

Safety Evaluation of New Hemostatic Agents, Smectite Granules, and Kaolin-Coated Gauze in a Vascular Injury Wound Model in Swine

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Background: In 2007, a potent procoagulant mineral called WoundStat (WS), consisting of smectite granules, received clearance from the Food and Drug Administration for marketing in the United States for temporary treatment of external hemorrhage. Previously, we found that microscopic WS particles remained in the injured vessels that were treated, despite seemingly adequate wound debridement. Thus, we investigated the thromboembolic risk of using WS when compared with kaolin-coated gauze, Combat Gauze (CG); or regular gauze, Kerlix (KX) to treat an external wound with vascular injuries in pigs.

Methods: The right common carotid artery and external jugular vein of pigs were isolated and sharply transected (50%). After 30 seconds of free bleeding, the neck wounds were packed with WS, CG, or KX and compressed until hemostasis was achieved ($n = 8$ per group). Wounds were debrided after 2 hours, and vascular injuries were primarily repaired with suture. Blood flow was restored after infusing 1 L of crystalloid (no heparin or aspirin) and the wounds were closed. Two hours later, computed tomographic angiography was performed, and the wounds were reopened to harvest the vessels. The brains and lungs were recovered for gross and microscopic examination after euthanasia.

Results: No differences were found in baseline measurements. Thrombelastography showed similar hypercoagulability of the final blood samples when compared with baselines in all groups. All vessels treated with KX or CG were patent and had no thrombus or blood clot in their lumen. In contrast, seven of eight carotid arteries and six of eight jugular veins treated with WS developed large occlusive red thrombi and had no flow. Small clots and WS residues were also found in the lungs of two pigs. Histologically, significant endothelial and transmural damage was seen in WS-treated vessels with luminal thrombi and embedded WS residues.

Conclusion: WS granules caused endothelial injury and significant transmural damage to the vessels that render them nonviable for primary surgical repair. The granules can enter systemic circulation and cause distal thrombosis in vital organs. More relevant in vitro and in vivo safety tests should be required for clearance of new hemostatic agents.

Key Words: WoundStat, Combat gauze, Hemorrhage control, Hemostatic agent, Vascular injury, Animal model, Swine.

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Uncontrolled hemorrhage continues to be the number one cause of battlefield death.^{1,2} In 2003, two new Food and Drug Administration (FDA) approved hemostatic agents, QuikClot (QC) granules and the HemCon (HC) bandage, were deployed for treating compressible wounds to control hemorrhage refractory to tourniquet and gauze application. Despite a few positive anecdotal reports,^{3,4} other reports⁵ and personal communication with combat medics implied limited use or avoidance of these agents in the field because of either painful side effects (thermal injury with QC) or poor efficacy in controlling severe bleeding. Earlier experimental studies have also cast doubt about the efficacy of these agents in more challenging arterial hemorrhage.^{6–8} We recently reported two experimental studies in search of identifying novel and more effective topical hemostatic products than those previously deployed.^{9,10} These studies examined the efficacy and acute safety (tissue reaction) of four new hemostatic granules/powders and four new hemostatic dressings in comparison to QC and HC dressings. The test products were preselected among numerous new hemostatic products based on initial pilot testing. The selected products were tested in a lethal femoral artery hemorrhage model in pigs that could not be stopped by gauze or tourniquet application. Based on blood loss and survival results, WoundStat (WS; smectite granules) was found to be the most effective hemostatic with 100% survival rate followed closely by Combat Gauze (CG; kaolin-coated surgical gauze) with 80% survival rate with no significant difference in efficacy between the two products. Both products were significantly more effective than the recent HC bandage and QC beads (QC ACS⁺), with 10% and 16% survival rates, respectively.

Neither WS nor CG is biodegradable and must be removed from wounds before surgical repair and closure of the wounds. In our previous studies, the removal of CG was an easy procedure, but cleaning WS clay and removing all particles required extensive and meticulous debridement. Microscopic residues of WS, however, were seen in the majority of treated vessels, suggesting it had the potential to be the source for thrombosis if blood flow was restored. Traces of kaolin were also detected in one specimen.

This study was therefore designed to investigate the potential thrombogenicity of WS and CG when they are used to control external bleeding due to major vascular injury. For this purpose, a new wound model was developed in pigs that involved a neck injury with partial transection of the carotid artery and the jugular vein. Histologic changes and thrombo-

sis occurrence were examined in the treated vessels after surgical repair and 2-hour blood reflow. In addition, the distal organs (lung and brain) in which the residue may reside were examined for evidence of thromboembolism. Regular gauze (Kerlix, [KX]) was used as a control agent.

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of the U.S. Army Institute of Surgical Research. All animals received care and were used in strict compliance with the Guide for the Care and Use of Laboratory Animals.¹¹ WS and CG were purchased from commercial sources. Both products are approved by the U.S. FDA and are available for purchase without the need for prescription. These agents are indicated for temporary treatment of external wounds to control moderate-to-severe bleeding in patients.

Yorkshire cross-bred male pigs ($n = 24$) weighing 34 kg to 42 kg were purchased from Midwest Research Swine (Gibbon, MN) and used in this study. Before the surgery date, venous blood samples were collected from femoral veins (percutaneous catheter) and complete blood count and standard clotting tests (prothrombin time [PT], activated partial thromboplastin time [aPTT], and fibrinogen) were performed to ensure that these measures were within the normal range and met our inclusion criteria. After at least 1 week of acclimation, pigs were fasted for 12 hours to 18 hours before the surgery with free access to water. On the day of surgery, animals were premedicated, anesthetized, and ventilated as described previously.⁹ Anesthesia was maintained with 1% to 2% isoflurane in 100% oxygen gas administered by the automatic respirator. Maintenance fluid, lactated Ringer's (LR), was infused at 5 mL/kg/h through an 18-gauge catheter placed in an ear vein.

Surgical Procedures

A 5-cm incision was made in an inner thigh muscle, and a superficial branch of the femoral artery was isolated above the knee. The artery was cannulated with a gel-filled thin cannula attached to a small sensor device (TL 11M2-C70-PCT; Data Sciences International, St. Paul, MN), which was temporarily implanted in the subcutaneous groin. Vital signs (heart rate and systolic, diastolic, and mean arterial pressures) received by the sensor were transmitted remotely to a computer system (via a receiver plate) and displayed and recorded throughout the experiment. The left femoral vein was also cannulated for blood sampling and fluid (Hextend and LR) administration.

To create the injury and hemorrhage, a 10-cm incision was made in the lateral ventral region of the neck, and the underlying tissues were dissected to expose the vessels. Segments (~5 cm long) of the common carotid artery and the external jugular vein were isolated, and lateral branches were cauterized and divided with minimum trauma to the vessels. For clamping purposes, umbilical tape loops were placed loosely around the vessels and passed through small plastic tubing. After allowing 10 minutes of stabilization (with no surgical manipulation), baseline blood samples were collected; and the vessels were occluded by pulling the umbilical tapes through the tubing and then marked for transection.

With the use of an iris scissor, vessels were partially transected (~50% of their circumference). The vascular loops were then released, and free bleeding was allowed for 30 seconds (pretreatment blood loss). Animals were randomized and wounds were packed either with two packages of WS or CG, or one roll of (KX) gauze ($n = 8$ per group). The surgeons were blinded to the identity of the hemostatic agent until treatment started. Two packages of WS or CG were required to fill the relatively large wound space. The agents and the control (KX) were then covered with a laparotomy sponge and manually compressed for as long as needed until hemostasis was achieved. Compression, however, was interrupted after 2, 5, 15, 30, 45, 60, 90, and 120 minutes to check for hemostasis; and the laparotomy sponge was replaced if it had absorbed a significant amount of blood. These sponges were collected, and the absorbed blood was measured and recorded as posttreatment blood loss. At the start of compression, 500 mL of colloid fluid (Hextend) was also administered intravenously (IV; 50 mL/min) to compensate for pretreatment blood loss and to raise and maintain the pigs' mean arterial pressure at 60 mm Hg to 65 mm Hg.

Two hours after treatment, the hemostatic materials were taken out, the vessels were occluded again to prevent rebleeding, and the wounds were debrided according to standard clinical procedure using 1 L (CG and KX) to 2 L (WS) of saline with a bulb syringe in pulsatile fashion to flush and clean the wound thoroughly. No visible WS was left in the wound. Next, the loops were momentarily released to allow free bleeding, and the vessels were flushed with saline to remove any hemostat residue or clots in the lumens. The vascular injuries were then sutured (primary repair) using a monofilament nylon suture (7-0 Prolene). All of the anastomoses were performed by one investigator only (B.S.K.). During anastomosis, 1 L of LR fluid was administered IV to produce a mild hemodilution. Blood flow was restored first in the artery and then in the vein after the repair of each vessel and administration of LR. No anticoagulant or platelet inhibitor was given to any of the pigs during clamping, anastomosis, or the reflow period. The neck wounds were then closed in layers by suturing (2-0 Vicryl), and the pigs were monitored for an additional 2 hours under anesthesia.

After the monitoring period, final blood samples were collected for laboratory tests; and the pigs were transferred to the imaging room. Computed tomography (CT) angiography was performed, and images of the blood flow through the arteries and veins of the neck were taken seconds after infusion of a contrast agent (100 mL of Omnipaque, 300 mg/mL, IV). The neck wounds were then reopened, blood flow (or lack of it) through the repaired vessels was examined; and vessels were ligated and, along with the vagus nerve, collected for histology. At this time, the pigs were injected with 10 mL of heparin (1000 U/mL, IV) and then euthanized by an injection of sodium pentobarbital (4.5 mg/kg, IV). The purpose of the heparin injection at this point was to prevent postmortem blood clotting in the organs and tissues. During necropsy, the entire lung was harvested, sliced in 1-cm to 1.5-cm thickness, and carefully examined for any residues and blood clots in the vessels. Four tissue

samples from the upper and the lower lobes of the right and left lungs were collected for histology. Brains were harvested from the skull in intact form and placed in fixative solution for 48 hours. After partial hardening (fixation), the organs were sliced and observed for the presence of blood clots or foreign materials. Tissue samples were collected from three regions of the brain for histologic analysis. The necropsy procedure, tissue sampling, and examination of histologic slides were done by a board-certified veterinarian pathologist (J.S.E.) who was initially blinded to the treatment of the samples.

The blood samples collected during the experiments (baseline and final) were analyzed for blood gases, complete blood count, and plasma coagulation tests (PT, aPTT, and fibrinogen). In addition, whole-blood coagulation assays in response to tissue factor were also performed by using thrombelastography (TEG). Briefly, the TEG machines (TEG Hemostasis Analyzer 5000; Hemoscope, Niles, IL) were calibrated before use and set at 37°C. Ten microliters of 1:200 diluted tissue factor (Innovin; Dade Behring, Marburg, Germany), 2 μ L of corn trypsin inhibitor (3.8 mg/mL; Hematologic Technologies, Essex, VT), and 340 μ L of fresh venous blood sample (drawn within 2 minutes) were placed sequentially in each TEG cup, and coagulation tracing was recorded. Each sample was tested in quadruplicate, and tracing continued until 30 minutes after the clot reached maximum strength. The TEG parameters measured include reaction time (R-time, the time that the initial fibrin formation is detected); clotting time (K-time, the time from the R-time until a firm clot is formed); angle (α , the kinetics of clot formation); maximum amplitude (the maximum strength or firmness of the developed clot); and clot lysis (LY 30) measured at 30 minutes after the clot reached maximum strength and calculated as the percentage reduction of the area under the TEG graphs.

Data Analysis

Data are expressed as the mean \pm SEM and analyzed by *t* test and analysis of variance for parametric data. The nonparametric data were analyzed by using the Newman-Keuls multiple comparison test, and the bigroup comparison was done by using Dunnett's test. A *p* < 0.05 was considered statistically significant.

RESULTS

The baseline hemodynamic and hematological parameters measured before vascular injuries were within normal ranges and not different among treatment groups (Table 1). These parameters were also measured at the conclusion of the experiments (Table 2). Although some measures such as core temperature and pH remained essentially unchanged, others were affected as a result of the injury and hemorrhage. Hemoglobin (22%), platelet count (22.5%), and fibrinogen (13%) were significantly reduced (*p* < 0.05), whereas the standard clotting times (PT and aPTT) were essentially unaffected. No differences were found in the final blood measurements among the treatment groups.

TEG assays of whole blood suggested development of a hypercoagulable state after injury and repair of the blood

TABLE 1. Baseline Physiological and Hematological Measurements of the Operated Pigs

Measurement	Kerlix (n = 8)	Combat Gauze (n = 8)	WoundStat (n = 8)	Overall <i>p</i>
Body weight (kg)	37.0 \pm 1.0	37.1 \pm 0.9	36.6 \pm 0.9	0.9
Temperature (°C)	37.6 \pm 0.2	37.5 \pm 0.1	37.7 \pm 0.2	0.6
Mean arterial pressure (mm Hg)	68.6 \pm 2.3	66.0 \pm 2.2	64.8 \pm 2.1	0.5
HGB (g/dL)	9.4 \pm 0.2	9.2 \pm 0.2	9.4 \pm 0.2	0.7
HCT (%)	28.1 \pm 0.7	27.4 \pm 0.6	28.4 \pm 0.5	0.6
PLT (1,000/ μ L)	373 \pm 35	391 \pm 39	334 \pm 26	0.5
PT (s)	10.9 \pm 0.2	10.9 \pm 0.1	11.1 \pm 0.1	0.4
aPTT (s)	15.8 \pm 0.2	15.4 \pm 0.1	16.2 \pm 0.3	0.5
Fibrinogen (mg/dL)	230 \pm 20	239 \pm 17	248 \pm 19	0.8
pH	7.4 \pm 0.01	7.4 \pm 0.0	7.4 \pm 0.01	0.4
Lactate (mM)	1.6 \pm 0.3	1.4 \pm 0.4	1.5 \pm 0.2	0.7
Base excess (mM)	6.2 \pm 0.5	6.9 \pm 0.6	6.3 \pm 0.6	0.6

Data are expressed as mean \pm SEM and analyzed by one-way ANOVA test. No significant difference was found among the groups.

HGB, hemoglobin; HCT, hematocrit; PLT, platelet.

TABLE 2. Final Hematological Measurements in the Operated Pigs

Measure	Kerlix (n = 8)	Combat Gauze (n = 8)	WoundStat (n = 8)	Overall <i>p</i>
Temp (°C)	38.3 \pm 0.1	38.3 \pm 0.1	38.5 \pm 0.2	0.3
Mean arterial pressure (mm Hg)	47.8 \pm 1.4	48.8 \pm 1.6	48.5 \pm 1.4	0.9
HGB (g/dL)	7.2 \pm 0.2	7.2 \pm 0.2	7.4 \pm 0.2	0.9
HCT (%)	21.3 \pm 0.6	21.7 \pm 0.7	22.0 \pm 0.8	0.8
PLT (1,000/ μ L)	287 \pm 28	298 \pm 23	266 \pm 25	0.7
PT (s)	11.3 \pm 0.2	11.2 \pm 0.2	11.4 \pm 0.3	0.8
aPTT (s)	16.7 \pm 0.5	16.1 \pm 0.5	16.6 \pm 0.4	0.7
Fibrinogen (mg/dL)	200 \pm 18	195 \pm 13	228 \pm 12	0.3
pH	7.4 \pm 0.01	7.4 \pm 0.01	7.4 \pm 0.01	0.7
Lactate (mM)	0.7 \pm 0.1	0.8 \pm 0.1	0.8 \pm 0.2	0.7
Base excess (mM)	8.6 \pm 0.5	8.9 \pm 0.7	8.6 \pm 0.6	0.9

Data are expressed as mean \pm SEM and analyzed by one-way analysis of variance test. No significant difference was found among groups.

HGB, hemoglobin; HCT, hematocrit; PLT, platelet.

vessels when compared with baselines (Fig. 1). On average, R-time and K-time were reduced by 36% and 42%, respectively; and α angle was increased by 24%. The final clot strength (maximum amplitude) and the percentage fibrinolysis for the first 30 minutes (LY 30), however, were unchanged. The increase in coagulation rate was similar among treatment groups (Table 3).

The average pretreatment blood loss, a measure of uniformity of injury and bleeding response, was \sim 9 mL/kg with no differences among the groups (Table 4). Posttreatment blood loss, however, was significantly less in animals treated with CG and WS (*p* < 0.001) than in controls (KX), with no difference between the two test agents (Table 4). The total compression time to achieve hemostasis with each

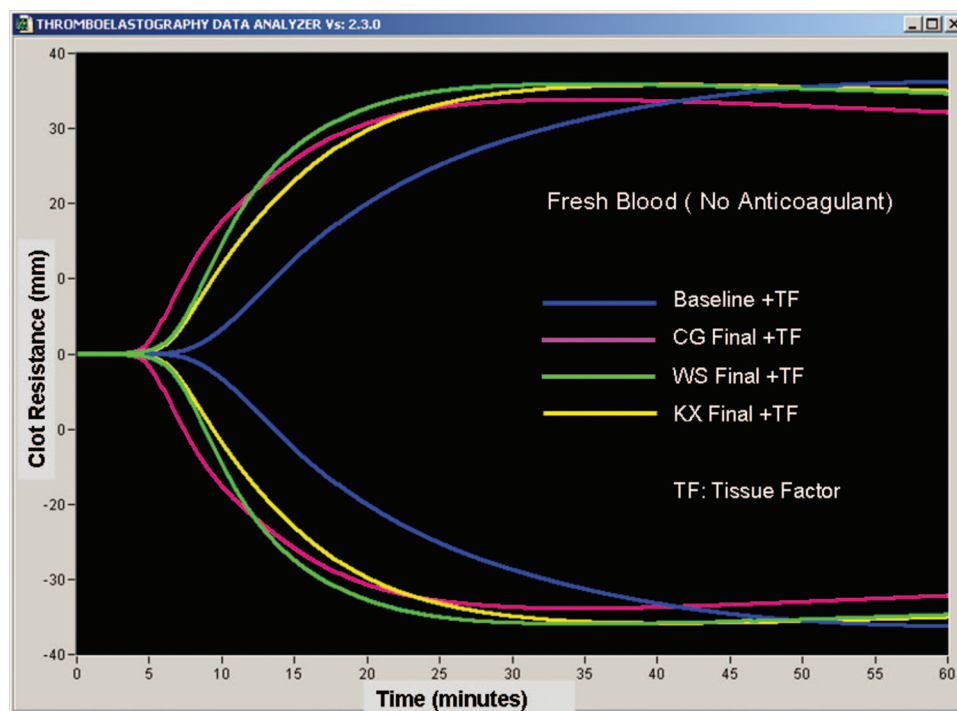


Figure 1. The average thrombograms of pigs' blood collected at the baseline and at the conclusion of the experiments (final). An increase in coagulation rate was measured in the final blood samples when compared with baselines in all groups.

TABLE 3. Thrombelastography (TEG) Analysis Blood Drawn From the Pigs Before Vascular Injury (Baseline) and 2 h After Repair and Reflow of the Vessels

TEG Parameter	Baseline	Kerlix	Combat Gauze	WoundStat	<i>p</i> (Among Groups)
R-time (min)	6.9 ± 0.3	4.7 ± 0.3*	4.0 ± 0.3*	4.7 ± 0.4*	0.5
K-time (min)	3.8 ± 0.3	2.3 ± 0.2*	2.0 ± 0.2*	2.3 ± 0.2*	0.5
Angle (°)	49.7 ± 1.6	61.0 ± 1.3*	64.2 ± 2.6*	60.1 ± 2.0*	0.4
MA (mm)	72.7 ± 0.5	70.3 ± 0.9	71.5 ± 1.2	72.4 ± 1.0	0.8
LY 30 (%)	0.8 ± 0.2	0.8 ± 0.3	1.0 ± 0.3	0.9 ± 0.3	0.7

Data are expressed as mean ± SEM and analyzed by *t* test (comparison with baseline) and one-way analysis of variance test.

* Values were significantly (*p* < 0.05) different from the respective baseline. Baseline values represent the average of three groups.

MA, maximum amplitude.

TABLE 4. Bleeding Outcomes Following Injury and Treatment With Different Agents

Outcomes	Kerlix	Combat Gauze	WoundStat	<i>p</i> (Among Groups)
Blood loss pretreatment (mL/kg)	9.3 ± 0.8	8.4 ± 0.7	9.6 ± 0.6	0.5
Blood loss posttreatment (mL/kg)	7.3 ± 0.7	3.9 ± 0.8*	2.9 ± 0.2*	<0.001
Compression time (min)	54.4 ± 12.6	13.9 ± 8.9†	9.1 ± 3.6†	<0.01

Data are expressed as mean ± SEM and analyzed by one-way analysis of variance (ANOVA) with posttest comparison of all pair groups using Newman-Keuls test.

* *p* < 0.01 vs. Kerlix controls.

† *p* < 0.05 vs. Kerlix controls.

No differences were found between CG and WS.

product was also shorter for the CG and WS groups than for the KX-treated animals (*p* < 0.01, Table 4).

The assessment of blood flow by CT images (Figs. 2 and 3) was confirmed by direct observation of the vessels when the wounds were reopened. The results showed that all the vessels treated with KX or CG were somewhat constricted but patent with no apparent difference between the two groups. No significant thrombus or blood clot was found in the lumen or on the suture line of these vessels after recovery (Fig. 4). In contrast, seven of eight carotid arteries treated with WS were occluded with thrombus and had no blood flow when examined at 2 hours postrepair. Similarly, six of the

eight jugular veins treated with WS developed large red clots with no blood flow through the vessels. A red thrombus layer covering the entire inner wall was also seen in a patent vein (Fig. 4, right lower panel).

Gross examination of the whole brain shortly after recovery and brain slices after fixation did not show any abnormal findings in all the pigs. Inspection of the lungs, however, revealed a blood clot (2–3 cm long and 2–3 mm thick) in a lower lobe of one lung and some residue similar to WS particles in the lung of another WS-treated pig. No gross abnormalities were detected in the lung of KX- or CG-treated animals.

The histologic changes of CG- and KX-treated vessels were equivalent in almost every way with minimal diffuse

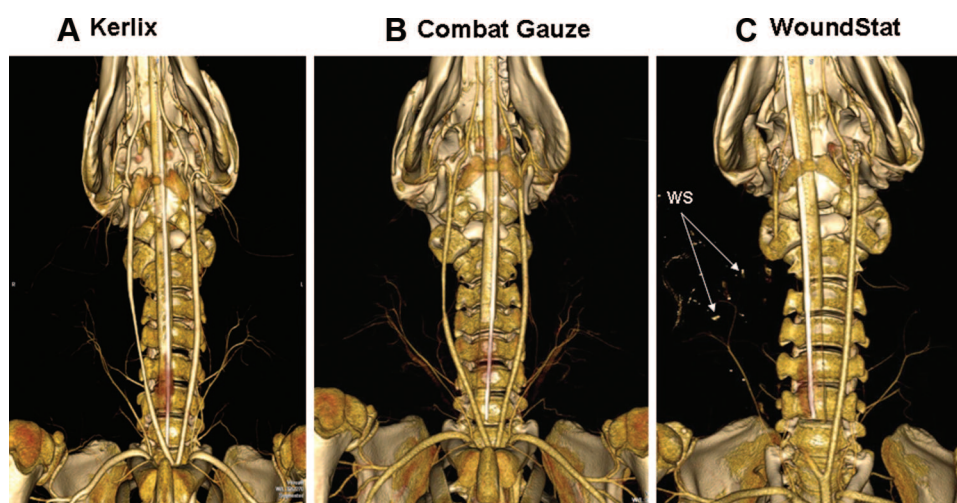


Figure 2. CT images of blood flow through the carotid arteries 2 hours after reflow. Note the narrow blood flow through the arteries treated with KX (A) or CG (B) and the lack of blood flow in a long segment of the right carotid artery treated with WS (C). Arrows show the WS residue in the wound.

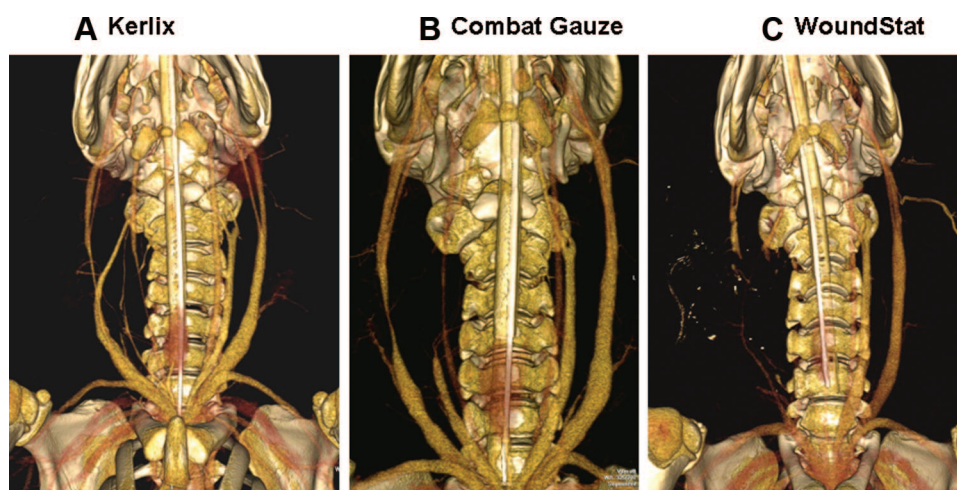


Figure 3. Typical CT images of blood flow through the external jugular veins 2 hours after reflow. Note the narrow blood flow (vasoconstriction) through the veins treated with KX or CG (A and B) and abolished flow in the vein treated with WS (C).

endothelial blebbing and no intraluminal thrombus. Pigs from the KX group had a high incidence of microthrombi in their lung (six of nine), and one animal had a small thrombus in a vessel of the brain. These microthrombi in capillaries are commonly formed in hemorrhage trauma models and considered clinically insignificant. The prevalence of microthrombi in this group may have been due to the larger blood loss and much longer compression time on the neck area proximal to lung to achieve hemostasis with regular gauze. In contrast, significant endothelial and transmural injury across the carotid arterial walls with large intraluminal thrombi were found in WS-treated vessels (Fig. 5). In the veins, WS caused significant delamination of the outer adventitia and necrosis of associated nuclei and inflammatory cells (Fig. 6). Within most of the luminal thrombi (eight of eight veins and six of eight arteries), gray granular materials were visible under polarizing light that was confirmed to be WS. The WS

residues were also found in several areas of the lung in one pig. A large piece of the residue was associated with an arterial thrombus in one lung (Fig. 7).

DISCUSSION

This study evaluated the short-term safety of using two new hemostatic agents to control external bleeding with major vascular injuries in pigs. The materials were applied to a neck wound with both arterial and venous injuries for 2 hours and subsequently removed. After surgical repair and 2 hours of blood reflow, the structure and function of the treated vessels were carefully examined. In addition, end organs distal to the vessels were inspected for evidence of embolization. The results showed essentially no difference in vascular function of the wounds that were treated with regular gauze (controls) or CG. The treatment of the wounds

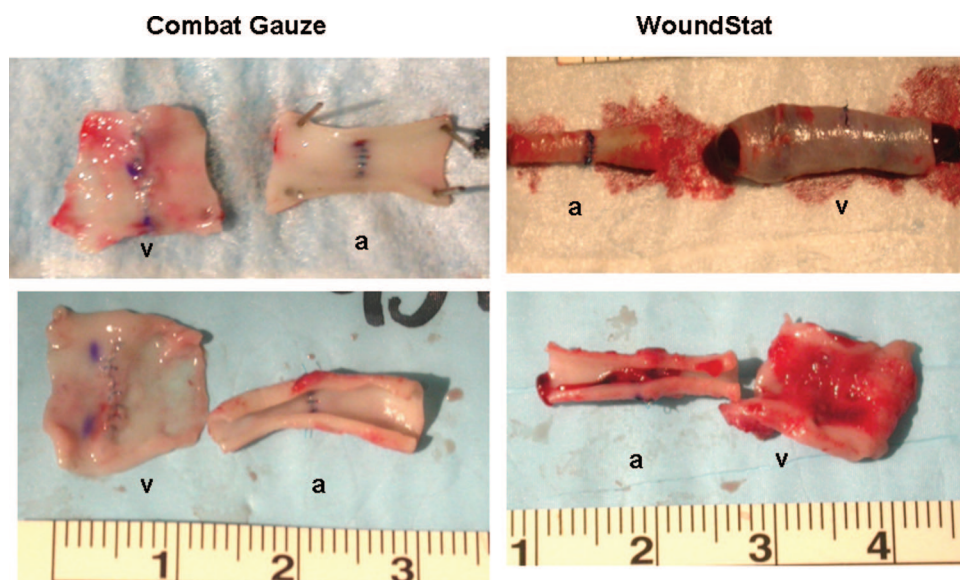


Figure 4. Views of representative CG- or WS-treated arteries (a) and veins (v) immediately after recovery from the pigs.

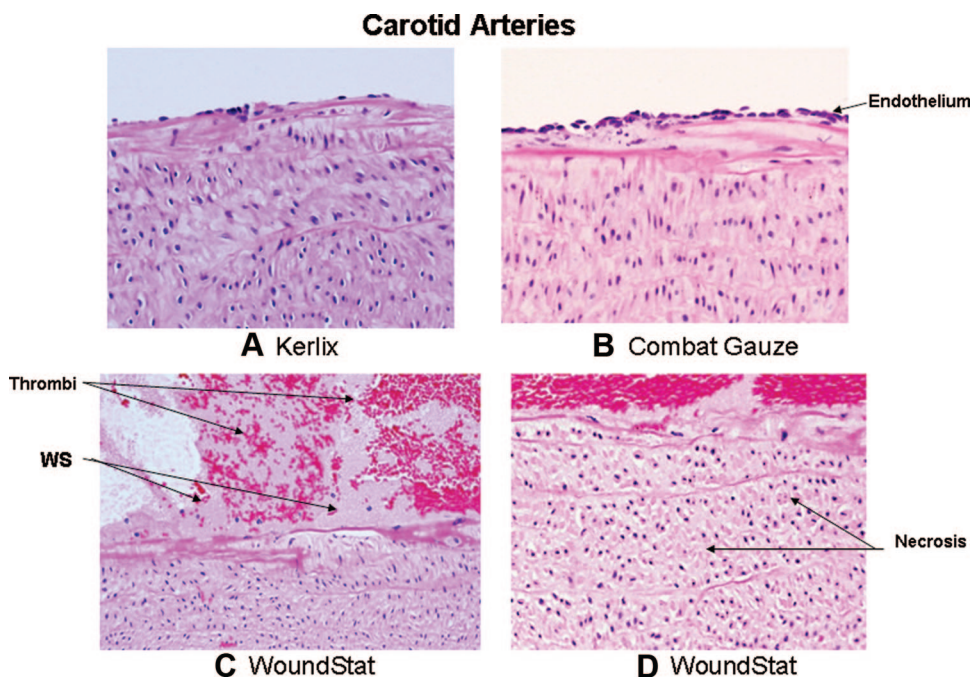


Figure 5. Composite micrographs of carotid arteries treated with KX (A), CG (B), or WS (C and D). Damaged endothelium, intraluminal blood clots, WS residue, and necrosis of smooth muscle cells are evident in the WS-treated vessels.

with WS, however, resulted in severe endothelial injury, significant transmural damage, massive thrombosis, and complete occlusion of the injured vessels following blood reflow. Despite our best effort for complete debridement, small microscopic residues of WS remained in the wound and in the lumen of vessels, providing additional sources for the development of thrombosis. Embolized WS particles associated with thrombi were also found in the lungs of two animals. Although the damaged blood vessels can be replaced with viable grafts, the likely chronic inflammation and potential

endothelial necrosis caused by embolized WS in distal organs could be a major problem.

WS is composed of smectite granules, an aluminum phyllosilicate mineral with high water absorbency, which concentrates the clotting factors in blood. The negative charges of the smectite granules also activate the clotting cascade and promote clot formation. The potent clotting activity of this agent was apparent when the WS-treated blood was analyzed by the TEG method.⁹ The main hemostatic mechanism of WS, however, appears to be due to

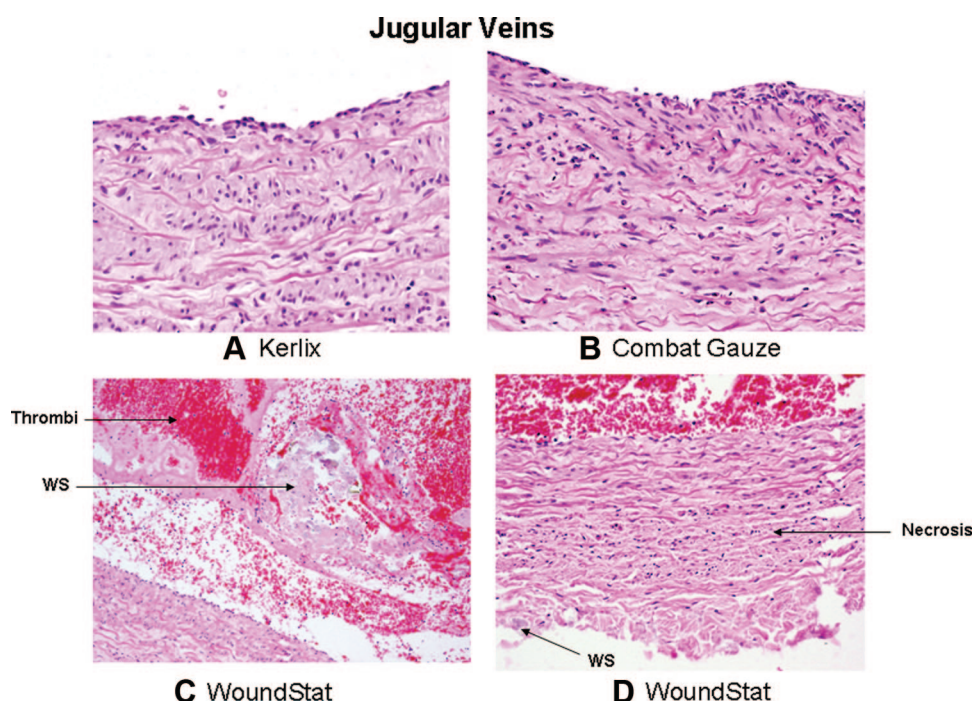


Figure 6. Composite micrographs of jugular veins treated with KX (A), CG (B), or WS (C and D). Large blood clots associated with WS residues are seen in the lumen of the vein (C). Necrosis of smooth muscle cells and delamination of outer adventitia are also apparent in the vessel (D).

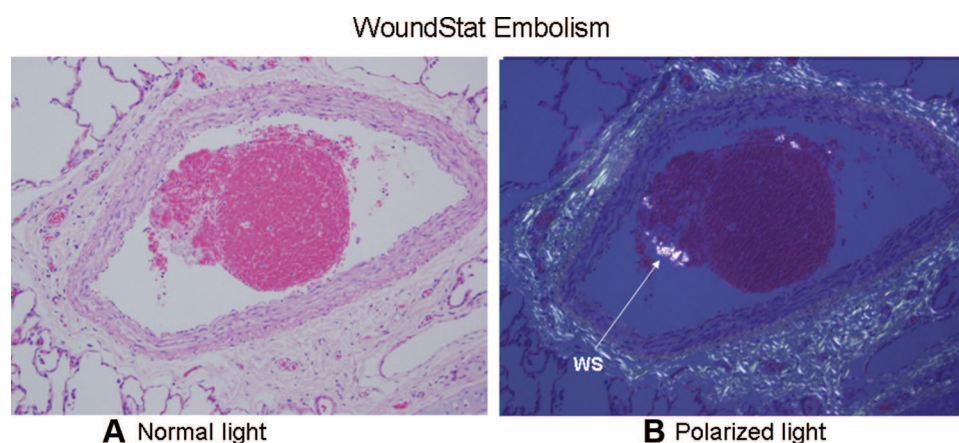


Figure 7. Embolized WS residues and associated arterial thrombosis in the lung. The hematoxylin and eosin stained tissue as seen under normal light (A) and under polarized light (B), which identifies the WS residue clearly.

the strong tissue-sealant properties of this mineral. Once the WS granules are mixed with blood, pliable clay is formed that on compression binds tightly to underlying bleeding tissues and provides immediate hemostasis in the wound.⁷

CG is a combination of special surgical gauze (50% Rayon and 50% polyester) with another aluminum phyllosilicate mineral, kaolin. Kaolin is a strong contact pathway activator agent initiating rapid clot formation in a wound. A substantial amount of kaolin powder (~10% of total weight) is incorporated into each CG; however, the product is indistinguishable from regular gauze because of the soft and fine

nature of the white kaolin powder. CG has all the advantages of normal gauze (flexibility, large coverage, ease of application, and ease of removal) plus increased hemostatic function. The strong clot formed within CG adheres the material to the bleeding site and stops the bleeding after some initial blood loss. The adherence of CG to the vessel injury was noted during debridement when the last layers of CG were removed from the wound.

In our previous studies examining the efficacy of WS and CG, we found microscopic traces of WS particles in the lumen of nearly all the treated arteries (9 of 10) despite adequate debridement and saline flushes of wounds before

sample collections. Traces of kaolin powder were also detected in one vessel specimen treated with CG. Similar to WS particles, kaolin fine residues can also be detected under a microscope with polarized light. Given that kaolin powder is not bound to the gauze, it was conceivable that when CG is placed in a wound filled with blood, kaolin could leach out and enter the systemic circulation and cause local or distal thrombosis. Therefore, this study examined these potential side effects of both products.

Both WS and CG have received FDA clearance (510[k] Premarket Notification) for marketing in the United States for temporary treatment of external wounds with moderate-to-severe bleeding. To obtain FDA clearance, WS was demonstrated to be essentially equivalent, if not superior, to QC, another previously approved hemostatic agent. The original QC was made of zeolite granules with high water absorbency which, by concentrating clotting factors in blood, promoted clot formation and produced hemostasis. The water absorption reaction, however, was exothermic, causing high temperature and occasionally significant thermal injuries that required skin grafting.⁴ Earlier animal studies clearly showed the thermal damage of QC on hepatocyte, nerve, muscle and vascular tissues.^{6,12} Full thickness burn as well as tissue necrosis and large abscesses were also found in the groin wounds of the pigs treated with QC 4 weeks after recovery.¹³ A few years after marketing, the QC manufacturing company (Z-Medica) recognized this serious side effect and modified the chemical structure of zeolite granules to minimize heat generation (cool formulation). In addition, Z-Medica has placed the new QC in small water-permeable bags to facilitate application and removal of granules and to eliminate the risk of emolization.

According to the manufacturer, WS was subjected to cytotoxicity (fibroblast cell culture), systemic toxicity, and intracutaneous irritation tests according to the International Organization for Standardization (ISO) guidelines and was found to be safe for intended use. However, none of these standard tests had exposed WS to endothelial cells or blood vessels to determine the true effect of WS on these targeted tissues. The risk of embolism also could not have been determined by these tests. Although the ISO tests may be sufficient for safety clearance of some products that are applied externally (over the skin or on a superficial wound), more specific safety tests should be required for approval of hemostatic agents that may be placed in deep penetrating wounds with potential internalization. Relevant information may be obtained by conducting an animal study with a typical wound that is treated with the test agent to assess the short- and long-term effects of the materials on the exposed tissues and the overall recovery and health status of treated subjects. It worth mentioning that WS, like other topical hemostatic products, has no package insert and all the information regarding indication, directions for use, and safety warnings are printed on the package itself. The intended use is "emergency use for external temporary traumatic wound treatment to achieve hemostasis in moderate-to-severe bleeding." The only safety warning printed on the package is "do not use in eyes or swallow."

The WS tissue damages, including endothelial degeneration and myofibril necrosis, may result from the toxicity of this mineral toward these sensitive cells. Murphy et al.¹⁴ have shown the significant cytotoxicity of aluminum phyllosilicate clay minerals, including montmorillonite (the main smectite mineral), bentonite (a smectite mineral), and kaolinite, on human umbilical vein endothelial cells. Incubation of 0.1 mg/mL of montmorillonite with human umbilical vein endothelial cells caused 100% cell lysis after 24 hours. The kaolinite also had a toxic effect on the cells but to a lesser extent. The lack of toxicity of kaolin in CG, as noted in this study, may be attributed to the fact that only a small amount of kaolin powder is incorporated into each roll of CG and that perhaps most of the mineral remains in the gauze when it is applied to the wound (indirect exposure). On the other hand, a large quantity of WS-smectite (150 g) is poured into the wound directly, exposing the tissues to the mineral at the highest concentration and maximizing the toxicity effect. In some primary neuronal cultures, addition of bentonite or montmorillonite at 0.1 mg/mL concentration caused complete cell lysis within 60 minutes.¹⁵ Interestingly, however, these minerals had no harmful effect on other cell lines such as oligodendroglia or neuroblastoma.¹⁴ This finding suggests that WS cytotoxicity may not have been detected when it was tested on the fibroblast cell culture as guided by ISO tests for device assessment.

Our initial study, which revealed the potential thrombogenicity of WS, was conducted in a porcine model with femoral artery injury and focused on determining the efficacy of the product.⁹ That model was not used for this study, in favor of developing a new model for testing the safety of the new hemostatic agents. The new model involved injuries to both an artery and a vein in the neck area. The expectation was that the risk of thromboembolism might be higher in the low-pressure venous circulation than in the arterial vessels and therefore injuries to both vessel types should be included. The model also allowed tracing and detecting the potential emboli in the distal end organs (lung and brain) of the treated vessels. Such follow-up would have been much more difficult and may be inconclusive if the extremity injury model was used. Heparin treatment was avoided during surgical repair and blood reflow, so that it would not mask thrombosis occurring in the damaged vessels. In addition, surgery either with a low dose of heparin or without heparin was consistent with standard vascular surgery practices in combat support hospitals.¹⁶

The main limitation of this study is that the model does not mimic a relevant battlefield wound. On the other hand, such wounds by their nature are so complex and heterogeneous that they do not allow performing a controlled study with reproducible and quantifiable outcomes. Nonetheless, the findings in this porcine model point out serious side effects of WS and should send strong warning to the care providers who may use this product as the last resort for control of severe hemorrhage.

In summary, the safety of two new and extremely effective hemostatic agents was tested in a surgical model that was able to reveal local tissue damage and embolization

to distal organs as a result of treatment with the agents. Although CG produced changes that were not different from regular gauze, WS treatment caused severe endothelial injury and significant transmural damage that rendered the vessels nonviable for primary surgical repair. The WS residues were also embolized in the venous circulation and were trapped in the lung with associated thrombosis. If used in a wound, it may be necessary to replace the injured vessels with interposition grafts to avoid thrombotic complications. The present findings also suggest that more stringent safety tests should be performed before approval of hemostatic products that are indicated for treating moderate-to-severe bleeding.

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DISCUSSION

Dr. Charles A. Adams (Providence, Rhode Island):

Thank you, Doctor Spain. I had the pleasure of just hosting a lunch symposium with Doctor Croce and he really is on his good behavior today. It was a pleasure.

Doctor Sise, members and guests of the association, Doctor Kheirabadi and his colleagues at the ISR, Fort Sam Houston, have continued their work evaluating topical adjuncts for the control of external hemorrhage.

They utilized a porcine model, as you can see, with combined venous and arterial injury which is an improvement on their previous models, I believe. And he explained the rationale behind that.

Following a brief period of uncontrolled hemorrhage they then tried these various products, specifically they used: Kaolin coated gauze and then they used the WoundStat which is the smectite containing product.

I was not familiar with smectite but it's actually an aluminum phyllosilicate and it absorbs like most of these adjuncts a lot of the plasma leaving the clotting factors in high concentration.

What they showed is that the QuickClot – correction, the WoundStat formulation resulted in hemostasis quite rapidly.

They monitored a whole host of parameters looking at blood loss, hematocrit, hemodynamics and they also used thrombo elastograms which he didn't show you in the presentation which was really nice in the manuscript.

After hemostasis was achieved they then underwent CT scanning with a CT angio which I thought was also impressive and they showed that there was a great deal of thrombosis.

And as you can see in his graphics they actually show that there was a significant amount of WoundStat retained in the wound. Following, these animals were sacrificed and tissues were taken for histology.

While WoundStat was the most effective at controlling hemorrhage it was also associated with the highest degree of thrombosis. And as he showed in his histologic data it really promoted a lot of intense degradation of the endothelium as well as localized damage inside the wound.

I have four questions. Actually I had a couple more but I'll trim it down in the interest of time. He mentioned that he performed bulb irrigation with one to two liters to try and remove that and that's a fairly relevant clinical situation.

But I was curious if you had tried any type of motorized mechanical pulse irrigation, sym pulse, lavage, such as that, because those are also clinically used quite frequently to, as an adjunct for debridement?

Although the model is consistent with a real-life clinical scenario, primary repair is probably not as common as interposition graphs with either venous interposition graphs or PTFE, especially when dealing with gunshot wounds where debridement of the artery is necessary otherwise there is a higher incidence of thrombosis.

I was curious, since the WoundStat remains in the wound have you done any of those venous interposition graphs? And if you have, what has been the effect on long-term vascular patency with that WoundStat still present

in the wound? If you haven't done those experiments, that might be something interesting to try next.

Next, WoundStat's major effect on hemostasis is the fact that this stuff really becomes a clay and your data was quite eloquent showing that it was very effective at obtaining hemostasis.

But since it's like a granule that needs to be poured in I was wondering if you have had any work or if you have considered trying to reformulate it with a non-adherent barrier, some type of interface, so that it didn't really incorporate in the wound; and that might be one of the ways to get rid of it so that it's not retained in the wound.

Again, there is also many products commercially available that have a similar process where there is a non-adherent barrier to keep it from adhering.

And, finally, as you pointed out, the data submitted to the FDA might be inadequate. Work by Murphy several years ago did indeed show that WoundStat was cytotoxic to endothelial cells and, as you pointed out, the FDA testing was done on fibroblast as well as oligodendro sites.

So in light of that fact and in light of your findings of your results, do you think that the FDA should impose a black-box warning talking about vascular repairs and anastomosis on this product?

Or to go to the even next step, do you think that this product should be recalled from the market if it has such dramatic negative effects on local vascular repairs?

In conclusion, I applaud the authors for their continued hard work on this clinically relevant topic.

And I'd like to thank the association for the privilege of reviewing this as well as for the privilege for myself to become educated on products that I frequently don't use since these, as I can see, are probably best used in the pre-hospital setting as well as in combat situations. Thank you.

Dr. Bijan S. Kheirabadi (Fort Sam Houston, Texas): Thank you, Doctor Adams, for insightful questions, comments. As far as whether we have used motorized pumps for debridement, Indeed we have done an earlier study in our institute comparing the two debridement methods.

Actually, we found that using a syringe to flush with the pulsatory fashion removed the material better and caused less tissue damage than using a high pressure motorized device.

As far as why did we use primary anastomosis and have we considered to do a interposition graft in this study, we have not done in these repair procedures. However, we have done additional experiments in which vessels were not injured but the wound were packed with WoundStat to see how the vessels may be affected.

These vessels did not develop occlusive thrombosis but the toxicity of WoundStat was still present on the outer (adventitia) and muscular layers of the vessels. Therefore, the damage is not limited to endothelial cells.

Even if the vessel was grafted, the area of the vessel that came in contact with the WoundStat would have developed some kind of tissue necrosis in the long-term.

Whether the product has been considered to have some kind of barrier between the mineral and the tissue, I think this approach is being persuaded by the company.

For instance, the QuickClot originally was marketed in granular form which produced a great deal of heat and tissue damage in wounds. Later on it was put in some kind of permeable bags that prevented its direct contact with the tissue and yet maintained hemostasis.

I think the same kind of modification is being considered by the company if they can maintain the same efficacies by putting the material in a small bag. And, finally, we have shared these information with FDA and they are aware these animal studies and the findings. We have also plan to meet with FDA officials to discuss these results and present our concerns. What would be the FDA final decision regarding the WoundStat approval is not known at this time.

The company is also aware of these findings and they are working their way of decreasing some of these side effects.

Again, thank the association for the privilege of the podium.