THE EFFECT OF APPLICATION OF LOCAL OXIDISED CELLULOSE POWDER ON HEMOSTASIS TIME IN A RAT MODEL WITH FEMORAL ARTERY BLEEDING

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ABSTRACT

***Aims**: Whether pre-hospital area or emergency department, the early diagnosis and control of bleeding is the first critical step in the treatment of trauma patients. This becomes increasingly critical as the victim-to-medical-personnel or equipment ratio becomes higher (disasters, wars and/or mass casualty events e.g.). We purposed to investigate the hemostatic effect of application of local oxidised cellulose powder on time to hemostasis time in a rat model with severe femoral artery bleeding.*

***Materials and methods**: The femoral arteries of 10 female Wistar albino-strain rats were punctured under ether anesthesia with 22G Branule. Whether in the control group we used only local compression, in the study group, we used compression along with application of standard weight compress together with oxidised cellulose powder. We investigated the time to hemostasis for the both groups.*

***Results**: In the study group, bleeding stopped at the 2nd minute in two and the 4th minute in three of five rats. In the control group, hemostasis could not be achieved in any of the rats even at 4th minute. The difference between two groups was statistically significant (p=0.004).*

***Conclusion**: Application of oxidised cellulose powder and compression both significantly decreased the time to hemostasis in this experimental rat model with femoral artery bleeding.*

***Key words**: Bleeding, oxidised cellulose, hemostasis, local hemostatic agent.*

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Introduction

Not all trauma patients will have the chance to be seen in an advanced trauma care center and the majority of patients with major bleeding will die in the first half-hour. According to recent literature, opinions regarding aggressive resuscitation with fluids and blood transfusion lean toward the result of coagulopathy and worsened bleeding. Due to this reason, definitive hemorrhage control has been emphasized, and resuscitation with blood and fluid is undermined[1-3]. Moreover, hemostasis must be ensured by the time of surgery, with serious consideration regarding the potential for coagulopathy, hypothermia and acidosis as a result of aggressive fluid resuscitation[4-6].

Despite the proliferation of state-of-the-art facilities, obtaining hemostasis in bleeding trauma patients remains one of the biggest challenges in emergency departments and in the field (e.g. wars, disasters, etc.). 51% of deaths occurring in the first 48 hours are due to unsuccessful hemostasis. Failure of hemorrhage control in the first 24 hours always results with high morbidity and mortality[4-6].

Use of local hemostatic agents started at the beginning of the 1900s, with the use of fibrin. Oxidised cellulose (OC) powder has been developed by Frantz and its initial reports of surgical use of that substance were around the 1940s[7].

The real mechanism of OC through cessation of bleeding is not clear yet. It is thought that OC accelerates the polymerization of fibrinogen and

works as a cofactor in hemostasis process due to its Ca+2 content. This substance swells after contact with fluids (It can absorb water as much as seven times its own weight) and fills up the wound effectively, mobilizing the coagulation system. Some authors define the function of OC as the structural skeleton of the physiological clot formation. At the same time, by decreasing pH and activating clot formation, OC destroys the structure of albumin and globulin. It is an absorbable material, with absorption taking place in 27 to 28 days when used as a local agent. No problems have been reported yet regarding possible systemic absorption of the material[8-11].

Matherials and methods

Ten Wistar albino female rats weighing 200g to 250g were used. We used the same methodology used in Ersoy et al's study, as it was shown to be effective[12]. Left femoral artery was cannulated by 24G Branule. Right femoral artery was perforated in order to obtain active bleeding by 22G Branule.

Following this phase, the rats were divided into two different equal groups; control ("standard pressure", n=5) and study (both "standard pressure" and "OC", n=5) groups. 0.5g of OC was applied to the area and then 200g standard weight pressure was applied and maintained. At that point, a chronometer was set to determine the timing of cessation of the bleeding.

At the end of the 1st minute, after removal of the scale weight, cessation of bleeding was checked by an emergency physician who is not an individual of the study design. In case of detection of cessation of bleeding at the 1st minute, it was recorded as the time of cessation. If the bleeding continued at the end of the 1st minute, a second, equal dose of OC was applied along with the same type/duration of pressure until the next minute. In case of hemorrhage cessation at the 2nd minute, it was recorded accordingly. If bleeding did not cease, the same set of aforementioned actions were repeated until the 4th minute. If cessation of bleeding was achieved at the third attempt, it was recorded as cessation occurred at the 4th minute. It was documented as an unsuccessful application if bleeding continued.

This same standard process mentioned above was performed on all 5 rats also in the study group. The same process was also repeated in the control group by only with the standard weight of 200g, but without application of OC.

The timing of bleeding cessation, mean arterial pressure (MAP) and heart rate (HR) were all recorded at the beginning, at the 1st, 2nd, and 4th minutes. Arterial blood gas samples were obtained just prior to creation of bleeding and at the end of the study. Moreover, samples were taken from the femoral artery at the beginning and at the end of the study (4th minute) and periodic times to check for partial carbon dioxide pressure ($PaCO_2$), saturation of O2 (SaO_2) partial oxygen pressure (PaO_2), pH, bicarbonate (HCO3), base excess (BE), hemoglobin (HB), hematocrit (HCT), sodium (Na^+), potassium (K^+) and calcium (Ca^{+2}).

Results

There was no statistically significant difference between groups on behalf of weight, vital and metabolic parameters of the rats. There was also no statistically significant difference among HR, MAP, $PaCO_2$, PaO_2, SaO_2, Na^+, K^+, Ca^{+2}, HCO_3, BE values (Mann Whitney U, $p>0.05$). However, statistically significant differences were detected in HB, HCT and pH values at the 4th minute between the two groups (Mann Whitney U, $p<0.05$) (Table 1).

Cessation of bleeding could not be achieved in any of the rats in control group, even at the end of the 4th minute. However, bleeding was stopped in three-out-of-five study group rats at the 2nd minute, and at the 4th minute in the other two rats. The difference between two groups was statistically significant ($p=0.004$) (Table 2).

Discussion

Till today, many experimental animal models have been used to in order to show the efficacy of topical homeostatic agents[11-18]. Unfortunately, we could not find a stable and universal experimental animal to compare the hemostatic properties of all similar agents already available on the market[19]. We used the same methodology used in Ersoy et al's study[12].

We could not find any article about the use of OC to create hemostasis in external arterial active bleeding. From that perspective, this is the first and unique study in which OC is used for that purpose on such an animal model. We were unable to find dosing information for the use of OC in capillary, small venous or parenchymal bleeding during surgical interventions[8-10, 20]. We planned to use OC in an amount necessary to cover the surface of the

wound. In this way, we determined a standard dose of 0.5 g of OC for this animal model. According to the package insert, hemostasis could be achieved in two-to-three minutes after utilization of OC[21]. By the way, we aimed to investigate time spent when hemostasis was achieved in zero, first, second, third and the fourth minutes.

	Control Group*	Study Group*	p Value
Weight (gr)	226±5	224±5	0.690
HR (rate/min)			
0. min	314±86	334±21	0.548
1. min	319±81	322±50	0.841
2. min	310±94	307±60	1.000
4. min	307±99	293±44	1.000
MAP (mmHg)			
0. min	90±8	86±12	0.421
1. min	80±15	75±4	0.151
2. min	80±15	71±5	0.151
4. min	78±18	63±6	0.151
pH			
0. min	7.41±0.03	7.45±0.03	0.056
4. min	7.40±0.03	7.30±0.06	0.008**
$PaCO_2$ (mmHg)			
0. min	35±5	29±1	0.151
4. min	34±8	42±7	0.056
PaO_2 (mmHg)			
0. min	95±31	128±7	0.151
4. min	107±31	86±41	0.151
SaO_2 (%)			
0. min	96±2	99±1	0.095
4. min	96±2	92±5	0.151
Hb (g/dl)			
0. min	11.2±1.8	10.2±1.2	0.310
4. min	6.0±2.0	8.5±0.3	0.008
Hct (%)			
0. min	33.0±5.3	30.0±3.5	0.222
4. min	17.6±5.9	24.9±0.9	0.008
Sodium (mmol/L)			
0. min	144.0±1.0	144.3±1.6	0.690
4. min	145.2±4.0	145.0±0.9	0.690
Potassium (mmol/L)			
0. min	3.6±0.5	3.6±0.6	0.690
4. min	3.6±0.7	4.4±1.3	0.310
Calcium (mmol/L)			
0. min	1.3±0.1	1.3±0.0	1.000
4. min	1.3±0.2	1.3±0.1	0.841
Bicarbonate(mmol/L)			
0. min	21.7±1.2	19.6±0.3	0.056
4. min	20.5±4.4	20.5±1.2	0.548
Base excess (mmol/L)			
0. min	-2.6±0.9	-2.8±0.9	0.841
4. min	-4.1±3.0	-5.5±1.6	0.151

Table 1: Comparison of weight, vital and metabolic values in the study and control group.
**Mean value±standart deviation, **Mann Whitney U (p<0.05).*

Current literature is dominated by studies on hemostasis in cases with internal bleeding. As ours, is the first and unique study, experimenting hemostasis in external arterial active bleeding after OC application, we can only compare our results with the studies with internal bleeding. As an example, Hanks et al. compared the hemostatic effects of fibrin sealant and OC in multiple surgical interventions in their multicenter, prospective, randomized controlled study[22]. They found that hemostasis time was shorter in the group which used the fibrin sealant compared with the group which used OC. Tugnoli et al. used OC in addition to the primary suturing for control of bleeding in one group and fibrin sealant in the other group[23]. Bleeding stopped in two-to-eight minutes in the OC group and OC was observed to be absorbed in 7 to 14 days without any complications.

		Bleeding at 0. minute	Bleeding at 1st minute	Bleeding at 2nd minute	Bleeding at 4th minute
Control Group	1. rat	Yes	Yes	Yes	Yes
	2. rat	Yes	Yes	Yes	Yes
	3. rat	Yes	Yes	Yes	Yes
	4. rat	Yes	Yes	Yes	Yes
	5. rat	Yes	Yes	Yes	Yes
Study Group	1. rat	Yes	Yes	Yes	*ceased*
	2. rat	Yes	Yes	Yes	*ceased*
	3. rat	Yes	Yes	*ceased*	*ceased*
	4. rat	Yes	Yes	*ceased*	*ceased*
	5. rat	Yes	Yes	*ceased*	*ceased*

Table 2: Comparison of cessations' time of bleeding in the study and control group.
Fisher's Exact Test; second minute p=0.083, fourth minute p=0.004.

In our experimental model on rats with femoral arterial bleeding, hemostasis was achieved in the first four minutes in the study group; however, hemostasis could not be achieved in the control group. These results were consistent with the literature.

OC is used fundamentally to create hemostasis in capillary, venous and small arterial bleeding cases[9, 10, 18, 20, 24]. Distinctively, here in this study, we have determined that topical application of OC was also effective in the control of external active bleeding in serious external arterial bleeding cases.

In all studies, including ours, on the use of hemostatic agents for external bleeding, vital and metabolic values were also studied. In their study with a similar model, Aydinoglu et al. did not find any significant statistical differences between the vital and metabolic parameters (HR, MAP, $PaCO_2$, PaO_2, O_2 Sat, HB, HCT)[13]. Ersoy et al. did not find any statistical differences between the control and study groups among the vital and metabolic parameters either (HR, MAP, $PaCO_2$, PaO_2, pH, glucose, Na^+)[12]. However, HCT values have been found to be significantly elevated in the study

group compared to the control group at 60 and 90 seconds[12]. In our study, we found low HB and HCT values at the 4th minute in both groups. However, the values in the control group were lower, and this difference was statistically significant. We inferred that this result was due to the continuation of bleeding in the control group even at the 4th minute. On the other hand, pH levels were higher in the control group, while one would expect a lower pH due to ongoing bleeding and its expected result "hypoperfusion". Nevertheless, it may not be possible to come to such a definite conclusion through a 4 minute experiment. Some more studies with longer duration of observation are needed to discover possible metabolic side effects of the use of OC. We did not find any statistical significant difference in the other metabolic or vital parameters.

Conclusions

OC could be a good option to control hemorrhaging in ambulances, at home (via first-aid kits) or in circumstances with limited medical personnel, such as natural disasters, wars and/or mass casualty events. On the other hand, we believe that to confidently recommend OC use for these purposes it must be validated by human studies.

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