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Fibrinogen and prothrombin complex concentrate in trauma coagulopathy

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ABSTRACT

Background: Coagulopathy after injury contributes to hemorrhage and death. Treatment with specific coagulation factors could decrease hemorrhage and mortality. Our aim was to compare fibrinogen and prothrombin complex concentrate (PCC) in a rabbit model of hemorrhagic shock.

Materials and methods: New Zealand white rabbits were anesthetized. Blood was withdrawn to a mean arterial pressure (MAP) of 30–40 mm Hg for 30 min. Animals were resuscitated with lactated Ringer to a MAP of 50–60 mm Hg and randomized to receive 100 mg/kg of fibrinogen, PCC 25 IU/kg, or lactated Ringer. A liver injury was created. A MAP of 50–60 mm Hg was maintained for 60 min. The primary outcome was blood loss, and secondary outcomes were fluid administered and coagulopathy as measured by plasma-based tests.

Results: There were eight animals in each group. Median blood loss was significantly higher in the fibrinogen group, at 122 mL (95% confidence interval [CI], 75–194), when compared with that in the control group, 35 mL (95% CI, 23–46; P value = 0.001), and the PCC group, 26 mL (95% CI, 4–54; P value = 0.002). Resuscitation fluid requirement was highest in the fibrinogen group, at 374 mL (95% CI, 274–519), and lowest in the PCC group, at 238 mL (95% CI, 212–309) (P = 0.01). Plasma-based coagulation tests were not different among groups.

Conclusions: In a rabbit model, PCC did not have a significant effect on blood loss. Fibrinogen increased blood loss and fluid requirements.

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1. Introduction

Hemorrhage is a leading cause of death in civilian trauma [1]. In military trauma, hemorrhage has also been shown to be the most common cause of death in potentially survivable injuries [2]. Coagulopathy after traumatic injury is a significant factor in blood loss. Coagulopathy is present early after injury and is associated with increased mortality [3,4]. The etiology of trauma-associated coagulopathy is complex and multifactorial. Consumption of coagulation factors, dilution from administration of fluids, hypothermia, shock, and acidosis all play a role [5]. It has also been postulated that hypoperfusion leads to increased activation of the anticoagulant protein C through the thrombin-thrombomodulin pathway [6]. Hypoperfusion also results in a decrease in plasminogen activator inhibitor-1, increasing tissue plasminogen activator and resulting in hyperfibrinolysis. These findings, and other efforts elucidating the specific coagulation

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defects causing acute traumatic coagulopathy, would allow resuscitation to focus on correcting these defects.

Fibrinogen has been shown to be one of the first coagulation factors to fall below critical levels with major blood loss [7]. Fries *et al.* [8] showed in a porcine model of hemodilution and liver injury that treatment with fibrinogen resulted in normalization of clot formation and decreased blood loss compared with controls. In trauma patients with evidence of hypoperfusion, there is also a significant decrease in activity level in factors II, VII, IX, X, and XI early after admission [9]. Prothrombin complex concentrate (PCC), containing factors II, VII, IX, and X, should correct this deficiency. This has been shown to be the case in a porcine model, where administration of PCC resulted in normalization of the prothrombin time (PT) and decreased blood loss [10]. We speculated that administration of fibrinogen would be as effective as PCC in controlling bleeding and correcting coagulopathy in a rabbit model of hemorrhagic shock and liver injury.

2. Materials and methods

This study was reviewed and approved by the Institutional Animal Care and Use Committee of Naval Medical Center San Diego. New Zealand white female rabbits in this study were purchased specific pathogen free (*Pasteurella multocida*) from a United States Department of Agriculture-licensed vendor. They were housed and studied in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited facility maintained on a 12:12-h light–dark cycle. All animals were housed in stainless steel, solid floor rabbit caging, and maintained on standard laboratory diet (Harlan, Sacramento, CA) and reverse osmosis purified drinking water. After an overnight fast, rabbits 2.9–4.0 kg in weight were anesthetized with an intramuscular injection of ketamine 39–46 mg/kg. A size 1.5 pediatric laryngeal mask airway was placed, and general anesthesia was maintained with isoflurane. A 4-cm scalpel incision was performed in the right jugular furrow with subsequent blunt dissection to the depth of the carotid sheath. The carotid artery was exposed, and a 20G catheter was placed in the carotid artery for blood pressure monitoring and blood withdrawal. Two 2-0 silk sutures were placed around the artery to secure the catheter. A second incision was performed on the opposite side in a similar fashion, and a 20G venous catheter was inserted and secured in the internal jugular vein for infusion of fluids and medication. Mean arterial blood pressure, oxygen saturation, and heart rate were monitored and recorded every 5 min. After stabilization, blood was withdrawn until a mean arterial pressure (MAP) of 30–40 mm Hg was achieved. After a 30-min hypotensive period, animals were resuscitated with lactated Ringer (LR) solution to a MAP of 50–60 mm Hg. Animals were prerandomized to the control group or one of two treatment groups. Treatment groups received fibrinogen (RiaSTAP; CSL Behring GmBH, Marburg, Germany) at a dose of 100 mg/kg or PCC (Profilnine SD; Grifols Biologicals Inc, Los Angeles, CA) at 25 IU/kg. The control group received only LR. Animals in the fibrinogen and PCC groups received the drug in 20 mL of LR as part of the resuscitation phase. This was also included in the total volume of resuscitation fluid for these animals. A laparotomy was performed with a scalpel incision, and a standardized 15-mm left medial lobe hepatectomy injury was created. The abdomen was closed with surgical staples. Animals were maintained with a MAP of 50–60 mm Hg for an additional 60 min. Animals surviving to 60 min after drug infusion were humanely euthanized with a lethal injection of sodium pentobarbital (0.28–0.30 mL/kg). The abdomen was then reopened, and all blood and blood clot was removed with preweighed gauze pads. The amount of blood loss was then determined by the weight of blood and clot evacuated from the abdomen. Four milliliters of blood was withdrawn for analysis at five time points throughout the experiment as follows: (1) at the beginning of the experiment after induction of anesthesia and stabilization, (2) after the 30-min hypotensive period, (3) after resuscitation, and (4) 30 and (5) 60 min after drug infusion. The total blood loss does not include the amount withdrawn for laboratory studies. PT, partial thromboplastin time (PTT), international normalized ratio (INR), and fibrinogen tests were performed on a Stago Evolution (Diagnostica Stago, Inc, Parsippany, NJ). Complete blood counts were performed using a Sysmex XE-5000 (Sysmex Corporation, Kobe, Japan). Blood tests for pH, PCO_2, HCO_3, PO_2, and base excess were performed on an i-STAT machine (Abbott Laboratories, Abbott Park, IL). Our primary outcome was blood loss, and secondary outcomes were fluid administered and coagulopathy as measured by plasma-based laboratory values.

2.1. *Statistical analysis*

Based on data from previously published studies in porcine models, we estimated that blood loss would be 20–40 mL per animal. Using an alpha = 0.05 and power = 0.80, we calculated a requirement for 11 rabbits per group to detect a 10% difference in blood loss among groups. The rank-sum test with Bonferroni adjustment was used to compare blood loss and fluid given among the three groups. Thus, a P value of <0.017 was required for significance. A two-way repeated measures analysis of variance was used to compare hemoglobin, hematocrit, and platelets among groups over time. A Wilcoxon signed-rank test was used to compare PT, PTT, and INR over time.

3. Results

There were eight animals in each group that survived the entire 60-min experiment. One animal in the fibrinogen group expired 42 min into the experiment and was not included in the analysis. At baseline, the median MAP did not differ among groups. In the control group, MAP was 60.0 mm Hg (95% confidence interval [CI], 47.8–66.7), in the fibrinogen group, MAP was 57.5 mm Hg (95% CI, 40.1–70.5), and in the PCC group, MAP was 60.5 mm Hg (95% CI, 53.0–66.6; P value = 0.7). Median animal weights at baseline were not different between groups. The median weight in the control group was 3.65 kg (95% CI, 3.07–3.95), the fibrinogen group was 3.56 kg (95% CI, 3.10–3.70), and in the PCC group was 3.57 kg