EXPERIMENTAL STUDY

Hemostatic efficacy of local chitosan linear polymer granule in an experimental sheep model with severe bleeding of arteria and vena femoralis

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ABSTRACT

BACKGROUND: The aim of the present study was to evaluate the hemostatic effect of chitosan linear polymer in a sheep model with femoral bleeding.

METHODS: Following induction of anesthesia and intubation of sheep, groin injury was induced to initiate hemorrhage. Animals were randomly assigned to study and control groups. In the control group, absorbent pads were packed on the wound, and pressure was supplied by a weight placed over the dressing. In the study group, chitosan linear polymer was poured onto the bleeding site; absorbent pads and pressure were applied in the same manner. At 5-min intervals, bleeding was evaluated. Primary endpoint was time to hemostasis.

RESULTS: Bleeding had stopped by the 1st interval in 5 members of the study group, and by the 2nd interval in 1 member. One sheep was excluded. The bleeding stopped after the 1st interval in 1 member of the control group and after the 2nd interval in 4 members. Bleeding stopped in 2 cases following ligation of the bleeding vessel. Hemostasis was achieved earlier in the study group, compared to the control group, and the difference was statistically significant.

CONCLUSION: Hemostasis was achieved earlier following application of chitosan linear polymer.

Keywords: Chitosan linear polymer; external bleeding; trauma; uncontrolled hemorrhage.

INTRODUCTION

Uncontrolled hemorrhage continues to be the leading cause of death due to military trauma, and the second leading cause of death in a civilian setting. Bleeding treated with blood transfusion comes with inherent risk of complication, as well as significant expense. Rapid and immediate intervention provided by the victim or an on-scene first-responder is one of the most effective means of reducing morbidity and mortality. Even if bleeding is eventually controlled, sufficient blood loss leaves victims vulnerable to hypothermia, coagulopathy, infection, and multiple organ failure. Rapid hemostasis is essential as a strategy, not only for initial survival, but also for optimal recovery.[1–16]

A recent review of autopsies of 982 combat deaths from Iraq and Afghanistan revealed that nearly 24% could have been prevented with effective treatment. Of those potentially preventable deaths, 85% occurred as a result of hemorrhage, 1/3 of which were compressible, 2/3 of which were not, em-

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phasizing the need for improvement in hemorrhage control therapy in the field and in combat hospitals.[17–20]

In the past 1–15 years, significant research has been invested in developing new hemostatic agents to rapidly and securely stop severe incompressible bleeding that cannot be controlled with standard gauze dressing or tourniquet placement. Although tourniquets are vital in control of certain extremity injuries, efficacy in treating wounds in areas such as the neck, abdomen, or groin is limited or nil. When the source of bleeding is in any such area, very little can be done on the battlefield or in the street to control the hemorrhage. In contrast, bleeding from an extremity can potentially be controlled by direct compression and application of dressing by the soldier or first-responder. In response, hemostasis research has been a major emphasis of cooperative efforts by military and civilian trauma researchers of different countries.[1,6,7] The need for hemostatic agents is greater in the event of large-scale disaster such as terrorist attack, bombing, or devastating earthquake, though in such austere conditions, proper hemostasis of all those wounded is often impossible to achieve.[1–5,8–11,14–16,18,20]

The aim of the present study was to test the hemostatic effect of chitosan linear polymer and compression, compared with standard gauze dressing and compression in an experimental young sheep model with severe bleeding of femoral artery and vein. The hypothesis was that chitosan linear polymer would stop the bleeding more quickly and effectively than the standard treatment.

MATERIALS AND METHODS

The University of Dokuz Eylül, institutional review board for the care and use of animals approved the present study. Research was conducted in compliance with the Animal Welfare Act and other national statutes and regulations relating to animals and experiments involving animals. The study adhered to the principles stated in the Guide for the Care and Use of Laboratory Animals.

Study design

The present study was performed in the Laboratory of Animal Sciences of the University of Dokuz Eylül, School of Medicine, İzmir, Turkey with the collaboration of the departments of emergency medicine, anesthesiology, and intensive care.

As no universally accepted animal model to observe the hemostatic effects of a new promising local hemostatic agent presently exists, the following references were used with a few modifications.[1,10,15] The design implemented was unblinded and randomized, with an accepted swine groin injury model (though juvenile sheep were used). The efficacy of 2 treatments for surgically created, lethal, groin arterial and venous bleeding injury were tested. Treatment consisted of chitosan linear polymer (Celox™; MedTrade Products Ltd., Crewe, UK) granule and compression. The control group was treated only with standard gauze and compression to the site of injury.

Animal Subjects

Fourteen young Kıvırcık sheep of both genders were purchased from the Bulut sheep farm in Kemalpaşa, İzmir. Mean weight was 31.8±1.9 kg (range 30–34 kg). No pilot study was conducted. All were fed on a standard diet and observed for at least 1 week to ensure good state of health. Food was withheld the night before the experiment, though free access to water was permitted. Animals were maintained on a 12-h light/dark cycle at a room temperature of 20–22°C.

Regarding the modifications presently implemented, young sheep were utilized instead of swine, as swine are quite difficult to procure, due to religious reasons and recent cases of trichinosis. Sheep are docile and large, facilitating surgical manipulation, and physiology is similar to that of humans. Sheep have been well-established as models of choice in the field of heart pathology, owing to similarities to humans in heart size and hemodynamic flow parameters.[21–23]

Study Protocol

The present study was carried out in the Therapy Pet Hospital in İzmir, as the physical and architectural facilities of the present research center were inadequate.

Anesthesia was induced with intramuscular injection of ketamine hydrochloride (10 mg/kg), xylazine (0.15 mg/kg), and inhaled isoflurane (4–5%). After intubation, isoflurane concentration was reduced to 0.5–1% for the remainder of the experiment. Each animal was placed in the supine position on the operating table and allowed to breathe spontaneously using a mixture of 21% oxygen and air administered through an anesthesia ventilator (Model 84, M1166A; Hewlett Packard, Palo Alto, CA, USA). The right carotid artery and a branch of the external jugular vein were cannulated with 16-gauge intravenous catheter and 9-F introducer sheath, respectively, using a cut-down technique. Catheters were attached to a Viridia CMS hemodynamic monitoring system (Hewlett Packard, Palo Alto, CA, USA) for continuous monitoring of carotid artery pressure and heart rate. Each intravenous catheter was continuously flushed with 0.9% saline solution (5 mL/h) to maintain patency (Figures 1a–c).

Measurements

Mean arterial pressure, heart rate, pH, $PaCO_2$, PaO_2, serum lactate, glucose, sodium, base deficit, hematocrit, hemoglobin, and rectal core temperature were measured every 5 minutes for the first 15 minutes, and every 15 minutes thereafter.

Arterial blood samples, (except lactate levels) were analyzed with Irma Trupoint Blood Analysis System (ITC Medical Supplies, Inc., San Francisco, CA, USA). Whole blood lactate lev-

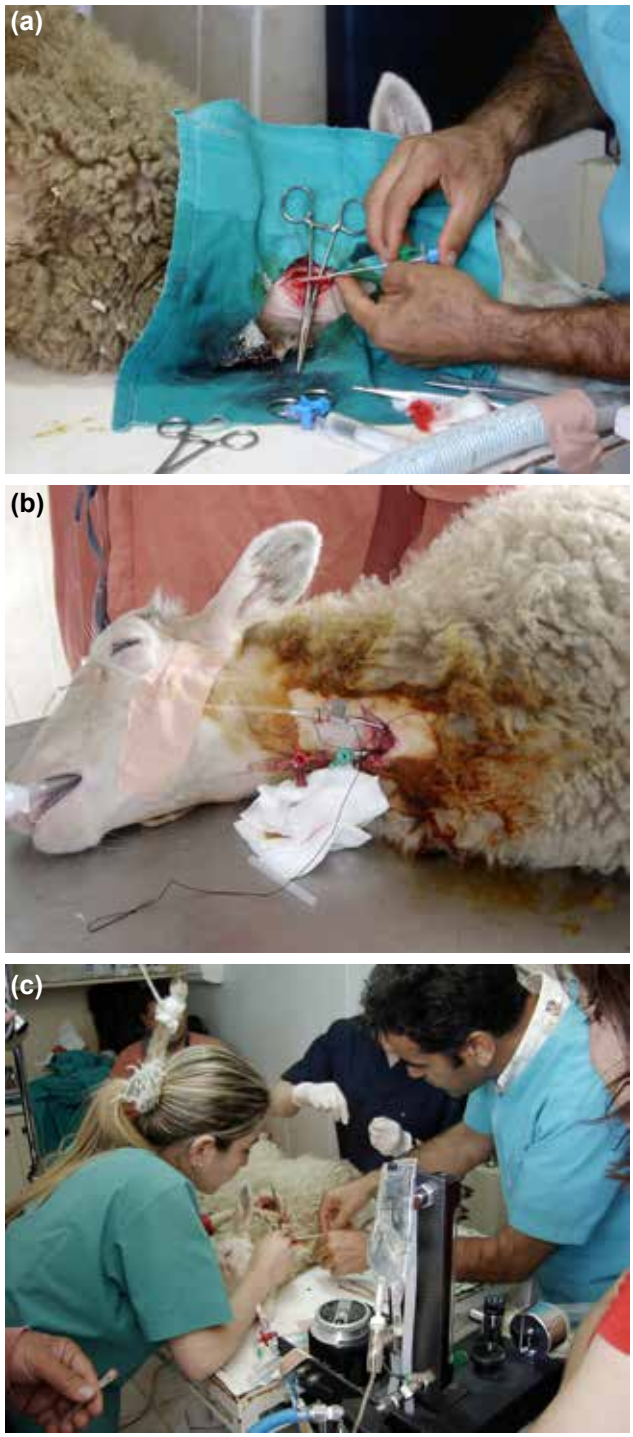

Figure 1. (a) Cannulation of intubated sheep. **(b)** Intubated and cannulated sheep. **(c)** Sheep under anesthesia.

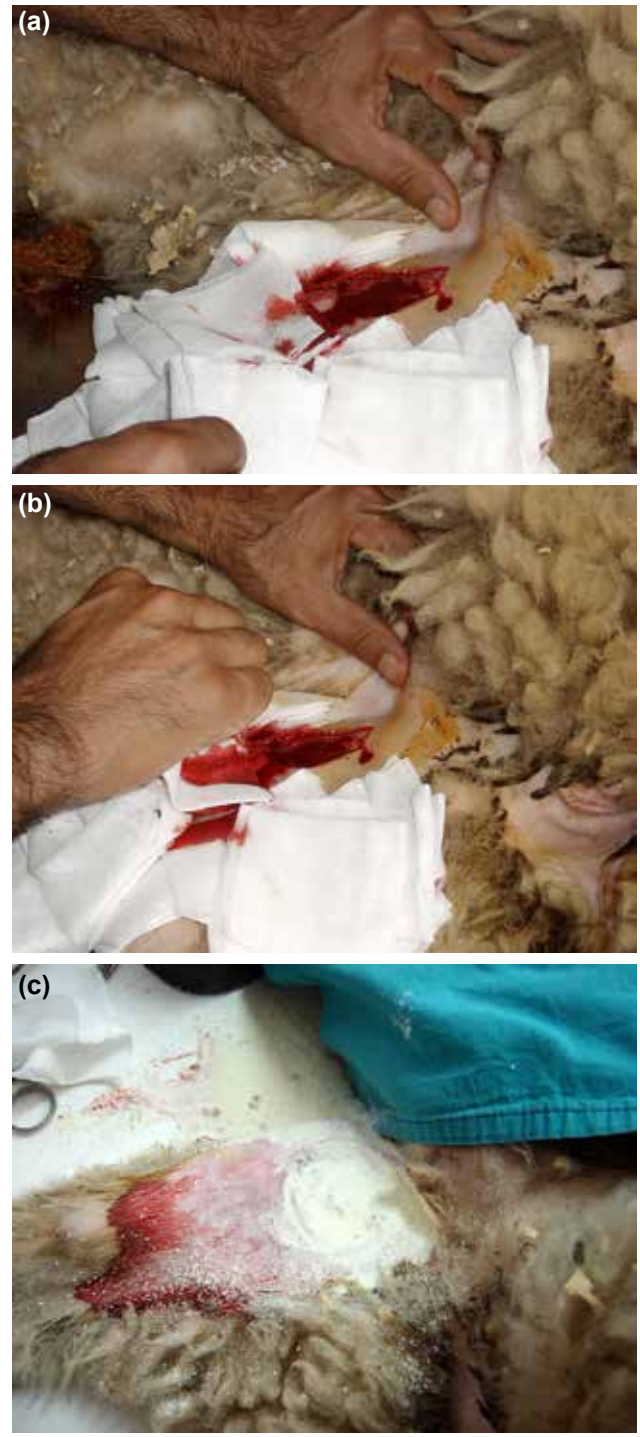

Figure 2. (a, b) Incised and active bleeding in the groin site of sheep. **(c)** Chitosan linear polymer on cut area, with no active bleeding.

els were analyzed using YSI Sport 1500 lactate analyzer (YSI, Inc., Yellow Springs, OH, USA) with a cell lysing agent added to the buffer. Prior to blood analysis, the analyzer was calibrated with standard solutions.

Induction of Uncontrolled Hemorrhagic Shock

A large area of the groin was shaved with an electric razor and cleansed with povidone-iodine. Complex right groin injury was induced to produce uncontrolled hemorrhage, as described by Alam et al.[15,16] Injury included dissection of proximal thigh soft tissues (skin, quadriceps, and adductor muscles) and later, complete division of dissected femoral artery and vein just below the inguinal ligament. This was achieved by incising the vessels with a sharp no. 22 scalpel. After 30 seconds of free bleeding (simulating the response time of the helper), the animals were randomized into 2 groups (Figure 2a, b).

Group 1. Control group (compression only) (n=7)

Group 2. Study group (chitosan linear polymer+compression) (n=7)

In both groups, excess blood was evacuated from the wound with gauze, without dislodging the clot at the vascular injury site after the cut. In the study group, 15 g of chitosan linear polymer (1 package), was poured over the vascular injury site so as to completely cover the transected femoral vessels (Figure 2c), after which the wound was packed with 8x8-cm absorbent gauze (code EES110; Kuteks Sanayii, İzmir, Turkey). Following this, a 5-kg scale weight was attached to achieve a standard level of compression. As application of circumferential compressive dressing would not have been appropriate to the type of injury, gauze was used to cover the wound, like a blanket (Figure 3a, b). At 5-min intervals, the bleeding from the site was evaluated by an unaffiliated and unblinded emergency physician with a stopwatch, who reported whether or not it had stopped. Every 5 minutes, scale weight and gauze were removed, and hemostasis was assessed (Figure 4). If the bleeding had stopped, the test was scored as "passed at 5th minute." If the bleeding continued, the same quantity of chitosan linear polymer granule and the same amount of compression were similarly applied for an additional 5 minutes. If the bleeding stopped after the second application, the test was scored as "passed at 10th minute." If the bleeding continued after the second application, a third trial of the same procedure was repeated for a final 5 minutes. If the bleeding had stopped by the final assessment, the test was scored as "passed at 15th minute." If hemostasis had not been achieved by the third application, the test was scored as "failed," and the bleeding was stopped by ligation of the vessel. Failure of hemostasis was defined as blood pooling outside of the wound.

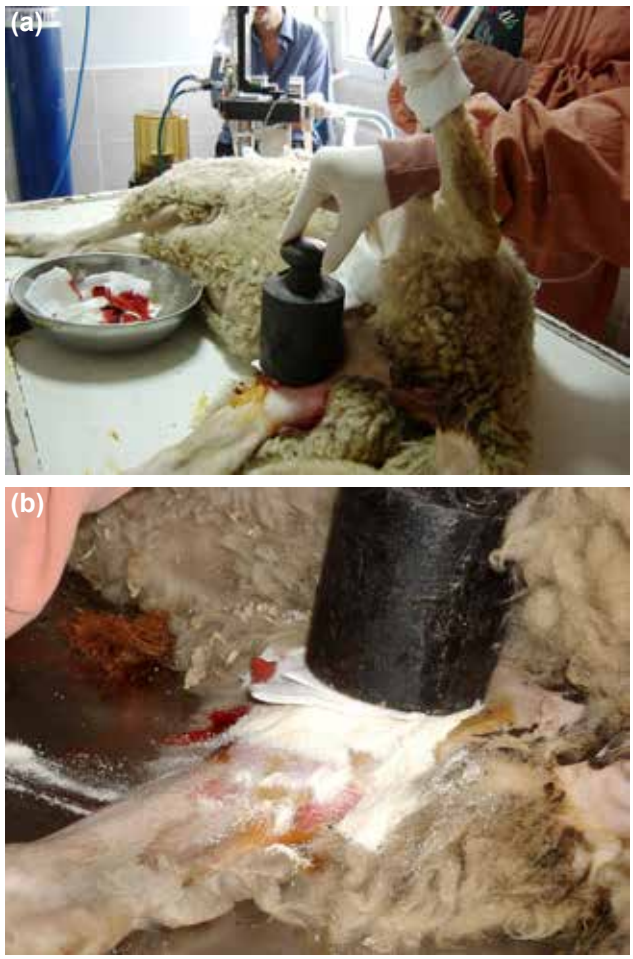

Figure 3. (a, b) Application of scale weight over bleeding groin area.

In the control group, the same procedures were performed, with the exception of chitosan linear polymer application. Hemostasis was similarly assessed at the 5th, 10th, and 15th minutes after removal of compression.

Primary outcome was time to hemostasis. At the end of the 15th minute, the study was finalized, but intubation was maintained for an additional 3 hours in order to observe the hemostatic status of the cut area. Following this, the sheep were extubated and transported back to the farm at which they had been purchased. Fifteen days later, the animals were revisited. All were alive, and no complications were observed. After all were sacrificed, 5x5-cm samples of skin were excised to aid in future studies of local histopathological effects of chitosan linear polymer, prior to the animals being sold to butchers.

Resuscitation Protocols

To simulate the austere battlefield environment, the volume of resuscitation fluid was limited to 500 mL of Haemaccel™ (Polygeline 17.5 g/500 mL, Dem İlaç, İstanbul, Turkey), which was intravenously administered. The infusion was initiated 15 minutes after the induction of injury (simulating delay in establishing intravenous access).

Data Analysis

Physiologic variables were analyzed using SPSS software for

Figure 4. Formation of clot (chitosan linear polymer and blood).

Table 2. Timetable for the hemostasis

	Study group			Control group		
	Bleeding stopped in the first trial	**Bleeding stopped in the second trial**	**The vessel needed to be sutured**	**Bleeding stopped in the first trial**	**Bleeding stopped in the second trial**	**The vessel needed to be sutured**
1st sheep	+					+
2nd sheep		executed				+
3rd sheep	+				+	
4th sheep		+			+	
5th sheep	+			+		
6th sheep	+				+	
7th sheep	+				+	

Windows (version 10.0; SPSS Inc., Chicago, IL, USA). Hemodynamic data, and blood gas and temperature results were expressed as means±SD. Mann-Whitney U test was used for group comparison, and $p<0.05$ was considered significant.

RESULTS

No complications were observed in the execution of study protocol. No mortality was observed in the 14 juvenile sheep at the end of the study (180th minute). In 1 sheep in the control group, tracheotomy was successfully performed after intubation attempts were unsuccessful. One sheep from the treatment group was excluded after severe bleeding could not be induced.

Achievement of Hemostasis

While hemostasis was achieved in the first interval in 5 of 7 sheep in the treatment group, it was achieved in only 1 sheep in the first interval in the control group. Hemostasis was achieved in the second interval in 4 sheep in the control group (Table 1). Difference in time to hemostasis between groups was statistically significant ($p<0.05$).

Monitorized Physiological Parameters

Difference between variables (MAP, heart rate, hemoglobin, hematocrit, pH, $PaCO_2$, PaO_2, blood lactate levels, oxygen saturation, and rectal temperature) between the groups was not statistically significant ($p>0.05$) (Table 2).

DISCUSSION

Results of the present study demonstrate that application of chitosan linear polymer granule and compression of the bleeding femoral artery and vein significantly reduced time to hemostasis, compared to the control group.

Depth and irregular geometry of combat wounds make uniform application and acceptable usage of hemostatic agent difficult, even under the best of conditions, but even more so when applied by non-medical personnel. When additional circumstances are present, such as wounds in places not amenable to tourniquet application and inability to hold pressure for extended periods of time, challenges to hemostatic agent application are daunting. The aim of the present study was to develop a safe and effective treatment that would address a number of unique challenges experienced on the battlefield and in major civilian traumas, as the use of tourniquet, not always effective, may cause complications and is not without controversy.[2,3,7,18–20]

While many local hemostatic agents have been tested, no clear consensus exists regarding optimal hemorrhage control strategy for external bleeding.[1,4,8,9,13–17,20,24–30] However, Pusateri and Grissom[10,19] outlined ideal qualities of hemostatic agents for prehospital and battlefield use.

The focus of the present study was on a granular hemostatic agent heavy enough to be poured into the wound without being rapidly flushed away by ongoing bleeding or easily blown away in adverse weather conditions. Product contact with site of bleeding and conformance to the wound were important priorities. Recognizing the potential limited access to additional product in emergency evacuation situations, it was likewise important that the product could be re-applied if bleeding recurred.[1,3,13,15,16, 21–28,31–33]

While some reports on human use have been published, live tissue studies and animal trials have been highly encouraging. Chitosan linear polymer has a long shelf life and requires no special assembly (or mixing of components) at the time of application. It has reportedly been effectively used to control major arterial bleeding, is non-allergenic, can be used effectively at temperature extremes, doesn't generate heat, is lightweight, portable, and easy to administer without specialized training, and is low in cost. Chitosan linear polymer is available in granular form that gels to conform to the wound once poured over the bleeding site, and can be easily removed. It is heavy enough to be poured into the wound without being rap-

Table 2. The list of the monitorized physiological parameters

Parameters	Time (min)	Experimental groups	
		Control group	Study group
Mean arterial pressure (mmHg)	Baseline value	95±28	95±17
	Following dissection	189±259	82±35
	5th minutes	90±13	78±37
	10th minutes	91±17	89±15
	15th minutes	90±17	62±44
	30th minutes	95±14	48±46
	45th minutes	28±50	11±29
Heart rate (beat/minutes)	Baseline value	92±24	86±21
	Following dissection	94±14	81±40
	5th minutes	92±21	89±15
	10th minutes	88±17	80±15
	15th minutes	90±16	56±39
	30th minutes	80±14	40±38
	45th minutes	24±42	14±37
Hemoglobin (g/dL)	Baseline value	7.3±1.7	6.0±2.8
	Following dissection	6.9±2.2	4.9±3.9
	5th minutes	4.5±3.3	4.1±2.9
	10th minutes	4.5±2.0	10.6±6.8
	15th minutes	4.1±2.9	4.9±2.4
	30th minutes	3.5±3.4	2.9±3.8
	45th minutes	13.9±37.0	0.7±1.9
Hematocrit (%)	Baseline value	21.7±4.5	16.9±6.4
	Following dissection	20.6±6.5	16.3±8.8
	5th minutes	16.2±5.7	15.2±4.0
	10th minutes	14.6±2.58	31.1±20.2
	15th minutes	25.1±24.5	14.5±7.2
	30th minutes	14.8±5.2	10.2±10.6
	45th minutes	2.7±7.2	2.1±5.7
pH	Baseline value	6.35±2.80	7.39±0.07
	Following dissection	7.38±0.11	4.21±3.93
	5th minutes	7.37±0.10	7.42±0.10
	10th minutes	7.42±0.09	7.43±0.09
	15th minutes	7.40±0.06	6.34±2.80
	30th minutes	7.39±0.04	4.26±3.99
	45th minutes	1.06±2.82	1.06±2.80
Partial carbon dioxide pressure(mmHg)	Baseline value	38±18	43±6
	Following dissection	47±12	27±26
	5th minutes	42±7	36±5
	10th minutes	40±10	40±13
	15th minutes	44±7	39±19
	30th minutes	42±7	23±25
	45th minutes	5±14	6±17

Table 2. The list of the monitorized physiological parameters (Cont)

Parameters	Time (min)	Experimental groups	
		Control group	Study group
Partial oxygen pressure (mmHg)	Baseline value	285±200	258±227
	Following dissection	214±141	215±169
	5th minutes	299±149	238±181
	10th minutes	287±191	215±154
	15th minutes	330±209	237±207
	30th minutes	142±146	134±175
	45th minutes	14±38	58±154
Blood lactat levels	Baseline value	1.49±1.60	0.85±0.62
	Following dissection	1.05±1.23	0.73±0.55
	5th minutes	1.80±1.48	0.76±0.33
	10th minutes	1.49±1.28	0.77±0.45
	15th minutes	1.37±1.60	0.31±0.32
	30th minutes	1.32±1.25	0.34±0.35
	45th minutes	0.04±0.12	0.24±0.44
Saturation (%)	Baseline value	83±37	83.72±37.18
	Following dissection	96±4	96.88±4.39
	5th minutes	98±2	98.48±2.60
	10th minutes	98±2	98.05±2.13
	15th minutes	98±2	98.52±2.17
	30th minutes	95±3	95.61±3.97
	45th minutes	13±37	13.98±37.00

idly flushed away by ongoing bleeding or easily blown away in adverse weather conditions.[6,13,14,17,19,24,25,27,28,31,32] These properties approximate ideal qualities of hemostatic agents for prehospital and battlefield use.[10–19]

Various hemostatic products have been tested in a number of animal models, ranging from liver to complex groin injuries, with disparate results, depending on the model and the standard of cost-effectiveness. It can be argued that no agent has met all ideal criteria, though chitosan linear polymer may be a candidate.[6,7,10,13,17,19,24,25,27,28,31,32]

The groin injury model presently utilized took into account realities of modern combat injuries, and has previously been tested.[1–3,13,15,16,27] Salient features include lethal but potentially salvageable injury, anatomic location unsuitable for application of tourniquet, combined arterial and venous injuries with uncontrolled hemorrhage, large soft tissue injury, realistic delays in application of dressing and start of resuscitation, and resuscitation approach consistent with new recommendations for the military. However, it should be emphasized that certain modifications were made. First, although similar studies used swine, the present was only the second to be conducted on young sheep.[21,24,25,27,28,31] In the majority of studies, compression over the bleeding site was performed with finger pressure. In an effort to determine how a standard level of pressure could be achieved by use of manual compression, the technique was presently modified by placing a scale weight (weighing 5 kg) over the gauze on the bleeding site, a modification used for the second time.[1–3] In a recent paper by Ward et al.[26] a similar method of achieving standard pressure was reported. To standardize the amount of pressure applied on the wound, a pediatric blood pressure cuff was used. The aim in both studies was to maintain a standard amount of pressure over the bleeding site. It is presently believed that the method described may be more efficient than that used in the present study, as more diffuse and stable pressure could be transferred to the bleeding site.[8,15]

Few studies concerning hemostatic effect of chitosan have been reported. Hamid et al.[21] conducted a study with Persian Gulf Chitosan (PGC) in a carotid puncture hole sheep model, and the results suggested that PGC was safe and effective means of achieving hemostasis. This was the 1st such study conducted with sheep, and the present was the 2nd. Kozen et al.[28] compared Celox™ with HemCon and QuickClot in a lethal hemorrhagic groin injury porcine model. Celox™ improved hemorrhage control and survival, and has

been accepted as a viable alternative for the treatment of severe hemorrhage. Also reported was the lifesaving use of Celox™ in 2 patients undergoing cardiothoracic surgery during which conventional techniques failed. This was the first reported surgical use.[34] However, it is difficult to understand how Celox™ was used internally, as it is indicated for external use only, though the authors may have been urged to use it due to force major.

Though use is not frequently described, it may be wise to keep local hemostatic agents, such as chitosan linear polymer, on hand, to be used in the event of emergencies including mass casualty events, such as those caused by terrorism, natural disasters, devastating earthquakes, and industrial accidents. Hemostatic agents may also aid in patient triage, temporizing otherwise lethal hemorrhage.[24] After more human studies have been conducted, these agents may be used as a first aid agent in homes, as well.[33] However, more studies are needed before these agents can be recommended for general use.

Limitations

It must be noted that the study design precluded the need for blinded investigation. The present study was also limited by the lack of universally accepted optimal model for comparison of hemostatic effects produced by wound dressings. It should be emphasized, however, that the model used was not intended to precisely mimic a clinical situation, but was meant to serve as a platform for the evaluation of dressings used to control high-pressure arterial and venous profuse bleeding. The bleeding source was directly visible and accessible, unlike those likely to occur in the field. Finally, a scale weight was used for compression, though manual compression is generally provided in real-life situations. Complete transection of the femoral vessel could have caused retraction of the artery with resultant vasospasm that artificially contributed to improved hemostasis. Removal of a portion of the anterior arterial wall would eliminate the effect of arterial retraction. However, patients with penetrating injuries to large vessels more commonly have complete transection of vascular structures, not glancing injures that blow out only 1 side of the vessel wall. Study protocol and parameters may be improved by the investigation of clot formation over the artery and vein. Markedly decreased fibrinolytic activity, and increased platelet number and adhesiveness in sheep may affect the hemostatic system. These factors could increase the tendency toward hypercoagulability.[22,23]

Conclusion

In the present experimental young sheep model with severe bleeding of arteria/vena femoralis, application of chitosan linear polymer granule and compression over the bleeding site significantly reduced time to hemostasis, compared to the control group.

While further translational research is required, one can imagine this intervention providing a prehospital hemostatic bridge for severely bleeding casualties, who would otherwise bleed to death in the field, allowing for arrival to a surgical treatment facility.

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Conflict of interest: None declared.

REFERENCES

1. Ersoy G, Kaynak MF, Yilmaz O, Rodoplu U, Maltepe F, Gokmen N. Hemostatic effects of microporous polysaccharide hemosphere in a rat model with severe femoral artery bleeding. Adv Ther 2007;24:485–92.
2. Ozgur D, Gurkan E, Osman Y, Ozlem D, Necati G. The Effect of Application of Local Oxidised Cellulose Powder on Hemostasis Time in a Rat Model With Femoral Artery Bleeding. Acta Medica Mediterranea 2015,31:179–82.
3. Aydinoglu A, Ersoy G, Dogan A, Akay H, Yilmaz O, Gokmen N. Hemostatic effect of microporous polysaccharide hemosphere in a heparinized rat model with femoral artery bleeding. European Journal of Emergency Medicine 2008;15:286–306.
4. Eryilmaz M, Ozer T, Menteş O, Torer N, Durusu M, Günal A, et al. Is the zeolite hemostatic agent beneficial in reducing blood loss during arterial injury? Ulus Travma Acil Cerrahi Derg 2009;15:7–11.
5. Eryilmaz M, Mentes O, Ozer MT, Ersoy G, Durusu M, Rodoplu U, et al. Current principles of use of topical hemostatic agents in trauma cases. Turkish Journal of Emergency Medicine 2007;7:136–43.
6. Bulger EM, Snyder D, Schoelles K, Gotschall C, Dawson D, Lang E, et al. An evidence-based prehospital guideline for external hemorrhage control: American College of Surgeons Committee on Trauma. Prehosp Emerg Care 2014;18:163–73. Crossref
7. Taillac PP. Stop the bleeding. New external hemorrhage control evidence-based guideline. JEMS 2014;Suppl:18–21.
8. Velmahos GC, Tabbara M, Spaniolas K, Duggan M, Alam HB, Serra M, et al. Self-expanding hemostatic polymer for control of exsanguinating extremity bleeding. J Trauma 2009;66:984–8. Crossref
9. Kilbourne M, Keledjian K, Hess JR, Scalea T, Bochicchio GV. Hemostatic efficacy of modified amylopectin powder in a lethal porcine model of extremity arterial injury. Ann Emerg Med 2009;53:804–10. Crossref
10. Pusateri AE, Holcomb JB, Kheirabadi BS, Alam HB, Wade CE, Ryan KL. Making sense of the preclinical literature on advanced hemostatic products. J Trauma 2006;60:674–82. Crossref
11. Brown MA, Daya MR, Worley JA. Experience with chitosan dressings in a civilian EMS system. J Emerg Med 2009;37:1–7. Crossref
12. Recinos G, Inaba K, Dubose J, Demetriades D, Rhee P. Local and systemic hemostatics in trauma: a review. Ulus Travma Acil Cerrahi Derg 2008;14:175–81.
13. Özlem K, Fatma Ö, Betül ÇE, Naciye İB, Deniz S. Hemostatic effect of a chitosan linear polymer (Celox®) in a severe femoral artery bleeding rat model under hypothermia or warfarin therapy. Ulus Travma Acil Cerrahi Derg 2011;17:199–204. Crossref
14. Kheirabadi BS, Edens JW, Terrazas IB, Estep JS, Klemcke HG, Dubick MA, et al. Comparison of new hemostatic granules/powders with cur-

rently deployed hemostatic products in a lethal model of extremity arterial hemorrhage in swine. J Trauma 2009;66:316–28. Crossref
15. Alam HB, Chen Z, Jaskille A, Querol RI, Koustova E, Inocencio R, et al. Application of a zeolite hemostatic agent achieves 100% survival in a lethal model of complex groin injury in Swine. J Trauma 2004;56:974–83.
16. Alam HB, Uy GB, Miller D, Koustova E, Hancock T, Inocencio R, et al. Comparative analysis of hemostatic agents in a swine model of lethal groin injury. J Trauma 2003;54:1077–82. Crossref
17. Bennett BL, Littlejohn L. Review of new topical hemostatic dressings for combat casualty care. Mil Med 2014;179:497–514. Crossref
18. Kragh JF Jr, Nam JJ, Berry KA, Mase VJ Jr, Aden JK 3rd, Walters TJ5, et al. Transfusion for shock in US military war casualties with and without tourniquet use. Ann Emerg Med 2015;65:290–6. Crossref
19. Grissom TE, Fang R. Topical hemostatic agents and dressings in the prehospital setting. Curr Opin Anaesthesiol 2015;28:210–6. Crossref
20. Pekcan M, Eryilmaz M. New hemostatic agents to be used in trauma cases. Scientific literature of GESDAV 2006;1:3–55.
21. Hamid M, Nakisa AM, Ali M, Farzin H. Preparation of chitosan derived from shrimp's shell of Persian Gulf as a blood hemostasis agent. Iranian Polymer Journal 2002;11:63–8.
22. Gajewski J, Povar ML. Blood coagulation values of sheep. Am J Vet Res 1971;32:405–9.
23. Scheerlinck JP, Snibson KJ, Bowles VM, Sutton P. Biomedical applications of sheep models: from asthma to vaccines. Trends Biotechnol 2008;26:259–66. Crossref
24. Sohn VY, Eckert MJ, Martin MJ, Arthurs ZM, Perry JR, Beekley A, et al. Efficacy of three topical hemostatic agents applied by medics in a lethal groin injury model. J Surg Res 2009;154:258–61. Crossref
25. Burgert J, Gegel B, Neal AR, Kammer KE, Paul ME, Schwartz DJ, et al. The effects of arterial blood pressure on rebleeding when BleedArrest, Celox and TraumaDex are used in a porcine model of lethal femoral injury. Mil Med 2012;177:340–4. Crossref
26. Ward KR, Tiba MH, Holbert WH, Blocher CR, Draucker GT, Proffitt EK, 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;63:276–84. Crossref
27. Littlejohn LF, Devlin JJ, Kircher SS, Lueken R, Melia MR, Johnson AS. Comparison of Celox-A, ChitoFlex, WoundStat, and combat gauze hemostatic agents versus standard gauze dressing in control of hemorrhage in a swine model of penetrating trauma. Acad Emerg Med 2011;18:340–50. Crossref
28. Kozen BG, Kircher SJ, Henao J, Godinez FS, Johnson AS. An alternative hemostatic dressing: comparison of CELOX, HemCon, and QuikClot. Acad Emerg Med 2008;15:74–81. Crossref
29. Shanmugam V, Robinson MH. Case report of uncontrollable pelvic bleeding-managed by a previously unreported method (QuikClot). Colorectal Dis 2009;11:221–2. Crossref
30. Yüce S, Çandirli C, Yenidünya S, Muslu B. New hemostatic agent: the effect of Ankaferd Blood Stopper on healing wounds in experimental skin incision model. Turk J Med Sci 2014;44:288–94. Crossref
31. Burgert JM, Gegel BT, Austin R 3rd, Davila A, Deeds J, Hodges L, et al. Effects of arterial blood pressure on rebleeding using Celox and TraumaDEX in a porcine model of lethal femoral injury. AANA J 2010;78:230–6.
32. Official web site for Celox™. http://www.celoxmedical.com. (Accessed March 08, 2015).
33. Markenson D, Ferguson JD, Chameides L, Cassan P, Chung KL, Epstein J, et al. Part 17: first aid: 2010 American Heart Association and American Red Cross Guidelines for First Aid. Circulation 2010;122(18 Suppl 3):934–46. Crossref
34. Millner RW, Lockhart AS, Bird H, Alexiou C. A new hemostatic agent: initial life-saving experience with Celox (chitosan) in cardiothoracic surgery. Ann Thorac Surg 2009;87:13–4. Crossref

DENEYSEL ÇALIŞMA - *ÖZET*

Ciddi femoral arter ve ven kanaması olan deneysel koyun modelinde, lokal "chitosan linear polymer" granül uygulamanın, hemostatik etkinliği

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AMAÇ: Femoral kanamalı, deneysel koyun modelinde, "chitosan linear polymer" uygulamasının, hemostatik etkinliğini araştırmayı amaçladık.
GEREÇ VE YÖNTEM: Koyunların anestezi ve entübasyonunu takiben, kanamayı başlatmak için kasık yaralanması oluşturuldu. Denekler, çalışma ve kontrol grupları şeklinde randomize edildiler. Kontrol grubunda, kanama alanı üstüne absorban pedler yerleştirildi ve üstüne ağırlık konarak bası oluşturuldu. Çalışma grubunda ise kanama alanına "chitosan linear polymer" döküldü, absorban pedler ve ağırlık aynı şekilde yerleştirildi. Her beş dakikada bir kanama değerlendirildi. Ana çıktı kanama duruncaya kadar geçen zamandı.
BULGULAR: Çalışma grubunda kanama, beş denekte ilk, diğer denekte ise ikinci girişimde durdu. Bir denek çalışmadan çıkarıldı. Kontrol grubunda, kanama bir denekte ilk girişimde, dört denekte ikinci girişimde, iki denekte ise, yerin dikilmesinden sonra durdu. Hemostaz çalışma grubunda, kontrol grubuna oranla, daha erken dönemde sağlandı ve her iki grup arasındaki fark, istatistiksel olarak anlamlı idi.
TARTIŞMA: Bu koyun modelinde "chitosan linear polymer" uygulaması hemostazı kontrol grubuna göre daha kısa sürede sağladı.
Anahtar sözcükler: Eksternal kanama; kitosan lineer polimer; kontrolsüz kanama; travma.

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