Published in final edited form as:

Shock. 2014 November ; 42(5): 456–463. doi:10.1097/SHK.0000000000000238.

Fibrinogen Concentrate Improves Survival During Limited Resuscitation of Uncontrolled Hemorrhagic Shock in a Swine Model

Nathan J. White¹, Xu Wang¹, W. Conrad Liles², and Susan Stern¹

¹Division of Emergency Medicine, University of Washington, Seattle WA, USA

²Division of Allergy and Infectious Diseases, University of Washington, Seattle WA, USA

Abstract

The purpose of this study was to evaluate the effect of fibrinogen concentrate, as a hemostatic agent, on limited resuscitation of uncontrolled hemorrhagic shock. We use a swine model of hemorrhagic shock with free bleeding from a 4mm aortic tear to test the effect of adding a one-time dose of fibrinogen concentrate given at the onset of limited fluid resuscitation. Immature female swine were anesthetized and subjected to catheter hemorrhage and aortic tear to induce uniform hemorrhagic shock. Animals (N=7 per group) were then randomized to receive either; 1. No fluid resuscitation (Neg Control), 2. Limited resuscitation in the form of two boluses of 10ml/kg of 6% hydroxyethyl starch solution (HEX) given 30 minutes apart, or 3. The same fluid regimen with one dose of 120mg/kg fibrinogen concentrate given with the first HEX bolus (FBG). Animals were then observed for a total of 6 hours with aortic repair and aggressive resuscitation with shed blood taking place at 3 hours. Survival to 6 hours was significantly increased with FBG (7/8, 86%) vs. HEX (2/7, 29%), and Neg Control (0/7, 0%) (FBG vs. HEX, Kaplan Meier LR $p=0.035$). Intraperitoneal blood loss adjusted for survival time was increased in HEX (0.4ml/kg/min) when compared to FBG (0.1mg/kg/min, $p=0.047$) and Neg Control (0.1ml/kg/min, $p=0.041$). Systemic and cerebral hemodynamics also showed improvement with FBG vs. HEX. Fibrinogen concentrate may be a useful adjunct to decrease blood loss, improve hemodynamics, and prolong survival during limited resuscitation of uncontrolled hemorrhagic shock.

Keywords

Trauma; Hemorrhage; Hemostasis; Fibrinogen; Resuscitation

INTRODUCTION

Fluid resuscitation of hemorrhagic shock prior to definitive hemostasis is a great challenge. Fully 30% of hemorrhagic deaths from trauma occur prior to hospital arrival and definitive surgical hemostasis (1,2) Limited-volume fluid resuscitation and early hemostatic therapy

Corresponding Author: Nathan White MD, Harborview Medical Center, Emergency Department, Box 359702, 325 9th Avenue, Seattle, WA 98104, whiten4@uw.edu, Phone: 206-744-8465, Fax: 206-744-4095.

Conflicts of Interest

The remaining authors report no relevant financial disclosures or conflicts of interest associated with this study.

using coagulation protein-containing blood products are now used as hemostatic resuscitation in combination with damage-control surgery (3). When combined with early damage control surgery to stop bleeding, hemostatic resuscitation can reduce morbidity and mortality (4). However, these approaches require both rapid blood product transfusion and rapid transport to surgical facilities. Often times, these preconditions cannot be met, especially during combat when there can be significant transport delays due to terrain, weather conditions, and remoteness. Fully 90% of combat casualties in recent US conflicts died prior to arrival at surgical facilities, and approximately 90% of potentially survivable deaths were attributed to hemorrhage (5). Truncal hemorrhage is often the case because tourniquets for control of extremity hemorrhage are now standard of care. Therefore, there remains a great need for hemostatic resuscitation strategies that can both decrease blood loss from Truncal hemorrhage and provide resuscitation to prolong survival time during transport to hospital facilities.

Two critical components of coagulopathy and blood loss after injury are the rapid decrease of fibrinogen concentration in plasma and endothelial activation and dysfunction. Fibrinogen is the first hemostatic protein to reach critically low levels during surgical blood loss (6). Fibrinogen can be consumed by its activation to fibrin and hyperfibrinolysis during shock, diluted by administration of crystalloid resuscitation fluids, and can be lost to the extravascular compartment by neutrophil-mediated endothelial permeation (7–9). Early replacement of fibrinogen after hemodilution and bleeding in swine models of solid organ injury can improve clot strength, reduce blood loss, and prolong survival time (10,11). There is also evidence that a higher fibrinogen/red blood cell transfusion ratio may provide a survival benefit in combat casualties (12). In addition, there is a suggested mortality benefit of the antifibrinolytic tranexamic acid for trauma patients (13). However, it is unclear if fibrinogen supplementation can decrease blood loss from high-pressure truncal wounds and enhance resuscitation during limited resuscitation lasting several hours.

Disruption of endothelial barrier function and loss of the protective endothelial glycocalyx have further been implicated as initiators of coagulopathy after trauma (14–16). During endothelial homeostasis Angiopoietin-1 (Ang-1) is continuously secreted and activates the Tie2 receptor to promote a local anti-inflammatory and an impermeable endothelial barrier. However, preformed Ang-2 is released from endothelial Weibel-Palade bodies upon endothelial activation and competitively inhibits Ang-1/Tie2 interactions, thus promoting endothelial barrier disruption and leakage. Plasma levels of Ang-2 have correlated with clinically-relevant organ dysfunction and markers of endothelial cell activation in septic humans and have been implicated in regulation of vascular reactivity during hemorrhagic shock (17–19).

The primary objective of this study was to test the effect of fibrinogen concentrate during limited resuscitation of hemorrhagic shock with truncal bleeding from a high-pressure arterial wound. We hypothesized that fibrinogen concentrate will reduce blood loss, prolong survival time, and improve clot formation. Secondly, we aimed to evaluate the performance of this model in terms of endothelial activation and its sensitivity to bleeding during fluid resuscitation of hemorrhagic shock. We used a two-phase swine model of hemorrhagic shock with free bleeding from aortic tear under conditions of limited fluid

resuscitation to test the hemostatic effect of fibrinogen concentrate during limited resuscitation (phase I) followed by survivability during surgical hemostasis and aggressive resuscitation (phase II).

MATERIALS AND METHODS

Animals

The protocol was approved by the Animal Care and Use Committee of the University of Washington and animals were cared for at the AAALAC-accredited Department of Comparative Medicine facility. Compliance with the Animal Welfare Act was maintained according to the *Guide for the Care and Use of Laboratory Animals*, National Academy of Science, 2011.

This research protocol was previously published in detail (20). Briefly, Immature female mixed breed Yorkshire swine were first sedated using intramuscular Ketamine (Bioniche Pharma, Galway, Ireland) and anesthetized throughout the protocol using inhaled 2% isoflurane (VetOne, Boise, ID). A one-time Buprenorphine (0.01mg/kg) (Ben Venue Laboratories Inc. Bedford, OH) injection was given for analgesia and to reduce the concentration of isoflurane needed to achieve and maintain a surgical-plane of anesthesia. Animals were placed on a volume cycled ventilator and ventilated (ANESCO SAV 2500 Anesthesia Ventilator, ANESCO, Inc. Georgetown, KY) with a tidal volume of 5 to 10 mL/kg adjusted to maintain pCO₂ between 35 and 40 mmHg (Capnomac Ultima, Datex, Madison, WI). Bilateral femoral artery and femoral vein catheters were placed and a Swan-Ganz thermodilution catheter (Edwards Life Sciences, Irvine, CA) was advanced from the external jugular to the pulmonary artery for measurement of cardiac output (CO), pulmonary artery pressure, central venous pressure, and sampling for central venous blood gas. Placement was confirmed by characteristic pressure wave form. A laparotomy incision was then made, splenectomy, and bladder cannulation performed, and a 5-0 monofilament stainless steel surgical wire was inserted traversing 4mm longitudinally into the infrarenal aorta. The aortotomy wires were externalized through the midline abdominal incision and the abdominal skin was then closed with staples.

To monitor cerebral resuscitation parameters, the brain was also instrumented for monitoring using a neonatal intraventricular catheter (Phoenix Biomedical Corp. Valley Forge PA) to measure intracranial pressure (ICP). All animals re-equilibrated for 30 minutes after instrumentation. Body temperature was maintained at 37±1°C with a warming blanket throughout the protocol.

Controlled hemorrhage begun at an initial rate of 35ml/kg/min from the right femoral artery catheter with a MasterFlex® roller pump (Cole-Parmer, Vernon Hills, IL). The rate was pre-programmed to decrease exponentially over time. The shed blood from the femoral artery was collected into an agitated blood collection bag containing citrate anticoagulant (1:9 volume ratio of citrate to blood) for later autologous whole blood transfusion. When mean arterial pressure (MAP) reached 50 mmHg, the infrarenal aortotomy wire was pulled, creating a 4mm aortic tear for uncontrolled hemorrhage in the closed abdomen. Once MAP

equaled 30 mmHg, the roller pump was paused and restarted to maintain MAP equal to 25–30 mmHg for 15 minutes.

Animals were randomized to one of three groups, each group containing seven animals. Negative controls (Neg Control) received no fluid resuscitation; Fibrinogen treated animals (FBG) received 120mg/kg of purified human fibrinogen (Product #F4883, Sigma Aldrich Co.) dissolved to final volume of 50 ml in 0.9% Normal Saline concomitantly with a 10 mL/kg bolus of 6% hydroxyethyl starch solution in lactated electrolyte solution (Hextend™, Hospira Inc. Lake Forest, IL, USA) infused over 10 minutes; and the hydroxyethyl starch group (HEX) received the same 10ml/kg of Hextend™ infused over 10 minutes concomitantly with 50 ml of Normal Saline as a fibrinogen volume control. A second identical 10ml/kg HEX infusion was given to all animals in the FBG and HEX groups 40 minutes later. The fibrinogen product used is a highly purified lyophilized human fibrinogen from human plasma containing 90% clottable fibrinogen protein and 0.03 Units/mg solid plasminogen and plasmin. The product was not tested for the presence of endotoxin. Previous studies of fibrinogen supplementation after hemodilution and hemorrhage have reported efficacy from 70–200mg/kg doses (10,11). We chose 120mg/kg as a reasonable dose considering the presence of a high-pressure aortic injury that we assumed would require more than minimum dosing for a hemostatic effect. Hextend was used because it is a popular colloid volume expander used for combat casualty care and remains the recommended initial resuscitation fluid for tactical combat casualty care despite recent reports of increased need for renal replacement when similar hydroxyethyl starch-based colloids were used for resuscitation in ICU patients (21). Hextend was also used in this model to induce a greater degree of coagulopathy than could be obtained from simple hemorrhage to counteract the robust coagulation system of swine (22). Resuscitation was divided into two phases: I) pre-hospital resuscitation (180 minutes) and II) in-hospital surgical repair of aortic tear at 180 minutes and further goal-directed care up to 360 minutes.

The animals received no further interventions or fluids during Phase I. At Phase II, the abdomen of all surviving animals was opened, bleeding from the aortic tear was controlled and the aortic injury was repaired primarily using 6-0 prolene suture. Intraperitoneal hemorrhage was measured using pre-weighed gauze sponges. The peritoneum, midline fascia, and skin were then closed. All surviving animals were then aggressively fluid resuscitated to restore normal physiological parameters using normal saline to achieve MAP 70 mmHg and shed blood to achieve Hb 7g/dl. Mechanical ventilation was titrated to achieve arterial blood oxygen saturation >92% and end tidal CO₂ between 35 to 40 torr. Resuscitation was continued in this manner for an additional 180 minutes for a total of 360 minutes after initial hemorrhage. Surviving animals were euthanized humanely under anesthesia using intravenous pentobarbital overdose (Euthanasia III solution, Med-Pharmex, Inc. Pomona, CA). Criteria for death prior to 360 minutes were defined as MAP 10mmHg with loss of arterial waveform for one minute. Animals meeting these criteria were promptly euthanized.

Measurements

The hemodynamic, cerebral, metabolic, and endothelial state of the animal was serially measured during the protocol. Vital signs, central hemodynamics, and cerebral parameters were measured and recorded serially using Biopac Software. Standard metabolic markers including lactate, base excess, and blood gas, were measured using the Radiometer blood gas analyzer ABL 805 (Radiometer, Copenhagen, Denmark). We measured Angiotensin-2 (Ang-2) in heparinized plasma to evaluate endothelial activation and barrier function. Ang-2 was measured at baseline, 30, and 105 minutes and was staggered from other measurements to reduce the overall volume of blood taken at each time point to prevent phlebotomy from impacting resuscitation. Plasma concentrations (dilution factors indicated in parentheses) of Ang-2 (1:5) by ELISA using validated, paired monoclonal antibodies (R&D Systems DuoSet kits, Minneapolis MN, USA) according to manufacturers' instructions with modifications: (1) assays were performed in 50 μ L per well; (2) plasma samples were incubated overnight at 4°C; and (3) ELISAs were developed using ExtravidinR-Alkaline Phosphatase (Sigma-Aldrich; 1:1000 dilution, 45-minute incubation) followed by addition of p-nitrophenyl phosphate substrate (Sigma-Aldrich) before optical density reading at 405 nm. Concentrations were interpolated from 4-parameter-fit standard curves. Background levels were determined from blank wells included on each plate (assay buffer added instead of sample), and the subsequent optical density was subtracted from all samples and standards prior to analysis. Samples with optical densities below the lowest detectable standard were assigned the value of that standard. The lower limit of detection was 27.34 pg/mL.

To measure coagulation and clot formation, fibrinogen concentration was measured in plasma by the modified method of Clauss using a bedside coagulation monitor (STArt-4, Diagnostica Stago Inc., Asnières, France). Whole blood clot formation was measured using thrombelastography (TEG, TEG-5000, Haemonetics Corp, Braintree, MA). Thrombelastographs of activated whole blood were performed by recalcification of standard citrated whole-blood samples in the presence of 1 IU/ml of human thrombin (Diagnostica Stago Inc., Asnières, France) according to manufacturer's guidelines. Fibrin-specific clot strength was measured in recalcified samples in the presence of 10 μ g/ml cytochalasin-D to paralyze the cytoskeleton of platelets, preventing platelet-induced clot contraction. The commercially-available TEG functional fibrinogen assay was not used because pig platelets are resistant to this assay.

Intraperitoneal blood loss was measured at 180 minutes when the abdomen was reopened for aortic repair. Pre-weighed gauze sponges were used to collect and quantify the blood loss within the abdominal cavity.

Statistical analysis

Normally-distributed continuous data was expressed as mean with SEM. The primary outcome of interest was survival. Univariate Kaplan-Meier survival analysis was performed to compare survival rates for each intervention. Normally distributed continuous outcome variables that were measured at a single point in time (e.g. blood loss, fluid requirement) were compared using analysis of variance (ANOVA). We calculated and compared both total hemorrhage volume as well as hemorrhage volume adjusted for survival time. A mixed

model repeated measure two-way ANOVA (rmANOVA) was used to identify the effect of FBG on continuous data measured longitudinally over time (e.g. MAP). For all analyses two-sided $p < 0.05$ was considered to be statistically significant for each overall effect. Individual comparisons were made after Tukey Kramer adjustment for multiple comparisons. Pearson product moment correlation was calculated to determine bivariate associations between variables.

All analyses were performed using JMP-9 statistical package (SAS Inc., Cary, NC).

RESULTS

Survival and Blood Loss

Basic characteristics and outcomes are given in Table 1. A total of 21 swine ($N=7$ per group) were studied. Baseline weights, initial catheter hemorrhage volumes, and end-of-catheter-hemorrhage lactate concentrations were similar in all groups indicating a uniform degree of hemorrhagic shock at the onset of prehospital fluid resuscitation. We found this model to be universally and rapidly lethal after approximately one hour without fluid resuscitation. Intraperitoneal blood loss was decreased and survival time was longest with FBG. Kaplan Meier survival analysis is given in Figure 1. FBG significantly improved survival (6/7, 86%) compared to HES (2/7, 29%), or Neg Control (0/7, 0%) (KM LR $p=0.035$). All deaths occurred during the 'prehospital' phase I of fluid resuscitation when the aortic injury was allowed to freely bleed.

Resuscitation

Systemic and cerebral hemodynamic resuscitation parameters are plotted in Figure 2. Mean arterial pressure (MAP) with FBG was similar to HES during the first 60 minutes of fluid resuscitation, but was increased above standard hypotensive resuscitation goals (MAP = 65mmHg) during the latter portion of phase I, achieving near baseline levels after approximately 70 minutes of resuscitation. MAP in the HES animals remained at-or-below hypotensive resuscitation goals throughout phase I. After aortic repair and aggressive resuscitation (phase II), all surviving animals achieved a near-baseline MAP. After 60 minutes of fluid resuscitation, cardiac output tended to be higher with FBG vs. HES. Cerebral resuscitation parameters responded similarly to systemic resuscitation parameters. Cerebral perfusion pressure (CPP) was uniformly decreased after catheter hemorrhage and did not recover when fluid resuscitation was withheld in the Neg Control group. CPP increased rapidly with treatment in both FBG and HES groups. Cerebral perfusion pressure tended to be higher with FBG during the latter half of phase I. Animals receiving FBG also experienced an increase in PAP above other groups between 10–60 minutes of resuscitation. PAP was increased compared to other treatment groups for approximately 60 minutes during fluid infusion in phase I and returned to approximate baseline levels thereafter. There was no discernable decrease of MAP or cardiac output when PAP was elevated.

Arterial lactate concentration was increased similarly in all groups during the initial hemorrhage period. (Figure 3) Lactate increased steadily until death without fluid resuscitation. There were two lactate peaks during the prehospital period in the HES group.

The first was early, between 15–60 minutes, and the next was later between 105–150 minutes of resuscitation. With FBG, arterial lactate peaked early at 60 minutes and declined thereafter, achieving near baseline levels during the latter portion of the prehospital period. Arterial lactate concentration returned to near baseline levels in all surviving animals after aortic repair and aggressive resuscitation, indicating that these animals had not yet reached a state of irreversible hemorrhagic shock by the end of the prehospital period. Statistical comparison of animals surviving to phase II was severely limited because only N=2 HEX animals reached phase II vs. N=7 FBG animals. The 2 surviving HEX animals required more normal saline after aortic repair (mean= 20ml/kg (SE=0.1)) compared to FBG (1.5ml/kg (0.6)). We were also unable to identify any differences in the volume of shed blood required to reach the predetermined physiological parameters (HEX=20ml/kg (6.4) vs. FBG=9ml/kg (4), $p=0.2$)).

Clot Formation and Endothelium

Mean plasma fibrinogen concentration was similar in all groups at baseline and after catheter hemorrhage. At 60 minutes of phase I fluid resuscitation, fibrinogen levels were significantly reduced compared to baseline only in the HES group. Thrombin-activated whole blood TEG parameters and fibrin-specific TEG parameters are given in Table 2. There were no differences in thrombin-activated TEG parameters between treatment groups. MA was reduced at 60 minutes of resuscitation compared to baseline only in the HEX group. Fibrin-specific clotting Angle was significantly decreased at 60 minutes of phase I compared to time=0 and MA was significantly reduced for both HEX and FBG groups at 60 minutes compared to baseline. MA was not reduced from baseline levels at 60 minutes in the Neg Control group. Ang-2 levels tended to increase over time in all groups during resuscitation and were not significantly different between groups (See Figure 4.).

Model Performance

To assess model performance, animals were classified as survivors and nonsurvivors at six hours and blood loss, fibrin-specific thrombelastography, and endothelial activation by the concentration of Ang-2 in plasma were compared. (Figure 5.) Intraperitoneal blood loss was significantly greater in nonsurvivors. Total unadjusted intraperitoneal blood loss ($R=-0.75$, $p<0.001$) and adjusted blood loss for survival time ($R=-0.88$, $p<0.001$) were also significantly and negatively associated with survival time in those animals receiving fluid resuscitation. At 60 minutes of prehospital resuscitation, fibrinogen concentration was decreased and fibrin specific clot formation was significantly impaired in nonsurvivors. Intraperitoneal blood loss was strongly and negatively correlated with both 60 minute fibrinogen concentration ($R=-0.85$, $p<0.001$) and fibrin-specific MA ($R=-0.65$, $p=0.005$) in the fluid resuscitation groups. Ang-2 levels steadily increased over time in nonsurvivors during Phase I, achieving an average 319% increase from baseline at 30 minutes, and 780% increase from baseline at 105 minutes. Plasma Ang-2 levels were also increased in nonsurvivors compared to survivors by 30 minutes of prehospital resuscitation and remained elevated at 105 minutes of resuscitation indicating early endothelial activation that was amplified in nonsurvivors.

DISCUSSION

Survival and Blood Loss

Standard TCCC limited resuscitation using HES was clearly enhanced in this model with the addition of one dose of 120mg/kg of fibrinogen concentrate. Survival was improved, rate of internal bleeding was reduced, and global and cerebral resuscitation was enhanced with fibrinogen. In addition, plasma fibrinogen concentration was preserved and the blood loss adjusted for survival time was decreased with fibrinogen. However, there was no clear increase in fibrin-specific clot strength associated with fibrinogen-supplementation.

Other swine models of hemodilution and bleeding from solid organ injury have produced similar results to ours. Fries et al, demonstrated that high-dose fibrinogen concentrate (200mg/dl) given after 65% hemodilution with gelatin could reduce blood loss from a liver injury and restore fibrin clot architecture (10). Similarly, Grottke et al., induced coagulopathy using HES and lactated ringers to replace 80% of blood volume in a swine model. They found that as little as 70mg/kg of fibrinogen supplementation could prolong survival and reduce blood loss after blunt liver injury (11). These studies provide strong evidence for the critical role of fibrinogen to counteract coagulopathy and slow bleeding after injury. Our data also support a beneficial effect of fibrinogen supplementation to reduce life-threatening bleeding after injury. We add that the favorable effect of fibrinogen supplementation is preserved in the setting of high-pressure arterial bleeding.

Resuscitation

Limited resuscitation strategies are not typically capable of providing for full hemodynamic or metabolic resuscitation. This limitation dictates that current low-volume fluid resuscitation strategies may be used only briefly, and must be followed by rapid surgical hemostasis prior to more aggressive resuscitation. This situation is commonly encountered by both civilian and military settings. However in our study, markers of both global and cerebral resuscitation were improved and even normalized with the addition of fibrinogen concentrate to limited fluid resuscitation in this model. The benefit may be due to reduced blood loss that appears to be associated with fibrinogen supplementation during resuscitation and may be due to its hemostatic effect. Others have demonstrated beneficial effects of hemostatic enhancement on blood loss and blood pressure at rebleeding in swine models. Sondeen et al used activated recombinant Factor VII (rFVIIa) to demonstrate a significant increase in blood pressure rebleeding threshold and a decrease in blood loss in a similar swine aortic injury model (23). Honickel et al showed that prothrombin concentrate (PCC) could also reduce blood loss from liver injury and improve thrombin generation in a swine model of dilution and hypothermia (24). However, rFVIIa failed to improve outcomes in a large randomized clinical trauma trial and there appears to be a narrow therapeutic range for PCC in trauma (25, 26). Increasing dosage from 35mg/kg to 50mg/kg was associated with increased thromboembolism and onset of disseminated intravascular coagulation after blunt liver injury in a swine model (26). Our results suggest that fibrinogen concentrate may offer similar benefits, but specific studies of rebleeding are required. Fibrinogen concentrate use in afibrinogenemic patients and bleeding patients also report very few adverse events (27,28). A 22-year pharmaco-surveillance program of a single fibrinogen concentrate

(Haemocompletan CSL-Behring GmbH, Germany) reported only nine spontaneous reports of thrombotic adverse events (3.48 events per 105 treatment episodes) (29). Further testing to identify a hemostatic effect of fibrinogen concentrate on resuscitation and potential for pathological thrombosis after trauma is indicated.

Clot Formation

Survivors demonstrated preserved fibrin-specific clot strength at 60 minutes post-fluid resuscitation in this model. However, clot strength was similarly decreased in both FBG and HEX groups after fluid administration. Fibrinogen concentration was also preserved at baseline levels with sham resuscitation when both fibrinogen supplementation and fluid resuscitation were withheld. These results suggest that the primary effect of fibrinogen supplementation at the chosen dosage was to counterbalance the dilutional and anticoagulant effects of Hextend rather than a supranormal effect of fibrinogen on clot formation. Hextend has direct coagulopathic effects when given in similar dosage to ours (20ml/kg) that are above and beyond hemodilution (22). The direct coagulopathic effect is due to the hydroxyethyl starch-induced interference with clot propagation and strength rather than impairment of thrombin generation and is recoverable with fibrinogen treatment (22,30). We suspect that fibrinogen concentrate did preserve clot strength in this model. However, inter-animal variation was too wide and mortality too early in the HES group to definitively conclude a specific effect of fibrinogen concentrate because of the limited number of animals tested in each group (N=7). Further studies powered to show differences in clot formation with fibrinogen concentrate are needed.

Functional fibrinogen measurements by TEG have demonstrated good correlation with plasma Clauss-based measurements of fibrinogen concentration in trauma patients (31). However, we found a discordance between these measurements in the FBG group in which fibrinogen concentration was preserved when measured by the method of Clauss in plasma, yet fibrin-specific clot strength was reduced when measured by TEG. A possible explanation for this discordance is the tendency for some coagulation analyzers to report falsely-elevated fibrinogen concentrations in the presence of colloid volume expanders when employing the method of Clauss (32).

Model Performance

The goal of this model was to create favorable physiological conditions for severe hemorrhagic shock and endothelial dysfunction in the setting of free bleeding from a non-compressible internal wound that would be responsive to hemostatic interventions. Ideally, severe shock and endothelial dysfunction would be induced during phase I, yet animals would remain recoverable during phase II with surgical hemostasis and aggressive resuscitation. In keeping with these goals, we found that survival time in the resuscitated animals in this model was closely associated with the amount of internal blood loss, making it relevant to study the impact of hemostatic and fluid resuscitation strategies. The animals in the Neg Control group clearly died from hemorrhagic shock primarily from the initial catheter hemorrhage because intraperitoneal blood loss was very low and lactate rose consistently until death. Nonsurvivors in the HEX group initially benefited from fluid resuscitation, but continued to bleed and succumbed during phase I. Therefore, these

animals represent a situation where fluid resuscitation promotes rebleeding and exsanguination. The two surviving animals in the HEX group likely survived because they demonstrated very little internal blood loss (mean=10.2ml/kg STD=6.7). A single animal in the FBG group died quickly with a large amount of blood loss, while the remaining survivors in the FBG group bled very little, achieving a volume of adjusted intraperitoneal blood loss that was comparable to those receiving no fluid resuscitation. Overall, the survival benefit seen in the fibrinogen group may be attributed to decreased blood loss during fluid resuscitation. However, it is not clear if this effect can be attributed to an increase in fibrin-dependent clot strength.

Fibrinogen levels were clearly compromised early after hemorrhage in this model. This effect was primarily due to HES-induced dysfunction and dilution, since fibrinogen concentration was not reduced after hemorrhage in the Neg Control group. Endothelial activation was also likely initiated early during phase I as indicated by increased plasma Ang-2 levels over time. Other animal models have suggested a role for Ang-2 in the regulation of vascular reactivity during hemorrhagic shock and early death after experimental injury (33,34). Ang-2 has also been shown to be increased in the serum of trauma patients with septic complications (34). Overall, our model performed adequately to test the effect of a hemostatic intervention. In addition, a metabolic milieu was created by this model that reflected important components of trauma induced coagulopathy (TIC). However, the magnitude of decreased clot strength in whole blood in the Neg Control and HEX groups was limited and not consistent with that reported in humans with TIC (35). Therefore, we cannot conclude that the coagulopathy created in this model was similar to native TIC reported in humans.

Limitations

Several limitations must be considered when interpreting the results of this study. First, this is a tightly-controlled model of hemorrhage with clearly-defined wound geometry, and close monitoring of physiology. These conditions, along with obvious differences between humans and swine, does not allow for direct application of these results to the human trauma population. Next, the model does not fully reproduce native primary TIC where coagulopathy spontaneously develops after injury and hemorrhage without the influences of secondary coagulopathies. We suspect that the lack of additional tissue trauma precluded our ability to fully reproduce native TIC-like conditions in this model. We also are unable to separate protein colloidal and hemostatic effects of fibrinogen concentrate in this study. There may have been specific effects of fibrinogen as a colloidal protein that were independent of its hemostatic effects. We are planning future studies on this topic that will include a comparable non-hemostatic protein control such as albumin to investigate this possibility. We also noted an increase in pulmonary artery pressure that coincided with fibrinogen infusion. The cause of this reaction and if it occurs similarly during fibrinogen infusion in humans remains unclear. Pigs are known to have similar adverse reactions to perfluorocarbon, particulate, and endotoxin infusions (36,37). This response may be attributed to an acute inflammatory reaction causing pulmonary vascular constriction mediated by pulmonary macrophages because depletion of pulmonary macrophages in swine has also been shown to eliminate the increase in PAP associated with endotoxin

infusion (38). There was also no simultaneous increase in systemic vascular resistance (data not shown), decrease in blood pressure, or decreased cardiac output to suggest that the PAP response was a product of systemic vasoconstriction similar to that seen with infusion of hemoglobin-based oxygen carriers (HBOC's) (20). The response may also be specific to fibrinogen because isolated rabbit lungs have also demonstrated vasoconstriction and a sharp rise in PAP when perfused with soluble fibrin/fibrinogen-oligomers (39). This response was attenuated when pulmonary thromboxane generation was blocked. While, there were no adverse effects of the increased PAP on hemodynamics or outcomes in this study, the relevance to humans remains unclear and further investigation is required. We also did not specifically investigate for pathological thrombosis of vessels or organs that might be associated with fibrinogen infusion. This is an important potential adverse effect of fibrinogen concentrate and requires further study using animal models specifically designed to assess thrombosis as a safety outcome.

In summary, a single dose of fibrinogen concentrate improved limited resuscitation and survival in this model of high-pressure arterial hemorrhage. Further investigation of fibrinogen concentrate as an adjunct to limited resuscitation strategies is warranted.

Acknowledgments

We would like to acknowledge the special contributions of Nicole Bradbury B.S. who was an integral member of our research team who is no longer with us after unfortunately passing away unexpectedly, may she rest in peace. This study was supported by the Naval Medical Research Center (NMRC) (CDMRP grant W81XWH-08-2-0166). NJW was supported in part, by the National Center for Advancing Translational Sciences (NCATS) (grant KL2 TR000421), a component of the National Institutes of Health (NIH). The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Departments of the Navy, Army, and Defense or the US Government and do not necessarily represent the official view of NCATS or NIH.

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Figure 1.

Kaplan Meier Survival analysis of limited resuscitation with 6% hydroxyethyl starch given as two 10 ml/kg boluses 30 minutes apart at the onset of fluid resuscitation (time = 0 min) with 120mg/kg of fibrinogen concentrate (FBG) and without (HEX). A negative resuscitation control arm (Neg Control) was included to define the lethality of the model without fluid resuscitation. FBG demonstrated significantly improved survival compared to HEX. *=FBG vs. HEX Kaplan Meier Log Rank $p=0.035$. Vertical interrupted line marks time of aortic repair and onset of aggressive resuscitation using shed blood.

Figure 2.

Hemodynamic profiles of animals resuscitated with 6% hydroxyethyl starch (HES), in addition to 120mg/kg human fibrinogen concentrate (FBG) compared to no fluid resuscitation (Neg Control). Mean arterial pressure (**Panel A.**) Cardiac output (**Panel B.**) and cerebral perfusion pressure (**Panel C.**) responded favorably in the FBG group. Pulmonary artery pressure actually increased for a short period of time during fibrinogen infusion (**Panel D.**) The aortic injury was repaired and aggressive resuscitation using shed blood was initiated at 180 minutes.

Figure 3. Arterial lactate profiles

of animals resuscitated with Hextend™ (HEX), with 120mg/kg human fibrinogen concentrate (FBG) compared to no fluid resuscitation (Neg Control). Arterial lactate concentration tended to return towards baseline levels more consistently in the FBG group. The aortic injury was repaired and aggressive resuscitation using shed blood was initiated at 180 minutes.

Figure 4. Fibrinogen concentration and endothelial activation

Plasma fibrinogen concentration was maintained at baseline levels in the FBG and Neg Control groups and was significantly reduced from baseline levels with HEX alone when measured 60 minutes after the onset of fluid resuscitation (**Panel A.**). Angiopoietin-2 (Ang-2) levels in plasma tended to increase over time during fluid resuscitation but were not significantly different between groups (**Panel B.**).

Figure 5. Model Performance

Survivors demonstrated less intraperitoneal blood loss (**Panel A.**) improved fibrin-specific clot formation angle and maximal amplitude by thrombelastography at 60 minutes of fluid resuscitation (**Panel B.**), and significantly less endothelial activation by Ang-2 concentration in plasma (**Panel C.**).

Table 1

Baseline characteristics, blood loss, and survival time.

	Neg Control		HES		FBG	
	Mean	SE	Mean	SE	Mean	SE
Weight (kg)	20.3	0.6	20.3	0.6	20.3	0.6
Catheter Hemorrhage volume (ml/kg)	7.6	0.4	10.3	1.7	9.0	1.0
Total Hemorrhage volume (ml/kg)	30.6 [#]	3.0	47.4 [*]	3.0	38.0	3.0
End of hemorrhage period lactate conc. (mmol/L)	4.1	0.5	4.0	0.5	3.4	0.5
Intraperitoneal blood loss (ml/kg)	6.8 [#]	3.2	22.0 [*]	3.2	12.6	3.2
Adjusted Intraperitoneal Bleeding (ml/kg/min)	0.1 [#]	0.1	0.4 [*]	0.1	0.1 [#]	0.1
Survival Time (min)	72.7	39.9	155.3	39.9	318.7 ^{**#}	39.9

Total Hemorrhage volume is the sum of initial catheter hemorrhage and intraperitoneal blood loss. Intraperitoneal blood loss was measured at the end of the 180 minute prehospital phase during aortic repair. Adjusted intraperitoneal blood loss is the volume of blood in the peritoneum divided by prehospital survival time. One way ANOVA with Tukey Kramer HSD for individual comparisons.

^{*} =Sig. Different vs. No Resuscitation group,

[#] =Sig. Different vs. HES group. Neg Control = no fluid resuscitation. HES = two 10ml/kg boluses of 6% hydroxyethyl starch given 30 minutes apart. FBG= 120mg/kg fibrinogen concentrate given with the first HES bolus. SE= Standard Error.

Table 2

Fibrin-specific and thrombin-activated viscoelastic clot formation.

Fibrin-Specific Clot Formation		Neg Control		HEX		FBG	
TEG Parameter	Protocol Time (min)	Mean	SE	Mean	SE	Mean	SE
R(min)	Baseline	18.5	2.9	9.2	2.7	10.7	2.7
	0	9.7	2.9	6.1	2.9	4.6	2.7
	60	6.3	3.2	9.2	3.2	10.9	2.9
K(min)	Baseline	N/A		N/A		N/A	
	0	N/A		N/A		N/A	
	60	N/A		N/A		N/A	
Angle(deg)	Baseline	22.9	6.6	27.7	5.5	24.3	5.5
	0	21.0	6	43.5	6	49.1	5.5
	60	36.2	6.6	14.2 [*]	6.6	16.4 [*]	6
MA(mm)	Baseline	13.9	2.1	17.7	2.3	17.3	1.9
	0	14.1	2.1	18.4	2.3	20.3	1.9
	60	15.2	2.3	6.3 ^{*#}	2.6	6.9 ^{*#}	2.3

Thrombin-Activated Whole Blood Clot Formation		Neg Control		HEX		FBG	
TEG Parameter	Protocol Time (min)	Mean	SE	Mean	SE	Mean	SE
R(min)	Baseline	1.1	0.2	1	0.2	1.1	0.2
	0	1.1	0.2	1	0.2	0.9	0.2
	60	1.2	0.2	1	0.2	1.4	0.2
K(min)	Baseline	2.4	0.2	1.6	0.2	1.6	0.2
	0	1.6	0.2	1.2	0.2	1.2	0.2
	60	1.2 [#]	0.2	1.4	0.2	1.9	0.2
Angle(deg)	Baseline	62.8	2.5	71.7	2.3	71	2.3
	0	71.1	2.5	76	2.3	76.2	2.3
	60	73.7	2.7	71.5	2.7	66.7	2.5
MA(mm)	Baseline	68.9	2.4	75.6	2.2	76.1	2.2
	0	70.6	2.4	76.9	2.2	79.1	2.2

Thrombin-Activated Whole Blood Clot Formation		Neg Control		HEX		FBC	
TEG Parameter	Protocol Time (min)	Mean	SE	Mean	SE	Mean	SE
	60	71.5	2.6	65.4 [*]	2.6	69.9	2.4

Two-way ANOVA with Tukey Adjustment for multiple comparisons.

^{*} =sig different vs. Protocol Time=0

[#] =Sig different vs. Protocol Time=Baseline

N/A= Criteria not met for measurement