Long-term preclinical evaluation of the intracorporeal use of advanced local hemostatics in a damage-control swine model of grade IV liver injury

Kenji Inaba, MD, Bernardino C. Branco, MD, Peter Rhee, MD, Bradley Putty, MD, Obi Okoye, MD, Galinos Barmparas, MD, Peep Talving, MD, *and* **Demetrios Demetriades, MD, PhD,** *Los Angeles, California*

BACKGROUND: The purpose of this study was to evaluate the long-term efficacy and safety of kaolin- and chitosan-based hemostatic agents for hemorrhage control in a 14-day survival, damage-control swine model of Grade IV liver injury.

METHODS: A total of 48 anesthetized pigs (40 kg) underwent a 35% total blood volume bleed, cooling to 34°C and a standardized liver injury. The animals were randomized to standard gauze control (SG, n = 12), QuikClot Combat Gauze (QCCG, n = 12), Celox (CX, n = 12), or Celox Gauze (CXG, n = 12) packing. At 15 minutes, shed blood was calculated, followed by damage-control closure. At 48 hours, pack removal and definitive closure was performed. At 14-day sacrifice, the liver, kidney, heart, lung, and small bowel standard intra-abdominal organs were sampled for histopathological examination.

RESULTS: Uncontrolled blood loss at 2 minutes demonstrated internal consistency of the injury. Blood loss at 15 minutes was significantly lower in the CX and QCCG arms (SG, 11.1 ± 1.1 mL/kg; QCCG, 5.3 ± 1.2 mL/kg; CX, 5.7 ± 1.2 mL/kg; and CXG, 10.1 ± 1.3 mL/kg; $p = 0.002$). Forty-eight–hour survival was 50.0% for SG, 58.3% for QCCG, 83.3% for CX, and 41.7% for CXG ($p = 0.161$). Fourteen-day survival was 41.7% (5) for SG, 50.0% (6) for QCCG, 58.3% (7) for CX, and 41.7% (5) for CXG ($p = 0.821$). Four CX and two QCCG deaths were caused by bowel obstruction; one SG death was caused by sepsis; the remainder was caused by blood loss. Histopathology in one CX animal demonstrated eosinophilic material within a coronary vessel consistent with granule embolization.

CONCLUSION: Celox and QuikClot Combat Gauze were effective hemostatic adjuncts to standard intracavitary damage-control packing. The hemostasis was durable, facilitating pack removal, and definitive closure at reoperation. There was however an increase in the development of intra-abdominal adhesions resulting in small bowel obstruction. The potential for distant embolization of granular agents warrants further investigation. (*J Trauma Acute Care Surg*. 2013;74: 538–545. Copyright © 2013 by Lippincott Williams & Wilkins)

KEY WORDS: Topical; hemostatic; liver; damage control; long-term.

For injured patients who survive to receive medical care, the most common cause of preventable death is uncontrolled bleeding.[1–3] For these patients, the rapid identification and control of truncal internal hemorrhage are critical. Although a surge in contemporary research has emphasized the importance of normalizing the systemic coagulopathy associated with injury, local surgical control remains to be the priority. Unless this is achieved rapidly, ongoing blood loss will cause progressive derangement of the intrinsic coagulation system, increasing the need for nonnative blood component therapy and decreasing the ability to achieve this control.

The rapid evolution of advanced local hemostatic agents[4,5] has been driven largely by the practical needs of military combat casualty care providers. Several are now approved by the US Food and Drug Administration for external use and are available commercially in the civilian sector. Increased experience with many of these hemostatic agents is being gained for external hemorrhage control, both by prehospital care providers as well as by US troops on the ground in Afghanistan.[6]

As part of a series of studies investigating the extension of these advanced local hemostatic products into internal use, a 48-hour damage-control model of Grade IV liver injury was developed and used to test two of these products, Celox and QuikClot ACS+, against standard gauze.[7] Both agents were demonstrated to be effective adjuncts to standard damage-control packing, improving hemorrhage control. Celox was able to provide durable control, facilitating the process of packing removal at the time of take-back laparotomy. This mirrors the published off-label human experience with these hemostatics in cases of intractable traumatic internal bleeding refractory to traditional hemostatic measures where the use of these products has resulted in several uncontrolled case series of survivors.[6,8–10] When put into context with the available short-term preclinical data,[11–17] the results are promising. The long-term impact of these products, however, has not yet been scientifically validated.

Submitted: June 16, 2012, Revised: August 11, 2012, Accepted: August 14, 2012.
From the Division of Trauma and Surgical Critical Care (K.I., B.P., O.O., G.B., P.T., DD), University of Southern California, Los Angeles, California; and Division of Trauma, Surgical Critical Care and Emergency Surgery (P.R.), Department of Surgery (B.C.B.), University of Arizona, Tucson, Arizona.
This study was presented at the International Association for Trauma Surgery and Intensive Care Annual Meeting, August 28–September 1 2011, Yokohama, Japan.
Address for reprints: Kenji Inaba, MD, Division of Trauma and Surgical Critical Care, University of Southern California, 1200 North State St, Room C5L100, Los Angeles, CA 90033-4525; email: kinaba@surgery.usc.edu.

DOI: 10.1097/TA.0b013e31827d5f5f

The purpose of the current study was to evaluate the long-term safety and efficacy of these hemostatic agents for intracorporeal hemorrhage control by testing QuikClot Combat Gauze, Celox, and Celox Gauze against a standard gauze dressing to (1) compare blood loss, ability to perform packing removal at reoperation, and 14-day mortality; (2) identify any deleterious effects of the hemostatic agents on distant organs (heart, lungs, small bowel, and kidney); and (3) identify any negative impact of the hemostatic agents on tissues directly adjacent to the injury (noninjured liver tissue and small bowel). Our hypothesis was that these advanced local hemostatic agents will provide safe and durable control of local bleeding resulting in decreased blood loss without collateral tissue damage in a large animal damage-control model of high-grade liver injury.

MATERIALS AND METHODS

This is a randomized controlled preclinical trial conducted with the approval of the USC Institutional Animal Care and Use Committee. All animals were cared for according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. A total of 48 female Yorkshire-Hampshire swine weighing approximately 40 kg were purchased from IFPS Inc. (Norco, CA) and were housed in quarantine for 7 days before the experiment. Food was withheld the night before the procedure with free access to water.

The experimental protocol and animal preparation has been described in detail by Schnuriger et al.[18] Briefly, all animals were premedicated with an intramuscular injection of tiletamine/zolazepam (4 mg/kg each) and xylazine (300 mg), followed by 0.01 mg/kg of glycopyrrolate. A 20-gauge catheter was placed in the marginal ear vein for peripheral venous access. The animals were intubated using a 7 Fr endotracheal tube. General anesthesia was maintained using sevofluorane 1% to 5% in 100% oxygen (Drager Narkomed 4 anesthesia system, Drager Medical, Inc., Telford, PA). Continuous core body temperature monitoring was performed using an esophageal thermistor probe.

Through a neck dissection, the right carotid artery was cannulated with a 20-gauge angiocatheter, used for continuous invasive arterial blood pressure monitoring and blood draws. The right external jugular vein was cannulated with a 9 Fr introducer sheath for administration of resuscitative fluids and blood draws. All animals underwent a standardized 35% blood volume (25 mL/kg, based on 7% body weight blood volume) withdrawal through the external jugular vein sheath. Animals were resuscitated with lactated Ringer's solution (LRS) at room temperature to achieve a 3:1 fluid-to-blood withdrawal resuscitation.

Through a midline laparotomy, two frozen 500-mL LRS bags wrapped in sterile towels were placed into the abdomen away from the liver surface to achieve a core temperature of 34°C. A standardized Grade IV American Association for the Surgery of Trauma - Organ Injury Scale liver injury was created. Using a scalpel blade, a 3 × 10 cm, 0.5-cm-deep rectangular injury was created 3 cm from the medial edge of the left middle lobe. This segment was then avulsed off with a crushing clamp, disrupting the left middle hepatic vein.

Following 2 minutes of uncontrolled bleeding, free intraperitoneal blood was quantified using preweighed gauze to assess injury uniformity. Animals were then randomized to standard gauze control (SG), QuikClot Combat Gauze (QCCG), Celox (CX) and Celox Gauze (CXG) for hemorrhage control. Standard gauze or hemostatic was applied to the injury followed by damage-control liver packing with 30, 4 × 4-cm gauze pads. After 15 minutes, these packs were removed for weighing, and free intraperitoneal blood was quantified. Damage-control packing was repeated, and the abdomen was closed.

All animals were then resuscitated with warm LRS to maintain a minimum invasive mean arterial pressure (iMAP) of 60 mm Hg.

Forty-hours hours following initial damage-control operation, the animals were returned to the operating room. The packing and local hemostatic was removed, and the liver injury was assessed for bleeding and categorized as to whether the animal could undergo definitive closure or would need to be repacked for hemorrhage control. For those with ongoing bleeding, cautery and suture ligation were used to achieve hemostasis before definitive abdominal closure. Fourteen days following initial damage-control operation, survivors were sacrificed with an overdose of 120 mg/kg intravenously administered sodium pentobarbital. Samples of the liver and small bowel (for depth of necrosis) as well as the kidney, lung, small bowel and the left anterior descending coronary artery (for evidence of thrombi or emboli) were evaluated by an independent pathologist blinded to the treatment arm.

Hematocrit, platelet count, pH, lactate, base excess, and prothrombin time (PT) were performed at baseline, after blood withdrawal and induction of hypothermia, closure, 48 hours, and sacrifice by the USC Clinical Reference Laboratory. Thromboelastography (TEG) was performed with citrated blood at baseline, after blood withdrawal and induction of hypothermia, closure, and at 48 hours.

Primary outcome measures included shed blood at 15 minutes, need for repacking at the 48-hour second-look operation, and 14-day survival. Secondary outcomes included depth of liver and small bowel necrosis and evidence of distant emboli.

Standard statistical analysis was performed using the SPSS version 18 (SPSS Inc, Chicago, IL). Values are reported as mean ± SEM. Proportions were compared using the Fisher-Freeman-Halton's test, and means across the three different arms were compared with one-way analysis of variance (ANOVA). Bonferroni's correction was used for post hoc analysis.

RESULTS

Damage-Control Surgery

A total of 48 animals were randomized (12 in the SG, 12 in the QCCG, 12 in the CX, and 12 in the CXG group). Average weight was 42.0 ± 3.0 kg, with 1,067 ± 59.6 mL of blood withdrawn per animal. Hemodilution and cooling reduced iMAP (78.8 ± 12.9 mm Hg to 57.5 ± 11.9 mm Hg, $p < 0.001$), core body temperature (35.1°C ± 1.1°C to 32.9°C ± 0.5°C, $p < 0.001$), hematocrit (30.0% ± 2.3% to 22.8% ± 0.5%, $p < 0.001$), and platelet count (352.4 ± 39.7 × $10^3/\mu L$ to 215.3 ± 30.1 × $10^3/\mu L$, $p < 0.001$) (Table 1). No

TABLE 1. Hematocrit and Platelet Count at Different Phases of the Experiment, Stratified by Treatment Arm

	SG (n = 12)	QCCG (n = 12)	CX (n = 12)	CXG (n = 12)	*p*
Hematocrit, %					
Baseline	29.5 (2.4)	30.7 (2.2)	30.5 (2.1)	29.3 (2.3)	0.343
End of blood draw	22.5 (2.3)	23.2 (2.5)	22.4 (4.6)	22.6 (2.7)	0.922
Closure	20.4 (2.5)	21.2 (2.5)	21.2 (2.8)	21.1 (3.2)	0.886
Platelets, $\times 10^3/\mu L$					
Baseline	349.0 (43.7)	365.0 (41.0)	351.4 (41.9)	347.9 (41.2)	0.883
End of blood draw	258.8 (34.2)	291.8 (39.8)	263.1 (32.5)	251.2 (39.3)	0.521
Closure	159.1 (29.7)	231.9 (30.6)	249.2 (30.7)	188.5 (26.8)	0.179

p values were derived from one-way ANOVA. Values are reported as mean (SD).

change in PT (11.1 ± 0.8 to 11.7 ± 1.0, $p = 0.379$) or TEG was noted (Table 2). There was a minor decrease (Table 3) in arterial pH (7.478 ± 0.036 to 7.407 ± 0.046, $p = 0.043$), and increase in lactate (1.3 ± 0.7 mmol/L to 4.3 ± 1.3 mmol/L, $p < 0.001$). Changes in the iMAP during surgery were consistent across all the study arms and are shown in Figure 1. No significant differences in physiologic parameters, TEG, or laboratory values were noted between arms at baseline, after hemodilution, or at closure (Tables 1–3).

The average weight of the liver segment avulsed was 36.8 ± 8.0 g and did not differ between arms (SG, 38.4 ± 7.4 g; QCCG, 36.8 ± 7.8 g; CX, 33.3 ± 9.1 g; and CXG, 38.7 ± 7.6 g; $p = 0.336$). As a marker of the internal consistency of the injury, the blood loss at 2 minutes did not differ between arms (SG, 3.6 ± 1.8 mL/kg; QCCG, 3.4 ± 1.8 mL/kg; CX, 3.2 ± 1.5 mL/kg; and CXG, 4.4 ± 1.6 mL/kg; $p = 0.324$). Fifteen minutes after packing, blood loss was significantly higher in the SG and CXG arms, (SG, 11.1 ± 1.1 mL/kg; QCCG, 5.3 ± 1.2 mL/kg; CX, 5.7 ± 1.2 mL/kg; and CXG, 10.1 ± 1.3 mL/kg; $p = 0.002$; Fig. 2). Post hoc analysis did not show a significant difference between the QCCG and CX arms ($p = 1.000$).

Average lactate levels increased from 3.2 ± 0.7 mmol/L to 4.3 ± 1.0 mmol/L ($p < 0.001$). No significant physiologic or laboratory parameter differences were noted between arms at this phase of the experiment (Tables 1–3).

Second-Look Laparotomy

All animals survived initial damage-control packing. A total of 16 animals died before 24 hours (six in the SG, six in the CXG, three in the QCCG, and one in the CX group) all owing to uncontrolled intra-abdominal bleeding (Table 4). Four animals died between 24 hours and 48 hours, two in the QCCG (one of uncontrolled intra-abdominal bleeding and one

TABLE 2. Coagulation Parameters Stratified by Arm and Phase of the Experiment

	SG (n = 12)	QCCG (n = 12)	CX (n = 12)	CXG (n = 12)	*p*
PT (sec)					
Baseline	10.9 (1.0)	11.1 (0.8)	11.5 (0.7)	11.0 (0.6)	0.335
End of blood draw	11.4 (0.9)	11.7 (0.8)	11.8 (0.9)	11.3 (0.9)	0.362
Closure	11.5 (1.0)	11.9 (0.9)	11.8 (1.0)	11.7 (0.9)	0.418
TEG					
Split point, min					
Baseline	4.9 (2.7)	4.9 (1.8)	4.7 (2.2)	4.3 (2.2)	0.922
End of blood draw	5.8 (2.4)	4.4 (1.7)	6.1 (3.8)	4.9 (2.7)	0.512
Closure	4.6 (1.9)	3.9 (1.2)	4.8 (2.0)	4.6 (1.6)	0.674
r Time, min					
Baseline	6.0 (2.9)	6.7 (2.2)	6.3 (1.9)	5.8 (2.9)	0.887
End of blood draw	7.1 (2.9)	5.1 (1.8)	7.4 (4.6)	6.0 (2.3)	0.367
Closure	5.7 (2.3)	4.8 (0.9)	5.9 (2.1)	5.7 (1.8)	0.598
K, min					
Baseline	1.4 (0.6)	1.7 (0.5)	1.6 (0.5)	1.6 (0.8)	0.850
End of blood draw	2.1 (1.0)	1.5 (0.4)	2.6 (2.3)	1.7 (0.5)	0.242
Closure	2.0 (1.0)	1.5 (0.3)	2.3 (1.7)	1.7 (0.4)	0.324
MA, min					
Baseline	75.1 (3.8)	75.0 (3.3)	73.5 (5.1)	75.0 (4.4)	0.805
End of blood draw	70.8 (4.4)	69.6 (5.1)	68.4 (8.5)	71.5 (4.3)	0.656
Closure	66.7 (5.6)	69.0 (4.4)	65.9 (9.4)	68.4 (6.6)	0.725

p values were derived from one-way ANOVA. Values are reported as mean (SD).

TABLE 3. Arterial Blood Gas Values Stratified by Arm and Phase of the Experiment

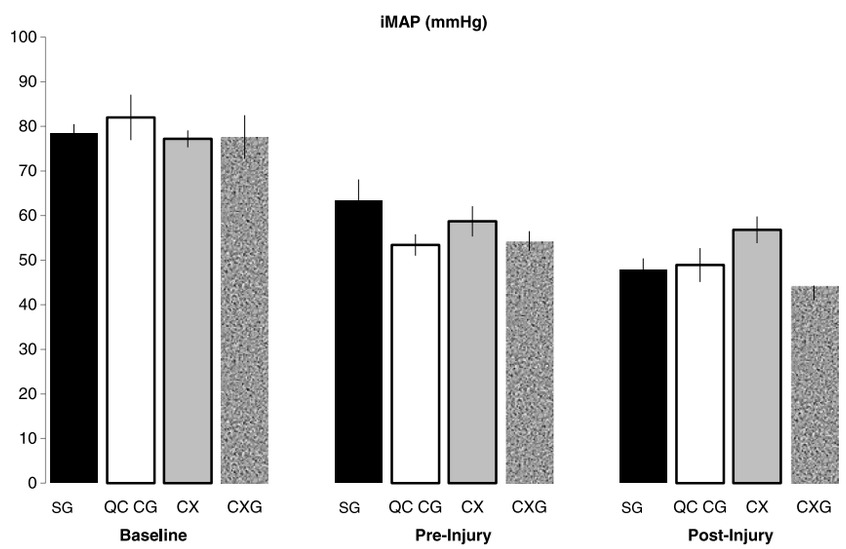
	SG (n = 12)	QCCG (n = 12)	CX (n = 12)	CXG (n = 12)	*p*
pH					
Baseline	7.479 (0.034)	7.477 (0.030)	7.478 (0.051)	7.476 (0.037)	0.998
End of blood draw	7.424 (0.058)	7.422 (0.043)	7.433 (0.043)	7.442 (0.049)	0.870
Closure	7.407 (0.060)	7.406 (0.041)	7.401 (0.049)	7.414 (0.042)	0.954
Lactate, mmol/L					
Baseline	1.5 (1.1)	1.2 (0.3)	1.5 (1.1)	1.3 (0.8)	0.868
End of blood draw	3.2 (0.8)	3.4 (0.3)	3.2 (0.8)	2.9 (0.6)	0.562
Closure	4.1 (1.2)	4.6 (1.6)	4.1 (1.2)	4.4 (1.0)	0.913
Base excess, mEq/L					
Baseline	10.0 (3.6)	11.4 (1.5)	10.1 (3.2)	9.8 (2.3)	0.540
End of blood draw	4.4 (2.1)	4.2 (1.5)	5.1 (2.6)	4.7 (2.0)	0.799
Closure	3.2 (2.7)	2.3 (1.4)	3.6 (2.6)	3.3 (1.8)	0.575

p values were derived from one-way ANOVA. Values are reported as mean (SD).

of small bowel obstruction [SBO]), one in the CX (SBO), and one in the CXG group (bleeding). At 48 hours following injury, 28 animals (10 in the CX, 7 in the QCCG, 6 in the SG, and 5 in the CXG group) were brought back to the operating room to evaluate liver hemostasis. Physiologic and laboratory parameters are depicted in Table 4. On average, 262.3 ± 242.4 mL of free blood was found inside the abdominal cavity. There was significantly less free blood in the CX and QCCG arms (SG, 550.2 ± 351.8 mL; QCCG, 180.4 ± 64.3 mL; CX, 150.5 ± 124.2 mL; and CXG, 260.8 ± 140.1 mL; $p = 0.003$). Post hoc analysis showed significant differences in the SG group versus QCCG ($p = 0.018$) and SG versus CX arms ($p = 0.003$). None of the other pairwise comparisons were significantly different. All SG and CXG animals started bleeding once packs were removed. Cauterization to achieve hemostasis was required for all SG and CXG animals. However, whereas all SG animals also required repacking for upward of 15 minutes and complex suture repair of the liver for hemostasis, only three CXG animals required suture repair or repacking ($p < 0.001$). The abdomen was eventually closed in all animals without packs left in place. During the next 12 days, a total of 5 animals died (three in the CX, one in the SG, and one in the QCCG group). Four were caused by SBO (three in the CX and one in the QCCG group), and one was caused by intra-abdominal sepsis (SG group).

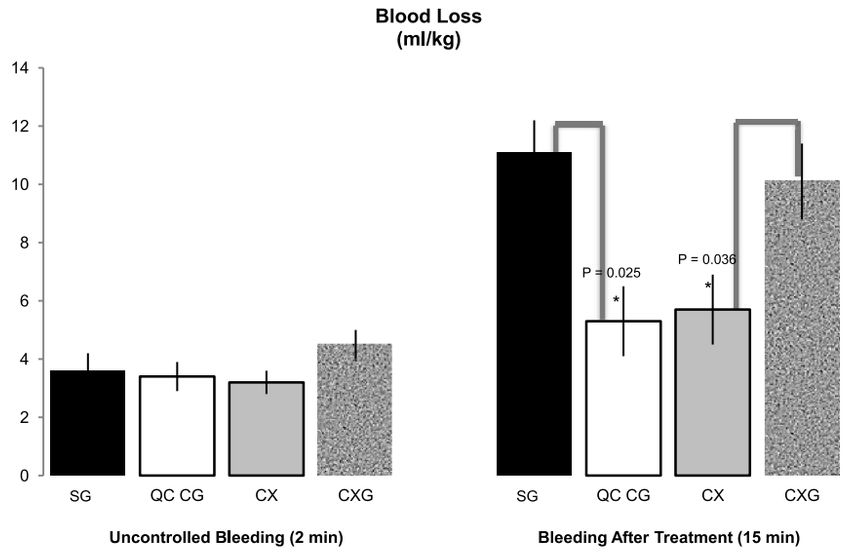
Long-Term Survival and Histopathologic Evaluation

Twenty-three animals survived to sacrifice (five in the SG, six in the QCCG, seven in the CX, and five in the CXG group). All CX and CXG animals had small bowel adhesions, whereas only two QCCG and one SG animal developed adhesions. All adhesions were thick and fibrous in nature. There was no difference in depth of necrosis of liver or small bowel between arms. Histopathologic examination in one CXG animal demonstrated impregnation of eosinophilic material into the



SG, Standard gauze ; QC CG, QuikClot® Combat Gauze; CX, Celox™; CXG, Celox™ Gauze.

Figure 1. iMAP at different time points of the experiment.



SG, Standard gauze; QC CG, QuikClot® Combat Gauze; CX, Celox™; CXG, Celox™ Gauze.
Group comparisons performed using ANOVA, Post-hoc pair-wise comparisons done using Bonferroni corrections. *statistically significant at p<0.05

Figure 2. Uncontrolled blood loss and blood loss at 15 minutes after treatment, stratified by arms of the experiment.

TABLE 4. Outcomes of the Experiment

	SG (n = 12)	QCCG (n = 12)	CX (n = 12)	CXG (n = 12)	*p*
24 h					
Mortality, % (n)	50.0 (6)	25.0 (3)	8.3 (1)	50.0 (6)	0.059
48 h					
Mortality, % (n)	50.0 (6)	41.7 (5)	16.7 (2)	58.3 (7)	0.161
Weight loss from initial operation, kg	2.6 (1.7)	1.7 (1.6)	1.4 (0.5)	2.0 (0.5)	0.520
MAP, mm Hg	58.9 (18.6)	67.7 (18.8)	61.6 (12.2)	45.2 (4.5)	0.153
Temperature, °C	38.5 (0.8)	38.4 (1.0)	38.6 (0.7)	38.6 (0.6)	0.974
AST	104.0 (29.1)	89.7 (45.1)	49.2 (20.0)	93.6 (45.7)	0.139
ALT	36.6 (11.0)	47.3 (22.3)	28.4 (13.4)	33.2 (8.0)	0.236
Free intra-abdominal blood (mL)	550.2 (351.8)	180.4 (64.3)	150.5 (124.2)	260.8 (140.1)	0.003
Cauterization of bleeding liver surface, % (n)	100.0 (6)	28.7 (2)	20.0 (2)	100.0 (5)	<0.001
Need for repacking, % (n)	100.0 (6)	0.0 (0)	0.0 (0)	60.0 (3)	<0.001
14 d					
Mortality, % (n)	58.3 (7)	50.0 (6)	41.7 (5)	58.3 (7)	0.821
Deaths caused by bleeding, % (n)	50.0 (6)	33.3 (4)	8.3 (1)	58.3 (7)	0.038*
Deaths caused by small bowel obstruction, % (n)	0.0 (0)	16.7 (2)	33.3 (4)	0.0 (0)	0.039*
Deaths caused by sepsis, % (n)	8.3 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.382
Weight gain from initial operation, kg	6.4 (1.6)	2.1 (2.9)	1.6 (4.5)	4.5 (4.3)	0.299
AST	51.3 (38.7)	75.4 (62.1)	48.3 (36.1)	167.5 (167.5)	0.456
ALT	29.3 (4.3)	38.2 (19.7)	36.8 (8.1)	38.3 (13.2)	0.573
Small bowel adhesions, % (n)*	20.0 (1)	33.3 (2)	100.0 (7)	100.0 (5)	0.004*
Depth of liver necrosis, cm*	0.6 (0.4)	0.3 (0.1)	0.4 (0.3)	0.4 (0.1)	0.887
Depth of small bowel, cm*	0.09 (0.03)	0.10 (0.05)	0.08 (0.03)	0.07 (0.04)	0.796
Deposit of agent in adjacent organs, % (n)	0.0 (0)	0.0 (0)	0.0 (0)	20.0 (1)	0.435
Emboli of agent to distant organs, % (n)	0.0 (0)	0.0 (0)	14.3 (1)	0.0 (0)	0.441

*Only animals that survived 14 days were evaluated for these outcomes.

p values for categorical variables were derived from the Fisher-Freeman-Halton's exact test; *p* values for continuous variables were derived from one-way ANOVA. Values are reported as mean (SD).

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

small bowel surface with surrounding fibrinosuppurative serositis (Table 4). In one CX animal, similar bright eosinophilic material was found in a coronary vessel. The surrounding myocardium demonstrated a pyogranulomatous inflammatory reaction. The coronary deposits could not be definitively confirmed to be CX; however, they were similar in appearance to the CX left on the surface of the injured liver. There were no clinical sequelae caused by either the foreign material or surrounding tissue inflammation.

DISCUSSION

During the initial resuscitation and treatment of critically injured patients, there are numerous competing goals that must be achieved to ensure optimal outcomes. Primary among these is the control of internal truncal hemorrhage. An inability to control bleeding will result in the most common cause of preventable death after injury. The surgical control of traumatic hemorrhage is often made difficult by the architecture of the damaged tissue, especially if not amenable to reapproximation with sutures or direct control with packing. This challenge is further increased if there is associated hypothermia, acidosis, or systemic coagulopathy, a problem seen in approximately a quarter of injured patients[19–21] on arrival.

Many advanced local hemostatic agents have been developed to address local hemorrhage control. Tested in preclinical models of external hemorrhage, these agents have been demonstrated to decrease blood loss, resulting in improved survival.[22–35] There are multiple external agents now commercially available and in use, both in the military as well as in civilian prehospital settings.[4,5] For internal use, however, only uncontrolled, off-label experiences for refractory bleeding have been reported,[6,8–10] and rigorous, preclinical studies assessing the safety and efficacy of these agents are required. The importance of this has been highlighted by recent work done by the group at the US Army Institute for Surgical Research,[35] which demonstrated that smectite granules from a product called WoundStat, can be embolized through the systemic circulation.

For this study, we used a previously developed model of high-grade liver injury,[18] designed to test the hemostatic efficacy of these agents under the same conditions they would be expected to perform in human patients. It uses packing and damage-control sequencing, a more clinically relevant test of hemostatic ability, with outcome measures that include blood loss and the need for repacking at the time of reoperation. Building upon a solid base of previous work demonstrating short-term (60–90 minutes)[12,13,15,16,36] hemostatic efficacy for internal use, we recently tested several agents in this damage-control model with 48-hour follow-up and reoperation.[7] Validating the previous studies, these agents, QuikClot ACS$^+$ and Celox, were able to improve hemostasis when compared with standard gauze packing. Celox in particular also demonstrated durable hemorrhage control, facilitating packing removal at the time of the take-back laparotomy.

In this current study, three agents were tested against a standard gauze dressing. QuikClot Combat Gauze is a 3-inch × 4-yard rolled gauze that has been impregnated with kaolin. Kaolin is an inert mineral that activates the clotting cascade via factor XII. Celox is a powdered formulation that is derived from chitosan, which is a nontoxic, biodegradable complex carbohydrate. Celox Gauze is a gauze base with Celox bonded to the surface. There are no intrinsic coagulation components integrated into these products, but they act to trigger the native coagulation system to promote clot formation.

Tested against standard gauze, as in our previous study, these agents outperformed the control. For acute hemorrhage control, the blood loss was halved for the QCCG- and CX-treated animals. Hemostasis occurred rapidly, and once the bleeding stopped, it remained stopped for the duration of the experimental run. The CXG however did not improve hemostasis when compared with standard gauze. This was caused in part by the architecture of the gauze itself, which was firm and did not conform to the irregular contours of the liver injury. Although not powered to delineate a survival impact, CX in particular improved early survival owing to hemorrhage. During the initial 48 hours of packing, the CX- and QCCG-treated animals also bled significantly less than the control as measured at the take-back operation. Finally, for CX and QCCG, there was a significant improvement in the durable hemostasis outcome measure. After acute hemorrhage control, this is the next most important outcome measure for a topical hemostatic agent. In clinical practice, once the initial damage-control procedure is completed, all efforts are targeted at normalizing any physiologic defects. This would then allow safe return to the operating room where pack removal, definitive repair, and abdominal closure can be achieved. Both CX and QCCG facilitated removal of the packing and hemostatic agent in all cases without the need for the repacking or complex suture repair that was required for the standard gauze control.

This long-term evaluation was designed to address two additional clinically relevant outcome measures not tested in any of the previous preclinical series. First, impact on adjacent organs was examined. There was no histologic difference between treatment groups in the depth of necrosis of the liver or small bowel in direct contact with the hemostatic agents. However, for both the CX and CXG arms, all animals had macroscopic evidence of adhesions. In the QCCG and standard gauze animals, this was rare. These adhesions directly impacted outcomes, with several deaths attributed to bowel obstruction. This was most pronounced in the animals treated with CX because the powdered agent became dispersed throughout the peritoneal cavity. The translation of these findings to human patients packed with these agents is unknown. Unlike animals that are up and walking soon after extubation, human patients undergoing damage-control packing are usually nursed in the supine position and remain so, until their take-back procedure. This would keep the packing intact, isolating the hemostatic agents to the area where they were placed. For granular agents, the second concern driving the long-term follow-up in this study was the risk of embolization. In the work done at the US Army Institute for Surgical Research using a 50% common carotid artery and external jugular injury in swine, smectite granules were seen to embolize from the area of injury to the lung.[35] In their study, QCCG was also studied, but no evidence of distal embolization was found. As suggested by the authors, this may have been caused by the small total load of kaolin and the strength of adherence to the gauze vehicle. In this current

study, likewise, although no distal emboli were found with the QCCG or CXG, for the powdered CX, in one animal, material consistent with the hemostatic agent was found in the coronary vessels. Although this finding was confined to only one animal, this may have been caused by sampling error. No thromboembolic complications or negative local clinical sequelae were noted; however, the potential for this could not be ruled out.

The model as designed for this analysis was able to test hemorrhage control, damage-control packing, definitive pack removal, and collateral organ damage. The injury demonstrated internal consistency, and the rate of blood loss at 2 minutes was highly reproducible. Achieving coagulopathy, however, as previously described,[37] is challenging in swine. The animals were hemodiluted, cooled to 34°C, and resuscitated with only crystalloid in a 3:1 ratio. Despite this, although a measurable decrease in hemoglobin mass and platelet count was noted, both the traditional measures of coagulopathy as well as the TEG results remained unchanged from baseline. As the induction of coagulopathy and acidemia is difficult in a swine model, although the comparative anatomy facilitates the creation of clinically relevant injuries and allows the testing of interventions, the direct applicability of these results to human patients is unknown. Despite these limitations, when compared with standard gauze, a significant improvement in both acute hemorrhage control and packing removal at reoperation was able to be demonstrated using this model. Perhaps more importantly, the propensity to form adhesions and the potential for collateral organ damage was also detected.

SUMMARY

In this model of high-grade liver injury, Celox and QuikClot Combat Gauze were effective adjuncts to standard intracavitary damage-control packing for achieving hemostasis. The hemostasis was durable, facilitating pack removal and definitive closure at the time of reoperation. There was however an increase in the development of intra-abdominal adhesions, resulting in small bowel obstruction. The potential for distant embolization of granular hemostatic agents highlights the need for further investigation.

AUTHORSHIP

K.I., B.C.B., P.R., B.P., O.O., and G.B. contributed in the study conception and design. K.I., B.C.B., O.O., and G.B. performed the acquisition of data. K.I., B.C.B., P.R., O.O., B.P., G.B., P.T., and D.D. performed the analysis and interpretation of data. K.I., B.C.B., O.O. drafter the article. P.R., B.P., P.T., and D.D. provided critical revisions.

ACKNOWLEDGMENTS

We thank P. Kirkman, Anesthesia Technician, E. Kirkman, DVM, and D. Casebolt, DVM. Without their support and technical assistance, this project would not have been possible.

DISCLOSURE

The authors declare no conflicts of interest.

REFERENCES

1. Sauaia A, Moore FA, Moore EE, et al. Epidemiology of trauma deaths: a reassessment. *J Trauma*. 1995;38:185–193.
2. Gruen RL, Jurkovich GJ, McIntyre LK, et al. Patterns of errors contributing to trauma mortality: lessons learned from 2,594 deaths. *Ann Surg*. 2006;244:371–380.
3. Teixeira PG, Inaba K, Hadjizacharia P, et al. Preventable or potentially preventable mortality at a mature trauma center. *J Trauma*. 2007;63:1338–1346; discussion 1346–1347.
4. Alam HB, Burris D, DaCorta JA, et al. Hemorrhage control in the battlefield: role of new hemostatic agents. *Mil Med*. 2005;170:63–69.
5. Pusateri AE, Holcomb JB, Kheirabadi BS, et al. Making sense of the preclinical literature on advanced hemostatic products. *J Trauma*. 2006;60:674–682.
6. Cox ED, Schreiber MA, McManus J, et al. New hemostatic agents in the combat setting. *Transfusion*. 2009;49(Suppl 5):248S–255S.
7. Inaba K, Rhee P, Teixeira PG, et al. Intracorporeal use of advanced local hemostatics in a damage control swine model of grade IV liver injury. *J Trauma*. 2011;71:1312–1318.
8. Wright FL, Hua HT, Velmahos G, et al. Intracorporeal use of the hemostatic agent QuickClot in a coagulopathic patient with combined thoracoabdominal penetrating trauma. *J Trauma*. 2004;56:205–208.
9. Rhee P, Brown C, Martin M, et al. QuikClot use in trauma for hemorrhage control: case series of 103 documented uses. *J Trauma*. 2008;64:1093–1099.
10. Shanmugam V, Robinson MH. Case report of uncontrollable pelvic bleeding—managed by a previously unreported method (QuikClot). *Colorectal Dis*. 2009;11:221–222.
11. Arnaud F, Tomori T, Carr W, et al. Exothermic reaction in zeolite hemostatic dressings: QuikClot ACS and ACS+. *Ann Biomed Eng*. 2008;36:1708–1713.
12. Pusateri AE, Delgado AV, Dick EJ Jr, et al. Application of a granular mineral-based hemostatic agent (QuikClot) to reduce blood loss after grade V liver injury in swine. *J Trauma*. 2004;57:555–562; discussion 562.
13. Pusateri AE, McCarthy SJ, Gregory KW, et al. Effect of a chitosan-based hemostatic dressing on blood loss and survival in a model of severe venous hemorrhage and hepatic injury in swine. *J Trauma*. 2003;54:177–182.
14. Bochicchio G, Kilbourne M, Kuehn R, et al. Use of a modified chitosan dressing in a hypothermic coagulopathic Grade V liver injury model. *Am J Surg*. 2009;198:617–622.
15. Bochicchio GV, Kilbourne MJ, Keledjian K, et al. Evaluation of a new hemostatic agent in a porcine Grade V liver injury model. *Am Surg*. 2010;76:317–320.
16. Millner R, Lockhart AS, Marr R. Chitosan arrests bleeding in major hepatic injuries with clotting dysfunction: an in vivo experimental study in a model of hepatic injury in the presence of moderate systemic heparinisation. *Ann R Coll Surg Engl*. 2010;92:559–561.
17. Wright JK, Kalns J, Wolf EA, et al. Thermal injury resulting from application of a granular mineral hemostatic agent. *J Trauma*. 2004;57:224–230.
18. Schnuriger B, Inaba K, Barmparas G, et al. A new survivable damage control model including hypothermia, hemodilution, and liver injury. *J Surg Res*. 2011;169:99–105.
19. Niles SE, McLaughlin DF, Perkins JG, et al. Increased mortality associated with the early coagulopathy of trauma in combat casualties. *J Trauma*. 2008;64:1459–1463; discussion 1463–1465.
20. Brohi K, Singh J, Heron M, et al. Acute traumatic coagulopathy. *J Trauma*. 2003;54:1127–1130.
21. MacLeod JB, Lynn M, McKenney MG, et al. Early coagulopathy predicts mortality in trauma. *J Trauma*. 2003;55:39–44.
22. Alam HB, Chen Z, Jaskille A, et al. Application of a zeolite hemostatic agent achieves 100% survival in a lethal model of complex groin injury in swine. *J Trauma*. 2004;56:974–983.
23. Acheson EM, Kheirabadi BS, Deguzman R, et al. Comparison of hemorrhage control agents applied to lethal extremity arterial hemorrhages in swine. *J Trauma*. 2005;59:865–874; discussion 874–875.
24. Ahuja N, Ostomel TA, Rhee P, et al. Testing of modified zeolite hemostatic dressings in a large animal model of lethal groin injury. *J Trauma*. 2006;61:1312–1320.

25. Ward KR, Tiba MH, Holbert WH, et al. Comparison of a new hemostatic agent to current combat hemostatic agents in a swine model of lethal extremity arterial hemorrhage. *J Trauma*. 2007;63:276–283; discussion 283–284.
26. Arnaud F, Tomori T, Saito R, et al. Comparative efficacy of granular and bagged formulations of the hemostatic agent QuikClot. *J Trauma*. 2007; 63:775–782.
27. Englehart MS, Cho SD, Tieu BH, et al. A novel highly porous silica and chitosan-based hemostatic dressing is superior to HemCon and gauze sponges. *J Trauma*. 2008;65:884–890; discussion 890–892.
28. Kheirabadi BS, Edens JW, Terrazas IB, et al. Comparison of new hemostatic granules/powders with currently deployed hemostatic products in a lethal model of extremity arterial hemorrhage in swine. *J Trauma*. 2009;66:316–326; discussion 327–328.
29. Sohn VY, Eckert MJ, Martin MJ, et al. Efficacy of three topical hemostatic agents applied by medics in a lethal groin injury model. *J Surg Res*. 2009;154:258–261.
30. Devlin JJ, Kircher S, Kozen BG, et al. Comparison of ChitoFlex(R), CELOX, and QuikClot(R) in Control of Hemorrhage. *J Emerg Med*. 2011;41:237–245.
31. Sambasivan CN, Cho SD, Zink KA, et al. A highly porous silica and chitosan-based hemostatic dressing is superior in controlling hemorrhage in a severe groin injury model in swine. *Am J Surg*. 2009;197:576–580; discussion 580.
32. Arnaud F, Parreno-Sadalan D, Tomori T, et al. Comparison of 10 hemostatic dressings in a groin transection model in swine. *J Trauma*. 2009;67:848–855.
33. Arnaud F, Teranishi K, Okada T, et al. Comparison of Combat Gauze and TraumaStat in two severe groin injury models. *J Surg Res*. 2011;169: 92–98.
34. Kheirabadi BS, Scherer MR, Estep JS, et al. Determination of efficacy of new hemostatic dressings in a model of extremity arterial hemorrhage in swine. *J Trauma*. 2009;67:450–459; discussion 459–460.
35. Kheirabadi BS, Mace JE, Terrazas IB, et al. Safety evaluation of new hemostatic agents, smectite granules, and kaolin-coated gauze in a vascular injury wound model in swine. *J Trauma*. 2010;68:269–278.
36. Kheirabadi BS, Acheson EM, Deguzman R, et al. Hemostatic efficacy of two advanced dressings in an aortic hemorrhage model in Swine. *J Trauma*. 2005;59:25–34; discussion 34–35.
37. Parr MJ, Bouillon B, Brohi K, et al. Traumatic coagulopathy: where are the good experimental models? *J Trauma*. 2008;65:766–771.

