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A pilot study of the use of kaolin-impregnated gauze (Combat Gauze) for packing high-grade hepatic injuries in a hypothermic coagulopathic swine model

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ABSTRACT

Background: Severe hepatic injuries may be highly lethal, and perihepatic packing remains the mainstay of treatment. This is not always successful, particularly in the setting of hypothermia and coagulopathy. Kaolin-impregnated Combat Gauze (CG) is an effective hemostatic dressing used primarily to treat external wounds. The objective of this study was to determine the ability of CG to control severe hemorrhage in hypothermic, coagulopathic swine with a high-grade hepatic injury.

Methods: Anesthetized animals underwent splenectomy and were cooled to 32°C while undergoing a 60% exchange transfusion with Hextend. A grade V liver injury was created in the left middle hepatic lobe. Animals were allowed to freely bleed for 30 s and then randomized to treatment with CG or plain gauze laparotomy pads (PG) applied to the injury site. Animals were then resuscitated with warmed Hextend.

Results: There was no difference between groups in preinjury hemodynamic or laboratory values. Animals packed with CG had less blood loss when compared with standard packing (CG = 25 mL/kg *versus* PG = 58 mL/kg, $P = 0.05$). There was a trend towards lower hetastarch resuscitation requirements in the CG group (CG = 7 mL/kg *versus* PG = 44 mL/kg, $P = 0.06$) but no statistically significant difference in mortality (CG = 13% *versus* PG = 50%, $P = 0.11$). Histology of the injury sites revealed more adherent clot in the CG group, but no inflammation, tissue necrosis, or residual material.

Conclusion: In pigs with severe hepatic injury, Combat Gauze reduced blood loss and resuscitation requirements when compared with plain laparotomy pads. Combat Gauze may be safe and effective for use on severe liver injuries.

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1. Introduction

Over the last decade, many new hemostatic agents designed to treat life-threatening extremity hemorrhage in the combat environment have been introduced. Most have demonstrated significantly improved control in models of severe arterial and venous hemorrhage [1–4]. Unfortunately, limitations due to the composition of these products (granular agents) and, in some cases, side effects (heat generation) have tempered surgeons' enthusiasm for these products.

Combat Gauze (CG; Z-Medica Corporation, Wallingford, CT) was first marketed in 2007 and has shown significant promise for controlling severe arterial hemorrhage, prompting the Department of Defense Committee on Tactical Combat Casualty Care to endorse the product for external use in Iraq and Afghanistan [5]. It consists of gauze impregnated with kaolin, a layered silica mineral well known for its ability to initiate the clotting cascade [6]. Although there have been few published data regarding Combat Gauze, several investigators have demonstrated its hemostatic efficacy in lethal models of extremity and visceral hemorrhage [3,4]. Anecdotal reports have suggested CG may be effective in the operating room setting where hypothermia and coagulopathy are common occurrences. We sought to determine if CG was effective in controlling hemorrhage in a swine model of severe liver hemorrhage that included hypothermia and dilutional coagulopathy, and whether such use would result in local tissue injury.

2. Materials and methods

This study was approved by the Animal Care and Use Committee at David Grant USAF Medical Center, Travis Air Force Base, California. All animals received care and were used in strict compliance with the Guide for the Care and Use of Laboratory Animals in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International [7].

2.1. Animal preparation

Intact female and castrated male Yorkshire cross-bred swine were obtained from the University of California Davis and acclimated for at least 10 d before use. Prior to surgery, each animal was fasted for 8–12 h but had unlimited access to water. Animals were premedicated with 4.4 mg/kg tiletamine/zolazepam (Telazol; Fort Dodge Animal Health, Fort Dodge, IA) intramuscularly and 0.1 mg/kg glycopyrrolate intramuscularly. Following isoflurane induction and endotracheal intubation, maintenance anesthesia consisted of 2% isoflurane in 100% oxygen. Animals were ventilated with tidal volumes of 7–10 mL/kg and a respiratory rate of 10–15 breaths/min sufficient to maintain end-tidal CO_2 between 35 and 45 mm Hg. Arterial blood pressure was monitored through a catheter placed in the carotid artery and fluids were administered through an internal jugular vein catheter, both placed through a longitudinal neck incision. Exsanguination was performed through a catheter placed in the median saphenous branch of a superficial femoral artery. Splenectomy was carried out through a midline laparotomy, the spleen weighed, and a volume of lactated Ringer's solution equal to three times the spleen weight was infused prior to obtaining baseline hemodynamic and laboratory data [8,9].

2.2. Hypothermia and dilutional coagulopathy protocol

Dilutional coagulopathy was induced through a 60% exchange transfusion using 6% hetastarch in lactated Ringer's solution (Hextend; Hospira, Lake Forest, IL) as described by Delgado and others [10–12]. Briefly, the total blood volume was calculated and 60% of this value was removed through the femoral artery catheter during three successive 10-min periods with a 3-min equilibration interval between each period. Simultaneously, an equal volume of room-temperature (25°C) Hextend was infused through the internal jugular vein catheter at the same rate as the blood removal. Hypothermia was achieved through a combination of the room-temperature Hextend infusion, external cooling, and intra-abdominal ice packs to achieve a preinjury core temperature of 32°C ± 0.5°C. The minimal blood loss that occurred during instrumentation and splenectomy was measured but was not considered part of the preinjury hemorrhage volume or part of the calculated Hextend replacement volume.

2.3. Liver injury

Through the midline laparotomy, the left medial lobe of the liver was identified and resected using a wire saw placed at the central posterior cleft and advanced anteriorly to sever the lobe. This resulted in a reproducible cut surface area incorporating a central hepatic vein and multiple portal venous and hepatic arterial branches. In our laboratory, this injury has a mortality of approximately 50% by 1 h when treated with standard gauze packing in the absence of hypothermia or coagulopathy [12]. The injuries were made by the primary investigator, who was masked to the treatment arm at the time of injury.

Following lobectomy, the cut surface was allowed to bleed freely for 30 s. Intraperitoneal blood was not suctioned so that packing would be applied through a pool of blood. After the 30-s period, the cut surface was packed using either CG or a single plain gauze laparotomy pad (PG) randomly assigned for each animal. Randomization was performed by an individual not directly involved in the procedure, with the primary surgeon masked to the treatment arm until the point of application. Application of CG and PG were immediately followed by the placement of two additional PG laparotomy pads and manual pressure for 3 min. After 3 min of manual pressure, between six and 10 additional PG were placed directly on top of the injury as well as above and below the injury in a manner consistent with perihepatic packing of human injuries (total of 9–13 pads). All materials placed in the abdomen were weighed prior to surgery. The abdomen was then closed using towel clamps and the animals were observed for up to 2 h or until they expired.

2.4. Resuscitation

Animals were resuscitated with warmed Hextend to achieve a target mean arterial pressure (target MAP) of 80% of the preinjury MAP [10]. After the 30 s bleeding period and for 1 h post injury, the animal received a Hextend bolus whenever the MAP was 10% below the target MAP and stopped when the target MAP was achieved. This continued until 60 min post-injury, at which point no further resuscitation fluid was given regardless of the MAP.

2.5. Data collection

In addition to observing MAP, core temperature, electrocardiogram, mean arterial pressure, and blood oxygenation were monitored throughout the experiment. Arterial blood samples were initially obtained for complete blood count, prothrombin time/International Normalized Ratio (PT/INR), fibrinogen, and arterial blood gas pre-injury, and PT/INR, fibrinogen, and arterial blood gas assays were repeated immediately after the liver injury and at 30-min intervals until the end of the experiment. Subsequently, each animal was humanely euthanized by intravenous injection of a lethal dose of pentobarbital (390 mg/mL and 1 mL/3 kg). All materials were weighed at the end of the experiment to determine the amount of blood lost into the pads. Pictures were taken of the injury and adjacent tissues, and the surface area of each liver injury was measured with image processing and analysis software (Image-Pro; MediaCybernetics, Bethesda, MD). Tissue samples from the injured liver and adjacent tissues were obtained and saved in 10% buffered formalin solution for later analysis by histologists masked to treatment group and survival duration.

2.6. Data analysis

Numerical data are presented as medians and interquartile ranges. Because this was a pilot study and sample sizes were small, all data were analyzed using nonparametric methods, including the Wilcoxon signed rank test for matched data and the Wilcoxon-Mann-Whitney 2-sample statistic [13]. Results were considered statistically significant when P was less than 0.05.

3. Results

Eight animals were randomly assigned to each treatment. There were no significant differences in preinjury hemodynamic and laboratory parameters between the CG and PG groups (Table 1). All animals achieved the target temperature range of 32°C ± 0.5°C prior to creation of the liver injury. Complete blood count and coagulation parameters for both groups were within reference ranges for our laboratory. The small amounts of preinjury blood loss were similar.

By 5 min post-injury, animals in both groups experienced a significant decrease in MAP when compared with baseline values. However, on average, animals treated with CG remained at or above their target MAP throughout the entire study period, whereas animals receiving PG were generally below their target MAP (Fig. 1). By 30 min post-injury, there was a demonstrable difference in the hematocrit between the CG and PG groups; however, this did not achieve statistical significance ($P = 0.06$). All parameters—MAP, hematocrit, PT/INR, and fibrinogen concentration—were notably lower than baseline values (Table 2), as would be expected following the injury and Hextend exchange transfusion.

Table 1 – Baseline physiology and hematology parameters by treatment group at baseline.

Value	Combat Gauze	Plain gauze	P value
Body weight (kg)	46 (9)	48 (13)	0.79
Sex (male:female)	5:3	5:3	1.00
Temperature (°C)	32.1 (0.4)	31.8 (0.7)	0.06
MAP (mm Hg)	77 (13)	76 (14)	0.49
WBC (1000/μL)	12.2 (4.2)	11.0 (3.6)	0.40
RBC (10^6/μL)	5.9 (0.9)	5.5 (0.8)	0.83
HCT (%)	26 (4)	26 (2)	0.67
PLT (1000/μL)	234 (73)	199 (193)	0.82
PT (s)	10 (1)	11 (1)	0.11
INR	1 (0)	1 (1)	0.12
Fibrinogen (mg/dL)	357 (83)	376 (103)	0.48
Blood loss (mL/kg)	3.5 (3.9)	2.1 (2.7)	0.34

HCT = hematocrit; INR = International Normalized Ratio; MAP = mean arterial pressure; PLT = platelets; PT = prothrombin time; RBC = red blood count; WBC = white blood count.
Both groups $n = 8$, median (interquartile range), except for sex.

The liver injury was reproduced in all animals, as demonstrated by a similar cut surface area in the CG and PG groups (Table 3). Survival for the CG group (88%) exceeded the PG group (50%), although the results were not statistically significant ($P = 0.11$). Animals treated with CG maintained a higher MAP following injury (55 *versus* 50 mm Hg, $P = 0.27$) and required less Hextend per unit of body weight to maintain their target MAP than did those treated with PG (7 *versus* 44 mL/kg, $P = 0.06$). Most notably, animals in the CG group lost considerably less blood than those in the PG group (25 *versus* 58 mL/kg, $P = 0.05$).

Multiple liver sections from each animal were evaluated in a masked fashion by two independent histologists, who each

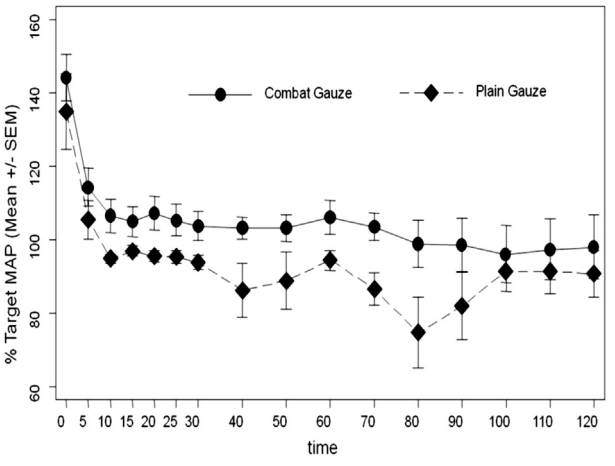

Fig. 1 – Percentage of target MAP over time for CG and PG groups.

Table 2 – Physiology and hematology parameters by treatment group 30 min following injury.

Value	Combat Gauze	Plain gauze	P value
Temperature (°C)	32 (1)*	32 (1)	0.34
MAP (mm Hg)	55 (13)*	50 (11)*	0.27
HCT (%)	15 (4)*	12 (4)*	0.06
PT (s)	12 (4)*	13 (2)*	0.90
INR	1 (0.1)	1 (0.1)	0.26
Fibrinogen (mg/dL)	174 (44)*	156 (50)*	0.27

HCT = hematocrit; INR = International Normalized Ratio; MAP = mean arterial pressure; PT = prothrombin time.
Both groups $n = 8$, median (interquartile range).
* Significantly different from baseline value in Table 1.

graded the entire wound surface of the specimen on surface clot formation, subsurface congestion in adjacent liver sinusoids, and evidence of hepatocyte necrosis. There was good agreement between observers (data not shown). Qualitatively, liver sections exposed to CG were more likely to contain surface clot than those exposed to PG, although congestion in adjacent sinusoids was similar in both groups. Neither hepatocyte necrosis nor increased inflammatory response was observed in sections exposed to either product, and there was no evidence of a residual foreign matter in sections exposed to CG by either visible or polarized light (Fig. 2).

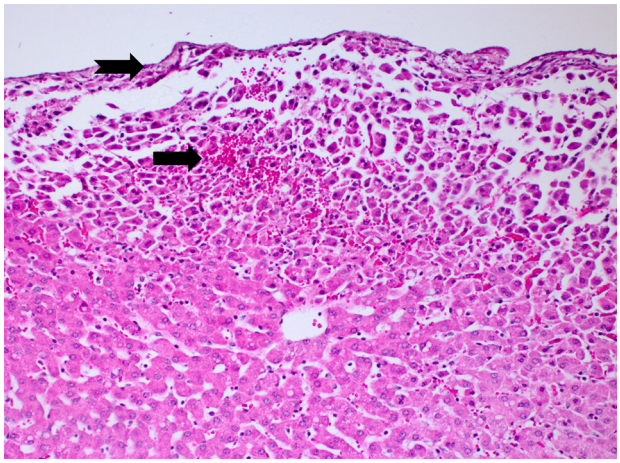

Fig. 2 – Photomicrograph of a representative liver section treated with CG. Note fibrinous clot formation on cut surface (*notched arrow*) and subsurface hemorrhage (*solid arrow*). (Color version of figure is available online.)

4. Discussion

In this pilot study, we evaluated the use of Combat Gauze for the management of a severe liver injury. Despite the small sample size, we were able to demonstrate a trend for lower blood loss and lower resuscitation volumes to maintain a predetermined mean arterial pressure after liver packing was initiated.

Uncontrolled hemorrhage is the leading cause of death following trauma. Although tourniquets have been a major improvement in the treatment of severe extremity injuries, injuries not amenable to tourniquets, including proximal limb and truncal injuries, continue to present a significant problem for medics and surgeons. Newer topical hemostatic agents have been developed to treat these injuries, and there is some evidence that these products have saved lives in combat [14]. At this time, the products are labeled for external use only, presumably due to difficulty handling the material and the potential for tissue injury. Although available data for CG do not suggest obvious concern for internal use, other agents may produce thermal injury (granular Quik Clot) and have the potential for local thrombosis and particle embolization (WoundStat) [15,16].

Kaolin-impregnated gauze (Combat Gauze) is a topical hemostatic agent that functions through direct activation of the intrinsic and common pathways [3,4,6,17]. The kaolin is impregnated into standard gauze and is therefore easy to apply and remove. Several investigators have presented data supporting the use of this agent in extremity and visceral injuries [4,18,19]. To date, Combat Gauze has not been evaluated for the treatment of visceral injuries in the setting of hypothermia and coagulopathy. Despite aggressive warming and early plasma transfusion, these conditions may still occur with severe hemorrhage from hepatic and other visceral injuries. Our results suggest that Combat Gauze may be effective under these conditions, as we observed a trend towards reduced blood loss, lower resuscitation requirements, and less mortality in this highly lethal swine liver injury model.

In this study, we made no effort to suction blood away from the cut surface prior to applying CG in order to determine if this agent remained effective when applied through a pool of blood. This characteristic is particularly important given that severe hepatic injuries often result in rapid hemorrhage that is difficult to evacuate prior to packing. In addition, injuries to areas such as the dome (Couinaud segments 7 and 8) or caudate (segment 1) are not easily visualized. In these cases, manual packing is generally done prior to complete evacuation of the blood. This is not unique to liver injuries, and Combat Gauze has the potential to significantly reduce blood loss from other visceral and possibly truncal vascular injuries.

The most obvious limitation to our study is the sample size. Preliminary work in our laboratory suggested that the injury, combined with hypothermia and coagulopathy, would produce a much higher mortality in the PG group.

Table 3 – Outcome measures.

Value	Treatment	Control	P value
Injury area (cm^2)	37 (6)	36 (8)	0.46
Hextend administered (mL/kg)	7 (29)	44 (45)	0.06
Blood loss (mL/kg)	25 (25)	58 (56)	0.05
Survival to 120 min (yes:no)	7:1	4:4	0.11

Both groups $n = 8$, median (interquartile range), except for survival.

Unfortunately, we were not able to reproduce the results of others with respect to coagulopathy, and this likely contributed to the higher-than-anticipated survival in the PG group. Although the reasons for this are not entirely clear, differences in premorbid variables such as nutritional status or sex makeup of the experimental cohort could potentially influence their response to an induced coagulopathy. For example, the hematocrit and postdilutional fibrinogen levels in our study showed a 40%–50% decline from baseline, suggesting that the dilutional effect of Hextend was effective. Unfortunately, the postdilutional fibrinogen remained well within normal range in both groups (median values: CG 174 mg/dL and PG 156 mg/dL, Table 2), which translated into a modest 20% increase in the prothrombin time (Tables 1 and 2). This is notably different from the results published by Delgado *et al.*, who reported a decline from approximately 160 mg/dL at baseline to approximately 97 mg/dL post-hemodilution, which translated to a nearly 50% increase in the prothrombin time [10].

It remains to be demonstrated that Combat Gauze is free of long-term side effects, as has been found with some granular hemostatic products [15,16]. Although Combat Gauze is not itself a granular agent, it is possible that residual kaolin on the liver surface could initiate a local foreign body or inflammatory response, placing the patient at risk for postoperative complications. Both gross and microscopic tissue evaluation in our study found no evidence of residual kaolin on the liver surface. Ultimately, survival studies will need to address this concern.

High-grade liver injuries can present a significant operative challenge to experienced trauma surgeons, and surgical packing is the mainstay of therapy. Adjuncts such as angioembolization are effective, but they require patient transport and result in a significant time delay at most institutions. In addition, this procedure is not universally available and addresses only one component of hepatic bleeding. Other, currently available topical hemostatic agents such as thrombin, topical collagen (Avitene), and cellulose (Surgicel) are frequently employed, but data supporting the efficacy of these products are lacking. In addition, some of these are expensive and require preparation, making them less than ideal for frequent or routine use. Newer, biologically active dressings are being studied and include the Fibrin patch and Fibrin Adhesive STat (FAST) dressing (dried fibrinogen, thrombin, Factor XIII) [10,19,20]. Their theoretical advantage over mineral-based hemostatic dressings, particularly in systemic coagulopathy, is obvious and has been demonstrated experimentally. Although potentially effective, they are not currently FDA approved and are likely to have significant cost constraints that may limit availability or application. CG is comparatively inexpensive (approximately $30–$40 for a 3 inch × 4 yard roll) and can be applied in an identical fashion to traditional packing.

In this model of severe liver injury, intracorporeal packing with Combat Gauze reduced blood loss and resuscitation requirements. Although mortality was lower, the study size was relatively small and there was no significant difference mortality. Further studies are necessary to ultimately prove efficacy. Combat Gauze has the potential to be a useful adjunct for treatment of severe liver injuries.

Disclaimer

The animals involved in this study were procured, maintained, and used in accordance with the Laboratory Animal Welfare Act of 1966, as amended, and NIH 80-23, Guide for the Care and Use of Laboratory Animals, National Research Council. The views expressed in this material are those of the authors and do not reflect the official policy or position of the U.S. Government, the Department of Defense, or the Department of the Air Force. The work reported herein was performed under United States Air Force Surgeon General–approved Clinical Investigation No. FDG20090045A.

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