Application of a Zeolite Hemostatic Agent Achieves 100% Survival in a Lethal Model of Complex Groin Injury in Swine

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Background: **Techniques for better hemorrhage control after injury could change outcome. We have previously shown that a zeolite mineral hemostatic agent (ZH) can control aggressive bleeding through adsorption of water, which is an exothermic process. Increasing the residual moisture content (RM) of ZH can theoretically decrease heat generation, but its effect on the hemostatic properties is unknown. We tested ZH with increasing RM against controls and other hemostatic agents in a swine model of battlefield injury.**

Methods: **A complex groin injury was created in 72 swine (37 ± 0.8 kg). This included semitransection of the proximal thigh and complete division of the femoral artery and vein. After 3 minutes, the animals were randomized to 1 of 10 groups: group 1, no dressing (ND); group 2, standard dressing (SD); group 3, SD + 3.5 oz ZH with 1% RM (1% ZH); group 4, SD + 3.5 oz ZH with 4% RM (4% ZH); group 5, SD + 2 oz ZH with 1% RM (1% ZH 2oz); group 6, SD + 3.5 oz ZH with 8% RM (8% ZH); group 7, SD + chitosan-based hemostat, HemCon (HC); group 8, SD + 3.5 oz nonzeolite mineral hemostat, Quick Relief (NZH); group 9, SD + bovine clotting factors-based hemostat, Fast Act (FA); and group 10, SD + 30 g of starch-based hemostat, TraumaDex (TDex). Resuscitation (500 mL of Hespan over 30 minutes) was started 15 minutes after injury and hemodynamic monitoring was performed for 180 minutes. Primary endpoints were survival for 180 minutes and blood loss. In addition, maximum wound temperatures were recorded, and histologic damage to artery, vein, nerve, and muscle was documented.**

Results: **Use of 1% ZH decreased blood loss and reduced mortality to 0% ($p < 0.05$). Increasing the RM adversely affected efficacy without any significant decrease in wound temperatures. Minimal histologic tissue damage was seen with ZH independent of the percentage of RM.**

Conclusion: **The use of zeolite hemostatic agent (1% residual moisture, 3.5 oz) can control hemorrhage and dramatically reduce mortality from a lethal groin wound.**

Key Words: **Hemostatic dressing, Battlefield injury, Uncontrolled hemorrhage, Chitosan, Zeolite, Swine, Femoral vessels, Groin, Extremity.**

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Uncontrolled blood loss is the major cause of death in civilian and military trauma,[1] and early hemorrhage control can improve outcome.[2,3] Because more than 90% of the combat deaths take place before reaching the field hospital,[4] the ideal method for hemorrhage control on the battlefield should be suitable for application by the injured soldier or the first responder. It should control bleeding from a variety of sources within minutes, with a low complication rate. When the source of bleeding is in the chest or abdomen, not much can be done to control the hemorrhage in the prehospital phase. In contrast, bleeding from an extremity injury (most common site of battlefield injury) can potentially be controlled by direct compression and application of a dressing. Some of the new hemostatic agents have shown promising results in animal trials, and two of these agents (QuikClot and HemCon) have been deployed to the ongoing military conflict in Iraq. To date, there are no published studies that have compared these two agents in a clinically relevant model of uncontrolled hemorrhage.

We have previously shown that use of a mineral zeolite hemostatic agent (ZH) can dramatically improve survival in a swine model of complex groin injury.[5] In that experiment, ZH (commercial name, QuikClot) performed better than two other hemostatic dressings and had a mortality rate of 0% compared with 83% in the no-dressing group ($p < 0.05$). The mechanism of action of ZH depends primarily on adsorption of water, which is an exothermic process. The maximum temperatures were noted to be between 42° and 44°C in the

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wounds, but could reach as high as 65°C in vitro, depending on the ratio of blood to ZH and the degree of hemodilution. Altering the residual moisture content (RM) of ZH can theoretically decrease the generation of heat, but its effect on hemostatic properties in vivo is unknown.

Therefore, the current study was designed to answer the following questions: What effect does increased residual moisture have on the exothermic reaction and efficacy of ZH? How does ZH compare to other promising hemostatic agents in decreasing blood loss and improving early survival?

MATERIALS AND METHODS

The institutional laboratory animal review board for the care and use of animals approved this study. All 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* (National Research Council, 1996 edition).

Animal Preparation

Yorkshire swine (weight, 36.96 ± 0.75 kg; Tom Morris Farms, Reisterstown, MD) were fed a standard diet and observed for at least 1 week to ensure a good state of health. Food was withheld the night before the experiment, but access to water was allowed. Anesthesia was induced with intramuscular injection of ketamine (10 mg/kg) and inhaled isoflurane 4% to 5%. After placement of endotracheal tubes, the isoflurane concentration was reduced to 0.5% to 1% for the remainder of the experiment. The animals were allowed to breathe spontaneously using a mixture of 21% oxygen and air administered through a Narkomed M ventilator (North American Dräger, Telford, PA). After placing the animals supine on the operating table, the right carotid artery and the external jugular vein were cannulated with a 22-gauge angiocatheter and a 9-Fr introducer sheath, respectively, using a cutdown technique. A 7.5-Fr oximetric thermodilution pulmonary artery catheter (Baxter Health Care Corp., Irvine, CA) was positioned in the pulmonary artery through the introducer sheath. The catheters were attached to a hemodynamic monitoring system (Hewlett Packard, Palo Alto, CA) for continuous monitoring of the pulmonary and carotid artery pressures. A Baxter system (Explorer, Baxter, Edwards Critical Care, Irvine, CA) was used for continuous monitoring of mixed venous oxygen saturation and for recording the measured and derived pulmonary artery catheter parameters. All of the catheters were continuously flushed with 0.9% saline solution (5 mL/h) to maintain patency. Arterial and mixed venous blood samples were analyzed on Nova Stat Profile Ultra (Nova Biomedical, Waltham, MA). The cardiac output, blood pressure, serum lactate, base deficit, and hemoglobin levels (arterial blood gases) were measured every 5 minutes for the first 15 minutes, and every 15 minutes thereafter.

Induction of Uncontrolled Hemorrhagic Shock

A complex groin injury was created in 80 swine (36.96 ± 0.75 kg) to produce uncontrolled hemorrhage. This injury included transection of the proximal thigh soft tissues (skin, and quadriceps and adductor muscles), and complete division of the femoral artery and vein just below the inguinal ligament. This was achieved by incising these structures with a sharp scalpel. After 3 minutes of bleeding (simulating the response time of the helper), the animals were randomized to the different treatment groups. Of 80 animals, 8 (10%) died because of massive exsanguination before the application of the dressing and were excluded from the study. The remaining 72 animals were randomly assigned to the different experimental groups.

Hemostatic Agents

For this experiment, hemostatic agents were selected that were considered suitable for a large and rapidly bleeding wound. To avoid duplication of effort, it was decided not to include established products that are commercially available for control of mild to moderate intraoperative bleeding. Because the goal of this study was to identify an effective agent for use by soldiers (without any medical training) under battlefield conditions, we also screened the agents from a logistic standpoint (e.g., ease of application, time to achieve hemostasis, training required, weight and volume of dressing, storage needs). The experimental groups were as follows:

Group 1: no dressing (ND) (n = 8).
Group 2: standard dressing (SD) (n = 7).
Group 3: SD + 1% RM zeolite hemostat 3.5 oz (QuikClot, Z-Medica, Newington, CT) (n = 7).
Group 4: SD + 4% RM zeolite hemostat 3.5 oz (n = 7).
Group 5: SD + 1% RM zeolite hemostat 2 oz (n = 7).
Group 6: SD + 8% RM zeolite hemostat 3.5 oz (n = 7).
Group 7: SD + HemCon hemostat (HC) (Hemorrhage Control Technologies, Lake Oswego, OR) (n = 7).
Group 8: SD + nonzeolite mineral hemostat (NZH) 3.5 oz (Quick Relief, Biolife, LLC, Sarasota, FL) (n = 8).
Group 9: SD + bovine clotting factor hemostat, Fast Act (FA) (Wortham Lab, Chattanooga, TN) (n = 6).
Group 10: SD + TraumaDex 30 g (TDex) (Medafor, Inc., Minneapolis, MN) (n = 7).

All the hemostatic agents were provided by the manufactures free of charge and without any restrictions. It was decided to apply standard doses (as determined by the manufacturer after observing the animal model) of each agent. The tested agents had very different physical properties and mechanisms of action, thus making it impractical to use identical doses. QuikClot is a granular zeolite that adsorbs water (exothermic reaction) and promotes clot formation. The same manufacturer supplied all the different formulations of QuikClot with increasing residual moistures. HemCon is a chitosan (deacetylated poly-*N*-acetyl glucosamine)-based dressing that was supplied as a 4 × 4-inch sponge with

medical foam backing. Each sponge contained 5 g of chitosan acetate salt. It works primarily by sticking to the tissues and sealing the injury. Quick Relief is a nonzeolite mineral (mixture of hydrophilic polymer and potassium salt) that has the appearance of coarse black sand. It interacts with blood to form a scab that stops bleeding. Fast Act is a hemostatic product derived from bovine clotting factors. It activates factors II, V, VIII, and XIII at the bleeding site, thus promoting clotting through the intrinsic and extrinsic pathways. This agent was supplied as a 4½ × 5-inch impregnated gauze (300 units/square inch). Finally, TDex (30 g/dose, volume equal to 3.5 oz of ZH) is a powder-like agent that consists of bioinert microporous particles. These particles absorb water and promote clotting by producing a gelling action. All of these agents are reported to have long shelf lives and require no special assembly (or mixing of components) at the time of application.

Standard dressings for this project consisted of an 8 × 10-inch absorbent pad and an elastic bandage (Cinch Tight, H&H Associates, Bena, VA). Because this injury was not suitable for application of circumferential compressive dressing, the pad and bandage were used as a bulky dressing for packing the wound. Excess blood was evacuated from the wound without dislodging the clot at the vascular injury site, hemostatic agents were applied (groups 3–10) to the vascular injury site, followed by packing of the wound with SD (groups 2–10). During application of hemostatic agents, the granular and powder formulations (groups 3–6, 8, and 10) were poured into the wound over the vascular injury site, whereas the sponges (group 7) and impregnated gauzes (group 9) were used to completely cover the transected femoral vessels. Once the hemostatic agents were in place, the standard dressing was used to pack the wound and manual compression was applied for 5 minutes in all animals.

Resuscitation Protocols

To simulate the austere battlefield environment, we decided to limit the volume of resuscitation fluids and follow the recommendations of the Combat Fluid Resuscitation 2001 Consensus meeting.[6] A total of 500 mL of 6% hetastarch in 0.9% sodium chloride (Abbott Laboratories, North Chicago, IL) was administered intravenously over 30 minutes. The infusion was started 15 minutes after injury (simulating delay in establishing intravenous access) in all groups. The animals were monitored for a total of 180 minutes after injury. Primary endpoints were blood loss after application of dressing and early (180 minutes) mortality. For the purpose of this study, no measurable blood pressure or apnea for a period of 5 minutes was taken as the time of death. All the animals that survived for 180 minutes were killed by exsanguination under inhaled anesthesia.

Measurement of Blood Loss

Blood loss was measured in a continuous fashion by gently suctioning the blood that oozed around the dressings into a collection container. All the dressings and hemostatic agents were weighed before application and at the end of the experiment, and the difference was added to the total blood loss.

Measurement of Exothermic Reaction

In all animals, two standard mercury thermometers were placed at the interface between the hemostatic agents and incised muscles. The higher of the two readings was recorded every minute for 15 minutes, every 5 minutes for 15 minutes, and every 15 minutes thereafter until return of wound temperature to baseline.

Histologic Examination of the Tissues

At the end of the experiment (180 minutes), segments of femoral artery, vein, nerve, and piece of quadriceps muscle were obtained from the site of dressing application as well as the opposite side (control). The tissue samples were fixed in 10% buffered formaldehyde solution. Representative sections (5 μm) were sliced from the leading edge of the samples (in contact with the hemostatic agent), attached to glass slides, and stained with hematoxylin-eosin for microscopic examination. The sections were examined qualitatively for morphologic changes by an experienced pathologist who was blinded to the group allocation of the samples.

Testing of a New Delivery Method

Because of its physical properties (granules), complete removal of zeolite from a large wound can be time consuming. We reasoned that this can be facilitated if the zeolite material is packaged in fabric pouches (similar to tea bags). In a separate experiment, five Yorkshire swine were subjected to the groin injury and resuscitated as described before. Three minutes after the injury, 3.5 oz of 1% residual moisture zeolite was applied, the wound was packed with the standard dressing, and manual compression was exerted for 5 minutes. The zeolite material for this experiment was specially packaged in a bilayer pouch (15–20 g/pouch). The inner layer was made of nonwoven Ahlstorm 4971 filter paper (Ahlstrom Corporation, Windsor Locks, CT), and the outer layer was cotton gauze. The rationale behind this approach was that the pouch would allow free adsorption of water into the zeolite and make it easy to remove the material from the wound at the end. The monitoring of hemodynamic parameters, blood loss, and exothermic reaction were identical to the previous experiment.

Statistical Analysis

All data presented as group means ± SEM. The SPSS statistical software program (SPSS/Windows, SPSS Inc., Chicago, IL) was used for intergroup comparisons. One-way analysis of variance with Dunnett's test for multiple comparisons was used to compare blood loss, wound temperature, and hemodynamic data. Unless specified otherwise, the ND group was used as a control for statistical

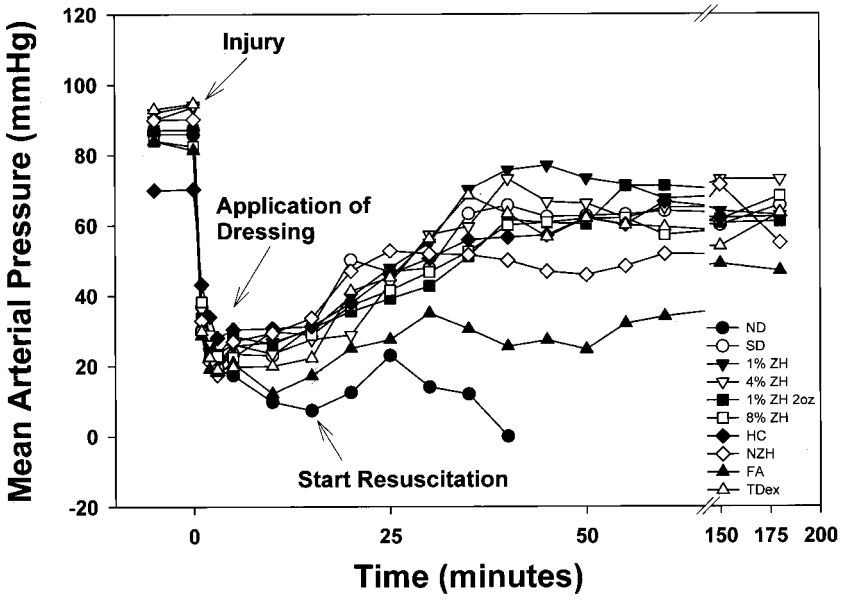

Fig. 1. *Mean arterial blood pressures (MAP) during the experiment. Data presented as group means. ND, no dressing; SD, standard dressing alone; 1% ZH, SD + 3.5 oz of zeolite hemostat with 1% residual moisture; 4% ZH, SD + 3.5 oz of zeolite hemostat with 4% residual moisture; 1% ZH 2oz, SD + 2 oz of zeolite hemostat with 1% residual moisture; 8% ZH, SD + 3.5 oz of zeolite hemostat with 8% residual moisture; HC, SD + HemCon hemostatic dressing; NZH, SD + 3.5 oz of nonzeolite hemostat; FA, SD + Fast Act bovine hemostatic dressing; TDex, SD + 30 g of TDex hemostatic powder.*

analysis. The χ^2 test was used to compare mortality rates against ND and SD groups. Significance was defined as $p < 0.05$.

RESULTS

Hemodynamic and Physiologic Parameters

Selected parameters are presented in Figure 1 and Table 1. The injury caused a rapid drop in blood pressure (mean arterial blood pressure between 15 and 30 mm Hg), a decrease in cardiac output to almost 25% of baseline, and a doubling of serum lactate levels within 5 minutes. Resuscitation resulted in improvement in blood pressure and cardiac output and clearance of lactic acidosis, especially when the hemostatic dressing was effective. The ND group had the lowest blood pressure and the worst cardiac output, and none of these animals survived until the end of the experiment.

Table 1 Selected Physiologic Parameters[a]

Parameters	Time (min)	Experimental Groups									
		ND	SD	1% ZH	4% ZH	1% ZH 2oz	8% ZH	HC	NZH	FA	TDex
Cardiac output (L/min)	0	4.6 ± 0.7	4.0 ± 0.4	4.4 ± 0.2	4.5 ± 0.4	4.2 ± 0.2	4.6 ± 0.3	5.6 ± 0.6	3.9 ± 0.3	3.7 ± 0.1	4.0 ± 0.4
	5	0.4 ± 0.2	1.1 ± 0.3	1.3 ± 0.3	1.2 ± 0.4	0.9 ± 0.1	1.4 ± 0.4	1.9 ± 0.2	1.0 ± 0.3	0.7 ± 0.2	1.0 ± 0.2
	60		4.9 ± 0.9	4.6 ± 0.5	4.8 ± 0.8	4.3 ± 0.3	4.3 ± 0.2	4.4 ± 0.3	2.8 ± 0.5	2.9	3.64 ± 0.7
	180		4.7 ± 0.8	3.9 ± 0.4	4.5 ± 1.0	3.5 ± 0.3	4.6 ± 0.5	5.0 ± 0.3	4.0 ± 0.2	2.0	3.34 ± 0.6
Hemoglobin (g/dL)	0	10.2 ± 0.5	9.8 ± 0.4	9.0 ± 0.2	9.0 ± 0.3	8.9 ± 0.4	9.2 ± 0.3	9.2 ± 0.3	9.1 ± 0.4	8.7 ± 0.3	9.6 ± 0.4
	60		6.8 ± 0.5	6.2 ± 0.4	5.6 ± 0.4	5.9 ± 0.3	7.0 ± 0.4	5.8 ± 0.4	6.7 ± 0.6	4.9	6.1 ± 0.4
	180		6.8 ± 0.2	6.3 ± 0.5	6.3 ± 0.5	6.5 ± 0.4	7.4 ± 0.1	5.4 ± 0.5	8.0 ± 0.9	6.2	7.1 ± 0.7
Lactate (mmol/L)	0	1.2 ± 0.2	1.1 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	1.4 ± 0.6	0.8 ± 0.2	1.0 ± 0.1	0.6 ± 0.2	1.2 ± 0.3
	5	2.1 ± 0.4	2.3 ± 0.8	2.5 ± 0.6	2.5 ± 1.5	2.7 ± 0.9	2.9 ± 1.1	1.2 ± 0.2	2.5 ± 0.4	3.5 ± 0.8	2.9 ± 0.4
	60		2.1 ± 0.4	2.4 ± 0.5	3.7 ± 0.9	2.0 ± 0.4	1.1	1.3 ± 0.3	4.6 ± 1.3	2.4	3.6 ± 0.6
	180		1.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	1.8 ± 0.6	1.4	0.4 ± 0.2	1.4 ± 0.8	1.6	1.6 ± 0.2

Data presented as group mean ± SEM. ND, no dressing; SD, standard dressing; 1% ZH, 3.5 oz of zeolite hemostat with 1% residual moisture; 4% ZH, 3.5 oz of zeolite hemostat with 4% residual moisture; 1% ZH 2 oz, 2 oz of zeolite hemostat with 1% residual moisture; 8% ZH, 3.5 oz of zeolite hemostat with 8% residual moisture; HC, HemCon hemostatic agent; NZH, nonzeolite mineral hemostat Quick relief; FA, bovine hemostatic agent Fast Act; TDex, starch-based hemostat TDex. Standard dressing was used with all hemostatic agents. Time 0 = Just prior to injury; Time 5 = 5 min postinjury; Time 60 = 60 min postinjury; Time 180 = 180 min postinjury. Empty cells show no surviving animals at that time point, and absence of SEM values shows only one surviving animal at that time point.

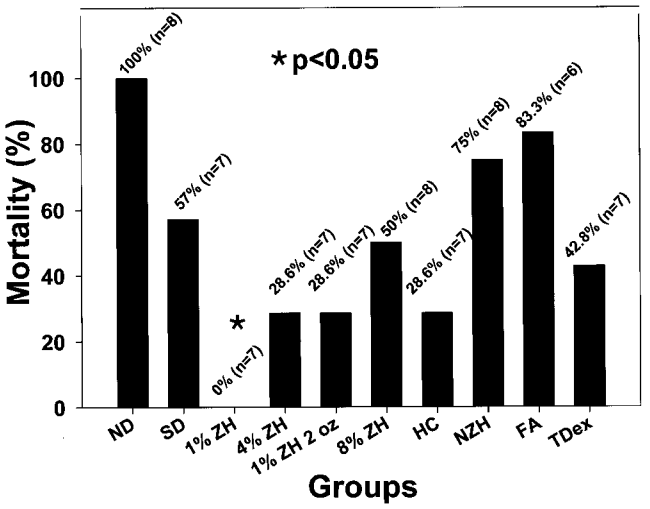

Fig. 2. *Mortality rates in different groups. Data shows the percent of animals that survived for 180 minutes.* *p $<$ *0.05* χ^2 *test compared with ND group. ND, no dressing; SD, standard dressing alone; 1% ZH, SD + 3.5 oz of zeolite hemostat with 1% residual moisture; 4% ZH, SD + 3.5 oz of zeolite hemostat with 4% residual moisture; 1% ZH 2oz, SD + 2 oz of zeolite hemostat with 1% residual moisture; 8% ZH, SD + 3.5 oz of zeolite hemostat with 8% residual moisture; HC, SD + HemCon hemostatic dressing; NZH, SD + 3.5 oz of nonzeolite hemostat; FA, SD + Fast Act bovine hemostatic dressing; TDex, SD + 30 g of TDex hemostatic powder.*

Mortality

This complex vascular and soft tissue injury resulted in 100% mortality in the control (ND) group. Application of standard dressing decreased the mortality to 57%. Addition of the zeolite hemostat (QuikClot) agent offered a statistically significant ($p = 0.02$) advantage by decreasing the mortality rate to 0%. When compared with standard dressing, it decreased the mortality from 57.4% to 0% ($p = 0.07$). The mortality rates in other experimental groups are shown in Figure 2. None of these were significant (or close to significant) when compared with the ND or SD groups. Majority of the deaths were within an hour of the injury, with very few deaths after 2 hours (Fig. 3).

Blood Loss

All the groups had similar blood loss before application of dressing (Fig. 4) except for the HemCon group (significantly lower blood loss compared with the NZH and TDex groups). The injury resulted in an initial brisk exsanguination of approximately 500 mL/min. With the onset of hypotension, arterial spasm, and formation of clot at the injury site, the rate of arterial hemorrhage typically decreased over the 3-minute period. However, bleeding from the venous injury was not influenced by the onset of hypotension. The application of standard dressing (without any hemostatic agent) and manual compression was effective in controlling hemorrhage in 43% of the animals. In the hemostatic agent groups, five of the groups lost less blood than the SD alone group, with the lowest blood loss noted in the 3.5 oz 1% residual moisture ZH group (7.5 ± 2.4 mL/kg). However, this did not reach statistical significance compared with the control (ND) group. The failure of dressings (renewal of hemorrhage) typically occurred when the blood pressure improved after resuscitation. It should be noted that treatment with 1% ZH (3.5 oz) and HemCon dressings displayed some interesting features. In the other treatment groups, application of hemostatic agents slowed down, but rarely stopped, the hemorrhage. Treatment with 1% ZH stopped bleeding in all the animals, whereas HC was unique because of the marked variability in effectiveness from animal to animal. In five of seven animals, the HC dressing adhered very well to the tissues, resulting in superb hemorrhage control, and none of these animals died. The postdressing blood loss in this subgroup ranged from 0.02 to 10.4 mL/kg. In two of seven (28.6%) animals, the dressing failed completely (poor adherence to the tissues), and both of these animals died. The blood loss in the HC group after application of dressing (13.0 ± 5.9 mL/kg) was largely because of the two failures (39.8 mL/kg and 29.3 mL/kg).

Exothermic Reaction

All the different formulations of mineral hemostatic agents tested in this experiment generated heat. The maximum temperature in the wounds treated with NZH (Quick Relief, group 8) was 49 ± 2.7°C, whereas in the ZH groups (groups 3–6) it ranged from 51° to 57°C. These numbers were not statistically different from each other, but groups 3, 4, and 8 showed significantly higher temperatures compared with ND and SD control groups (Fig. 5).

Histologic Evidence of Tissue Damage Caused by Exothermic Reaction

The histologic examination of tissues from the ZH and NZH groups showed changes consistent with exposure to heat. Representative experimental (site of dressing application) and control (opposite side) sections of artery, vein, nerve, and muscle from a 1% ZH 3.5 oz animal are shown in Figure 6. The sections of artery at the site of laceration typically demonstrated the following changes: disruption of the arterial wall, pink coagulum intermixed with red blood cells filling vessel lumen and surrounding the artery, and a variable degree of arterial wall necrosis (Fig 6*B*). The findings were more subtle in the veins and consisted of patchy areas of coagulative necrosis of the vessel wall (Fig 6*D*). These findings were limited to the end of the transected artery/vein that was in contact with the hemostatic agents. Sections taken 0.5 cm proximal to the transected end demonstrated an intact vessel wall without necrosis. The section of muscle showed mild necrosis at the periphery of the section that was in contact with the hemostatic material. In some animals, the skeletal muscle fibers were vacuolated

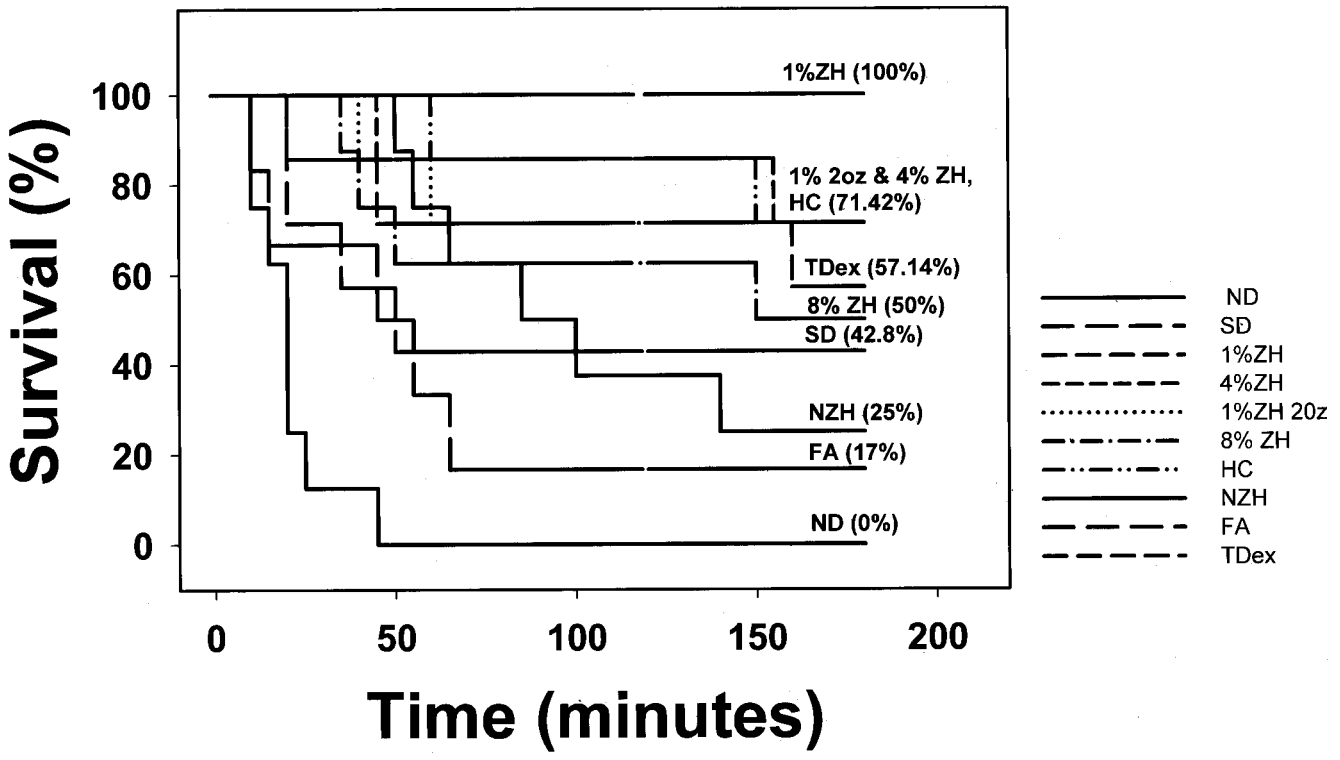

Fig. 3. *Time to death. Data presented as the percentage of surviving animals over time. ND, no dressing; SD, standard dressing alone; 1% ZH, SD + 3.5 oz of zeolite hemostat with 1% residual moisture; 4% ZH, SD + 3.5 oz of zeolite hemostat with 4% residual moisture; 1% ZH 2oz, SD + 2 oz of zeolite hemostat with 1% residual moisture; 8% ZH, SD + 3.5 oz of zeolite hemostat with 8% residual moisture; HC, SD + HemCon hemostatic dressing; NZH, SD + 3.5 oz of nonzeolite hemostat; FA, SD + Fast Act bovine hemostatic dressing; TDex, SD + 30 g of TDex hemostatic powder.*

with loss of striations and a pink fluid was present between muscle fibers, whereas the nerve fibers showed mild edematous changes (Fig 6*F*). Tissue ischemia caused by transection of the artery and hypotension may also account for some of the changes seen in the muscle and surrounding tissues. Examination of control sections of artery, vein, and muscle (Fig. 6*A*, *C*, and *E*) showed no significant pathologic changes except variation in staining in the muscle and limited extravasation of red cells that most likely took place at the time of tissue procurement.

Testing of Zeolite Hemostat in Pouches

The packaging of zeolite in pouches clearly facilitated the removal of hemostatic material at the end of the experiment. The removal of pouches was easier and faster compared with loose granular zeolite. However, packaging the zeolite in the pouches decreased its effectiveness. The blood loss before and after application of dressing was 26.9 ± 2.4 mL/kg and 10.3 ± 4.6 mL/kg, respectively, which was comparable to loose zeolite, but the 180-minute survival was only 60%. The maximum wound temperature was 48.2° ± 3.3°C. In two of five animals, the bleeding restarted after resuscitation and these animals died at 41 and 177 minutes after injury. The postdressing blood loss in these two animals was 18.9 mL/kg and 24.4 mL/kg, respectively. However, in three animals, the dressing was extremely effective, with postdressing blood losses of only 2.7 mL/kg, 3.5 mL/kg, and 2.8 mL/kg.

DISCUSSION

This experiment has demonstrated that in a model of lethal groin injury, hemorrhage control with a mineral zeolite hemostat (QuikClot) can significantly reduce the mortality from 100% to 0% ($p = 0.02$). When compared with standard dressing, the mortality was decreased from 57.4% to 0% ($p = 0.07$). The use of this agent was also associated with the lowest volume of blood lost. Other hemostatic agents tested in this experiment displayed mixed results. Although not statistically significant, the zeolite hemostats with increased residual moisture (4% and 8%), smaller dose (2 oz) of ZH with 1% residual moisture, HemCon hemostat, and TDex dressings all performed better than the standard dressing control (group 2). In contrast, application of two hemostatic dressings (Fast Act and Quick Relief) was associated with a mortality rate that was higher than the standard dressing group. This study has also shown that increasing the residual moisture content of ZH adversely affects efficacy without any meaningful decrease in exothermic reaction, or the histologic

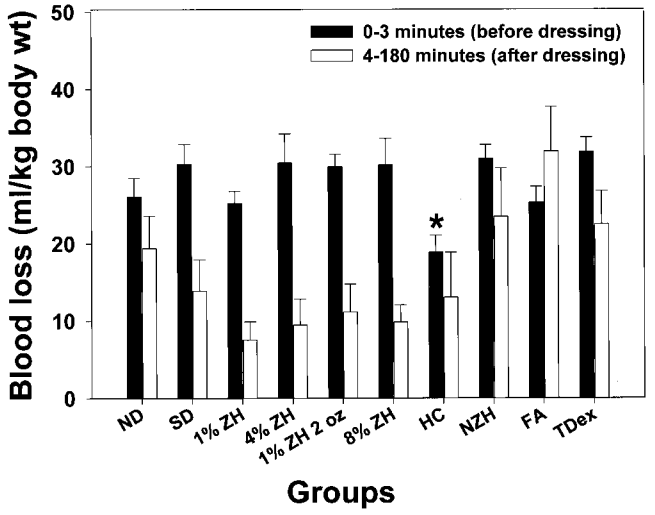

Fig. 4. *Blood loss before and after application of wound dressings. Data presented as group means ± SEM (milliliters of blood per kilogram of body weight).* Black bars *represent blood lost before application of dressing and* white bars *display volume of blood lost after application of dressing.* *$p < 0.05$ *using analysis of variance and Dunnett's test for multiple comparisons (HC against NZH and TDex). ND, no dressing; SD, standard dressing alone; 1% ZH, SD + 3.5 oz of zeolite hemostat with 1% residual moisture; 4% ZH, SD + 3.5 oz of zeolite hemostat with 4% residual moisture; 1% ZH 2oz, SD + 2 oz of zeolite hemostat with 1% residual moisture; 8% ZH, SD + 3.5 oz of zeolite hemostat with 8% residual moisture; HC, SD + HemCon hemostatic dressing; NZH, SD + 3.5 oz of nonzeolite hemostat; FA, SD + Fast Act bovine hemostatic dressing; TDex, SD + 30 g of TDex hemostatic powder.*

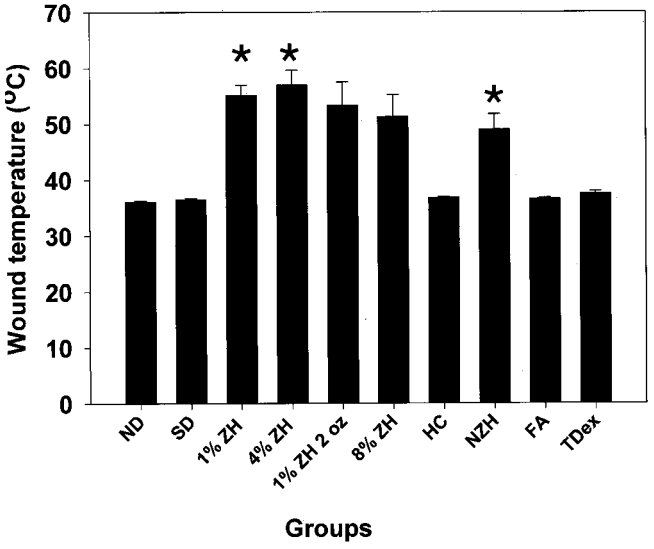

Fig. 5. *In vivo measurement of exothermic reaction. Data presented as group mean ± SEM.* *$p < 0.05$ *using analysis of variance and Dunnett's test for multiple comparisons against the ND group. ND, no dressing; SD, standard dressing alone; 1% ZH, SD + 3.5 oz of zeolite hemostat with 1% residual moisture; 4% ZH, SD + 3.5 oz of zeolite hemostat with 4% residual moisture; 1% ZH 2oz, SD + 2 oz of zeolite hemostat with 1% residual moisture; 8% ZH, SD + 3.5 oz of zeolite hemostat with 8% residual moisture; HC, SD + HemCon hemostatic dressing; NZH, SD + 3.5 oz of nonzeolite hemostat; FA, SD + Fast Act bovine hemostatic dressing; TDex, SD + 30 g of TDex hemostatic powder.*

evidence of tissue injury. On testing a new delivery method, we discovered that packaging the ZH in fabric pouches facilitated its application and removal. However, the pouch material acted as a barrier and decreased the effectiveness of ZH. It is possible that further refinement in packaging may overcome this problem.

Historically, approximately 20% of all injured soldiers die, with 90% of the deaths taking place before reaching the field hospital. The single major cause of potentially preventable death is hemorrhage. Prompt control of bleeding and resuscitation in the field can reduce the mortality rate by as much as 20%.[7,8] Although a number of promising hemostatic agents have been tested,[9–12] at present, there is no clear consensus regarding the optimal hemorrhage control strategy for combat casualties. The percentage of soldiers that are killed in action has remained fairly unchanged since the American Civil War at approximately 20%. However, the percentage of soldiers that die as a result of wounds after reaching medical facilities has steadily decreased.[13–15] During the ongoing conflict in Iraq, the emerging data so far suggest that the killed-in-action rate may remain unchanged. According to the information released by the Pentagon, by early September 2003 the total number of U.S. casualties in Iraq was 1,406, with 1,124 wounded (79.95%) and 282 dead (20.05%).[16] Anatomically, extremities have been the most common (up to 75% of penetrating wounds in World War II) sites of combat injuries over the last century.[17] This trend is even more pronounced during the recent conflicts because of the use of body armor and Kevlar helmets. With the evolution of body armor, fatal torso injuries have markedly decreased, whereas penetrating wounds to the face, groin, and pelvis are causing significant mortality.[18,19] This is important because compared with intracavity hemorrhage, bleeding from extremity wounds is potentially controllable in the field with appropriate and prompt use of tourniquets or hemostatic dressings. The use of tourniquets is not always effective, may cause complications, and is not without controversy.[20] Furthermore, injuries to proximal extremities (e.g., pelvis, groin, shoulder/axilla) are often not suitable for application of tourniquets. The nature of warfare is also rapidly changing, with terrorist activities and guerrilla warfare replacing the traditional combat between well-organized armies. Because of the overwhelming superiority of the U.S. military, the conventional war is likely to be brief, but the postwar period may be characterized by prolonged conflicts in urban areas or in hostile terrain between an ill-defined enemy and small, rapidly mobile U.S. combat units. This pattern of combat is currently evident in Afghanistan and Iraq. Compared with

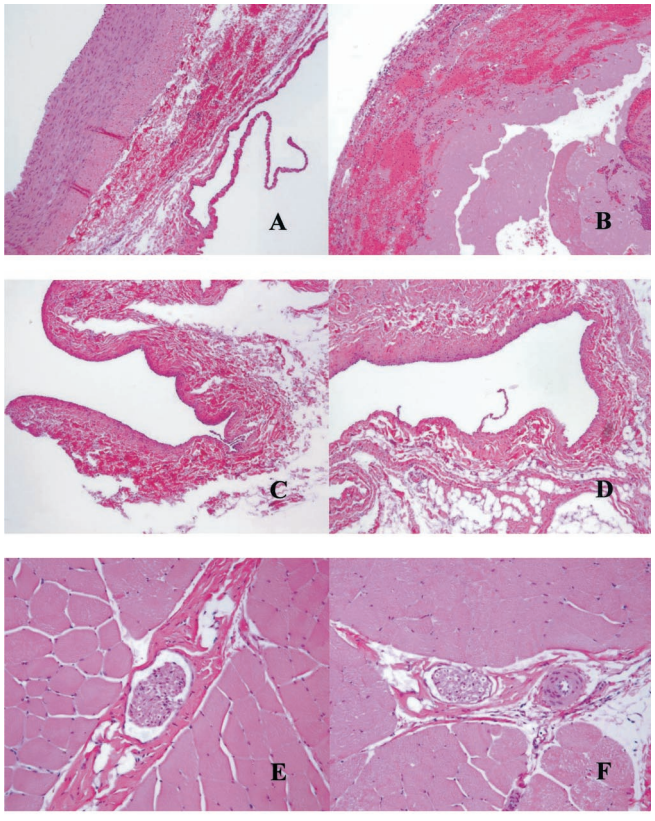

Fig. 6. *Histology sections. Representative sections of artery (*A *and* B*) vein (*C *and* D*) and muscle/nerve (*E *and* F*) are shown at 50× magnification after staining with hematoxylin and eosin. All samples shown here were obtained from the same animal. The tissues in panels* A, C, *and* E *were obtained from the noninjured side (control) and panels* B, D, *and* F *were from the site of 1% zeolite hemostatic agent (3.5 oz) application.*

previous wars (such as Korea and Vietnam) where the evacuation was rapid and field hospitals were close to the front lines, conflicts in urban or hostile environment are characterized by unforeseen delays in evacuation.[21] Early and effective hemorrhage control is even more important in these situations and could save more lives than any other measure. However, the best strategy for achieving this goal in austere and hostile combat situations is not clear.

The rationale for designing this model of groin injury takes into account the realities of modern combat injuries and has been published previously.[5] The salient features of this model include, lethal but potentially salvageable injuries, anatomic location unsuitable for application of tourniquet, combined arterial and venous injuries with uncontrolled hemorrhage, large soft tissue injury, realistic delays in application of dressing and start of resuscitation, and resuscitation approach consistent with new recommendations for the military. The obvious limitations include the lack of blast injury, burns, and so forth. We made some modifications in the model compared with the previously published study. The dressings were applied earlier (3 vs. 5 minutes postinjury), resuscitation was started earlier (15 vs. 30 minutes), and was more aggressive (500 mL of Hespan versus 1,000 mL of normal saline). These modifications were introduced to further increase the challenge for the hemostatic dressings: the dressings were applied while the wounds were still bleeding briskly and the resuscitation approach resulted in an earlier increase in blood pressure. As the exothermic reaction is influenced by the ratio of blood to ZH, the increased amount of bleeding may explain the higher wound temperatures (55°C) in the current study compared with the previous experiment (44°C). Typically, the increase in temperature was fast (within 30 seconds), reached the peak in 2 to 3 minutes, and stayed there for approximately 30 seconds. The decrease in the temperature back to baseline was slow and took 10 to 20 minutes. Increasing the residual moisture did not mitigate the exothermic reaction while adversely affecting the hemostatic properties of the ZH. The NZH dressing (group 8) caused a similar degree of exothermic reaction without controlling hemorrhage. These findings considered together seem to support fluid adsorption rather then exothermic reaction (cauterizing effect) as the main mechanism of action for the ZH. On gross examination, the exothermic agents (ZH and NZH) caused some discoloration of the tissues, but the tissues were clearly viable at 180 minutes. Histologic examination revealed coagulative damage to the arterial wall that extended up to 5 mm on serial sectioning. The vein and muscle were spared except for some minor coagulative and edematous changes. These changes were related to the degree of exothermic reaction and were seen in all ZH (regardless of the percentage of residual moisture) and NZH groups.

The ZH hemostatic agent is extremely effective, but there are potential risks involved with its use because of the exothermic reaction. The clinical relevance of the histologic tissue damage seen in our study is debatable, as most of the severe combat wounds routinely undergo debridement. The risks of exothermic reaction are clearly acceptable in the setting of a life-threatening injury. However, if used for nonlethal, minimal wounds, ZH may have an unfavorable risk/benefit ratio. The use of ZH in close proximity to heat-sensitive structures (e.g., nerves, cornea) should also be avoided until further testing.

Under ordinary circumstances, new drugs or devices are tested by multiple researchers through randomized clinical trials before widespread use. However, emergencies such as a large military conflict can create a unique challenge. The decision to use (or not to use) the product cannot wait for the "ideal" data (large randomized clinical trials), and may have to be made on the basis of the "best available" data. On February 21, 2003, at the request of U.S. Marine Corps, a panel of subject matter experts met at the Uniformed Services University of the Health Sciences (Bethesda, MD) and reviewed all available (published and unpublished) data about ZH. The panel recommended that ZH should be used when

the bleeding is unresponsive to standard of care therapy and is thus potentially life threatening.[22] These recommendations have been formalized into standard policy by the U.S. Marine Corps. Because the vast majority of limb injuries are nonlethal and hemorrhage is easy to control, the Marines are being trained to use standard therapy for initial control of hemorrhage (packing, circumferential dressing or tourniquet as indicated, depending on the nature and location of the injury). If the bleeding stops, the dressings should be left in place until evacuation to a higher level of care. However, if the bleeding continues after proper application of standard treatment, the responder should switch to the zeolite hemostat as a lifesaving measure. The panel also endorsed the use of this material by nonmedical personnel that have appropriate training. On the basis of these recommendations, two bags of ZH (3.5 oz each) have been added to the U.S. Marine Corps individual first aid kit. These new kits were deployed to the ongoing conflict in Iraq. According to early information from Iraq, ZH has been used in approximately 30 cases so far, with very good results. There have been occasional failures that were most likely caused by improper use (e.g., failure to apply manual compression), but more importantly, no serious complications have been reported. This anecdotal information has to be verified and analyzed before the full impact of this hemostatic approach can be ascertained.

Another hemostatic agent that has been deployed to the front lines in Iraq is HemCon. It has shown excellent results in an animal model of liver injury,[23] and has performed very well in limited cases (approximately four) reported from the battlefield in Iraq. In our experiment, HemCon either performed extremely well (five of seven animals) or failed completely (two of seven animals). All of the failures were caused by lack of adherence to the tissues and all of these animals died. As the test dressings were all from the same batch and looked identical, it was impossible to predict which dressing will not work. This is most likely a manufacturing/quality control issue that should be easy to resolve. In a pilot experiment (conducted in preparation for this study), the entire batch of HemCon failed to adhere. However, the new batch, tested in the current study, was markedly better and it is hoped that the manufacturer will soon be able to achieve a uniformly effective product. The obvious advantage of HemCon is the absence of any adverse effects, thus making it a very attractive choice. Both of these agents (QuikClot and HemCon) are relatively inexpensive (QuikClot, $20; HemCon, $100), are easy to apply, have extremely long shelf lives, and have no special storage requirements. These hemostatic agents have been approved by the Food and Drug Administration for clinical use and are being used by different branches of the U.S. military. Over the next few months, data gathered during the war will possibly support the concept that easy-to-use, rapidly acting hemostatic agents, when used early in the setting of battlefield injuries, can save lives. Although this discussion has focused on combat injuries, the rationale for the early use of hemostatic dressings applies equally well to the prehospital treatment of civilian trauma.

In summary, use of a zeolite hemostatic agent (QuikClot) in a swine model of lethal groin injury decreased blood loss and significantly improved early survival. This agent holds promise for the control of life-threatening external hemorrhage in the prehospital settings.

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