Effects of Celox and TraumaDEX on Hemorrhage Control in a Porcine Model

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The purpose of this study was to compare the effectiveness of 2 hemostatic agents, chitosan-based Celox and the biopolymeric, microporous particles TraumaDEX, with a control group in a porcine model of hemorrhage. The animals were randomly assigned to 1 of 3 groups: Celox (n = 5), TraumaDEX (n = 5), or a standard pressure dressing alone (n = 5). To simulate a battlefield injury, the investigators generated a compound groin injury with transection of the femoral artery and vein in 15 pigs. After 1 minute of uncontrolled hemorrhage, Celox or TraumaDEX was poured into the wound, followed by standard wound packing. The control group underwent the same procedures with the exception of the hemostatic agents. In all groups, 5 minutes of direct manual pressure was applied to the wound, followed by a standard pressure dressing (3M Coban). After 30 minutes, dressings were removed, and the amount of bleeding was measured.

There were statistically significant differences in bleeding between Celox and control (P = .01) and between TraumaDEX and control (P = .038), but no statistically significant difference in bleeding between Celox and TraumaDEX (P = .478). Celox and TraumaDEX may be effective hemostatic agents for use in civilian and military trauma.

Keywords: Celox, hemostasis, hemostatic agents, TraumaDEX, uncontrolled hemorrhage.

Certified Registered Nurse Anesthetists (CRNAs) are the sole anesthesia providers for the Army Forward Surgical Teams (FSTs) and serve as mission essential providers at the Combat Support Hospital (CSH). They are subject matter experts not only in the field of anesthesia but also in pain management, respiratory and critical care, and trauma resuscitation. In mass casualty situations in both military and civilian sectors, CRNAs may be the only or the most qualified provider to control massive hemorrhage and to initiate and manage trauma resuscitation. As such, evaluation and knowledge of the effectiveness of new and innovative measures of controlling hemorrhage is desirable.

Trauma represents one of the leading causes of morbidity and mortality in both the civilian and military populations, with uncontrolled hemorrhage the major cause of preventable death.[1-5] Historically, about 20% of the combat causalities were killed in action. Ninety percent of these deaths occurred before the soldier reached a field hospital, most often because of hemorrhage.[6-7] Additionally, almost 40% of the soldiers in Vietnam who died of exsanguination had a source of hemorrhage that may have been controlled by hemostatic measures.[8]

Hemorrhage is the leading cause of death on the battlefield. Even if hemorrhage is eventually controlled, sufficient blood loss leaves victims vulnerable to hypothermia, coagulopathy, infection, acidosis, and multiple organ failure.[5,9,10] Therefore, rapid hemostasis is essential for initial survival and optimal recovery.[11] Authorities emphasize that there are combat casualties who are potentially salvageable with improved methods of controlling early hemorrhage.[12,13]

This study investigated 2 new agents with the potential to assist in early control of hemorrhage: Celox and TraumaDEX. Celox (Medtrade Biopolymers of Crewe, United Kingdom, and distributed in the United States by SAM Medical Products, Portland, Oregon) uses chitosan, which is produced by deacetylation of chitin, a polysac-

charide, 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. The company states that it works independently of clotting factors.[14]

TraumaDEX (Medafor Inc, Minneapolis, Minnesota) is based on microporous polysaccharide "hemospheres" (MPH) technology that uses potato starch to create microscopic sponges that absorb the water in human blood. This process concentrates blood platelets and proteins, which form a gel matrix to slow blood flow and serve to enhance clotting. TraumaDEX is not part of the clot; it is just the catalyst that initiates the clot. The polysaccharides absorb the plasma without absorbing platelets, red blood cells, and/or coagulation factors. The engineered microporous material promotes gelling of the blood and concentration of platelets and coagulation factors at the site of injury. The agent does not interfere with the healing process and is completely biodegradable; therefore, it does not need to be removed from the body when no longer needed.[15]

The purpose of this study was to examine the effectiveness of these 2 hemostatic agents, Celox and TraumaDEX, in controlling hemorrhage in a swine model. The research question that guided the study was: Is there a statistically significant difference in the amount of bleeding between Celox, TraumaDEX, and control groups? According to the literature, there continues to be mixed results from studies relative to the effectiveness of hemostatic agents.[16,17]

Materials and Methods

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 Squadron.

Fifteen Yorkshire swine weighing between 70 and 87 kg were randomly assigned (5 per group) to 1 of 3 groups: Celox, TraumaDEX, or control group. The rationale for using swine of this size is that it represents the average weight of the US Army soldier.[18] The swine were, observed for at least 3 days to ensure good state of health, fed a standard diet, and restricted to nothing by mouth (NPO) 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 to 10 mL/kg) with a standard Narkomed anesthesia machine (Dräger, Telford, Pennsylvania) and continuously monitored with the following standard monitors: heart rate, blood pressure, electrocardiography, pulse oximeter measuring the oxygen saturation (Spo_2), end-tidal carbon dioxide ($ETCO_2$), and rectal temperature.

Animals were placed supine on a litter and transported to an operating room. An electronic scale (TIF 9010A, SPX Service Solutions, Owatonna, Minnesota) that measures pressure exertion was placed between the litter and operating room table. The scale was zeroed per the manufacturer's guidelines, and the pressure exerted on the wound was recorded. While an investigator applied manual pressure, the monitor was observed and pressure was maintained at 25 psi within ±14 g (0.5 oz) for 5 minutes. The pressure was reproducible from animal to animal. The TIF instrument is precise within 1.4 g (0.05 oz) and accurate within 0.5%.

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 a hemodynamic monitoring system (Hewlett-Packard Co, Palo Alto, California) for continuous monitoring of the arterial and central venous pressures. All of the catheters were continuously flushed with 0.9% saline solution (5 mL/h) to maintain patency.

Following line placement, the NPO fluid deficit replacement was initiated with normal saline according to the 4-2-1 method (Holliday-Segar formula) with an NPO period after midnight. A complex groin injury as described by Alam and colleagues[19] was generated to simulate a blast-type injury. 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.[19] Subjects were then monitored for 30 minutes to ensure hemodynamic stability, during which time the replacement of NPO fluid deficits and blood specimen collection were completed. Investigators evaluated hemoglobin, hematocrit, platelet count, prothrombin time (PT), partial thromboplastin time (PTT), and fibrinogen before intervention and euthanization. Temperatures were monitored via a rectal temperature probe and maintained at greater than 36.0°C using a forced-air warming blanket when needed. All subjects were hemodynamically stable before the intervention.

After the stabilization period, a scalpel was used to simultaneously transect the femoral artery and vein. The animals were allowed to hemorrhage for 1 minute, simulating the response time of a combat lifesaver, medic, or healthcare provider. Blood was collected from the wound by use of a suction catheter placed distal to the transected vessels. After 1 minute of hemorrhage, proximal pres-

Preparation (30 min)	Stabilization (30 min)	Hemorrhage (40 min)	Observation of hemostasis and blood loss (5 min)
Group 1—Control 1. Premedication 2. Inhalation induction with isoflurane 3. Peripheral IV started 4. Animal intubated 5. Transported to operating room 6. Carotid artery cannulated 7. Right subclavian triple-lumen catheter inserted 8. Complex groin injury inflicted without transecting femoral artery 9. Forced-air warming blanket started when needed to sustain temperature at 36°C 10. Blood specimens collected and NPO IV fluid deficit initiated	1. Remainder of NPO fluid deficit replenished (total 1-hour infusion time) 2. Subjects monitored: HR, BP, ECG, Spo_2, $ETCO_2$, and rectal temperature 3. Central venous and arterial pressures monitored 4. Above monitoring continued throughout protocol	1. Femoral artery and vein transected 2. Animals allowed to hemorrhage for 60 seconds 3. Suction catheter placed in distal part of wound 4. Blood loss calculated 5. Standard wound packing 6. Pressure applied for 5 minutes 7. Pressure dressing (3M Coban) applied for 30 minutes 8. 6% hetastarch in lactated electrolyte injection (Hextend) administered over 5 minutes	1. After 35 minutes, standard packing and pressure dressings 2. Blood (if any) collected by suction catheter in distal part of wound 3. Blood loss calculated 4. Hemostasis observed (defined as no more than 2% blood volume over a 5-minute period)
Group 2—Celox Same as above	Same as above	1. Same as above steps 1-4 2. Wound blotted with 4 × 4-in gauze pads 3. Celox poured into wound 4. Repeat steps 5-8 above	Same as above
Group 3—TraumaDEX Same as above	Same as above	1. Same as above steps 1-4 2. Wound blotted with 4 × 4-in gauze pads 3. TraumaDEX poured into wound 4. Repeat steps 5-8 above	Same as above

Table. Summary of Procedures

IV indicates intravenous; NPO, nothing by mouth; HR, heart rate; BP, blood pressure; ECG, electrocardiography; Spo_2, oxygen saturation; $ETCO_2$, end-tidal carbon dioxide.

sure was applied to the injury, and 4 × 4-in (10.1 × 10.1-cm) gauze pads were used to blot the blood from the wound according to the hemostatic agent manufacturer's guidelines. At this time in the Celox and TraumaDEX groups, the hemostatic agent was poured into the wound, followed by the standardized wound packing with petroleum gauze and roller gauze (Kerlix, Covidien, Mansfield, Massachusetts). The control group received proximal pressure and the standardized wound packing. Firm manual compression consisting of 25 psi (measured by the TIF scale) was applied for 5 minutes to the injury site in all animals. After the 5-minute time interval, all groups had a standard pressure dressing applied (3M Coban, 3M, St Paul, Minnesota), and 500 mL of 6% hetastarch in lactated electrolyte injection (Hextend, Hospira Inc, Lake Forest, Illinois) was administered. Hextend administration is recommended by the Tactical Committee on Combat Casualty Care treatment protocol for battlefield resuscitation.[20]

After 35 minutes of pressure on the wound (manual pressure and the pressure dressing), the standard pressure dressing was removed, with care taken to leave the clot intact. The rationale for using the petroleum gauze was that it allowed removal of the pressure dressing with minimal clot disruption. For the purposes of this study, hemostasis was defined as a clot formation with oozing of no more than 2% of the swine's total blood volume over a 5-minute period (approximately 100 mL in a 70-kg pig). According to Hugh Harroff, DVM, at the 59th Research Squadron, Lackland Air Force Base, swine blood volume approximates that of the adult human at 70 mL/kg (oral communication, 2008).

Blood loss was measured over 2 time periods: the initial injury to intervention and the intervention to the completion of the study. Measurement was accomplished through gentle suctioning of the blood in the distal part of the wound and collection on absorbent pads underneath the animal. In addition, all the dressings and hemostatic agents were weighed before their application and again at the conclusion of the experiment to determine the

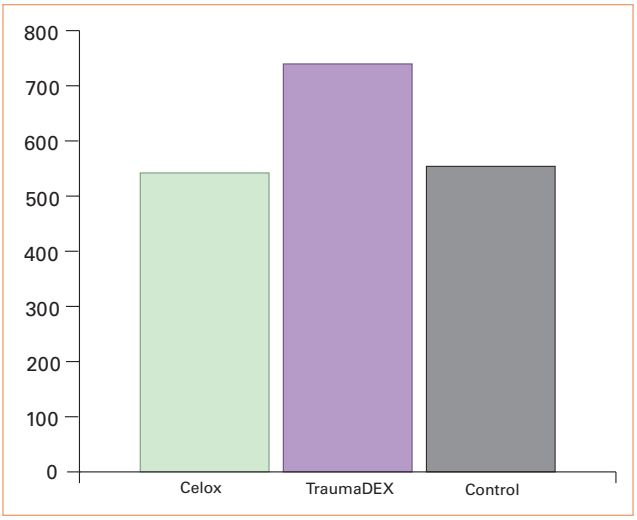

Figure 1. Initial 1-Minute Hemorrhage After Transection of Femoral Artery and Vein
Data are presented as mean, in milliliters.

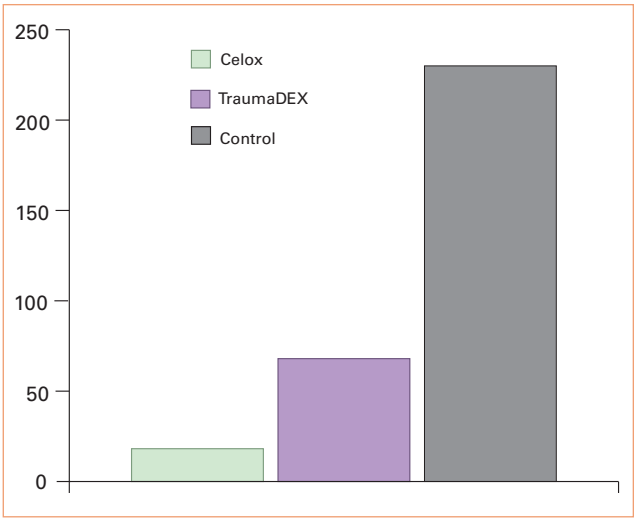

Figure 2. Comparison of Bleeding by Groups After Intervention
Data are presented as mean, in milliliters.

amount of exsanguination. The blood loss from the initial injury was determined by the weight of dressings before and after the transection of the femoral vessels, as well as any blood collected through suctioning of the wound. To determine the effectiveness of the hemostatic agents, the investigators determined blood loss in the same manner after the intervention. After measurement of blood loss from all sources, all animals were euthanized per protocol. See the Table for a summary of procedures.

For statistical analysis, the minimum number of animals was used to obtain a statistically valid result. A large effect size was determined for this experiment based on previous work.[16,17,19] Using a statistical power analysis software program (G*Power 3.0.10), an effect size of 0.6, a power of 0.80 and an α of 0.05, it was determined that a sample size of 5 swine per group (15 total) was needed for this study. The laboratory data, animal body weights, core body temperatures, amount of blood volume, and the amount of the initial 1-minute hemorrhage were analyzed using a multivariate analysis of variance (ANOVA). Both an ANOVA and a least significant difference (LSD) post hoc test were used to analyze the data for blood loss after 35 minutes of pressure on the wound.

Results

Investigators evaluated hemoglobin, hematocrit, platelet count, PT, PTT, and fibrinogen. All subject specimens were within normal limits. Pigs of similar size and weight were used in all 3 groups. The weight of the animals in the Celox group ranged from 70 to 87 kg (mean ± SD, 76.4 ± 8.4 kg), that in the TraumaDEX group ranged from 72 to 84 kg (mean ± SD, 75.8 ± 4.8 kg), and weight in the control group ranged from 70 to 84 kg (mean ± SD, 77.6 ± 5.6 kg).

There were no statistically significant differences between the groups in reference to the amount of initial 1-minute bleeding (P = .417). Hemorrhage at 1 minute in the Celox group ranged from 300 to 900 mL (mean ± SD, 541.6 ± 243 mL); in the TraumaDEX group it ranged from 400 to 954 mL (mean ± SD, 739.6 ± 208 mL); and in the control group this initial bleeding ranged from 205 to 862 mL (mean ± SD, 554.2 ± 305 mL) (Figure 1). Using multivariate ANOVA, there also were no statistically significant differences between the groups for the laboratory data, body weights, core body temperatures, and amount of blood volume (P > .05).

Blood loss after 35 minutes of pressure on the wound (manual pressure and the pressure dressing) was calculated for each group over a 5-minute period (Figure 2). The control group had the most bleeding, followed by the TraumaDEX group and the Celox group. The amount of bleeding for the Celox group ranged from 0 to 93 mL (mean ± SD, 18.l6 ± 41.6 mL); for the TraumaDEX group it ranged from 0 to 234 mL (mean ± SD, 68 ± 103.5 mL); and in the control group it ranged from 0 to 421 mL (mean ± SD, 230 ± 154 mL). An ANOVA and an LSD post hoc test indicated a significant difference between the groups (P = .025). There were statistically significant differences between Celox and control (P = .01) and between TraumaDEX and control (P = .038). However, there was no statistically significant difference between Celox and TraumaDEX (P = .478).

Discussion

The US Army's goal is that each soldier will carry a hemostatic agent, but research needs to be conducted to determine the most efficacious and cost-effective agent.[18] Pusateri and colleagues[17] outlined ideal qualities of hemostatic agents for civilian and military use. These include (1) being able to rapidly stop large-vessel arterial

and venous bleeding within 2 minutes of application when applied to an actively bleeding wound through a pool of blood; (2) no requirement for mixing or preapplication preparation; (3) simplicity of application by wounded victim, buddy, or medic; (4) lightweight and durable; (5) long shelf life in extreme environments; (6) safe to use with no risk of injury to tissues or transmission of infection; and (7) inexpensive.[17]

This study compared Celox and TraumaDEX against a standard pressure dressing, the control, in a hemorrhagic porcine model. A complex groin injury was generated simulating a blast-type injury that is common in combat, because the anatomical areas are not protected by conventional body armor, and a tourniquet cannot be placed to control hemorrhage. Both hemostatic agents, Celox and TraumaDEX, were able to rapidly stop large-vessel arterial and venous bleeding when applied to an actively bleeding wound through a pool of blood. This fulfilled the first of the requirements described by Pusateri et al. Celox performed clinically superior to TraumaDEX, and both were statistically and clinically superior at controlling hemorrhage compared with the standard pressure dressing control group.

The hemostatic agents in this study were easy to apply and did not require any premixing. Celox is packaged as loose granules, 35 g, in a waterproof pouch. It was easy to open and pour into the wound. On the other hand, TraumaDEX comes packaged in a plastic-tipped applicator containing 5 g of fine powder. Investigators noted that contact of the applicator with blood caused clotting within the applicator itself. Therefore, investigators removed the powder from the applicator, measured it into a weighed envelope, and poured it into the wound in the same manner as the Celox. An equivalent weight of the hemostatic agents was used for comparison in this study. The mean weight of Celox was 23.8 g compared with 24.8 g of TraumaDEX. The amount of hemostatic agent used filled the groin injury.

Standard packaging of the agents in waterproof, small packets allows soldiers and combat medics to easily carry these agents in pockets, backpacks, or medic rolls. These agents could also be easily used by physicians, nurses, and ordinary citizens in providing care in the civilian arena. Because of the nature of the products, exposure to heat or cold does not appear to be a factor in determining shelf life. Celox has a shelf life of 2 years compared with 3 years for TraumaDEX.[14,15]

Celox and TraumaDEX are approved by the US Food and Drug Administration (FDA). In this study, investigators anecdotally noted that both agents did not produce heat with application and had no obvious signs of tissue damage. There is substantial concern and reports of thermal injury to human tissue with other hemostatic agents.[16] Secondary to the mechanisms of action and the sterilization of these products, neither agent carries the risk of infection transmission, according to the manufacturers.[14,15]

Both hemostatic agents are relatively inexpensive compared with other available products. Both Celox and TraumaDEX cost approximately $20 for a single application, 35 g for Celox and 5 g for TraumaDEX, using the US General Services Administration (GSA) pricing index.[14,15] Investigators used approximately 5 applications of TraumaDEX at approximately $20 per application compared with 1 Celox application to ensure equivalent weights of the agents.

A new generation of dried fibrin dressing under investigation is effective but very expensive, costing approximately $500 to $1,000 per dressing, and is not FDA approved.[17] QuickClot (Z-Medica Corporation, Wallingford, Connecticut), whose active ingredient is kaolin, is available in a 3.5-oz granular powder retailing for approximately $10 per packet.[15] The HemCon bandage (HemCon Medical Technologies Corporation, Portland, Oregon) is a chitosan-based hemostatic dressing. The 4 × 4-in HemCon dressing is priced at approximately $100.[17] QuikClot and HemCon are FDA approved and in current use by the US Army.[17]

Conclusion

Celox and TraumaDEX were statistically and clinically superior at controlling hemorrhage compared with the standard pressure dressing in the control group. Both of these hemostatic agents are FDA approved, simple to use, lightweight, have a long shelf life, no known risk of injuries to tissues, and are relatively inexpensive.[14,15] Based on this study and the requirements outlined by Pusateri et al,[17] Celox and TraumaDEX are effective hemostatic agents for use in civilian and military trauma management.

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ACKNOWLEDGMENTS

We would like to thank the AANA Foundation for funding this research investigation, SAM Medical Products and Medafor Inc for supplying the hemostatic agents, and the faculty of the US Army Graduate Program in Anesthesia Nursing for their support of this project.

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