and promising agent to be used to stop bleeding. Early fibrinogen supplementation may simultaneously exert favorable hemostatic and cerebral resuscitative effects without increasing overall fluid requirements. In addition, direct inhibition of fibrinolysis by TXA may add benefit to fibrinogen-based resuscitation by reducing fibrinogen consumption and clot breakdown. We predict that combining fibrinogen, tranexamic acid, and Hextend into a single low-dose damage control resuscitation cocktail can meet the immediate need to limit blood loss and avoid secondary brain injury during the initial resuscitation of SOF casualties. We test this approach using a swine polytrauma model of combat injury with hemorrhagic shock, free bleeding from an aortic tear, femur fracture, and traumatic brain injury. Our rationale is that adding fibrinogen and TXA to initial TCCC fluid resuscitation will enhance hemostatic clot formation and reduce free bleeding, therefore, improving systemic and cerebral resuscitation of hemorrhagic shock and increasing survival time.

Hypothesis Statement*: A low-volume fluid resuscitation cocktail composed of Hextend, fibrinogen concentrate, and tranexamic acid will reduce blood loss, significantly increase survival time, and improve systemic and cerebral resuscitation in a swine polytrauma/uncontrolled hemorrhage model that includes TBI compared to Hextend alone.* To test this hypothesis, the following objectives were completed:

Objective 1. Identify the optimal dosing of fibrinogen required to reduce blood loss and extend survival time.

Objective 2. Establish what effects are unique to fibrinogen by comparing to a nonspecific protein control.

Objective 3. Determine the impact of tranexamic acid alone and in combination with fibrinogen on relevant outcomes.

KEY RESEARCH ACCOMPLISHMENTS

1. **Polytrauma Model Development:** Due to severe injury and the presence of traumatic brain injury, cardiovascular responses to Hextend bolus were found to be blunted. Blood pressure did not increase in response to the Hextend bolus similarly to that observed in previous simple hemorrhage models. Therefore, a change in the protocol was required. Namely, a small dose of Vasopressin was added to each fluid bolus to increase blood pressure and induce rebleeding

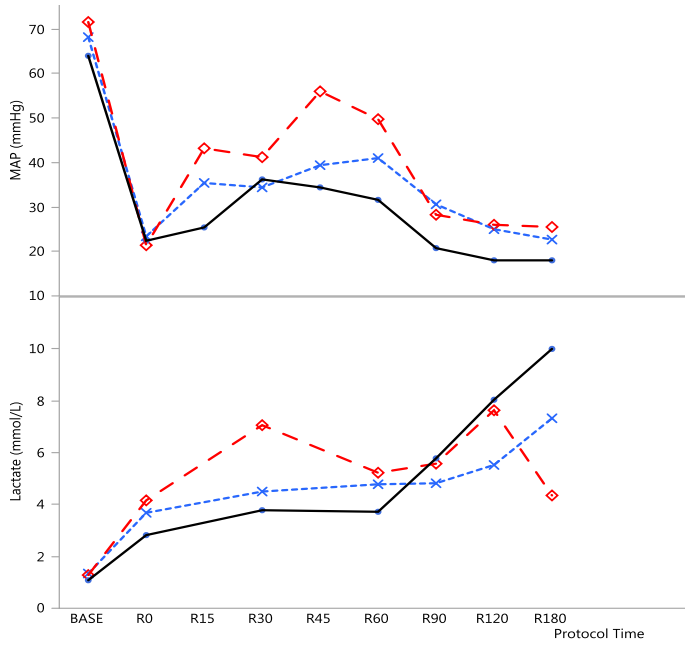


Figure 1. Mean arterial pressure (MAP) and arterial lactate concentration at baseline prior to hemorrhage (BASE) and during resuscitation (R) in minutes. Hextend with Vasopressin (Red Diamond), Hextend alone (Blue X), and no fluid resuscitation (closed circle) were compared.

from the aortic tear. With this addition, we found that internal blood loss was increased and the model became suitable for testing the effect of fibrinogen concentrate as a hemostatic agent in an active-bleeding situation. (**Figures 1 and Table 1**). Hextend alone was not suitable as a stand-alone resuscitation fluid, because even though blood loss was less, Hextend alone was unable to provide resuscitation required for survival to 6 hours in this model.

Table 1. Effect of Hextend (7ml/kg per bolus) and Hextend with Vasopressin (0.4U/kg per bolus)on intraperitoneal blood loss and survival time.

	No Fluids		**Hextend**		**Hextend + Vasopressin**		
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	ANOVA P Value
Survival Time (min)	137.4	39.7	190.2	55.5	140	86.2	0.4
Catheter Hemorrhage Volume (ml/kg)	15.3	4.5	17.1	3.8	16	7.3	0.9
Intraperitoneal Hemorrhage (mL/kg)	5.4	2.8	13.5	10.7	25.8*	13.6	0.02
Total Hemorrhage Volume (ml/kg)	20.7	2.2	30.6	10.3	41.8*	10.2	0.007
**=ANOVA P <0.02 vs. No Fluids group with Tukey HSD for individual comparisons.*							

The addition of Vasopressin to Hextend blunted the rise of blood lactate for a short time and tended to increase vital organ blood flow to the brain, gut, kidney, and heart. (Figures 2 and 3) This effect was present even though blood loss was also increased and suggests a favorable effect of Vasopressin on vital organ blood flow during low-volume Hextend resuscitaion.

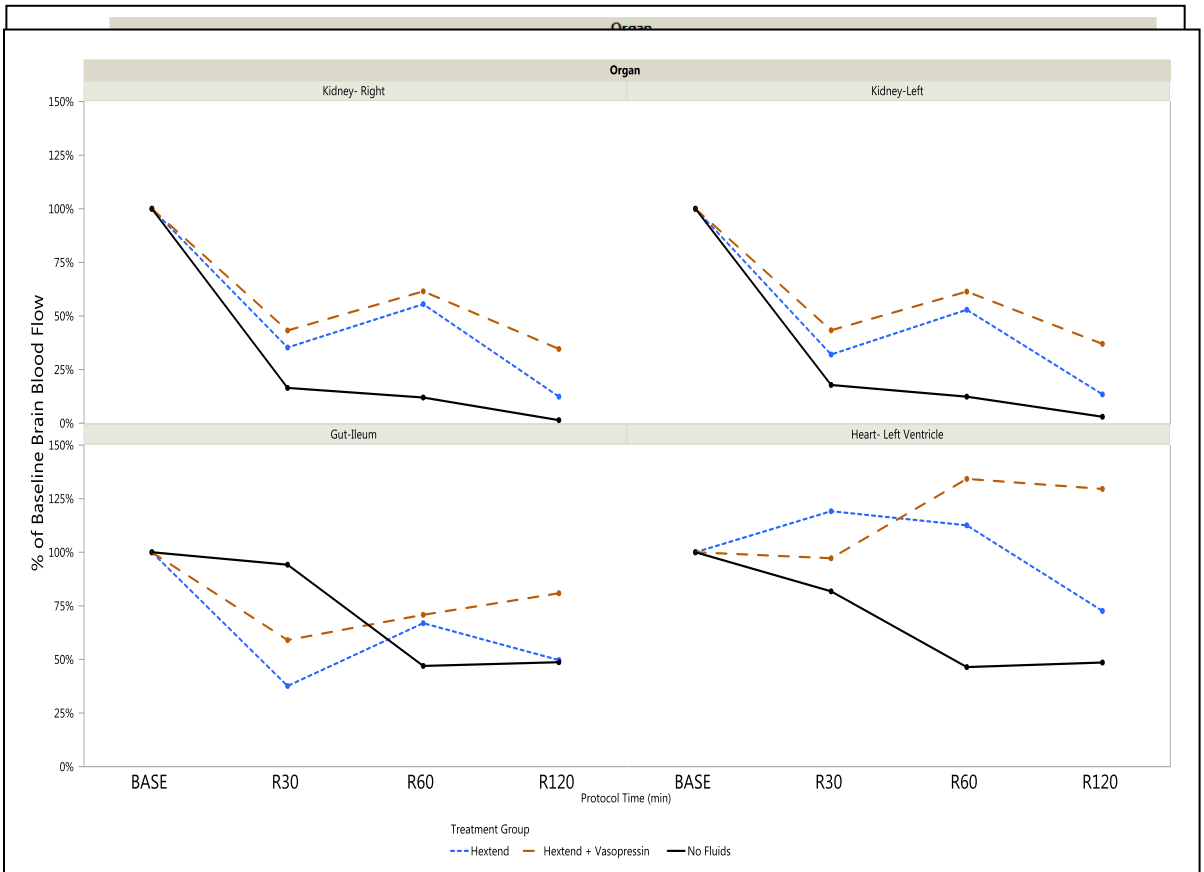


Figure 3. Effect of resuscitation regimen on vital organ blood flow measured by colored microsphere injection.

Model development has resulted in 2 major research accomplishments:

1. Successful development of a polytrauma model with TBI and internal blood loss suitable for testing of fibrinogen as a hemostatic agent.
2. Discovery of Vasopressin as an important agent to restore vascular tone and improve vital organ blood flow after polytrauma.

It is clear that Vasopressin-induced bleeding from the aortic injury is a key limiting factor for survival in this model. Therefore, we proceeded to evaluate fibrinogen concentrate as a hemostatic agent to counteract the increased blood loss. The model is now performs as predicted after the addition of Vasopressin to Hextend, and is capable of testing the stated hypotheses.

Objective 1. *Identify the optimal dosing of fibrinogen required to reduce blood loss and extend survival time.*

The goal of objective 1 was to determine an appropriate dose of human fibrinogen concentrate (Riastap™, CSL Behring) needed to reduce blood loss and extend survival in this model. Due to the addition of Vasopressin to the protocol, we now required 2 control arms during model development and must reduce the number of fibrinogen concentrations tested from 3 to 2. Therefore, we have tested a low dose (100mg/kg total) and high dose (200mg/kg total) of fibrinogen concentrate divided into two boluses given concurrently with Hextend (7ml/kg) and Vasopressin (0.4 ug/kg) separated by 30 minutes according to current TCCC guidelines.

- **Hextend + Vasopressin Control:** 7ml/kg Hextend + 0.4ug/kg Vasopressin given as two boluses separated by 30 minutes (N=5).
- **Hextend + Vasopressin + <u>High-Dose Fibrinogen</u>** (200mg/kg total): Hextend + Vasopressin + Fibrinogen 100mg/kg given as two boluses separated by 30 minutes (N=8).
- **Hextend + Vasopressin + <u>Low Dose Fibrinogen</u>** (100mg/kg total): fibrinogen

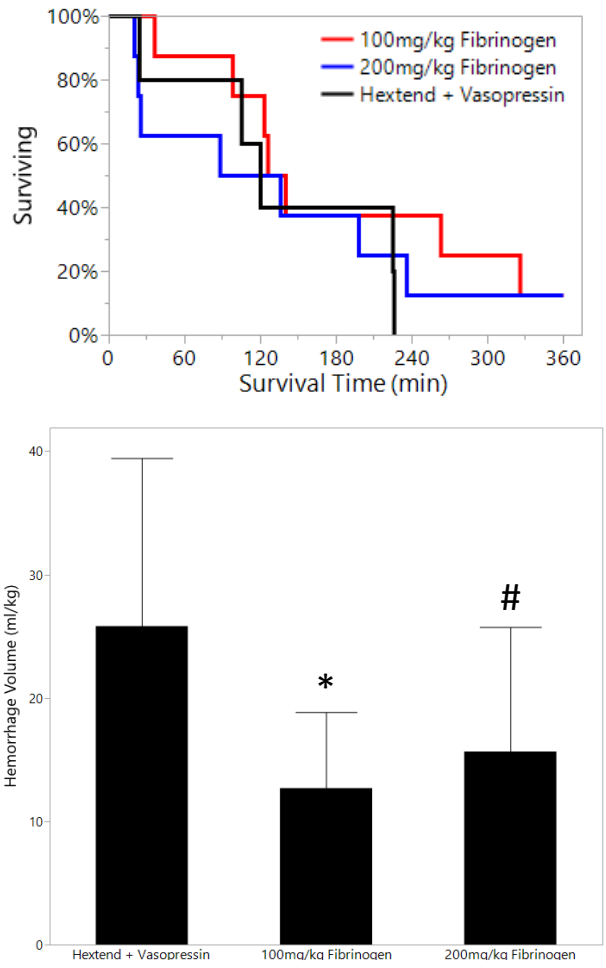


Figure 4. Effects of low and high-dose fibrinogen concentrate on survival time (top panel) and intraperitoneal blood loss (bottom panel) compared to control resuscitation using Hextend and Vasopressin. One animal survived in each fibrinogen group, however, there was no significant increase in survival with fibrinogen supplementation. Blood loss tended to be less in the 100mg/kg fibrinogen group. *= Dunnett's p=0.053 vs. control. # Dunnett's p=0.14 vs. control.

given as two 50mg/kg boluses separated by 30 minutes (N=8).

Results:

Survival and Blood Loss

Mean (SD) survival time was 140 (86.2) min for the Hextend/Vasopressin control group, 184 (116.9) min with the addition of 100mg/kg fibrinogen concentrate, and 136 (122.4) in with the addition of 200mg/kg fibrinogen concentrate. One of eight animals (13%) survived to 6 hours in each fibrinogen group. There were no significant overall differences in survival when compared to the Hextend/Vasopressin control group (0% survival) (Kaplan Meier Chi Square LR p=0.5). (**Figure 4**.). However, there was a strong but nonsignificant (p=0.053) effect of fibrinogen supplementation to decrease intraperitoneal blood loss with 100mg/kg fibrinogen concentrate. (**Figure 4**)

Resuscitation

There was a significant overall effect of treatment group on MAP during resuscitation to 240 minutes (ANOVA treatment group p<0.001). Supplementation with either 100mg/kg or 200mg/kg fibrinogen was associated with significantly increased MAP compared to control (ANOVA p values < 0.01 with Tukey adjustment for multiple comparisons). (**Figure 5**) There were no individual time points that differed significantly (ANOVA interaction p=0.7). There was a significant overall effect of treatment group on arterial blood lactate concentration up to 180 minutes
of survival time (ANOVA treatment group, p=0.02). Mean overall lactate concentration for the period was significantly less with 200mg/kg fibrinogen vs. control (p=0.02 with Tukey adjustment for multiple comparisons). There were no significant differences in MAP or lactate between fibrinogen groups.

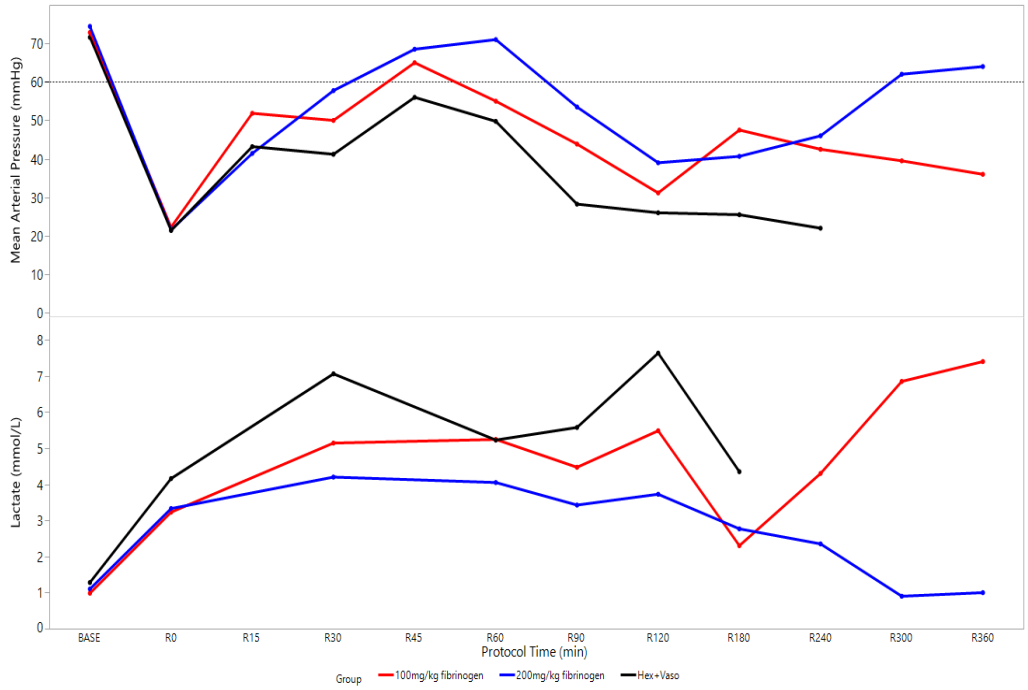


Figure 5. Mean arterial pressure (MAP) and arterial lactate concentration at baseline prior to hemorrhage (BASE) and during resuscitation (R) in minutes. MAP tended to be increased and lactate tended to be decreased in groups receiving fibrinogen. MAP=60mmHg is highlighted by the interrupted line for visual reference.

Vital Organ Blood Flow

There was a significant effect of treatment group on brain blood flow up to 120 minutes of resuscitation in all brain areas measured except for the cerebellum (all ANOVA treatment p values < 0.048). In each case brain blood flow was significantly increased in the 200mg/kg fibrinogen group compared to control (all individual p values ≤0.045 with Tukey adjustment for multiple comparisons). There was no difference between the fibrinogen groups when examining brain blood flow. Blood flow also tended to be increased in other vital organs such as the kidney (ANOVA treatment effect p<0.16 for right and left sides), ileum (ANOVA treatment effect p=0.07). Blood flow to the left ventricle of the heart was significantly increased with

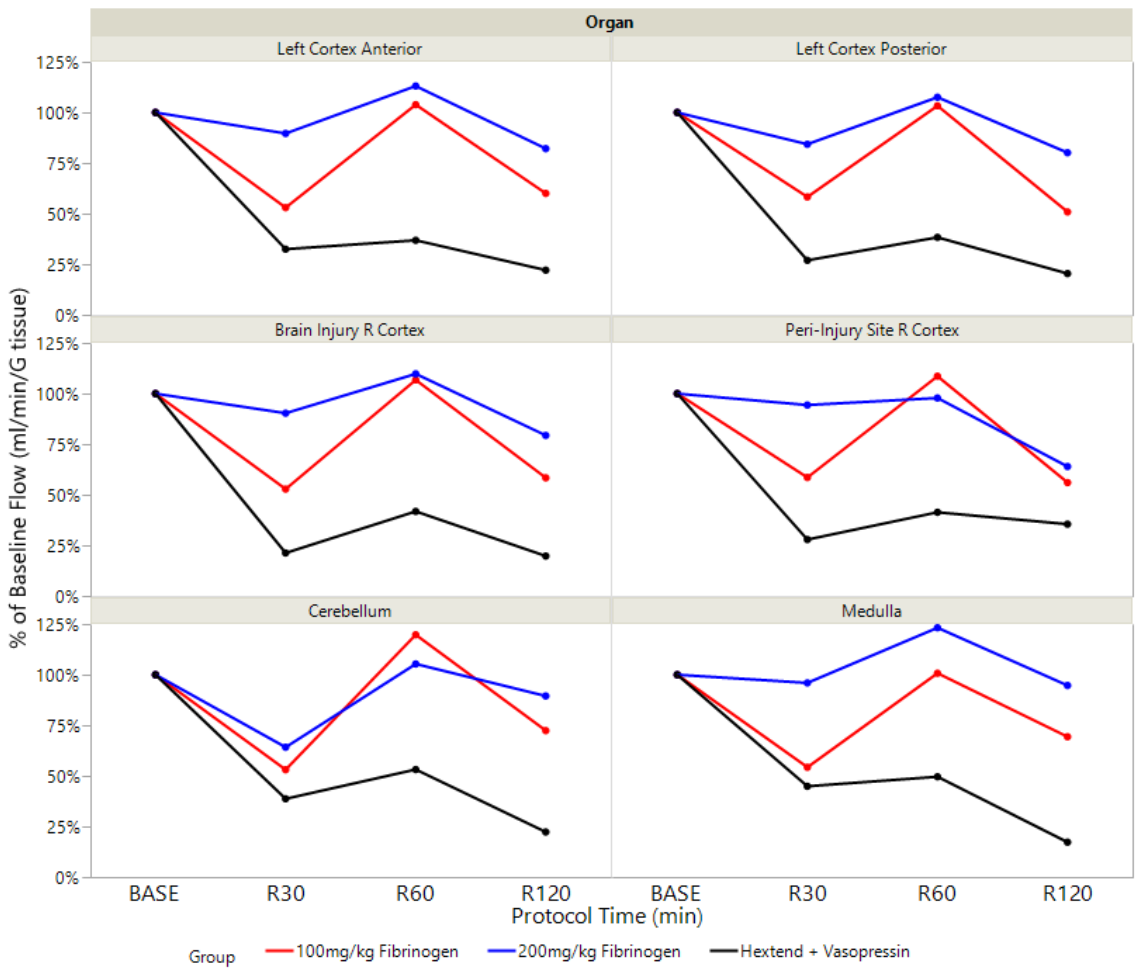
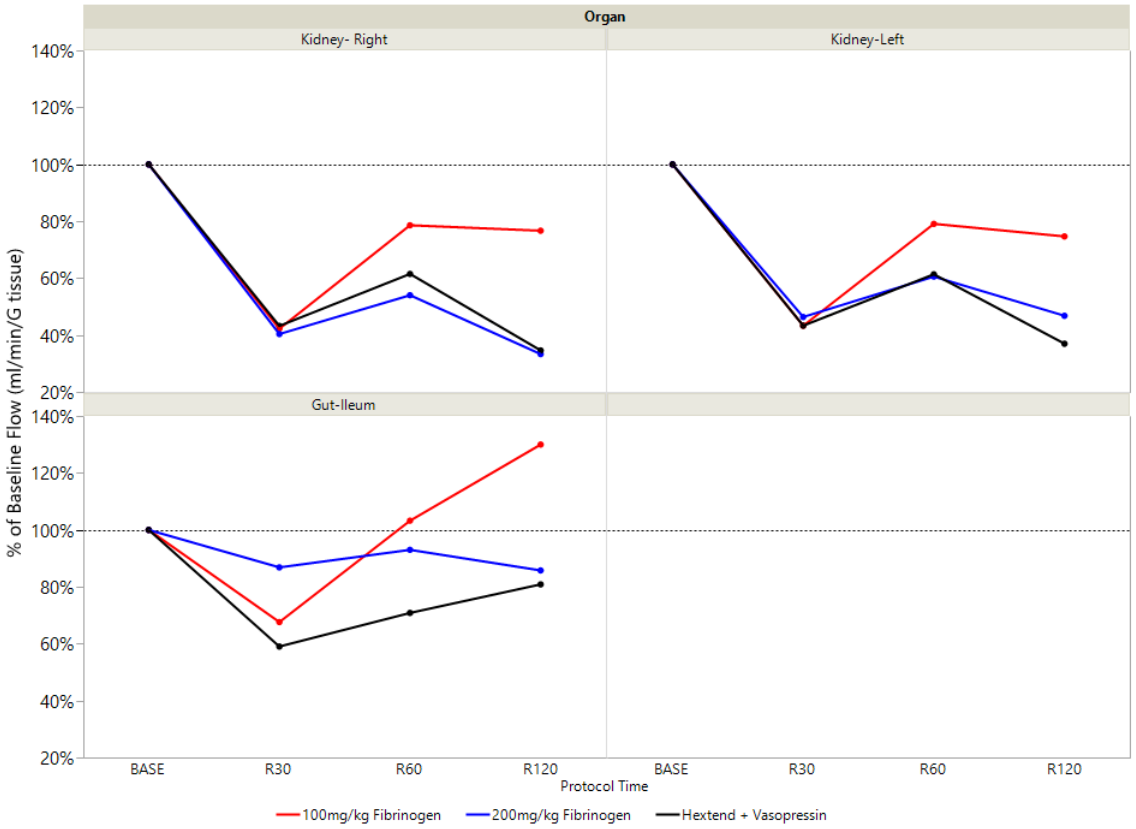


Figure 6. Effects of fibrinogen concentrate on regional brain blood flow. The right cortex was injured using fluid percussion.



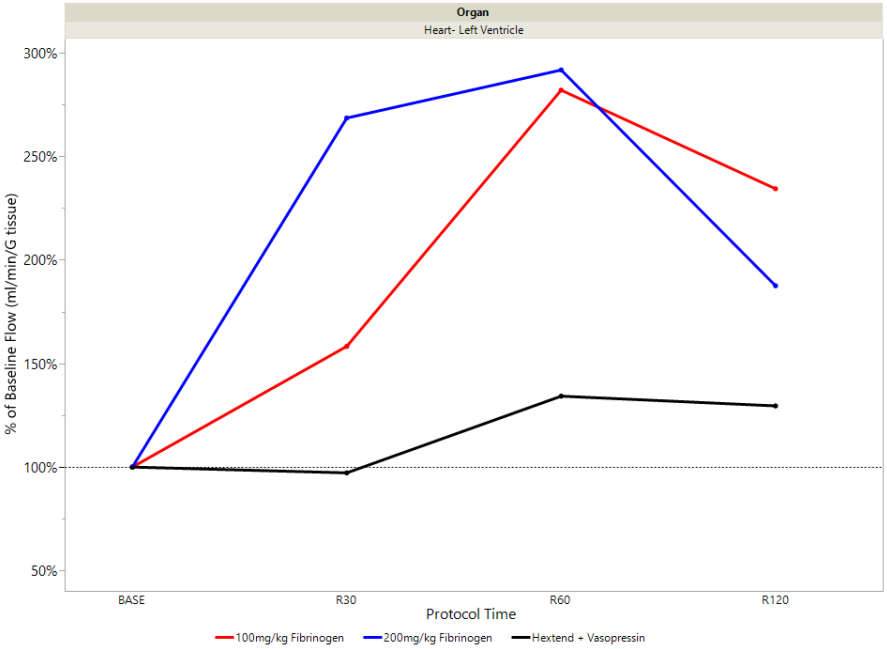


Figure 7. Effects of fibrinogen concentrate on vital organ blood flow to kidneys, gut, (top panel) and heart (bottom panel). Measured using injected colored microspheres. The horizontal interrupted line marks the 100% baseline blood flow for visual reference.

fibrinogen supplementation at 100mg/kg (ANOVA p=0.02) and at 200mg/kg (ANOVA p=0.007)

compared to control. (**Figure 7**). Compensatory cardiovascular responses typically increase blood to the heart during shock. Blood flow to the left ventricle was increased significantly in both fibrinogen groups compared to control (ANOVA p<0.02). (**Figure 7**) There were no significant differences in vital organ blood flow between fibrinogen groups and there were no individual differences at single time points.

Objective 2: *Non-hemostatic protein control:* The goal of this objective was to determine the hemostatic contribution of fibrinogen concentrate to resuscitation aside from its general protein oncotic effects. To test this effect, survival and blood loss was measured using a non-hemostatic control protein (albumin) instead of fibrinogen. The concentration of albumin is matched to the highest concentration of fibrinogen tested from Objective 1 (200mg/kg fibrinogen equivalent) and were standardized by molar concentration of protein.

Results:

Survival and Blood Loss

No animals survived in the albumin group (0/5), while 13% of animals (1/8) survived to 6 hours in each fibrinogen group. (Figure 8) Mean (SD) survival time was 123.4 (38.4) min for albumin, 135.8 (122.4) min for 200mg/kg fibrinogen, and 184 (116.9) min for 100mg/kg fibrinogen groups. There were no differences in overall survival between groups (Kaplan Meier Chi Square LR p=0.4). (**Figure 8**) Intraperitoneal blood loss was significantly increased in the albumin group compared to 100mg/kg fibrinogen (ANOVA p<0.001), and 200mg/kg fibrinogen groups (ANOVA, p=0.02). (**Figure 8**).

Objective 3: *Determine the effects of adding tranexamic acid (15mg/kg) alone and in combination with fibrinogen concentrate.*

- **Tranexamic Acid Control:** Hextend + Vasopressin + Albumin + TXA given as two boluses separated by 30 minutes (N=5)
- **Tranexamic Acid + Fibrinogen:** (Hextend +Vasopressin + 100mg/kg Fibrinogen + TXA given as two boluses separated by 30 minutes (N=8)

The tranexamic acid control group was compared to the albumin only group to examine for an

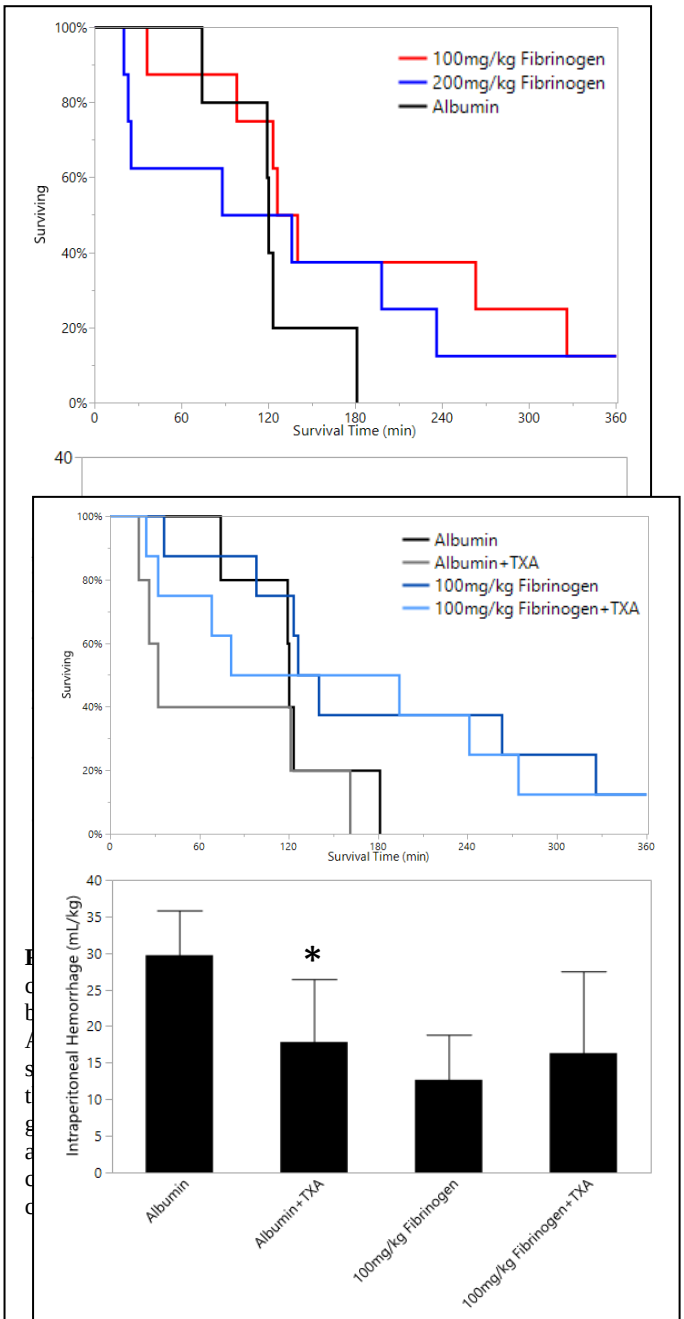


Figure 9. Kaplan Meier survival of tranexamic acid (TXA) alone (Albumin+TXA) and fibrinogen concentrate with TXA (100mg/kg fibrinogen + TXA), compared to their respective controls. Survival time (top panel) and intraperitoneal blood loss (bottom panel). All groups received Hextend and Vasopressin. One animal survived in each fibrinogen group and no animals survived in the albumin groups. Internal blood loss was significantly less with Albumin+ TXA compared to Albumin alone. There was no difference in blood loss between fibrinogen alone and fibrinogen + TXA. * p=0.04 compared to albumin.

independent effect of TXA on survival, clot formation, blood loss, and survival. The TXA+ 100mg/kg fibrinogen group was compared to the 100mg/kg fibrinogen group to examine for an additional benefit of TXA when added to fibrinogen concentrate. All groups received 2 boluses of Hextend (7ml/kg) and Vasopressin (0.4ug/kg) concurrently.

Results:

Survival and Blood Loss

None of the animals in the albumin alone or albumin+TXA groups survived to 6 hours (0% survival). One animal in each of the 100mg/kg fibrinogen and 100mg/kg fibrinogen+TXA groups survived to 6 hours (13% survival). Mean (SD) survival time was 123.4 (38) min for albumin, 71.8 (64.9) min for albumin+ TXA, 184 (116.9) min for 100mg/kg fibrinogen, and 159.3 (125.5) min for 100mg/kg fibrinogen+TXA. There was no difference in overall survival when comparing albumin alone to albumin+TXA (Kaplan Meier ChiSqr LR p=0.3) and when comparing fibrinogen alone to fibrinogen + TXA (Kaplan Meier ChiSqr LR p=0.7). (**Figure 9**). Blood loss was significantly less when TXA was added to albumin. Although, survival time was also shorter with TXA, so it is likely that blood loss was less simply because animals in this group did not have an equivalent time to bleed. There was no difference in blood loss when TXA was added to fibrinogen concentrate. (Figure 9).

TXA is an anfibrinolytic agent that inhibits the effect of the enzyme plasmin to cut fibrin fibers within a blood clot. Fibrinolysis is measured using rotational thromboelastometry by the percent recovery of clot strength when blood is clotted in the presence of the antifibrinolytic agent aprotinin (APTEM assay), an inhibitor of plasmin. To evaluate for significant fibrinolysis that might be treated by TXA, we calculated the percent clot recovery with APTEM during hemorrhage and resuscitation in each group. The average APTEM clot recovery was 0% for each group tested, including the TXA group. This result suggests that fibrinolysis was not present in this model, which explains the lack of an effect of TXA on blood loss.

Summary Analysis

To examine for an overall effect of fibrinogen regardless of concentration or TXA, animals were pooled into two separate groups segregated by the presence or absence of fibrinogen in the resuscitation fluid. The *Fibrinogen* group consisted of the 100mg/kg fibrinogen, 200mg/kg fibrinogen, and 100mg/kg fibrinogen+TXA groups (N=24 total). The *No Fibrinogen* group consisted of the Hextend+Vasopressin control, Albumin, and Albumin+TXA groups (N=15 total). All

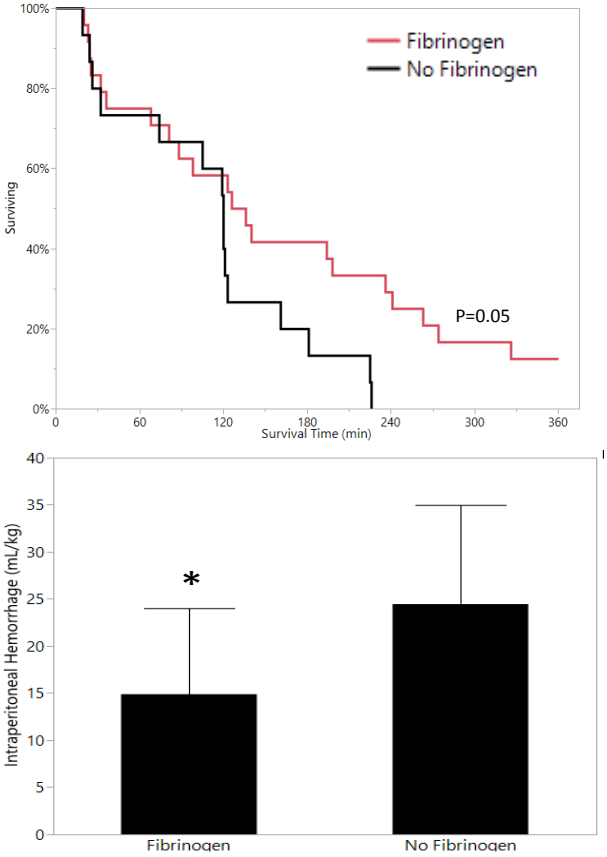


Figure 10. Kaplan Meier survival of pooled Fibrinogen and No Fibrinogen groups. Survival time (top panel) and intraperitoneal blood loss (bottom panel). All groups received Hextend and Vasopressin. Three animals survived in the Fibrinogen group and no animals survived in the No Fibrinogen group. Internal blood loss was significantly less with Fibrinogen compared to No Fibrinogen. * p=0.005 compared to No Fibrinogen.

animals received the same amount of Hextend and Vasopressin during resuscitation.

Survival and Blood Loss

Mean (SD) survival time was 111.7 (68) min for the No Fibrinogen group and was 159.7 (118) min for the Fibrinogen group. Survival was significantly greater in the fibrinogen group (Kaplan Meier Chi Sqr LR p=0.05). (**Figure 10**) Internal blood loss was significantly less for the Fibrinogen group. (Figure 10)

Resuscitation

There was a significant effect of fibrinogen on MAP measureable up to 240 minutes (ANOVA interaction effect p=0.03), cerebral perfusion pressure (CPP) up to 240 minutes (ANOVA treatment effect p< 0.001), and blood lactate concentration measureable up to 180 minutes (ANOVA interaction effect p=0.004). (Figure 11). MAP was significantly higher in the Fibrinogen group at 30, 60, 90, 120, and 180 minutes of resuscitation (R). CPP was increased overall during resuscitation, but was not different at any one individual time point. Lactate concentration was significantly decreased in the Fibrinogen group at 90 and 120 minutes of resuscitation time. (**Figure 11**)

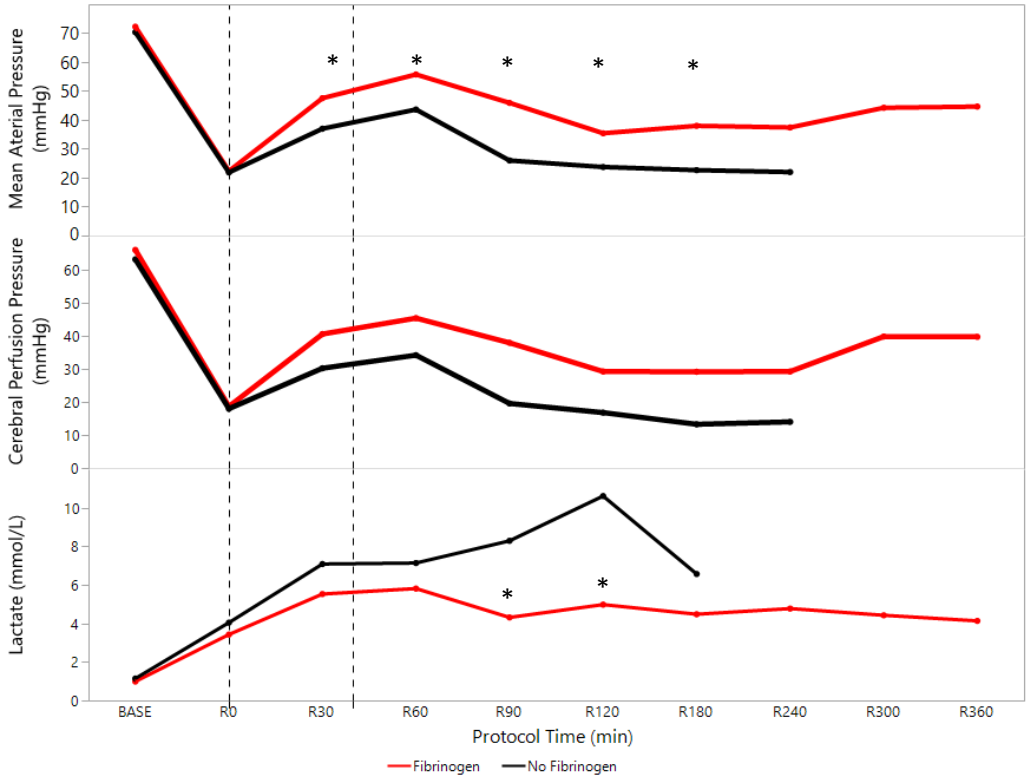


Figure 11. Pooled analysis of the effect of fibrinogen on mean arterial pressure, cerebral perfusion pressure, and arterial blood lactate concentration at baseline prior to hemorrhage (BASE) and during resuscitation (R) in minutes. Vertical interrupted lines denote the time of fluid boluses. * significantly different than No Fibrinogen group at that particular time point at p<0.05.

Vital Organ Blood Flow

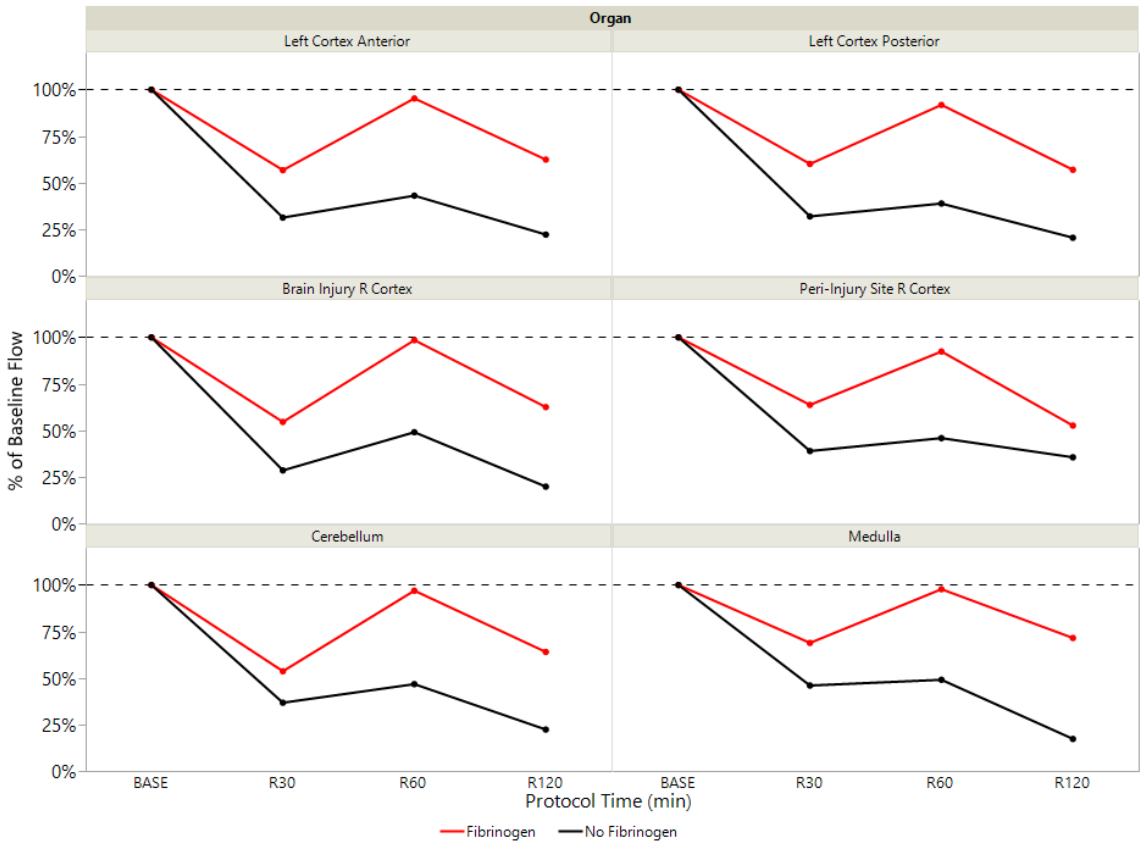


Figure 12. Pooled analysis of the effects of fibrinogen concentrate on regional brain blood flow. The right cortex was injured using fluid percussion. There was a significant effect of fibrinogen on brain blood flow in all regions tested. The horizontal interrupted line represents 100% of baseline flow for reference.

Pooled analysis of the effect of fibrinogen on brain regional blood flow revealed that there was a significant overall effect of fibrinogen on blood flow in all regions measured (all ANOVA treatment effect p values≤0.022)

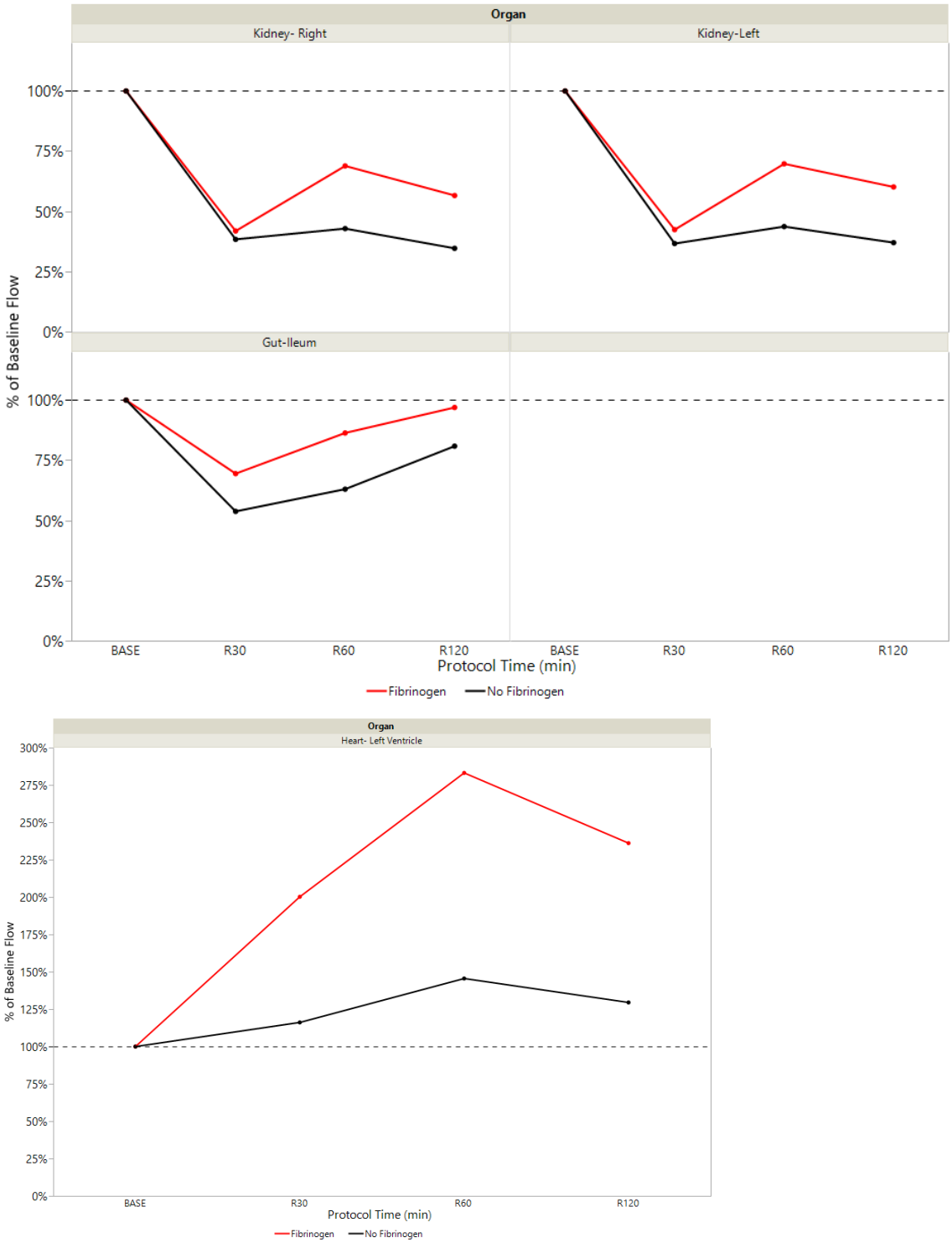


Figure 13. Effects of fibrinogen concentrate on vital organ blood flow to kidneys, gut, (top panel) and heart (bottom panel). Measured using injected colored microspheres. There was a significant effect of fibrinogen on blood flow in all organs tested. The horizontal interrupted line marks the 100% baseline blood flow for visual reference.

Pooled analysis of the effect of fibrinogen on kidney, gut, and heart blood flow revealed that there was a significant overall effect of fibrinogen on blood flow in all organs measured (all ANOVA treatment effect p values≤0.032) (**Figure 13**)

REPORTABLE OUTCOMES

1. We have successfully developed of a polytrauma model with TBI and internal blood loss suitable for testing of low volume damage control resuscitation cocktails.
2. Neurovascular support in the form of Vasopressin restored vascular tone and improved vital organ blood flow after polytrauma. However, internal blood loss was also increased in the presence of Vasopressin.
3. Fibrinogen concentrate at the dosages tested enabled survival to 6 hours and there was a significant increase in overall survival (p=0.05) in pooled analysis.
4. Fibrinogen concentrate counteracted the effects of vasopressin on bleeding by decreasing internal blood loss.
5. The effect of fibrinogen supplementation on internal blood loss is due to the specific hemostatic properties of fibrinogen rather than general protein oncotic effects.
6. Fibrinogen concentrate significantly increased brain blood flow and vital organ blood flow during limited resuscitation of polytrauma with TBI and hemorrhagic shock with free internal bleeding.
7. The current model does not induce hyperfibrinolysis and there was no effect of TXA in this model.
8. **The best performing damage control resuscitation cocktail in this model was Hextend (7ml/kg) + Vasopressin (0.4 ug/kg) + 50mg/kg fibrinogen concentrate given as two boluses per TCCC fluid resuscitation protocol.**

Animal Use:

Updated animal use protocol was approved on 12-04-2014.
We have used a total of 57 of 128 allotted animals for this study.
Model Development: 15/25 animals included
Objective 1: 24/29 animals included
Objective 2. 5/8 animals included
Objective 3. 13/16 animals included

Updated Study Schedule

Projects	Months 1-3-April-June '14	Months 4-12, July'14-April'15	Months 13-15, May-July'15	Months 16-22, Aug'15-Feb'16	Months 23-24, March-April'16
1.) Project Preparation: Goal- Preparation for experiments.	ACURO Approval Lab setup Acquire Animals	Model Development Protocol Revision 2nd ACURO Approval			
2.) Objective 1: (Year 1: Months 4-12) Goal – Identify optimal fibrinogen concentration needed to augment low volume Hextend field resuscitation.		FBG Dose Escalation Study Data Analysis, Submit Year-1 report			
3.) Objective 2: (Year 2: Months 13-15) Goal- Albumin control experiments to determine the specific effect of fibrinogen.		Albumin Control Experiments		Data Analysis supplementa Report, and Lab Resupply and setup	
4.) Objective 3: (Year 2: Months 16-22) Determine effect of adding tranexamic acid (15mg/kg) to the optimal fibrinogen dosage determined in objective 1.				TXA Dose Experiments	
Study Close (Year 2: Months 23-24). Final Report to USSOCOM, Final publication					Data Analysis and Publication

CONCLUSIONS

Experiments are complete for this project. The damage control resuscitation cocktail compromised of Hextend, Vasopressin, and 100mg/kg total fibrinogen concentrate performed the best in this model. We have discovered that Vasopressin can provide cardiovascular support without detrimental effects on vital organ microvascular blood flow. However, Vasopressin also increased blood loss from the aortic tear. The addition of fibrinogen concentrate at 100mg/kg total concentration successfully prevented the increased internal bleeding resulting in a strong effect of fibrinogen on overall survival to 6 hours. Given the severity of the model which

included TBI, femur fracture, 35% blood volume loss, and the very limited resuscitation provided (20ml/kg total), this model should be universally lethal at 6 hours. ***Therefore, any survival to 6 hours with the DCR cocktail is a remarkable achievement.*** In addition, our results indicate that fibrinogen also has favorable effects on brain and vital organ blood flow during volume-limited TCCC resuscitation in the presence of free bleeding. Fibrinogen has multiple effects outside of its hemostatic role. It is also the primary determinant of blood viscosity at the level of the microcirculation. This effect may explain the strong effects seen on brain and organ blood flow. This is an important result given the strong association between hypotension and increased secondary brain injury after trauma.

Next Steps

Further optimization of the DCR cocktail is indicated. Further testing should include a slower infusion rate that includes Vasopressin to support cardiovascular tone and fibrinogen to further reduce blood loss and support vital organ blood flow over a longer time period. TCCC guidelines have also changed in the interim to emphasize early infusion of fresh whole blood by buddy transfusion when possible. In this respect the DCR cocktail should be retested in combination with whole blood transfusion. Further considerations for testing and optimization include retesting in a coagulopathic bleeding model that includes overt hyperfibrinolysis. A pre-application containing these goals has been submitted to W81XWH-USSOCOM-BAA 15-1 to optimize the selected DCR cocktail.