Testing of Blood Products in a Polytrauma Model: Results of a Multi-Institutional Randomized Preclinical Trial

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Introduction: Trauma-induced coagulopathy, acidosis, and hypothermia form a "lethal triad" that is difficult to treat and is associated with extremely high mortality. This study was performed at three academic centers to evaluate whether resuscitation with blood components could reverse the coagulopathy in a complex polytrauma model.

Methods: Yorkshire swine (40 ± 5 kg) were subjected to a three-phase protocol: (a) "Prehospital" phase = femur fracture, hemorrhage (60% blood volume), and 30 minutes shock + infusion of saline (3× shed blood) + induction of hypothermia (33°C); (b) "Early hospital" phase = grade V liver injury; and (c) "Operative" phase= liver packing. After liver packing, the animals (n = 60) were randomized to the following groups: (1) Sham-instrumentation and anesthesia without hemorrhage/injuries, (2) fresh whole blood (FWB), (3) 6% hetastarch (Hextend), (4) fresh frozen plasma/packed RBCs in 1:1 ratio (1:1 FFP/PRBC), and (5) FFP alone. Treatment volumes were equal to the volume of shed blood. Hemodynamic and physiologic parameters and coagulation profile (thrombelastography, prothrombin time, activated partial thromboplastin time, international normalized ratio, and platelets) were monitored during the experiment and for 4 hours posttreatment.

Results: At the end of prehospital phase, animals had developed significant acidosis (lactate >5 mmol/L and base deficit >9 mmol/L) and coagulopathy. Posttreatment mortality rates were 85% and 0% for the Hextend and blood component treated groups, respectively ($p < 0.05$). Hemodynamic parameters and survival rates were similar in groups that were treated with blood products (FWB, FFP, and FFP:PRBC). Animals treated with FFP and Hextend had significant anemia compared with the groups that received red blood cells (FWB and FFP:PRBC). Treatment with FFP and FFP:PRBC corrected the coagulopathy as effectively as FWB, whereas Hextend treatment worsened coagulopathy.

Conclusions: In this reproducible model, we have shown that trauma-associated coagulopathy is made worse by hetastarch, but it can be rapidly reversed with the administration of blood components. Impressively, infusion of FFP, even without any red blood cells, can correct the coagulopathy and result in excellent early survival.

Key Words: Plasma, Hemorrhage, Shock, Coagulopathy, Liver injury, Polytrauma, Acidosis, Hypothermia.

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Development of coagulopathy, acidosis, and hypothermia in trauma patients is an ominous sign. Often labeled as the "lethal triad," these factors perpetuate each other, creating a vicious cycle that is difficult to interrupt.[1–7] Early coagulopathy is a marker of injury severity, as 25% of trauma patients are found to be coagulopathic on initial presentation, and is associated with significantly greater mortality.[8] Two retrospective studies (over 20,000 trauma patients) have reported similar findings despite differences in the definitions of coagulopathy and the volumes of prehospital resuscitation fluids.[9,10] In addition to higher mortality, patients with acute coagulopathy have longer length of stay (intensive care unit and hospital), fewer ventilator-free days,[8,11] and an increased likelihood of developing acute lung and renal injuries.[8] Similarly, early coagulopathy is present in more than one third of combat casualties that require transfusion, and it correlates with increased injury severity and mortality.[12]

Early and effective reversal of coagulopathy is clearly important[13] but the best method to achieve this goal remains controversial. Minimizing tissue ischemia, preventing excessive blood loss, avoiding large volume crystalloid resuscitation, rapid correction of hypothermia and acidosis, and appropriate blood component therapy are all logical steps. Mathematical models have been used to predict the ratios of fresh frozen plasma (FFP) and platelets that must be infused along with packed RBCs (PRBC) to prevent the development of coagulopathy.[14] Once coagulopathy is established, blood components are commonly transfused in an attempt to reverse the process. The optimal approach, however, remains controversial. Recent retrospective analysis of combat casualty data

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has provided some evidence in favor of early plasma transfusion by showing that FFP and PRBC transfusions were independently associated with increased survival and decreased survival, respectively.[15] Although some researchers have suggested that FFP should be given early,[16] and in a high ratio to PRBC (1:1),[17] this recommendation lacks prospective data. These retrospective studies have been criticized for having a "survival bias," where the most severely injured patients simply did not survive long enough to receive the plasma which is often not immediately available.

When component therapy is not available, military hospitals often resort to use fresh whole blood (FWB). According to recent reports, 13% of all transfused patients in the Operation Iraqi Freedom received FWB.[18] Although intriguing, all these studies suffer from the deficiencies of retrospective analyses and from a lack of appropriate control groups. Different ratios of blood components were not directly tested against each other in any of these studies. A large civilian trauma center has published their findings that suggest that establishment of an exsanguination protocol (emphasizing early FFP/platelet transfusion) significantly reduces mortality.[19] In that study, the protocol patients received FFP and PRBC in 1:2 ratio as well as a significantly lower volume of intravenous crystalloids, and the total number of PRBC and FFP transfused over 24 hours were nearly identical between the groups. Although this study advocated early (intraoperative) transfusion of FFP and platelets, it failed to identify whether there was an optimal dose of FFP. It also does not appear that the impressive improvement in mortality was because of a better correction of coagulopathy (and less blood loss) as there was no decrease in the overall red blood cell transfusion. On the other hand, another civilian center study has shown that the association between the FFP:PRBC and mortality follows a U-shaped curve, with higher mortality on either end and best results are achieved when the ratio is 1:2–1:3.[20] Interestingly, 1:1 FFP:PRBC ratio, while reducing coagulopathy, failed to improve survival in their retrospective analysis, prompting the authors to caution against adopting the 1:1 ratio without further testing. Many more clinical studies in the near future are expected to address this issue. Normal coagulation requires a fine balance between the pro- and anti-coagulation systems, and clearly an increase in clot breakdown can be equally detrimental. A prospective cohort study (>200 patients) has shown that acute coagulopathy of trauma is caused by tissue hypoperfusion and resultant anticoagulation/hyperfibrinolysis and not because of a deficiency of clotting factors.[21] This early coagulopathy can be seen in patients soon after arrival in the hospital, before they have received a large volume of resuscitation fluids (before hemodilution). This raises the question whether the benefits of plasma resuscitation noted by others were because of reversal of coagulopathy, better restoration of tissue perfusion, or other yet to be identified mechanisms? This is an important question because transfusion of blood products can be challenging: blood products are in limited supply, require careful screening, need specialized storage, have limited shelf life, and are sometimes associated with serious complications.[22–24] Clearly, a prospective randomized clinical trial would be required to conclusively answer these questions. However, conducting such a study in critically injured patients is challenging from legal, logistical, financial and ethical perspectives. Therefore, we decided to address some of these questions through a multi-institutional randomized preclinical trial. Our specific goal was to test whether trauma-associated coagulopathy could be reversed with the administration of different blood components in a clinically relevant polytrauma model.

MATERIALS AND METHODS

All the research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals. The study adhered to the principles stated in the Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (1996) and was approved by the appropriate Institutional Animal Care and Use Committees. All the procedures were performed under the supervision of a veterinarian. This highly reproducible model was developed and standardized by teams from Oregon Health and Science University, Portland, OR, the US Army Institute of Surgical Research, San Antonio, TX (funded by the US Army Medical Research and Materiel Command), and the Massachusetts General Hospital/Harvard Medical School, Boston, MA (funded by the Office of Naval Research) and included multiple injuries, large volume blood loss, hemodilution, hypothermia, acidosis, and coagulopathy.[25]

Animal Selection and Preparation

Female Yorkshire swine (n = 60) were used for the experiment. Food was withheld the night before the procedure, but access to water was allowed. Animals were sedated with 8 mg/kg Telazol (tiletamine hydrochloride 50 mg/mL and zolazepam hydrochloride 50 mg/mL; Fort Dodge Animal Health, Fort Dodge, IA) along with 1.2 mg of atropine intramuscularly. They were placed in the supine position, and anesthesia was induced with ~4% inhaled isoflurane in 100% oxygen. Animals were then intubated with a cuffed endotracheal tube 7.0 mm (i.d) × 55 cm, and mechanical ventilation was started with the initial settings of: tidal volume at 10 mL/kg body weight, peak pressure at 20 cm H_2O and 10 to 12 breaths per minute to maintain baseline (BL) end-tidal P_{CO_2} of 40 ± 2 mm Hg, and occasional sigh breaths. Reflexes and muscle relaxation were used to monitor the depth of anesthesia, and isoflurane dose titrated between 1 and 3%.

Instrumentation

A 5 French arterial catheter was placed via a cutdown technique in the right femoral artery and used for continuous monitoring of blood pressure. The right internal carotid artery and internal jugular vein were cannulated with 9 French introducer sheaths (Arrow International, Reading, PA). The venous sheath was used for fluid/blood product infusion and the arterial sheath was used for blood withdrawal (hemorrhage). The right external jugular was cannulated for the measurement of central venous pressure as well as infusion of calcium chloride during citrated blood product infusion. A midline laparotomy was performed and the falciform liga-

ment was divided to gain complete access to the liver. A Foley catheter was placed via a cystostomy for measurement of urine output. At this point, anesthesia was adjusted to allow animals to reach a mean arterial pressure (MAP) of 60 mm Hg or more. This marked the end of instrumentation phase and at this point, samples for the time-point BL were drawn.

Monitoring

Noninvasive monitoring included pulse oximetry and continuous electrocardiography. An esophageal thermometer was inserted to measure core temperature. Invasive hemodynamic monitoring (central venous and arterial pressures) was continuously performed, and blood pressure readings were recorded every 5 minutes. End tidal CO_2 was also measured throughout the experiment along with respiratory rate.

Blood Sampling and Analysis

Arterial blood samples were collected at BL to measure prothrombin time (PT), activated partial thromboplastin time (PTT), international normalized ratio (INR), fibrinogen, thromboelastography (TEG), complete blood count, and arterial blood gas. These measurements were done at eight time points during the experiment (Fig. 1): BL, postshock, postcrystalloid infusion (PC), end of administration of treatment (M_0), after 1 hour of monitoring (M_1), after 2 hours of monitoring (M_2), after 3 hours of monitoring (M_3), and after 4 hours of monitoring (M_4). A full description of these time points is presented in later sections. PT, PTT, INR, and fibrinogen were measured using the BCS Coagulation System (Dade Behring, Marburg, Germany). Thromboelastography was performed using the TEG 5000 Thromboelastograph Hemostasis Analyzer (Hemoscope, Niles, IL) using citrated kaolin samples and with temperatures adjusted to match the core temperature of the swine at the time of sample collection. Arterial blood gas analyses were performed serially. Based on our initial experience,[25] it was decided to centralize the measurement of coagulation parameters to eliminate interinstitutional variability. Therefore, all the samples during this study were analyzed at the US Army Institute of Surgical Research (Fort Sam Houston, TX) under the supervision of one key investigator (JLS).

Femur Fracture and Soft Tissue Injury

The left midshaft femur was localized using a 14 gauge needle, followed by a small cruciate incision at the site of localization, after which a captive bolt gun (Model RS22, Ramset—Powder Fastening System, Glendale Heights, IL) was applied to the femur and fired at the site to induce both a soft tissue injury and femur fracture. The midshaft of femur was palpated for confirmation of a fracture. Figure 2, A is a 3D computed tomography reconstruction of the type of femur fracture created by this method. Bleeding from the site was measured and taken into account when calculating the total hemorrhage volume.

Hemorrhage and Resuscitation Protocol

Total blood volume was estimated to be 70 mL/kg, and 60% of it was withdrawn using a Masterflex pump Model L/S Computerized Drive with a MF EasyLoad II Pumphead, Model 77201-60 (Cole-Parmer, Vernon Hills, IL). Blood was withdrawn at a rate of 100 mL/min and was captured in sterile blood bags (CPD/Optisol, Terumo Medical Corporation, Somerset, NJ). Isoflurane was decreased with falling blood pressure and turned off at a MAP of 30 mm Hg. Hemorrhage was also held briefly for MAP $<$25 mm Hg and 0.9% normal saline (Baxter Healthcare Corporation, Deerfield IL) was administered intravenously at a rate of 165 mL/min using Masterflex pump into the internal jugular vein. Once MAP reached 30 mm Hg, infusion was stopped and hemorrhage was reinitiated. Thus, the swine was kept within a MAP of 25–30 mm Hg until 60% of blood volume was withdrawn. After hemorrhage, the animal was left in shock for 30 minutes (representing time in the field before medical attention). The end of this period represented the postshock time point.

After 30 minutes of shock, the lost blood was replaced with three times the volume of normal saline (room temper-

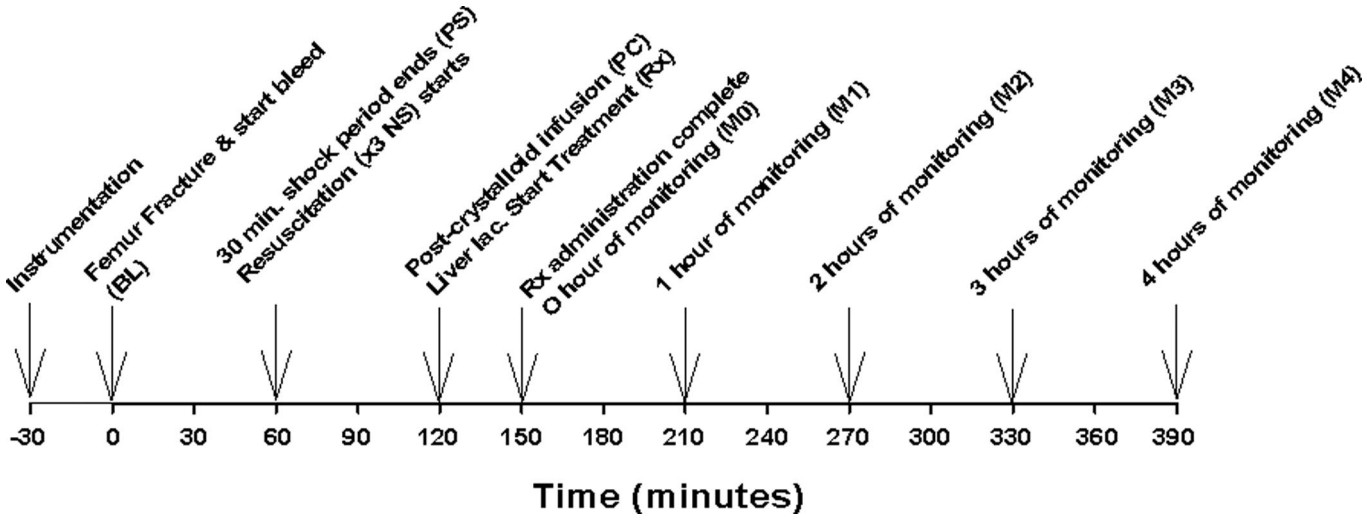

Figure 1. Timeline of the polytrauma/hemorrhagic shock model. NS, normal saline; Rx, treatment.

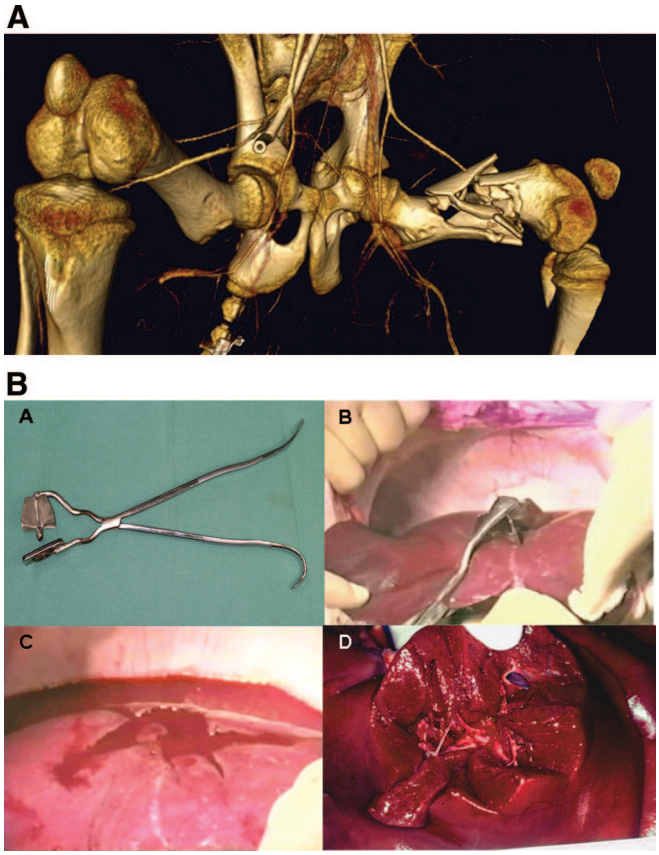

Figure 2. Injuries. Panel *A* shows a 3D reconstruction computerized tomography image of the femur fracture (provided by Dr. Jill Sondeen). Panel *B* shows the grade V liver injury (A: device, B: injury being created, C: actively bleeding liver laceration, D: postmortem view of the injury showing lacerated vascular structures).

ature) at a rate of 165 mL/min using the Masterflex pump through the internal jugular vein catheter. The amount of saline given during the hemorrhage phase was taken into account while calculating the volume of replacement saline. After resuscitation, animals were allowed to equilibrate for 15 minutes.

Mild Hypothermia

By the end of the 15-minute equilibration period after crystalloid infusion, most of the animals had spontaneously become hypothermic. However, if the target temperature of 33.0°C ± 0.3°C was not achieved spontaneously, intraperitoneal lavage with chilled crystalloid was performed to reach that temperature. At this stage, all the animals were equally hypothermic and had developed lactic acidosis and coagulopathy. This was designated as PC and was the end of the simulated "prehospital" phase of the protocol.

Liver Laceration

After 15 minutes of observation, preweighed laparotomy sponges were placed in both paracolic gutters and in the pelvis for blood collection. A grade V liver injury (simulating delayed rupture of a contained injury) was created[25] using a specialized clamp (Fig. 2, B). After the laceration, the liver was left to freely bleed for 30 seconds followed by packing with six dry laparotomy pads (4 cephalic and 2 caudal). The volume of shed blood was collected and measured during the initial 30-second hemorrhage period. Abdomen was then closed using a running suture. This marked the end of the "operative" phase of the trauma/hemorrhage protocol, and animals were randomized into different groups.

Treatment Protocol

Immediately after liver packing, transfusion of different blood products was started. The volume of transfusion matched the volume of withdrawn blood, and it was infused at a rate of 50 mL/min using the Masterflex pump. For those products that had been citrated, 2 g of $CaCl_2$ (50 mmol/L) was also infused over the same time period through the external jugular venous line. All the blood products were warmed in a 38°C water bath before administration.

Monitoring and Tissue Harvesting

Animals were monitored for 4 hours and blood samples were collected every hour. All the animals that survived for 15 minutes after completion of the treatment were included in the study. At the end of the monitoring period, or at the time of death, laparotomy sponges were weighed for contained blood. Tissue samples from lung, liver, kidney, and small bowel were harvested for analysis at a later stage, and placed in liquid nitrogen, 10% paraformaldehyde and RNAlater (Ambion, Inc., Austin, TX). Frozen tissues were then stored at −70°C and RNA later was placed in 4°C for 24 hours followed by permanent storage at −20°C. After tissue harvest, the animal was killed using Euthasol (sodium pentobarbital [100 mg/kg]).

Preparation of Blood Products

Whole blood withdrawn during arterial hemorrhage was collected in Teruflex triple blood bags (Terumo transfusion products). Animals in the FWB group received their own blood back as treatment. For other groups, blood was spun for 15 minutes at 5000 rpm with no brake (Model J-68 Centrifuge, Beckman Coulter, Fullerton, CA) separating the plasma (supernatant) from the cellular components. Fresh plasma was then extracted (Fenwal Plasma Extractor, Baxter, Deerfield, IL) and collected in the Terumo blood system satellite bag. It was stored in the −70°C freezer for future use. PRBC obtained through the centrifugation was stored at 4°C and used within 24 hours. All blood products were placed in a 38°C water bath for 1 hour before transfusion through a transfusion kit with filter (V2500, Y-Type Blood Set—170 μmol/L Blood Filter, Braun, Bethlehem, PA).

Model Development and Pilot Studies

To select the treatment groups for the final study and to establish the lethality of the model, a number of pilot studies were performed (n = 3–6/group) that included: controls for different individual injuries; FFP:RBC in ratios of 1:1, 2:1, 1:2, and 1:4; FWB; PRBCs alone; and plasma alone. Also, at one of the participating centers, animals (n = 6) were subjected to this protocol and left untreated (control: no treat-

TABLE 1. Baseline Comparison of Different Treatment Groups

Variables	Sham	FWB	Hextend	FFP:PRBC	FFP
Weight (kg)	41 ± 1.2	42 ± 1.5	40 ± 1.3	40 ± 1.4	45 ± 1.3
Blood loss (ml/kg body weight)	0	42 ± 0.5	43 ± 0.3	43 ± 0.4	42 ± 0.2
IVF (ml/kg body weight)	0	128 ± 1.2	129 ± 0.9	129 ± 1.1	126 ± 0.8

Data shown as means ± standard error of the means. Blood loss shown here represents the volume of blood that was withdrawn during the controlled hemorrhage phase (60% of estimated blood volume).

IVF, intravenous fluids; FWB, fresh whole blood; Hextend, 6% hetastarch; FFP:PRBC, fresh frozen plasma and packed red blood cells in a 1:1 ratio; FFP, fresh frozen plasma.

ment after the "operative" phase) to establish the lethality of the model.

Experimental Groups

After analysis of the pilot study data, the following five groups were selected for the multi-institutional randomized study:

1. Sham-instrumentation and anesthesia but no injuries and no treatment (Sham; n = 6, 2/center).
2. Treatment with FWB (n = 14, 4–5/center).
3. 6% Hetastarch in balanced lactated solution (Hextend; n = 14, 4–5/center).
4. FFP and PRBCs in 1:1 ratio (1:1 FFP/PRBC; n = 13, 4–5/center).
5. FFP alone (n = 13, 4–5/center).

Statistical Analysis

All data are presented as group means ± SEM. Statistical analysis was performed using the software package SAS v. 9.1 (SAS Institute, Cary, NC). Groups were compared using an analysis of variance with Dunnett's test for multiple comparisons on all continuous variables at each time point. Individual groups were analyzed using a one-way analysis of variance with repeated measures with a Dunnett adjustment back to BL. Survival rates were compared using Fisher's exact test. Statistical significance was defined as $p < 0.05$.

RESULTS

Model Validation and Pilot Studies

This was a highly lethal model where survival in the untreated animals (control group) was only 15%, and all the deaths were within 60 minutes of the liver injury (average time 55 ± 3 minutes). Although numbers were too small to perform statistical analysis, the various ratios of FFP:PRBC had similar outcomes in the pilot studies. Data analysis revealed that this model had excellent reproducibility at the three different centers (no difference in the severity of injuries, degree of shock, anemia, acidosis, and hypothermia).[25] Although animals at all the centers developed coagulopathy after administration of crystalloids, the coagulation studies (PT, PTT, INR, and TEG analysis) showed some interinstitutional variability. Thus, for this randomized study, all the samples were analyzed at one core facility (to eliminate variability introduced by differences in equipment and sample processing). We selected Hextend, FWB, 1:1 FFP:PRBC, and FFP treatments for the next phase of the study.

Survival

The BL characteristics for the different groups are shown in Table 1. There were no differences between the groups in terms of weight, controlled blood loss or volumes of crystalloids infused. Liver injury resulted in uncontrolled hemorrhage (400–600 mL) which was effectively controlled by liver packing (no difference between the groups). All the animals treated with blood products (FWB, FFP, and FFP:PRBC) survived the entire monitoring period, similar to the sham animals (100% survival). The survival in the Hextend group (15%) was statistically lower ($p < 0.05$) compared with all the other groups.

Hemodynamic and Physiologic Data

The MAP at the end of 60% hemorrhage decreased to less than 30 mm Hg ($p < 0.05$ versus BL) and was <40 mm Hg at the end of the 30 minutes of shock period ($p < 0.05$. versus BL). It remained significantly lower compared with the BL even after infusion of crystalloids (end of the "prehospital" phase). All the treatment modalities increased the MAP to near-BL levels for 2 hours, before it drifted down again into the 50 mm Hg to 55 mm Hg range (Table 2). In this model, injuries/hemorrhage and infusion of crystalloids resulted in the development of significant lactic acidosis, dilutional anemia, and thrombocytopenia (Tables 2, 3). As expected, treatment with FWB and FFP:PRBC (but not FFP and Hextend) corrected the anemia and thrombocytopenia (Tables 2, 3, M1 time point). Lactic acidosis persisted despite treatment. It worsened in the FFP treated animals toward the end of the experiment (Table 2), most likely as a result of decreased tissue oxygen delivery secondary to severe anemia.

Comparison of Coagulation and Hemostatic Parameters

After trauma, hemorrhage, and crystalloid infusion, all the animals developed significant ($p < 0.05$) coagulopathy with increased PT/INR compared with BL values (Table 3). Treatment with blood products rapidly corrected the PT/INR and the effect lasted for the entire period of monitoring, whereas Hextend failed to do so. All of the groups had similar TEG values at BL. The time to start of clot formation ("r" time), trended down in all groups during the protocol but reached statistical significance only in the FFP:PRBC (PC and M4 time points) and the FWB (M4 time point) groups. There was a decrease in the rate of clot formation (α) after crystalloid infusion in all animals (reached significance for the Hextend and FFP groups). This resolved in all the groups

TABLE 2. The Effect of Shock Protocol and Resuscitation Strategies on Hemodynamic Parameters

Parameter	Group	Baseline	Postshock	Postcrystalloid	1 h of Monitoring (M1)	2 h of Monitoring (M2)	3 h of Monitoring (M3)	4 h of Monitoring (M4)
Mean arterial pressure (mm Hg)	Sham	74.5 ± 2.4	73.2 ± 3.8	71.5 ± 6.4	76.2 ± 3.6	69.7 ± 2.3	67.8 ± 2.6	68.4 ± 4.2
	FWB	64.6 ± 3.4	38.3 ± 4.2*†	49.6 ± 3.5*†	60.1 ± 5.5	60.3 ± 3.7	56.0 ± 4.0	50.9 ± 4.2
	Hextend	68.1 ± 3.1	37.1 ± 3.9*†	45.0 ± 4.0*†	66.2 ± 3.1	59.1 ± 5.1	63.4 ± 3.6	54.2 ± 3.1
	FFP:PRBC	67.3 ± 4.0	38.9 ± 3.5*†	54.8 ± 3.7†	66.6 ± 4.5	60.9 ± 4.7	60.4 ± 4.2	54.3 ± 3.2
	FFP	70.1 ± 4.3	37.5 ± 3.9*†	49.2 ± 2.7*†	62.7 ± 1.8	60.9 ± 2.4	56.2 ± 3.0*	51.9 ± 3.4*
Lactate (mmol/L)	Sham	2.3 ± 0.4	2.6 ± 0.3	2.3 ± 0.3	1.8 ± 0.2	1.3 ± 0.1	1.1 ± 0.1*	1.3 ± 0.1*
	FWB	2.6 ± 0.5	6.2 ± 0.5*†	5.6 ± 0.6*†	5.9 ± 0.8*†	4.3 ± 0.6†	3.6 ± 0.7†	4.5 ± 1.1†
	Hextend	1.7 ± 0.2	6.6 ± 1.1*†	6.1 ± 0.9*†	7.2 ± 0.6*†	5.2 ± 1.5*†	5.5 ± 3.2*†	4.7 ± 3.4*
	FFP:PRBC	2.1 ± 0.3	6.9 ± 1.0*†	5.7 ± 0.8*†	5.4 ± 0.4*†	4.3 ± 0.4*†	3.7 ± 0.4*†	3.5 ± 0.7
	FFP	1.6 ± 0.2	5.3 ± 0.6*	5.3 ± 0.6*†	5.1 ± 0.6*†	4.7 ± 0.6*†	3.9 ± 0.6*†	5.6 ± 1.5†
Base excess (mmol/L)	Sham	3.6 ± 1.2	2.5 ± 1.8	5.0 ± 1.3	5.1 ± 1.3	5.0 ± 1.5	5.3 ± 1.2	5.3 ± 1.4
	FWB	4.0 ± 0.8	−5.6 ± 1.0*†	−9.4 ± 0.7*†	−7.5 ± 1.6*†	−4.9 ± 0.9*†	−3.2 ± 0.9*†	−3.5 ± 1.7*†
	Hextend	4.6 ± 1.1	−5.7 ± 0.9*†	−10.0 ± 1.1*†	−3.5 ± 3.8*†	−3.6 ± 4.3†	−3.3 ± 5.7†	−2.8 ± 4.2
	FFP:PRBC	4.4 ± 1.1	−5.6 ± 1.3*†	−9.4 ± 0.7*†	−7.4 ± 0.7*†	−4.4 ± 0.6*†	−3.8 ± 0.8*†	−3.0 ± 1.1*†
	FFP	5.4 ± 0.7	−4.5 ± 0.6*†	−8.8 ± 0.9*†	−4.8 ± 0.7*†	−1.7 ± 0.8*†	−1.4 ± 1.0*†	−1.1 ± 1.6*
Hemoglobin (g/dL)	Sham	8.6 ± 0.6	8.9 ± 0.7	9.3 ± 0.6	9.7 ± 0.6	9.7 ± 0.5*	10.1 ± 0.6*	10.8 ± 0.7*
	FWB	8.6 ± 0.4	6.4 ± 0.5*	4.9 ± 0.2*†	7.5 ± 0.4†	7.8 ± 0.4†	8.3 ± 0.3	9.0 ± 0.5
	Hextend	8.9 ± 0.5	7.2 ± 0.2*	4.4 ± 0.3*†	3.8 ± 0.2*†	4.0 ± 0.2*†	4.4 ± 0.1*†	4.3 ± 0.1*†
	FFP:PRBC	9.3 ± 0.4	7.0 ± 0.6*	4.8 ± 0.3*†	9.4 ± 0.5	9.1 ± 0.4	10.0 ± 0.6	10.7 ± 0.5
	FFP	9.5 ± 0.4	7.3 ± 0.4*	5.3 ± 0.2*†	4.2 ± 0.2*†	3.8 ± 0.2*†	4.1 ± 0.3*†	4.2 ± 0.2*†

Data shown as means ± standard error of the mean.

* $p < 0.05$ compared with own baseline. † $p < 0.05$ compared with Sham.

Sham, no hemorrhage/no treatment; FWB, fresh whole blood; Hextend, 6% hetastarch solution; FFP:PRBC, fresh frozen plasma and packed red blood cells in 1:1 ratio; FFP, fresh frozen plasma.

TABLE 3. The Effect of Shock Protocol and Treatment Strategies on Selected Hemostatic Parameters

Parameter	Group	Baseline	Postshock	Postcrystalloid	1 h of Monitoring (M1)	2 h of Monitoring (M2)	3 h of Monitoring (M3)	4 h of Monitoring (M4)
Prothrombin time (s)	Sham	11.7 ± 0.8	11.7 ± 0.9	11.4 ± 0.7	11.4 ± 0.6	11 ± 0.6	11.5 ± 0.9	11.3 ± 0.6
	FWB	11.9 ± 0.4	13.0 ± 0.6*	15.4 ± 0.7*†	12.6 ± 0.4#	12.6 ± 0.4	13.1 ± 0.5	13.4 ± 0.7
	Hextend	11.3 ± 0.4	12.2 ± 0.3*	15.4 ± 0.6*†	18.3 ± 0.6*†	19.3 ± 2.5†	19 ± 3†	18.7 ± 3.0†
	FFP:PRBC	11.8 ± 0.7	12.8 ± 0.6*	15.5 ± 0.7*†	12.1 ± 0.3#	12.2 ± 0.3	12.1 ± 0.3	12.4 ± 0.3
	FFP	12.4 ± 0.4	13.6 ± 0.3*	15.9 ± 0.4*†	12.1 ± 0.4#	12.5 ± 0.4	12.6 ± 0.3	12.5 ± 0.3
International normalized ratio (INR)	Sham	0.9 ± 0.01	1.0 ± 0.01	1.0 ± 0.01	1.0 ± 0.02	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	FWB	0.9 ± 0.04	1.1 ± 0.07*	1.3 ± 0.09*†	1.0 ± 0.06	1.0 ± 0.05	1.1 ± 0.07	1.1 ± 0.07
	Hextend	0.9 ± 0.03	1.0 ± 0.03	1.3 ± 0.07*†	1.7 ± 0.1*†	1.8 ± 0.3*†	1.8 ± 0.4*†	1.7 ± 0.4*
	FFP:PRBC	0.9 ± 0.06	1.1 ± 0.06	1.3 ± 0.08*†	1.0 ± 0.03	1.0 ± 0.03	1.0 ± 0.02	1.0 ± 0.04
	FFP	0.9 ± 0.04	1.1 ± 0.05*	1.3 ± 0.04*†	1.0 ± 0.04	1.0 ± 0.04	1.1 ± 0.04	1.0 ± 0.04
Platelet count ($\times 10^3$ μ/L)	Sham	297 ± 41	339 ± 35	292 ± 31	339 ± 42	334 ± 48	362 ± 40	353 ± 55
	FWB	372 ± 33	268 ± 29*	208 ± 37*	282 ± 32*	324 ± 32	315 ± 42*	339 ± 30*
	Hextend	333 ± 30	232 ± 20	173 ± 16*†	135 ± 30*	157 ± 10*	187 ± 7*	207 ± 6
	FFP:PRBC	350 ± 34	229 ± 41*	252 ± 22*	308 ± 52	300 ± 50	315 ± 47	272 ± 47*
	FFP	319 ± 45	241 ± 31	193 ± 40*	173 ± 26*†	197 ± 32*	206 ± 39*	280 ± 37
TEG-MA (mm)	Sham	70.6 ± 0.8	70.7 ± 1.1	62.9 ± 5.3	70.1 ± 1.1	71.7 ± 1.1	71.0 ± 1.4	71.7 ± 1.1
	FWB	71.9 ± 2.0	65.2 ± 2.3*	58.1 ± 1.2*	69.1 ± 1.5	68.6 ± 1.5	70.1 ± 1.1	70.7 ± 1.4
	Hextend	73.9 ± 1.7	67.6 ± 1.2*	60.5 ± 2.0*	36.7 ± 8.6*†	49.6 ± 1.8*†	49.4 ± 0.2*†	46.7 ± 4*†
	FFP:PRBC	70.1 ± 3.5	66.6 ± 1.2	59.1 ± 2.8*	64.5 ± 1.7	68.7 ± 1.4	68.7 ± 0.9#	68.5 ± 1.0
	FFP	72.1 ± 1.4	67.3 ± 1.2*	60.5 ± 2.2*	70.5 ± 1.1	71.3 ± 1.2	71.5 ± 1.1#	72.6 ± 1.5

Data shown as means ± standard error of the mean.

* $p < 0.05$ compared with own baseline. † $p < 0.05$ compared with Sham. # $p < 0.05$ compared with Hextend.

Sham, no hemorrhage/no treatment; FWB, fresh whole blood; Hextend, 6% hetastarch solution; FFP:PRBC, fresh frozen plasma and packed red blood cells in 1:1 ratio; FFP, fresh frozen plasma; TEG-MA, thromboelastography-maximum amplitude (clot strength).

that received blood products and got worse after treatment with Hextend (data not shown). Similarly, there was a significant decrease in the maximum strength of the clot (MA) by the end of the "prehospital phase" of the protocol (Table 3). The MA remained low in the Hextend group, but all the blood product treatment groups showed rapid return of clot strength back to normal. Importantly, there was no difference in the reversal of coagulopathy between the FWB, FFP:PRBC and the FFP groups. Animals treated with FFP demonstrated correction of coagulopathy as rapidly, and to the same degree, as the animals that were given FWB or 1:1 FFP:PRBC.

DISCUSSION

In this polytrauma model with acidosis, hypothermia, and coagulopathy, we have shown that coagulopathy worsens after hetastarch infusion, but it can be rapidly reversed with the administration of blood products. Impressively, infusion of FFP, even without any red blood cells corrected the coagulopathy and resulted in excellent early survival. Furthermore, we have shown that it is feasible to establish a reproducible (and clinically relevant) model for conducting a multi-institutional randomized trial at geographically disparate academic centers.

Trauma-associated coagulopathy is multifactorial in etiology. Hypothermia causes platelet dysfunction[2] and decreases the functional activity of clotting factors.[3–7] The enzyme complexes involved in clotting cascade are pH sensitive. Thus, degree of metabolic acidosis influences the magnitude of coagulopathy,[6] and the combination of hypothermia and acidosis has a synergistic effect.[7] Another critical contributing factor is the dilution of clotting factors from crystalloid and PRBC resuscitation. An analysis of German Trauma Registry patients revealed that nearly half of the patients who received more than 2 L of fluids were coagulopathic on arrival to the hospital.[9] Besides volume, the choice of resuscitation fluids also influences the degree of ensuing coagulopathy. In a swine model of hemorrhagic shock, resuscitation with normal saline resulted in a hypocoagulable state and increased blood loss as compared with resuscitation with lactated Ringers solution.[26] Similarly, large volume infusion of hetastarch solution along with hypothermia produces coagulopathy.[27] Exaggerated activation of anticoagulation/fibrinolytic cascades is equally important in the induction of posttrauma coagulopathy. Brohi et al.[11] have recently implicated tissue hypoperfusion and activation of the Protein C pathway in the development of traumatic coagulopathy, with a fairly stable level of coagulation factors.[21] Our model was designed keeping in mind these diverse etiologies of trauma-associated coagulopathy, including many risk factors such as long bone fracture, soft tissue injuries, large volume blood loss, a period of tissue hypoperfusion, hypothermia, acidosis, hemodilution, open body cavity, and delayed hemorrhage from a solid organ injury. Although all of the animals developed hypothermia because of shock, peritoneal irrigation with icecold saline was required in ~25% of the animals to bring the core temperature down to 33°C. This should be kept in mind while analyzing the findings of this study, as induced and spontaneous hypothermia are physiologically different. The protocol was found to be highly lethal during the model development phase, as 85% of the animals that received crystalloids (three times the volume of shed blood), but no blood products died. All groups developed significant metabolic acidosis following the hemorrhage/trauma protocol (Table 2). This acidosis persisted during the 4 hours of observation, which may be because of the severity of the insult or the relatively short observation period (clinically it could take up to 24 hours postresuscitation to clear significant lactic acidosis after multiple injuries). Also, the animals that were not given red blood cells (FFP and Hextend) developed anemia (Table 2), which clearly influenced the tissue oxygen delivery and the rate of lactic acid clearance. Although base deficit improved after FFP transfusion (plasma has excellent buffering capacity), the lactic acidosis persisted and actually started trending up toward the end of the monitoring period (Table 2). The various treatment groups in our experiment were selected to test the study question and not to represent the clinical standards of care. Clearly, infusion of FFP alone is not sufficient treatment for massive blood loss, and we expect red blood cells to be given in this clinical setting. However, one of the attractive features of plasma is that it can be transformed into a product suitable for prehospital use, and the resultant anemia could be corrected once the patient reaches a higher level of care.

Early use of FWB, PRBC, FFP, and platelets has some logistic limitations, especially in austere environments such as a battlefield. FWB (and fresh components such as apheresis platelets) carry similar risks of transmitting infections and requires availability of appropriate donors. Platelets need to be stored at 20 to 24°C and used within 5 to 7 days. Frozen plasma needs to be prepared within 8 to 24 hours and can only be stored at −18°C for a maximum of 1 year. It also requires refrigerated transportation, 30 minutes to thaw and should ideally be used within 6 hours of thawing.[28] However, thawed plasma can be stored refrigerated for 5 days with good preservation of most clotting factors (except factor VIII). PRBC also requires refrigeration and have a limited shelf life. As about 50% of potentially preventable hemorrhagic deaths take place before reaching a medical facility, there is a clear need for the development of innovative and effective strategies for the early (prehospital) treatment of coagulopathy. One solution is to convert FFP into shelf stable, lyophilized freeze dried plasma (FDP). Such a lyophilized product would have a number of advantages over FFP including: storage at ambient temperature, longer shelf life, quicker preparation time, ABO universality and reliable viral inactivation methods.[29] Based upon the findings of the current study that demonstrated the impressive hemostatic properties of plasma, we have proceeded to successfully develop and test (in the same model) lyophilized FDP.[30] In that study, we found the FDP to have levels of clotting factors, and in vivo effectiveness, identical to the FFP.

Comparison of different treatment strategies in this study revealed that infusion of the three different plasma rich products (FWB, FFP, and FFP:PRBC) rapidly corrected the PT/INR values. Immediately after the treatment agent had been administered, most of the animals returned to their preinjury PT/INR and remained so for the next 4 hours. None of these animals died during the observation period. This was in sharp contrast to the Hextend treated animals. Overall, there was a statistically significant drop in TEG-MA, at the post crystalloid infusion time point, which was reversible (Table 3). The animals treated with FFP alone were able to form a clot at the end of the experiment that was just as strong as the BL (Table 3). Classically, MA is attributed more to platelet function than to fibrinogen,[31] but our data suggest that this may not be entirely true as the platelet count in the plasma groups was very low. One possible explanation is that

the FFP infusion might supply enough additional fibrinogen to bind with the remaining platelets resulting in stiffer clots with more native viscoelastic properties.

In this experiment, we included the FWB treatment group as a control because of its increasing use in the combat environment.[18,32] Spinella et al.[33] studied 87 patients with 545 units of FWB transfusion at one combat hospital and reported significant improvement in both hemoglobin concentration and coagulation parameters with the use of FWB. Supporters of FWB claim that it acts as a ready source of RBCs, platelets, and clotting factors. The total concentration of these components when given as FWB is significantly higher than when each is given as a separate unit.[18] Also, FWB does not suffer from the problems of RBC storage, such as metabolic depletion and membrane loss. Safety of this practice related to the transmission of infections is clearly an issue, but a retrospective review of nearly 3,000 units of FWB donated in Afghanistan and Iraq revealed only three positive tests for hepatitis C antibody and one for human T lymphocyte virus.[34] Use of FWB for emergency transfusion requires availability of volunteer donors who are prescreened and typed.[28] Such constraints associated with FWB limit its use primarily to combat situations, and it is unlikely to gain wide acceptance in the civilian setting. Hextend was included in this study because it is widely used by the military in the current conflict, after being endorsed by a consensus conference on combat resuscitation.[35] The inclusion of 1:1 FFP:PRBC group reflected numerous recent studies that have proposed that trauma-associated coagulopathy should be managed with large volume infusion of FFP (higher ratios of FFP to PRBC).[13–17,36] The timing of component administration also seems to be important. The most recent study on this topic retrospectively analyzed data from 16 level I trauma centers (466 patients) and concluded that a high ratio of plasma and platelets to PRBCs in the first 6 hours of massive transfusion improved outcomes.[37] Although this study also has all the limitations of a retrospective analysis, the role of FFP in the treatment of coagulopathy is beyond doubt. The question is not whether FFP is beneficial, but rather what is the optimal dose and timing? Recognizing the benefits of increased plasma use, the Department of Defense has made available equipment and frozen plasma at all sites with surgical capabilities. But just like FWB, it too has logistical shortcomings which make it less than ideal for the prehospital combat environment. However, these logistical problems could be solved by the development of a product with a long shelf life that is stable at higher temperatures, is easily rehydrated, has ABO universality, and displays a good safety profile. We propose that lyophilized FDP may be such a product.[30]

Despite many attractive features, this model had some limitations that should be pointed out. We used laboratory measurements of coagulopathy rather than blood loss as the primary endpoint. The grade V liver injury in this model was lethal, and required packing to keep the animals alive. This effectively prevented additional blood loss, and thus we do not know the impact of INR/PT and TEG correction on clinical bleeding. In follow-up studies, a less lethal solid organ injury may be required to test this point. The dose of Hextend (equal to the 60% blood loss) was larger than what is clinically given to trauma patients. However, this was done to match the volume of infusion in this group to the other treatment groups. Smaller doses of Hextend may not worsen the coagulopathy and survival to the same degree. The improvement in outcome following treatment with blood products was not simply because of correction of coagulopathy, as the survival advantage was out of proportion to the improvement in the coagulation parameters. This is not surprising because resuscitation influences numerous cellular and subcellular cascades, and transfusion of blood has an impact that extends far beyond the coagulation system.[38] Tissue samples saved during this study are currently being evaluated to determine the cellular/molecular impact of the different treatment strategies.

In summary, we have shown the feasibility of conducting a multi-institutional preclinical trial utilizing a complex animal model. Our data demonstrate that trauma-associated coagulopathy is made worse by large volume hetastarch infusion, but can be rapidly corrected with the administration of blood products. Treatment with FFP can rapidly reverse coagulopathy and improve early survival, which justifies the development of shelf stable plasma products for prehospital use.

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