Effects of Arterial Blood Pressure on Rebleeding Using Celox and TraumaDEX in a Porcine Model of Lethal Femoral Injury

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This study was designed to identify the systolic blood pressure (SBP) and mean arterial pressure (MAP) at which rebleeding occurs when a clot is formed by a hemostatic agent, Celox or TraumaDEX, compared with a standard dressing. Fifteen pigs (5 each) were assigned randomly to 1 of 3 groups: Celox, TraumaDEX, or standard pressure dressing as a control.

In all animals, the femoral artery and vein were transected to simulate traumatic injury. Subjects were allowed to hemorrhage 1 minute before treatment. Direct pressure was held 5 minutes followed by application of elastic dressings for 30 minutes. Dressings were removed after 30 minutes, and the wound was observed for rebleeding. Animals demonstrating hemostasis received phenylephrine infusion to increase SBP in 10-mm Hg increments until SBP reached 210 mm Hg or hemorrhage recurred.

There were statistically significant differences between Celox (mean SBP, 166.4 mm Hg; mean MAP, 137.6 mm Hg) and the control (mean SBP, 88.25 mm Hg; mean MAP, 59.7 mm Hg), and between TraumaDEX (mean SBP, 152.2 mm Hg; mean MAP, 113.2 mm Hg) and the control (P < .05). However, no statistically significant difference existed between Celox and TraumaDEX. Celox and TraumaDEX effectively prevent rebleeding compared with standard dressing.

Keywords: Celox, hemorrhage, hemostatic agent, rebleeding, TraumaDEX.

Uncontrolled bleeding remains the leading cause of preventable death in trauma.1-3 Early and rapid control of hemorrhage after injury greatly improves outcome.4,5 Current initiatives to decrease preventable death specific to combat include the use of tourniquets and hemostatic agents at the point of injury. Current US military doctrine states that control of hemorrhage should precede airway management, with tourniquet use indicated as a primary intervention for hemorrhage control, followed by hemostatic agents.6

However, traumatic injuries occur regularly in anatomical sites where tourniquets cannot be used and hemorrhage control relies on traditional methods of wound management and/or hemostatic agents.7 Hemostatic agents are mineral, marine animal, or plant-based substances designed to be applied topically to a wound to stop hemorrhage. These agents are usually applied by a first responder at the point of injury.8 There are several hemostatic agents currently on the market, including the 2 agents that were investigated in this study: Celox and TraumaDEX.

Celox
Celox (Medtrade Biopolymers, Crewe, United Kingdom; distributed in the United States by SAM Medical Products, Portland, Oregon) uses chitosan produced by the deacetylation of chitin, a polysaccharide, derived from the exoskeleton of shrimp. Chitosan is positively charged and bonds readily to negatively charged red blood cell surfaces. The mechanism of action is the formation of an adhesive complex upon exposure to nega-
tively charged red blood cells. The manufacturer states that Celox works independently of coagulation factors based on proprietary research.9 Celox is currently in use by the US military.

**TraumaDEX**

TraumaDEX (Medafor Inc, Minneapolis, Minnesota) is based on microporous polysaccharide “hemospheres” (MPH) technology using potato starch to create microscopic sponges, which absorb the plasma in human blood. This process concentrates platelets and coagulation factors forming a gel matrix at the site of injury. The gel matrix slows blood flow and enhances clotting. TraumaDEX is not part of the clot; it is the catalyst that accelerates clot formation. The polysaccharide absorbs the plasma without absorbing platelets, red blood cells, or coagulation factors.10

Alam et al1 investigated the use of several agents and observed failure of hemostatic dressings, as indicated by renewal of hemorrhage after successful application of the dressing. This usually occurred following volume resuscitation. The mechanisms responsible for renewed hemorrhage are likely secondary to hemodilution and/or increase of arterial blood pressure that dislodge the established clot.11 Therefore, rebleeding after the application of a hemostatic agent remains a major concern.

Although hemostatic agents may be effective, there may be increased risk of rebleeding secondary to elevation of arterial pressures during resuscitation. To maximize the metabolic benefits of resuscitation without causing increased blood loss, it is critical to determine if there is a reproducible point at which rebleeding occurs after a clot is formed following the use of a hemostatic agent. There are no studies that have quantified the arterial blood pressures at which hemorrhage recurs with the use of hemostatic agents. Therefore, the purpose of this study was to determine the arterial blood pressures at which rebleeding occurred when 2 hemostatic agents were used separately to control hemorrhage compared with a standard pressure dressing in a porcine model. The following research question guided the study: Is there a statistically significant difference in the arterial blood pressures in which rebleeding occurs between the Celox, TraumaDEX, and control groups?

**Materials and Methods**

- **Study Design.** This study was a prospective, between-subjects experimental design using a porcine model. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at the 59th Medical Research Division, Lackland Air Force Base, Texas. The animals received care in compliance with the Animal Welfare Act, the Guide for the Use of Laboratory Animals, and the protocols of the 59th Medical Research Division. The minimum number of animals was used to obtain a statistically valid result. Using the data from previous work, the investigators calculated a large effect size of 0.6. Using statistical power analysis software (G*Power 3.0.10), with an effect size of 0.6, a power of 0.80, and an alpha of 0.05, we determined a sample size of 15 (5 swine per group) would be needed for this study.

Fifteen male Yorkshire swine weighing between 70 and 87 kg were assigned to 1 of 3 groups (5 in each group), Celox, TraumaDEX, or the control group (standard pressure dressing), using a set of computer-generated random numbers. Swine of this size were used because they represent the average weight of the US Army soldier.15 To minimize variability, all swine arrived at the 59th Research Division on the same day. They were purchased from the same vendor and the same lot number. Male pigs were used to avoid potential hormonal effects. The swine were observed for 3 days to ensure a good state of health. They were fed a standard diet and were fasted after midnight the day of the experiment.

Anesthesia was induced with an intramuscular injection of ketamine (20 mg/kg) and atropine (0.04 mg/kg), followed by inhaled isoflurane (4% to 5%). After placement of an endotracheal tube, a peripheral intravenous (IV) catheter was inserted, and the isoflurane concentration was reduced to 1% to 2% for the remainder of the experiment. The animals were ventilated (tidal volume, 8-10 mL/kg) with an anesthesia machine (Narkomed 2B, Dräger Medical, Telford, Pennsylvania). Heart rate, blood pressure, electrocardiography, oxygen saturation measured by pulse oximetry (SpO2), end-tidal carbon dioxide (ETCO2), and rectal temperature were continuously monitored using a Marquette Solar 800 monitor system (GE Marquette Medical Systems, Milwaukee, Wisconsin). Animals were placed supine on a litter and transported to an operating room table.

The left carotid artery was cannulated with a 20-gauge angiocatheter using a cut-down technique. A right triple-lumen central venous catheter was inserted using a modified Seldinger technique for central venous pressure monitoring, fluid volume management, and blood sampling. The catheters were attached to the Marquette Solar 800 system for continuous monitoring of arterial and central venous pressures. All invasive monitors were zeroed per manufacturer’s guidelines, and catheters were continuously flushed with 0.9% saline solution (5 mL/h) to maintain patency. Following line placement, the fasting fluid deficit was replaced using 0.9% saline solution according to the Holliday-Segar formula. A complex groin injury, as described by Alam and colleagues,12 was created to simulate a penetrating traumatic injury, a common occurrence on the battlefield. The injury included dissection of the proximal thigh soft tissues (skin, quadriceps, and adductor muscles) to the femoral artery and vein without transection just below the inguinal ligament within the femoral crease. Animals were stabilized for 30 minutes.
During that time, the replacement of fasting fluid deficits and collection of blood specimens were completed. Investigators evaluated hemoglobin level, hematocrit, platelet count, prothrombin time (PT), partial thromboplastin time (PTT), and fibrinogen level before intervention and again before euthanization. The body temperatures of all animals were maintained above 36°C.

After stabilization, a scalpel was used to simultaneously transect the femoral artery and vein. The animals were allowed to hemorrhage for 1 minute, simulating the minimum response time of a medic or healthcare provider. Blood was collected from the wound with a suction catheter placed distal to the transected vessels. After 1 minute, proximal pressure was applied and 4 x 4-inch gauze pads were used to carefully blot the blood. In the Celox and TraumaDEX groups, the hemostatic agent was poured into the wound, followed by standardized packing with petroleum and gauze (Kerlix, Kendall Medical Products, Mansfield, Massachusetts). The control group received only standardized wound packing. Firm manual pressure of 25 psi, measured by a TIF scale (model 9010A, SPX Service Solutions, Owatonna, Minnesota), was applied to the injury site for 5 minutes in all animals. This TIF scale is an electronic scale capable of measuring pressure exertion. The TIF instrument is precise within 1.4 g (0.05 oz) and accurate within 0.5%. The scale was placed between the litter and operating room table. The scale was zeroed and the pressure exerted on the wound was recorded. While one investigator was applying pressure, another investigator observed and ensured that manual pressure was maintained at 25 psi ± 0.5 oz/sq inch for 5 minutes. The pressure was consistent and reproducible from animal to animal. All times were measured with a stopwatch precise within 0.01 second and accurate within ± 0.1 second.

After 5 minutes of manual pressure, a standard pressure dressing was applied to all groups and secured with self-adhesive wrap (3M Coban, 3M, St Paul, Minnesota). Héostarch 6% in lactated Ringer's solution, 500 mL, was administered to simulate current battlefield resuscitation practice. 

After 35 minutes of wound pressure, 5 minutes of manual pressure, and 30 minutes with a pressure dressing, we carefully removed the pressure dressing, including the petroleum gauze, to avoid disturbing the established clot. The use of petroleum gauze allowed removal of the pressure dressing without adherence to the clot. Blood loss was recorded, from this point, with a new collection canister that was weighed and placed in use until the end of data collection. The definition of hemostasis used in this study was clot formation with blood loss of no more than 2% of the swine's total blood volume over a 5-minute period. The total blood volume of swine weighing 70 mL/kg approximates that of the adult human. Blood loss of 2% in a 70-kg swine is approximately 100 mL (oral communication, Hugh Harroff, DVM, 59th Research Squadron, Lackland Air Force Base, 2008).

Phenylephrine infusion was titrated to increase the systolic blood pressure (SBP) in increments of 10 mm Hg, in an attempt to dislodge the established clot. Each manipulation of SBP was maintained for 5 minutes while the investigators observed for rebleeding. If rebleeding occurred during this phase, arterial pressures were recorded and no further treatment was given or data collected. If no rebleeding occurred, the SBP was increased to a maximum of 210 mm Hg. Phenylephrine was used for arterial pressure manipulation because the drug is a direct α1 agonist and commonly used in clinical anesthesia practice. It is easily titratable and allows reproducible manipulation of blood pressure from subject to subject. See Table 1 for a summary of procedures.

During development of the animal model, large-volume crystalloid infusion was considered, instead of phenylephrine infusion, as a tool to manipulate arterial blood pressure. Based on previous experience with hypovolemic porcine models, the investigators noted the arterial blood pressures of animals receiving large-volume crystalloid infusion were highly variable and poorly reproducible between subjects. Secondly, large-volume crystalloid infusion could also produce dilutional coagulopathy and become a confounding variable. At the completion of data collection, all animals were euthanized per institutional protocol.

Results
The investigators evaluated hemoglobin level, hematocrit, platelet count, prothrombin time, partial thromboplastin time, and fibrinogen level in all animals. The findings from all specimens were within normal limits. The laboratory results, body weights, body temperatures, total blood volume, the total fasting fluid deficit resuscitation volume, and the amount and percentage of total blood volume of the initial hemorrhage were analyzed using a multivariate analysis of variation (MANOVA). There were no statistically significant differences between the groups before intervention (P > .05), indicating all groups were equivalent within these parameters.

One of the 5 animals in the control group achieved hemostasis and did not rebleed at a SBP of 200 mm Hg. The investigators have no explanation for this outlier. Because the outlier was so extreme, more than 3 standard deviations (SDs) from the mean SBP of rebleeding, it was decided to exclude the animal from the study.

The means for SBP and the mean arterial pressure (MAP) at which rebleeding occurred were calculated for each group. The range for the SBP and rebleeding for the Celox group was 118 to 200 mm Hg (mean ± SD, 166.40 ± 40.92 mm Hg); for the TraumaDEX group was 78 to 209 mm Hg (mean ± SD, 152.20 ± 59.05 mm Hg); and for the control group was 84 to 90 mm Hg (mean ± SD, 88.25
The range for MAP and rebleeding for the Celox group was 90 to 173 mm Hg (mean ± SD, 137.60 ± 41.19 mm Hg); and for the control group was 40 to 75 mm Hg (mean ± SD, 59.75 ± 14.54 mm Hg).

A MANOVA was used to determine the effect of SBP and MAP on rebleeding. Statistically significant differences were found between the groups (Wilks A, P = .01). A post hoc least significant difference test was used to determine where there were statistically significant differences. The mean difference between the SBP at which rebleeding occurred for the Celox group compared with the control group was 78.15 mm Hg (P = .021); for the TraumaDEX group compared with the control group was 63.95 mm Hg (P = .05); and for the Celox group compared with the control group was 2.80 mm Hg (mean ± SD, 137.60 ± 41.19 mm Hg; for the TraumaDEX group was 65 to 161 mm Hg (mean ± SD, 113.20 ± 41.19 mm Hg); and for the control group was 40 to 75 mm Hg (mean ± SD, 59.75 ± 14.54 mm Hg).
pared with the TraumaDEX group was 14.20 mm Hg (P = .615). The mean difference between MAP at which rebleeding occurred for the Celox group compared with the control group was 77.85 mm Hg (P = .008); for the TraumaDEX group compared with the control group was 53.45 mm Hg (P = .05); and for the Celox group compared with the TraumaDEX group was 24.40 mm Hg (P = .31). See Tables 2 and 3 for a summary of results.

Discussion

During the latter 20th century, aggressive high-volume resuscitation in the treatment of hemorrhagic shock with the goal of increasing arterial blood pressure to improve end-organ perfusion became a widely accepted practice in the military.17,18 As a result, large-volume crystalloid resuscitation became the standard of care for civilian trauma patients.19 The metabolic benefit of aggressive fluid resuscitation in animal models was demonstrated and used in patients experiencing uncontrolled hemorrhagic shock.20 However, subsequent studies of uncontrolled hemorrhage demonstrate that there is increased blood loss after aggressive resuscitation.21,22

Increased bleeding of vascular injuries may occur secondary to dilution of coagulation factors. More importantly, rapid, high-volume fluid resuscitation may increase blood pressure, which may in turn dislodge the clot.19,23-25 More recent studies suggest resuscitation to normal blood pressure may not be beneficial before definitive surgical control.20,26 Sondeen and colleagues11 investigated the effects of blood pressure at which rebleeding occurred after high-volume resuscitation in swine with aortic injury. They found the average pressure at rebleeding for all animals in the study was as follows: SBP = 94 ± 3 mm Hg, MAP = 64 ± 2 mm Hg, and diastolic blood pressure (DBP) = 45 ± 2 mm Hg. These investigators concluded there was a reproducible pressure at which rebleeding occurred in their swine model of uncontrolled hemorrhage. The optimal endpoint of resuscitation in patients without definitive hemorrhage control should be below this rebleeding pressure. The pressure at which rebleeding occurred in this aortotomy model was not affected by either time of resuscitation (5-30 minutes) or by the rate (100 vs 300 mL/min) of infusion.8

Several investigators have emphasized the metabolic benefits of fluid resuscitation. However, these benefits must be balanced against the deleterious effects of rebleeding.25,27,28 Blood loss associated with rebleeding results in increased morbidity and mortality. The US Military and many leaders in trauma management recommend low-volume resuscitation or permissive hypotension.

The goal of permissive hypotension is to keep the casualty alive through the use of low-volume colloid solution. Aggressive volume resuscitation is not recommended before definitive control of hemorrhage. Rather, colloid fluids are given only to achieve the therapeutic goals of SBP of approximately 90 mm Hg, MAP of approximately 60 mm Hg, palpable pulse, and/or consciousness. Thus, the blood pressure is maintained at a subnormal point, minimizing the chance of rebleeding and hemodilution.

Measurements of the control group support the findings of Alam and Rhee29 and others30 in that aggressive fluid resuscitation will likely result in hemodilution and/or increased blood pressure, which may dislodge an early soft clot. However, the SBP or MAP when rebleeding occurs was not quantified by previous investigators. In the current study, Celox and TraumaDEX demonstrated significantly better performance in the prevention of...
rebleeding, with Celox performing clinically better than TraumaDEX.

These findings support the contention that hemorrhage controlled with a hemostatic agent may provide an extra margin of safety in the presence of elevated blood pressures. The data from this study strongly suggest that clots formed with Celox and TraumaDEX may be stronger and less likely to fail as a result of higher systolic or mean blood pressures. These hemostatic agents may provide a protective benefit allowing more latitude with fluid resuscitation and, therefore, improve end-organ perfusion. This protective benefit may be advantageous in the prehospital patient with a delayed time from transport to definitive surgical care.

Celox and TraumaDEX are statistically and clinically more effective in preventing rebleeding compared with the standard dressing (control) in the presence of elevated systolic and mean arterial blood pressures. The major limitation of this study was the small sample size. With larger sample sizes, studies may have enough power to conclusively demonstrate statistically significant differences between the studied hemostatic agents. The investigators recommend that the study be replicated with a larger sample size. Another limitation of the study was that the effects of arterial blood pressure on rebleeding were determined solely with the use of phenylephrine. Further studies should be conducted to more closely identify a specific and reproducible point of rebleeding for clots formed with other currently used hemostatic agents. Finally, additional studies must be designed to investigate the effects of large-volume crystalloid infusion on rebleeding in clots formed with hemostatic agents.

REFERENCES


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