Original Article

CoolClot, a novel hemostatic agent for controlling life-threatening arterial bleeding

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BACKGROUND: Uncontrolled bleeding is the first leading cause of preventable death in the battlefield and the 2nd cause of mortality in civil accidents. Incompressible hemorrhage control is among the interventions that drastically increase the survival rate in wound individuals. We have previously shown that a certain mixture of bentonite and zeolite minerals can significantly decrease the bleeding in rats.

METHODS: In this study, nine healthy hybrid dogs were selected and after induction of anesthesia with ether, either arterial puncture by a needle or arteriotomy was performed on both groin regions of the dogs. For control arteries (either the right or left femoral artery), only pressure by sterilized gauze was performed, while for the femoral arteries of the opposite side, our invented hemostatic agent, namely CoolClot, was topically used before applying the pressure. In the second stage of the study, to assess the coagulation time, blood samples were collected from 10 volunteer students.

RESULTS: CoolClot significantly decreased the bleeding time in animals whose femoral arteries were cut or punctured. In the human phase of the study, the mean coagulation time in control blood samples was 253.4±44.1 seconds, whereas it was 149.5±50.0, 162.3±74.6 and 143.4±114.6 seconds, respectively in blood samples treated with bentonite, zeolite and CoolClot (P<0.05).

CONCLUSIONS: As controlling bleeding after a life-threatening arterial damage is critical for increasing the chance of survival, the results obtained in this study indicate the significant efficacy of CoolClot in shortening the bleeding time. Our experiments also indicate that CoolClot can significantly reduce the clotting time in human blood samples.

KEY WORDS: Bleeding; Hemostatic agent; CoolClot; Bentonite and zeolite

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INTRODUCTION

Uncontrolled bleeding is the first leading cause of preventable death in the battlefield injuries and the second cause of mortality in civil accidents.[1] Incompressible hemorrhage control is among the interventions that drastically increase the survival rate in wounded individuals.[2] Thus, presently with the clearance of US Food and Drug Administration, certain hemostatic agents are used for military and civil purposes.[3,4] Safety, efficacy, usability, cost, and approvability are among the major factors which should be considered in the evaluation of any potential hemostatic agent.[5] Substantial data indicate that some mineral-based hemostatic agents (capable of clotting blood) such as kaolin,[6–8] zeolite[9,10] or chitosan that is a linear polysaccharide[11–13] significantly decrease the hemostasis time. Spotnitz and Burks[14,15] have recently compared some major FDA-approved topical hemostatic agents including sealants that provide a sealing barrier

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and adhesives that bond tissues together.

We have previously shown that radioactive lantern mantle powder can cause a series of histological changes such as fibrinoid necrosis and neutrophil exudate which can facilitate the recovery of the wound. However, this study[16] could not show any significant difference between the tensile strength of the experimental and control groups. We have also reported that topical use of non-radioactive and radioactive lantern mantle powder identically showed significant alterations in the volume of blood loss, bleeding and coagulation time in Wistar rats.[17]

The first phase of the recent study was conducted in the Center for Radiological Research, Shiraz University of Medical Sciences (SUMS) in 2009.[18–20] The aim of that phase was to produce a hemostatic agent which was based on the use of designed minerals. The obtained results showed that a mixture of bentonite and zeolite minerals dramatically reduces the volume of blood loss, the bleeding and clotting time. Bentonite has a wide variety of pharmaceutical applications such as oral applications (capsules, tablets, suspensions, etc). According to FDA "There is no evidence in the available information on bentonite that demonstrates or suggests reasonable grounds to suspect, a hazard to the public when it is used in the manner now practiced or that might reasonably be expected in the future".[21] On the other hand, there are some recent reports on other medical applications of bentonite, such as bentonite nanoclay use in surgical threads.[22]

What made the results of our previous study different from those obtained in other studies was controlling the exothermic reactions which eliminated the risk of burning in the wound. At that time the use of known hemostatic agents such as QuikClot was associated with tissue damage caused by the exothermic reaction.[23–26] After the registration of CoolClot, the above-mentioned hemostatic agent (a specific mixture of bentonite-zeolite minerals), in the Iranian Industrial Property Registration Organization as a patent, the experiment was done continuously on laboratory animals to investigate the efficiency of this hemostatic agent in controlling life-threatening arterial bleedings. On August 21, 2011, the Iranian Food and Drug Administration issued the permit for clinical tests of CoolClot. In this study, the efficiency of CoolClot in reducing the clotting time in human blood samples was also investigated.

METHODS

First phase of the study

The ethics committee of SUMS approved the study. This study was conducted as an experimental research in the Center for Radiological Research, SUMS in 2011–2012. Nine adult healthy dogs weighing 20–30 kg and aged 12–18 months (male and female) were included in the study. According to SUMS ethical codes regarding the care and use of laboratory animals, all possible steps were taken to avoid animals' suffering at each stage of the experiment. After induction of anesthesia with ether in the first dog, by making an incision in the groin region and fixing the femoral artery, either arteriotomy or arterial puncture was performed by a standard needle No. 19–20 on the femoral artery of the animal on both sides. As shown in Table 1, for the first

Table 1. The bleeding time after either arterial puncture or arteriotomy in the right and left femoral arteries of the 5 dogs used in this study

Animals tested	Time for bleeding control (min)		Description
	No hemostatic agent	CoolClot	
Dog No.1 (M)	No stop, sutured after 4.55 min	1.5 min	Arterial puncture using needle No. 20–20 g CoolClot poured on puncture site
Dog No.2 (M)	Due to severe loss of blood pressure, it was not possible to check the opposite side	4 min	Arteritomy (2–3 mm) –35 g CoolClot poured on puncture site, The opposite side was not studied due to loss of blood pressure
Dog No.3 (F)	No stop, sutured after 15 min	5 min	Arterial puncture using needle No. 19. Local temperature controlled-pad was used instead of pouring the powder
Dog No.4 (M)	No stop, sutured after 15 min	7 min	Arterial puncture using needle No. 19. A new modified pad was used instead of pouring the powder. BP 116/53 decreased to 103/65
Dog No.5 (F)	No stop, sutured after 4 min	2 min	Arterial puncture using needle No. 19. A new modified pad was used instead of pouring the powder. BP 135/103 decreased to 76/50
Dog No.6 (F)	12 min	5 min	Arterial puncture using needle No. 19. A new modified pad was used instead of pouring the powder
Dog No.7 (F)	10 min	2 min	Arterial puncture using needle No. 19. A new modified pad was used instead of pouring the powder
Dog No.8 (F)	7 min	5 min	Arterial puncture using needle No. 19. A new modified pad was used instead of pouring the powder
Dog No.9 (F)	Due to animal's health condition, it was not possible to check the opposite side	2.5 min	Arterial puncture using needle No. 19. A new modified pad was used instead of pouring the powder

and second animals, 20 or 35 g of CoolClot hemostatic agent was put on the punctured site of tested femoral artery but no intervention was done on the opposite side. CoolClot was made by mixing zeolite (one third of the weight) and bentonite (two third of the weight) and a very small amount of some additives. These additives were all derived from other types of bentonite clay with different properties such as varieties in water absorption which was a cardinal factor in hemostatic efficacy of CoolClot. In the second animal, for making a severe arterial bleeding which could threaten the life of the animal, arteriotomy was performed by a surgery blade with a cleavage about 2–3 mL. On the other hand, for the third to 9th dogs, either CoolClot sterilized gauzes or ordinary sterilized gauzes were put and pressured on the punctured arteries of both sides. Every 30 seconds, gauzes were removed to check the bleeding condition. Animal's blood pressure was monitored carefully throughout the experiment. The bleeding time was measured by a standard calibrated chronometer.

Second phase of the study

In the next phase of the study that was performed on human subjects, blood sampling was done on 10 volunteers who were physically healthy and had no history of blood disorders. All patients participating in this study gave informed consent prior to blood donation. The volume of the blood sampling was 2 mL and it was done on the antecubital vein in a sterilized condition. Four separate test-tubes were prepared for each person. The first tube was named control tube and the other three tubes consisted of bentonite and zeoloite minerals and Cool Clot hemostatic agent. Based on the "Lee and White" method, the tubes were shacked simultaneously and with the equal angels. An experienced specialist diagnosed the formation of the clot. The clotting time was recorded by a chronometer.

Statistical analysis

The gathered data were analyzed by SPSS and analysis of variance (ANOVA) was also used for comparing the means of the five groups.

RESULTS

In the dog model, the termination time for bleeding in the artery tested with CoolClot of the first animal was 1.5 minutes, whereas in the opposite no stopping was observed after 4.55 minutes and a surgeon finally sutured the artery. The incision area was investigated after 4 days and it was found that there was no infection in the region CoolClot was used, whereas in the opposite side there were some obvious markers of infection.

In the second animal, following stopping the pressure after 4 minutes, there was no bleeding in the site CoolClot was used. Due to a dramatic decrease in the blood volume and pressure, it was impossible to conduct a similar experiment in the opposite femoral artery. After conducting the experiment, the mentioned animal regained consciousness and was alive without any treatment for 4 hours after arteriotomy.

In the third to 9th animals, following stopping the pressure after 5, 7, 2, 5, 2, 5, and 2.5 minutes, there was no bleeding in the puncture site CoolClot was used. However, in case of the opposite arterial punctures, the severity of the bleeding in animals either made the surgeon of our team to suture the arteries to save the animal's life or the bleeding times were higher than the opposite side (CoolClot side).

In the next phase of the experiment that was conducted on human blood samples, the average clotting time in the control blood samples was 253.4±44.1 seconds, whereas the average clotting time in the blood samples treated by bentonite-containing test tubes, zeolite-containing test tubes and the CoolClot hemostatic agent was reported respectively as 149.5±50.0, 162.3±74.6 and 143.4±114.6 seconds. The clotting time in blood samples of the control group compared to the samples treated by bentonite, zeolite, and CoolClot hemostatic agent is shown in Table 2.

Analysis of variance (ANOVA) showed that the observed differences were statistically significant (P<0.05). The significant level reported was related to the comparison of the mean clotting time of blood

Table 2. The clotting time in the blood samples of the control group in comparison with the samples treated by bentonite, zeolite, and CoolClot hemostatic agent

Clotting time (sec) (mean± SD)				P value (Friedman test)
Control	Intervention used			
	Bentonite	Zeolite	CoolClot	
235.4±44.1	149.5±50.0	162.3±74.6	143.4±114.6	P<0.01
Chi-square test 12.45	df=3	Asymp. Sig =0.006		
Pairwise comparisons				Wilcoxon's signed rank test
235.4±44.1	149.5±50.0			P<0.05
235.4±44.1		162.3±74.6		P<0.01
235.4±44.1			143.4±114.6	P<0.05
	149.5±50.0		143.4±114.6	NS
		162.3±74.6	143.4±114.6	NS

NS: not significant.

samples in the control group and in the groups treated with bentonite, zeolite and CoolClot hemostatic agent. On the other hand, when each intervention group was compared to the control group, the differences were again statistically significant ($P<0.05$ for control-bentonite groups, $P<0.01$ for zeolite-control groups, and $P<0.05$ for CoolClot-control groups).

DISCUSSION

This phase of the study revealed that CoolClot hemostatic agent plays a significant role in controlling life-threatening arterial bleeding that happens in battlefields or accidents. CoolClot not only significantly decreased the bleeding time in animal models whose femoral arteries were punctured by a needle, but also reduced the mean clotting time in human blood samples (253.4±44.1 seconds for control blood samples versus 143.4±114.6 seconds for those samples which were treated with CoolClot). The observed difference was statistically significant ($P<0.01$). Our findings indicated that CoolClot can be introduced as a novel cost effective hemostatic agent that may save human life in battlefield, accidents or sport fields. It should be noted that in battlefields, severe hemorrhage is the first leading cause of death which can be prevented by the standard damage control principles.[26,27]

A great advantage of CoolClot was lack of temperature rise in wound area. After the September 11th terrorist attacks, the US navy started wide studies in new technologies for stopping hemorrhages.[28] In these studies, the hemostatic agent which was at the focal point of interest was QuikClot. The main compound of QuikClot is zeolite mineral which like a mesh or a sieve keeps the particles in a molecular cage and holds them together by forming hydrogen bonds. It is assumed that QuikClot can stop bleeding by absorbing water from blood by concentrating the natural hemostatic factors in blood, or activating platelets and also by enhancing the phases of coagulation. Experiment conducted on the hemostatic properties of QuikClot showed the possibility of occurrence of exothermic reactions that could cause damage to the skin in prolonged contacts.[28] In this regard, as no exothermic reaction was observed when CoolClot was used, using this new hemostatic agent may not lead to skin damage.

On the other hand when economical factors are taken into account, it can be revealed that CoolClot is more cost effective than other hemostatic agents which are commercially available. For example, the current cost of fibrin glue per patient in middle east is approximately 700 US dollars, whereas CoolClot gauze costs about 5 US dollars.[29]

Based on the above mentioned advantages, the cardinal properties of CoolClot are: dramatic decrease in the volume of blood loss from the wounded area; dramatic decrease in bleeding time; decreasing the clotting time; lack of exothermic and tissue damage after topical use; no short-term or long-term side effects; cost effectiveness; wide range of applications from wounded soldiers or athletes to life-threatening accidents.

CoolClot rapidly absorbs water in the wounded area and it concentrates the cellular and protein components of blood and hence enhances the formation of clot. It should be noted that hemostatic agents such as QuikClot absorb water in an exothermic reaction and the high level of heat that is associated with its use, causes great concerns for its safety, while in case of CoolClot we had no exothermic reactions. The absence of exothermic reactions in CoolClot is highly indebted to the presence of bentonite.

In this study, bentonite mineral could have a significant role in reducing the topical temperature by controlling the exothermic reactions in bleeding place. The results of this study as well as the previous phrases indicate that topical use of CoolClot may have a significant role in reducing the bleeding time, clotting time and the volume of blood loss.

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