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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

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ABSTRACT

INTRODUCTION The purpose of this study was to explore the effectiveness of two chitosan formulations, Omni-Stat® granules and Celox Gauze®, in a model of major hepatic injury in the presence of clotting dysfunction.

MATERIALS AND METHODS Major hepatic injuries in moderately heparinised swine were treated with either Omni-Stat® granules or Celox Gauze® as compared to control plain gauze.

RESULTS Plain gauze control failed to stop the bleeding in 13 of 14 attempts. Omni-Stat® arrested the bleeding in 18 of 18 attempts, providing it was in contact with the bleeding surface. Celox Gauze® arrested bleeding in 5 out of 6 attempts initially, and with further pressure in the sixth.

CONCLUSIONS The results support the evidence that chitosan-derived products act independently of classical clotting pathways and should be effective in patients who suffer major liver injury even in the presence of clotting dysfunctions.

KEYWORDS
Animal model – Blood – Coagulation – Experimental surgery

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The recent conflicts in Iraq and Afghanistan, following on from critical analysis of causes of death in previous conflicts, have resulted in a considerable research effort to establish agents that are haemostatic in the presence of major vascular injuries.[1,2]

A number of different agents have been studied and/or deployed to the conflict zones. Amongst these agents are a number of chitosan-derived products where the reports of their use have been highly encouraging.[3,4]

Chitosans have wide-spread applications, have been widely studied in the biomedical field, and are highly biocompatible.[5] Their chemistry has been previously described.[6,7] The haemostatic activity of chitosan (the term used to describe a series of polymers derived from crustacean chitin with different degrees of de-acetylation) appears to be by direct electrostatic interaction between negatively charged cell membranes of the erythrocytes and positively charged chitosan, independent of classical coagulation pathways.[8]

The deployment of chitosan-based products in the military setting is supported by *in vivo* experimental work showing 100% effectiveness in an industry standard model of lethal groin haemorrhage in swine,[9] and improved outcome in a model of hepatic injury in swine.[10]

We have previously shown that Omni-Stat® (MedTrade Products Ltd, Electra House, Electra Way, Crewe Business Park, Crewe CW1 6GL, UK), a proprietary preparation of chitosan granules, has the ability to provide reliable haemostasis in a moderately heparinised modification of an industry standard model of femoral artery haemorrhage (manuscript submitted). We are also aware of anecdotal reports of successful haemostasis using chitosan granules and gauzes in the management of hepatic injuries in the current conflict in Afghanistan. Many patients with hepatic injuries can have an acquired coagulopathy and clearly there would be benefit in a product that reliably induces clot formation outside the normal clotting mechanisms in patients such as these. Therefore, we set out to explore whether Omni-Stat® would retain its effectiveness in a model of hepatic injury in the presence of clotting dysfunction. We also wished to explore the effectiveness of a single layer of Celox Gauze® in the management of liver injuries. Celox Gauze® (MedTrade Products Ltd) is a proprietary preparation of chitosan

granules bonded to synthetic gauze and is CE marked for temporary external use as a haemostatic agent.

Materials and Methods

All animals were treated in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH publication No. 86-23, revised 1996), and with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS no. 123). The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the host facility.

Landrace swine (*Sus scrofa domestica*) of either gender and weighing around 40 kg were premedicated with intramuscular atropine (2 mg). Anaesthesia was induced with intravenous Tiletamine and Zolazepam (Telazol®, Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA) reconstituted with 5 ml of Xylazine (0.1 mg/kg). Lidocaine (1 ml) was applied topically and the animals were intubated with size 7.0 cuffed endotracheal tubes. Anaesthesia was maintained with oxygen and 2% isoflurane through a re-breathing circuit. Maintenance fluids comprised normal saline at a rate of 100 ml/h.

Monitoring included an oesophageal stethoscope for temperature, heart rate and ECG, automated blood pressure reading by cuff and oxygen saturation by pulse oximetry. Information was displayed on 'VetSpecs' VSM7 monitors (VetSpecs, Inc. 111 Mountain Brook Drive, Suite 200, Canton, GA 30115, USA).

To simulate the effects of a clotting diathesis, heparin at a mean dose of 1.75 mg/kg was given intravenously prior to the start of surgery.

Surgical technique

Animals were positioned supine and the abdomen opened through a midline laparotomy. The liver was mobilised into the operative field by lifting up the left lateral and medial lobes and placing a sling around them to ease handling. The first injury created was to amputate the free edge of the left lateral lobe, approximately 2 cm from its tip over a length of about 8 cm. This produces a raw, freely bleeding area of approximately 8 cm by 1.5 cm. This section of the liver is held between index finger and thumb of the left hand and either a plain gauze compress or a treatment is applied.

In the control group, a plain gauze compress was applied to the raw surface and held in place with the right hand. Firm pressure was applied for a timed 5 min. The pressure was then released and the liver edge inspected for signs of on-going bleeding; if further bleeding occurred, a supplementary 2 min of pressure was applied followed by a further inspection. In any failure of the control group, the procedure was then repeated with an active treatment.

In the Omni-Stat® group, approximately 3 g of Omni-Stat® was used, being 50% of the contents of an applicator. The Omni-Stat® was dispensed from the applicator over the cut surface, covering the surface completely. A moist gauze compress was then held firmly over the Omni-Stat® for 5 min, with a further 2 min if there was evidence of bleeding after the initial compression. The moist gauze was then carefully peeled off to inspect the surface. Evidence of bleeding from the surface of the liver was noted.

In the Celox Gauze® group, a single layer of Celox Gauze® was cut to a size that covered the cut edge of the liver. It was placed over the cut surface, covering the surface completely. A moist gauze compress was then held firmly over the Celox Gauze® for 5 min; with a further 2 min if there was evidence of bleeding after the initial compression. The moist gauze was then carefully peeled off to inspect the surface. In particular, evidence was sought for continued bleeding through the single layer of Celox Gauze®.

The process was then repeated by taking one or more further sections from the lobe, each about 1 cm deeper to the first, and the process of control or treatment repeated. At the conclusion of the complete series of this and other experimental protocols, the animals were killed.

Results

A total of 38 treatments in 13 pigs were undertaken. Fourteen injuries were first used as gauze controls; all except one failed and were then treated with either Omni-Stat® or Celox Gauze®. There were 18 treatments with Omni-Stat® and six with Celox Gauze®. The haemodynamic data at the time of treatment are shown in Table 1. The comparison between the groups is shown in Table 2. The only significant differences between the groups were heart rate and body temperature for the comparisons control vs Omni-Stat® and Omni-Stat® vs Celox Gauze®. Blood pressure and haematocrit were similar for all groups. The differences in heart rate reflect the differences in core temperature and are, in turn, a reflection of the seasonal variations in ambient temperature as these procedures were not all undertaken at the same session, but at two different times of the year. We do not consider this to be of importance as the blood pressure and haematocrit were similar in all groups.

Thirteen of the 14 controls failed to achieve haemostasis. All Omni-Stat® treated injuries achieved haemostasis provided Omni-Stat® was in contact with the bleeding surface of the liver. Where the Omni-Stat® had not been applied accurately to the cut surface of the liver, or more usually had fallen off as the pressure was applied, there was generally continued bleeding from these small areas. Attention to removal of the gauze is important and it needs to be well moistened prior to removal. Where the Omni-Stat® was pulled off the liver as the gauze was removed, re-bleeding could occur.

In the Celox Gauze® group, haemostasis was successful at initial treatment in five out of six applications. The sixth achieved haemostasis following a further 2 min of compression. Even assuming we take this as a failure, comparison between

Table 1 Haemodynamic data

Product	Haematocrit	Heart rate (bpm)	Systolic pressure (mmHg)	Diastolic pressure (mmHg)	Mean pressure (mmHg)	Temperature (ºC)
Control	0.36 ± 0.05	82 ± 24	103 ± 33	70 ± 24	82 ± 27	37.1 ± 2.8
Omni-Stat®	0.36 ± 0.05	107 ± 30	102 ± 22	63 ± 13	75 ± 15	39.9 ± 2.7
Celox Gauze®	0.36 ± 0.02	71 ± 9	114 ± 38	81 ± 25	93 ± 31	36.5 ± 2.1

Values are mean ± SD.

Table 2 Statistical unpaired two-tailed *t*-test between groups

Comparison	Haematocrit	Heart rate	Systolic pressure	Diastolic pressure	Mean pressure	Temperature
Control vs Omni-Stat	0.816	0.020	0.966	0.312	0.383	0.008
Control vs Celox Gauze	0.933	0.155	0.528	0.391	0.480	0.614
Omni-Stat vs Celox Gauze	0.848	< 0.001	0.487	0.134	0.218	0.008

groups shows significant benefit for Omni-Stat® over control ($P < 0.001$) and for Celox Gauze® over control ($P < 0.001$), but no difference between Omni-Stat® and Celox Gauze® by chi-squared test (2*2 table with 1 df).

Discussion

Chitosan has previously been shown to be highly effective in experimental models of vascular trauma, both with transection of the femoral artery in the absence of heparin[9] and in our own experience with a 6-mm punch lesion of the femoral artery in heparinised swine. An earlier chitosan-based dressing has also been previously shown to be effective in an alternate model of hepatic trauma in non-heparinised swine.[10] The addition of a significant amount of heparin to this model makes it more challenging, but more useful given that patients who have had major liver trauma are liable to be coagulopathic. In spite of this, haemostasis was usually achieved, supporting both the *in vitro* laboratory evidence where clotting occurs in fully anti-coagulated blood and our own previous experience.

Omni-Stat® and Celox Gauze® would, therefore, seem to have potential for use in general and trauma surgery. As a note of caution, long-term stability of the clot is not assessed in these acute studies. Whilst the product is effective in producing haemostasis in dramatic situations, this should not be considered as definitive treatment but as an adjunct to conventional surgical therapies. Therefore, thought should be given to the management of situations where there is either a high risk of re-bleed, or a risk that a late re-bleed would produce a serious complication.

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References

1. Pusateri AE, Modrow HE, Harris RA, Holcomb JB, Hess JR, Mosebar RH *et al.* Advanced hemostatic dressing development program: animal model selection criteria and results of a study of nine hemostatic dressings in a model of severe large venous hemorrhage and hepatic injury in swine. *J Trauma* 2003; **55**: 518–26.
2. King K, Neuffer MC, McDivitt J, Rose D, Cloonan CC, Vayer JS. Hemostatic dressings for the first responder – a review. *Mil Med* 2004; **169**: 716–20.
3 Wedmore I, McManus JG, Pusateri AE, Holcomb JB. A special report on the chitosan-based hemostatic dressing: experience in current combat operations. *J Trauma* 2006; **60**: 655–8.
4. Pozza M, Millner RWJ. Celox™ (chitosan) for haemostasis in massive traumatic bleeding: experience in Afghanistan. *Eur J Emerg Med* 2010; In press.
5. Singh DK, Ray AR. Biomedical applications of chitin, chitosan, and their derivatives. *Polymer Rev* 2000; **40**: 69–83.
6. Roberts GAF. *Chitin Chemistry.* London: Macmillan, 1992.
7. Aranaz I, Mengibar M, Harris R, Panos I, Miralles B, Acosta N, *et al.* Functional characterization of chitin and chitosan. *Curr Chem Biol* 2009; **3**: 203–30.
8. Mirzadehl H, Yaghobi N, Amanpour S, Ahmadi H, Ali Mohagheghi M, Hormozi F. Preparation of chitosan derived from shrimp's shell of Persian Gulf as a blood hemostasis agent. *Iranian Polymer J* 2002; **11**: 63–8.
9. Kozen BG, Kircher SJ, Henao J, Fermin S, Godinez DO, Johnson AS. An alternative hemostatic dressing: comparison of Celox, HemCon, and QuikClot. *Acad Emerg Med* 2008; **15**: 74–81.
10. Pusateri AE, McCarthy SJ, Gregory KW, Harris RA, Cardenas L, McManus AT *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**: 177–82.