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Original Article

Ability of the ankaferd blood stopper® to prevent parenchymal bleeding in an experimental hepatic trauma model

Erhan Aysan, Hasan Bektas, Feyzullah Ersoz, Serkan Sari, Arslan Kaygusuz, Gulben Erdem Huq

Istanbul Educational and Research Hospital, Department of General Surgery, Istanbul, Turkey.

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Abstract: Hepatic parenchymal bleeding (HPB) is a major problem following both trauma and elective hepatic procedures. The present study investigated the effect of the Ankaferd Blood Stopper® (ABS) on HPB. Method(s): A total of 20 rats were used. After creating a laceration model in the left lateral hepatic lobe, the area was compressed for 3 minutes with the ABS in the rats in group 1 (n=10) and with 0.9% NaCl-soaked gauze in the rats in group 2 (n=10). Results: The mean change in haematocrit levels between baseline and the 24 hour values in group 1 was lower than group 2 (p=0.045). The mean perioperative bleeding in group 1 was lower than group 2 (p=0.003). The histopathologic evaluation revealed that there were no differences between the groups with respect to areas of necrosis (p=0.107) or inflammation (p=0.135). Conclusion: Although the ABS does not stop HPB completely, it ensures a statistically significant reduction in HPB.

Keywords: Bleeding, parenchyma, hepatic, trauma, elective, surgery, Ankaferd Blood Stopper

Introduction

Hepatic parenchymal bleeding (HPB) is a life-threatening clinical entity. The only purpose of emergency surgical intervention in hepatic trauma is to stop the bleeding [1]. The most common complication in all surgical interventions associated with the liver is haemorrhage. Mortality has been reported to be 3% to 14% in major hepatic surgery, with haemorrhage being the most common cause [1-3]. A number of studies have been conducted and various surgical approaches and technologies have been introduced to prevent HPB, including Pringle's maneuver, selective hilar vascular control [4], packing [5], cellulose compounds [6], gelatin sponges [7], microfibril collagen [8], collagen-based composites [9], water-jet scalpels [10], harmonic cutters [11], and microwave coagulators [12].

The liver is the most vessel-rich organ in the human body. The hepatic parenchyma does not have smooth muscle and has few collagen tissues. These characteristics are significant in terms of bleeding for two reasons: 1) vasoconstriction cannot occur since there is no smooth muscle contraction and 2) parenchymal stitches do not have the benefit of the resistance created by collagen fibers [13]. In various hepatic disorders, such as cirrhosis, the risk of bleeding, maintaining hemostasis, and re-bleeding after achieving hemostasis are further increased [14].

In this present study, we investigated the efficacy of the Ankaferd Blood Stopper® (ABS), a unique medicinal plant extract produced for stopping external bleeding, on preventing HPB.

Methods

This study was conducted at the Experimental Animal Breeding and Research Laboratory of Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey. Upon approval of Animal Ethics

Table 1. Histopathologic evaluation grading

Grade	Necrosis	Inflammation
0	No necrosis	No inflammation
1	Focal, minimal (%1)	Focal, minimal (%1)
2	Light (<%25)	Light (<%25)
3	Mild (%25-50)	Mild (%25-50)
4	Serious (>%50)	Serious (>%50)

Committee of the same laboratory, the procedure was initiated.

The rats were kept in laboratory animal cages with plastic bottoms and sides and a wire cage cover atop. The rats were fed with pellet-type laboratory animal feed. A total of 20 out-bred Wistar albino male rats (mean weight, 380±20g; mean age, 6 months) were used. First, the tail vein was cannulated and blood was drawn to fill two standard hematocrit (htc) tubes. The tubes were centrifuged at rpm for 5 minutes on a htc measurement device. The htc value obtained was recorded as the htc before the first laparotomy. After randomizing the rats into two equal groups we created a standard non-anatomic hepatic resection model on each group (as described below). On the surface where the resection had been performed, compression was applied for 3 minutes by using standard cotton gauze soaked in 1 ml of ABS (Trend-Tech Co.) in group 1. The same procedure was repeated in group 2 by soaking the gauze in 1 ml of saline solution (0.9% sodium chloride, Eczacibasi Co.). After 3 minutes, the gauze was removed and a plastic bag was placed under the liver. The bleeding from the surface that had been subjected to resection was drained into the plastic bag for 3 minutes. This amount was recorded as the perioperative haemorrhage volume (ml).

The rats were sacrificed by means of an extended ether inhalation 24 hours after the operation. Laparotomy was performed using an inverted U incision. The abdomen was explored. The liver surface where the resection had taken place and had contact with liquid-absorbed gauze was excised. Meanwhile, blood was collected into a htc tube from the bleeding hepatic parenchyma. All htc tubes were centrifuged on the same htc measurement device for an equal amount of time. The values obtained were recorded as htc values during the second laparotomy (24 hours after the first laparotomy). Resected hepatic parenchyma was fixed in formaldehyde to be kept for histopathologic evaluation. The resected hepatic parenchyma tissues were inserted into paraffin after dehydratation and 5 mm sections were stained with haematoxylin-eosin. They were graded at x100 enlargement in terms of the presence of necrosis and inflammation consistent with the histopathologic classification below (**Table 1**).

Operation and resection model

After an overnight fast, the rats were put in jars containing ether for 45-60 seconds to induce anesthesia. Anesthesia was maintained with intramuscular ketamine at a dose of 75 mg/kg. After shaving the midline of the abdomen, antisepsis was assured with povidone-iodine. Entry into the abdominal cavity was made through a 3 cm vertical midline incision, starting directly below the xiphoid. The right medial and the left lateral lobes of the rat liver, consisting of four lobes (right lateral, right medial, left lateral, and left medial) were discarded, revealing the left medial lobe. The longest transverse line was established without changing the anatomic position of the lobe, and a non-anatomic resection was performed on that line (**Figure 1**). Resected amount of liver were about 1 to 2g. After allowing 3 minutes for compression and 3 minutes for bleeding, the abdominal midline incision was closed by twice suturing with 3/0 polypropylene without interfering with the bleeding parenchymal surface.

This present study had two primary evaluation parameters. First, the volume of bleeding during the first laparotomy and the difference between htc values during the two laparotomies was established. The secondary evaluation parameter for the study was the histopathologic evaluation of the hepatic parenchyma that had been in contact with the ABS or saline solution.

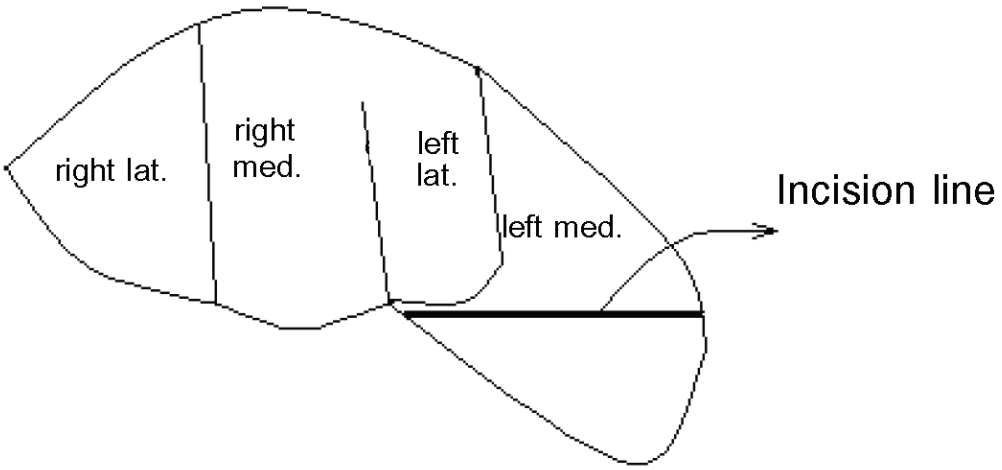

Figure 1. Rat liver anatomy and location of incision line of our non-anatomik resection model.

Table-2: Statistical analysis of htc. values

	Group 1 (ABS)	Group 2 (Control)	MW	p
1'st Lap. Htc.	59.5±2.99	58.8±3.12	39.5	0.422
2'nd Lap. Htc.	50.3±8.35	41.4±7.93	23	0.039
Z	-2.81	-2.81		
p	0.005	0.005		

Statistical evaluation

Statistical analyses in this study were performed utilizing GraphPad Prisma V.3 software. Along with descriptive statistical methods (mean and standard deviation), the Mann-Whitney U test was used for making comparisons between the groups. Comparison of qualitative data was made by using chi-square test and comparison of htc values obtained during the first and second laparotomy was made by using the Wilcoxon test. Statistical significance was set at $p<0.05$.

Results

No statistically significant differences were observed between groups 1 and 2 with respect to mean htc values before the first laparotomy (p=0.422). The mean htc values for both groups during the second laparotomy were significantly lower than before the first laparotomy (p=0.005). However, the mean htc value for group 1 during the second laparotomy was significantly higher than group 2 (p=0.039, **Table 2**). The mean difference in htc values between the two laparotomies in group 1 was significantly lower than group 2 (p=0.045). The mean perioperative bleeding during the first laparotopy in group 1 was shown to be significantly lower than group 2 (p=0.003; **Table 3**). Histopathologic evaluation revealed no statistically significant differences between the groups with respect to necrosis (p=0.107) or inflammation (p=0.135; **Table 4**).

Discussion

Few prospective randomized studies exist in the literature concerning the management of HPB. The chief reason behind this is the ethical issues regarding experimental human studies due to the serious morbid consequences and mortality that such hemorrhage poses. Conse-

Table 3. Statistical analysis of htc. difference and perioperative bleeding

	Group 1 (ABS)	Group 2 (Control)	MW	p
1'st – 2'nd Lap. Htc. Difference	9.2±7.93	17.4±8.32	23.5	0.045
Peroperative Bleeding (ml)	0.95±0.546	2.1±0.79	10.5	0.003

Table-4. Histopathologic evaluation results and statistical analysis

		Group 1 (ABS)		Group 2 (Control)		
Necrosis	Fokal, minimal (%1)	0	0%	4	40%	
	Light (<%25)	6	60%	5	50%	
	Mild (%25-50)	3	30%	1	10%	χ^2:6,09
	Serious (>%50)	1	10%	0	0%	p=0,107
Inflammation	Fokal, minimal (%1)	1	10%	5	50%	
	Light (<%25)	8	80%	4	40%	
	Mild (%25-50)	1	10%	1	10%	χ^2:4
	Serious (>%50)	0	0%	0	0%	p=0,135

quently, we carried out our experimental study on rats. We explored the effectiveness of ABS, which had not been previously investigated in any in vivo studies.

There are two fundamental approaches in the management of bleeding in all parenchymal organs, including the liver. Approaches for decreasing the volume of blood reaching the bleeding parenchyma include lowering the blood pressure, interrupting the blood flow into the vein proximally, vasoconstrictor agents with systemic effects, anatomic resection, and proximal vascular bypass. Approaches for preventing bleeding through the injured vessel include vein ligation, suturing the parenchyma, tissue glue, locally effective vasoconstrictor agents, mechanical compression, electrocoagulation, freezing, diathermy, laser, ultrasonic wave, and local hemostatic substances [15-17].

We aimed to create an effective HPB in the present study. Therefore, we performed a non-anatomic laceration on the widest transverse line of the left lateral lobe, which is the largest of the four lobes (right lateral, right medial, left medial, and left lateral) in the rat liver, and resected the distal piece (**Figure 1**). While the model we developed ensured widespread parenchymal capillary/arteriolar bleeding, it led to major acute bleeding by severing the main vascular structures (usually three) in the left lateral lobe.

In the control group, the volume of bleeding in the perioperative stage was 2,1±0,79 ml during the first laparotomy and a decrease by 17.4±8.32 ml was observed in the htc level during the second laparotomy, reflecting the severity of the bleeding created in the model. We had the option of creating blunt hepatic trauma as a parenchymal bleeding model; however, there are problems in blunt trauma models concerning the standardization of parenchymal damage and the volume of associated bleeding. Since the model we used allowed for a parenchymal incision of equal thickness and length, we were able to evaluate the results more objectively.

The ABS comprises a standardized mixture of five plants: Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum, and Urtica dioica. Each of these plants has specific effects on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics, and cell mediators [18-24]. For example, G. glabra inhibits angiogenesis, decreases vascular endothelial growth factor production, and cytokine-induced neovascularisation [21]. T. vulgaris has been shown to exhibit varying levels of antioxidant activity, which may help to prevent in vivo oxidative damage, such as lipid peroxidation associated with atherosclerosis [19]. V. vinifera has an anti-atherosclerotic effect [25]. A. officinarum inhibits nitric oxide production in lipopolysaccharide-activated mouse peritoneal

macrophages [18]. U. dioica produces hypotensive responses through a vasorelaxation effect mediated by the release of endothelial nitric oxide and the opening of potassium channels, and through a negative inotropic action [20].

ABS is a novel preparation, launched in 2007. The only study on ABS in the literature was by Goker et al. [25], which was an in vitro study demonstrating the mechanism of action of ABS. It revealed that when ABS is added to plasma or serum, it induces a very rapid (<1 sec) formation of a protein network and erythrocyte aggregation. The levels of coagulation factors II, V, VII, VIII, IX, X, XI, and XIII are not affected by ABS. Plasma fibrinogen activity and antigen levels decrease following the addition of ABS in parallel to the prolonged thrombin time. Total protein, albumin, and globulin levels decrease after the addition of ABS. Together; the findings suggest that ABS stimulates the formation of an encapsulated protein network, providing focal points for erythrocyte aggregation.

ABS is a local hemostatic. The agents in this group are called topical hemostatics or blood stoppers in the literature, and they can be found in powder, gauze, sponge, tampon, or liquid forms. Topical hemostatics are recommended for the management of low-volume bleeding rather than major hemorrhage.

The general mechanism of action of agents in the local hemostatics group is as follows: creating a plug by introducing a foreign object (gelatin sponge, oxidase cellulose, and oxidase regenerated cellulose) or activating thrombocytes upon contact and ensuring the release of mediators that trigger the natural hemostatic process (thrombin and microfibril collagen). Some agents have additional mechanisms of action, such as the adhesive effect of fibrin preparations and the vasoconstrictor effect of adrenalin [5, 7, 9].

ABS is unlike other local hemostatic agents because its mechanism of action is based on forming a polymerized protein network which becomes a focal point for sedimentation of erythrocyte aggregates. In other words, ABS causes neither a foreign object reaction nor thrombocyte activation. The unique mechanism of action of ABS offers a major advantage; not only does it provide hemostatis in patients with normal hemostatic parameters, it can also ensure hemostatis during bleeding episodes in patients with primary and secondary hemostasis impairment, unlike other agents.

This first in vivo study carried out with ABS demonstrated that when compared with the controls, ABS significantly reduced parenchymal bleeding occurring as a result of non-anatomic liver resection. ABS demonstrated its effect by revealing a statistically significant difference compared with the controls both in the early intraoperative stage (in the first 3 minutes after the bleeding started), and in the later stage (in the first 24 hours after the bleeding started).

Histopathologic evaluation demonstrated that although the presence of both necrosis and inflammation was slightly higher in the ABS group compared with the controls, the difference was not statistically significant. The slight difference in the presence of necrosis and inflammation in the ABS group can be attributed to the hemostatic effect and, therefore is not unusual. It is natural for liver parenchyma to have an inflammatory reaction upon contact with a mixture consisting of five different plants, and that more intense necrosis zones are observed at the microscopic level as a result of the hemostatic effect.

Karakaya et al were used hepatic laceration model to evaluate the hemostatic effect of ABS comparison with regenerated oxidized cellulose. They revaled that ABS is as effective as regenerated oxidized cellulose in achieving hemostasis [26].

In conclusion, although ABS did not stop liver parenchyma bleeding completely, it decreased the bleeding significantly. ABS is a promising novel and relatively effective agent in decreasing morbidity and mortality associated with HPB. Before starting human studies, the effect of the agent should be evaluated on larger laboratory animals. If the hemostatic effect observed is significant, the use of ABS in managing HPB may be considered. Consequently, the volume of bleeding can be decreased in surgical interventions on major parenchymal organs, such as the liver, lung, spleen, and kidneys (cholecystectomy, cystectomy, and nephrolithotomy) or in partial resections associated with these organs (partial hepatectomy, lung lobectomy, partial splenectomy, and partial nephrectomy).

Please address correspondence to: Erhan Aysan, PhD, Emirhan Cad. No: 3 Besiktas - Istanbul, Turkey. Post code: 80700. Telephone: +90-212-2279042, Fax: +90-212-8758620, E-mail: dr@webcerrah.com

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