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EVALUATION OF A HAEMOSTATIC AGENT IN RABBITS

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Abstract

Topical hemostatic agents are applied locally to areas of injured vascular endothelium to control local bleeding. Ankaferd Blood Stopper (ABS) has gained approval in Turkey and Bosnia-Herzegovina as a topical haemostatic agent for external post-surgical and post-dental surgery bleeding. The safety of topical use of ABS has been demonstrated in numerous *in vitro* and *in vivo* animal models, as well as in a clinical Phase I trial in humans. ABS, besides its haemostatic activity, also has *in vitro* anti-infectious and anti-neoplastic effects. To assess potential detrimental effects of intravenous administration of ABS into intact systemic circulation in a rabbit experimental model, one milliliter of ABS was administered intravenously into the systemic circulation of twelve rabbits which were included in the study via the marginal ear vein. Animals were observed for 1 hr before euthanasia was performed by administering 40 mg of intracardiac suxamethonium chloride. In the event of death (cardiopulmonary arrest) before the end of the planned observation period of 60 minutes, time of death was recorded and histopathological examination of the liver and spleen was commenced. Ten rabbits were alive by the end of the planned observation period, without showing any clear signs of discomfort, whereas two animals died within five minutes after systemic administration of intravenous ABS. Postmortem histopathological examination of the livers and spleens of all animals' revealed findings consistent with hepatic venous outflow obstruction. Systemic intravascular administration of ABS into intact vascular endothelium should never be performed in any setting. Further experimental and clinical studies on this liquid hemostatic agent should proceed by accepting ABS as purely a topical haemostatic agent, to be applied solely to areas of injured vascular endothelium.

Key words: Ankaferd blood stopper, rabbit, toxicity, intravenous administration.

Introduction

Ankaferd Blood Stopper (ABS) is a standardized herbal extract obtained from five different plants *Thymus vulgaris, Glycyrrhiza glabra*, *Vitis vinifera, Alpinia officinarum*, and *Urtica dioica* (Firat et al., 2009). ABS represents its unique local haemostatic effect by promoting the very rapid (<1 s) formation of a protein network which acts as an anchor for vital physiological erythrocyte aggregation, covering the classical cascade model of the clotting system without independently acting on coagulation factors and platelets (Goker et al., 2008a). ABS has been approved for local topical applications in dermal, external post-surgical and dental bleeding in Turkey and Bosnia-Herzegovina (Ercetin et al., 2010). ABS has been shown to be an effective hemostatic agent in both *in vivo* and *in vitro* studies (Goker et al., 2008a; Bilgili et al., 2009; Cipil et al., 2009; Huri et al., 2009a; Karakaya et al., 2009; Kosar et al., 2009; Ercetin et al., 2010). Bacteriostatic effects on gram positive and gram negative bacteria have also been demonstrated (Tasdelen Fisgin et al., 2009). It is currently approved for use in external and dental surgical bleeding (Ercetin et al., 2010). Its efficacy and safety as a haemostatic agent in dental surgery has been well documented, while at the same time contributing to wound healing (Baykul et al., 2010; Ercetin et al., 2010).

ABS has been used in the setting of gastrointestinal bleeding both as an adjuvant haemostatic agent and as a last resort where other conventional methods had failed (Ibis et al., 2008; Kurt et al., 2008a; Kurt et al., 2008b; Kurt et al., 2009; Ozaslan et al., 2009; Kurt et al., 2010). In these studies, the efficacy of ABS was once again demonstrated, particularly for bleeding from GIS tumors (Kurt et al., 2010). Furthermore, in two patients a decrease in tumoral microvessel density was observed, which may indicate a possible anti-angiogenic effect of ABS (Turhan et al., 2009). Similarly, ABS was beneficial in the management of chronic bleeding due to a solitary rectal ulcer (Ibis et al., 2008). Its efficacy was once again proven in a Klatskin tumor patient with severe GIS bleeding and epistaxis (Kurt et al., 2009). It has also been utilized as a bridge to

cyanoacrylate injection in a patient with active bleeding from a fundal varix (Tuncer et al., 2010). It has also been shown to be more effective than standard sterile sponges for controlling bleeding during port insertion, as well as being associated with lower rebleeding rates (Al et al., 2009). A recent study reported on an advantage of ABS over conventional methods in decreasing bleeding and shortening operation time in children undergoing tonsillectomies (Teker et al., 2009). It has also been used with success in a young girl with afibrinogenemia who presented with a finger cut (Ucar Albayrak et al., 2008). Favorable results have also been reported for prostatectomy (Huri et al., 2009b). ABS, besides its hemostatic activity, has also *in vitro* anti-neoplastic actions (Goker et al., 2008b; Goker et al., 2008c).

The safety of ABS has not yet been equivocally established, and its mechanism of action prompts serious concerns regarding a possible risk associated with intravenous leakage of the substance following gastrointestinal application. The aim of this study was to assess potential harmful effects of intravenous administration of ABS into intact systemic circulation in an experimental rabbit model.

Materials and methods

This experimental study was conducted with the approval of the Fatih University Medical School Ethics Committee. All procedures were in full compliance with Turkish Law 6343/2, Veterinary Medicine Deontology Regulation 6.7.26 and with the Helsinki Declaration of World Medical Association recommendations on animal studies.

Twelve rabbits, housed in metal cages with a wire netting bottom, maintained at a temperature of 23ºC (± 5ºC), were used in this study. The animals were allowed free access to a solid diet and tap water, and were allowed to roam freely for an hr in a small garden, twice daily.

Experimental design

Each of the rabbits, which were randomly assigned a number from 1-12, were weighed. Estimated total blood volume was calculated for each rabbit using the formula (60 ml x weight in kilograms), after which 1 ml of ABS was administered intravenously via the marginal ear vein. Animals were observed for 1 hr before euthanasia was performed by administering 40 mg of intracardiac suxamethonium chloride. In the event of death (cardiopulmonary arrest) before the end of the planned observation period of 60 mins, time of death was recorded and histopathological examination of the liver and spleen was commenced, performed by the same experienced pathologist (NT).

Table 1. Characteristics of the rabbits and histopathological findings

Rabbit	Weight (gr)	ABS		SUMMARY OF HISTOPATHOLOGICAL FINDINGS
		ml_{ABS}/ml_{TBV} ($x10^{-3}$)	ml_{ABS}/weight (kg)	**LIVER**
1	3900	4.598	0.256	• Sinusoidal dilatation and congestion (Rabbits 1-10) • Marked dilatation and congestion of the central and portal veins (All rabbits) • Early signs of perivenular necrosis (Rabbit 7) • Intraparenchymal herniation of a some of the central and portal veins (Rabbits 2-4, 6, 9-12) • Eosinophilic mass resembling fragmented erythrocytes, particularly within veins (All except rabbit 5) • Small- large subcapsular foci of confluent coagulative necrosis (Rabbits 2, 6-9,10-12) • Marked periductal inflammation, with lymphocytic infiltration of the biliary ductal epithelium (cholangitis) (Rabbit 4) • Hepatocellular cholestasis (Rabbit 6,9-11)
2	3100	5.9	0.323	
3	2900	6.349	0.345	
4	2850	6.472	0.351	
5	2900	6.349	0.345	
6	4500	3.945	0.222	
7	2350	8.032	0.425	
8	2400	7.843	0.417	**SPLEEN**
9	3500	5.168	0.286	• All rabbits had signs of mild splenic congestion. • Subcapsular necrosis (Rabbits 10-12)
10	4200	4.246	0.238	
11	3700	4.866	0.270	
12	3600	5.013	0.278	

* TBV: Total Blood Volume

Results

The characteristics of the rabbits and histopathological findings were summarized in table 1. The median weight of the animals was 3300 gr (2350-4500 gr). Animals 7 (2350 gr) and 8 (2400 gr) died within 5 minutes of administration of intravenous ABS, during which time they were in obvious distress. These two were the smallest rabbits of the study group. The remaining animals were still alive by the end of the planned observation period, without showing any clear signs of discomfort.

Pericentral congestion and sinusoidal dilatation, as well as varying levels of necroinflammation and coagulative necrosis were observed in the livers of all the animals, findings consistent with acute hepatic venous outflow obstruction, which in these cases was most probably due to congestive hepatopathy occuring as a result of right-sided heart failure (acute cor pulmonale) (Figure 1). Rabbit no. 4 had findings consistent with cholangitis. However, this was most likely unrelated to ABS administration as structures resembling bile stones were observed in the biliary tree.

Similarly histopathological examination of the spleens of all animals revealed signs consistent with mild congestion, probably as a result of portal hypertension.

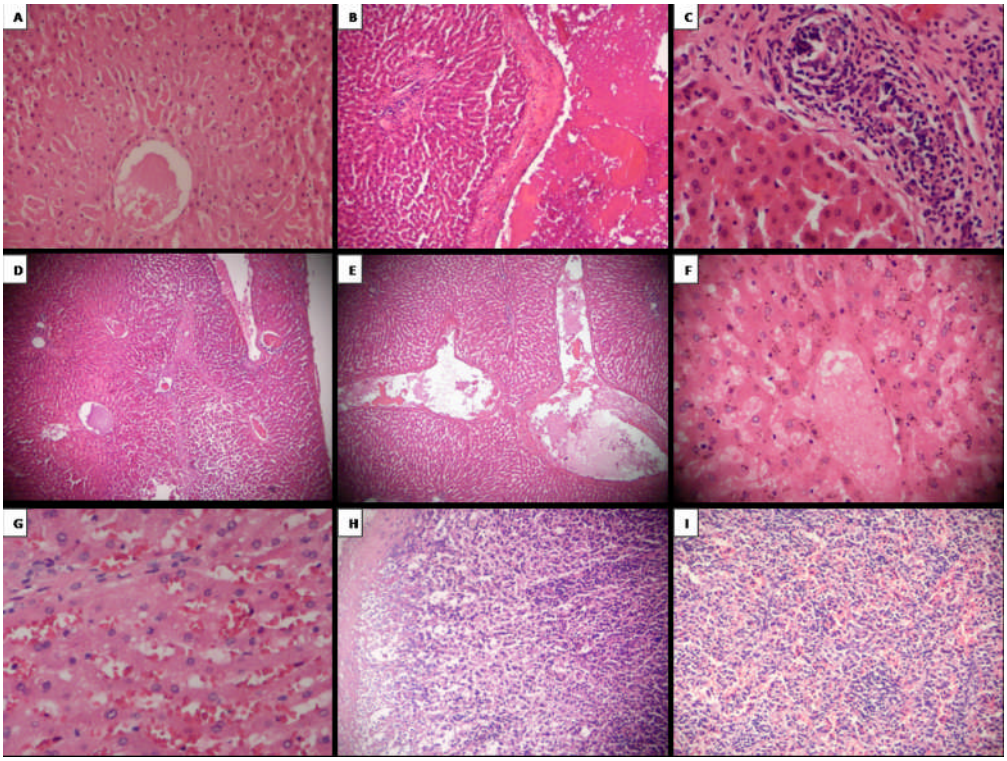

Figure 1. Histopathological examinations revealed A: Necrosis around the central vein. B: Eosinophilic mass. C: Cholangitis. D: Subcapsular necrosis and congestion of the central and portal veins. E: Intraparenchymal herniation of the central vein. F: Dilatation of the central vein and hepatocellular cholestasis. G: Sinusoidal dilatation and congestion. H: Subcapsular necrosis in the spleen. I: Mild splenic congestion.

Discussion

Ankaferd Blood Stopper is a unique standardized mixture of 5 plants: *T. vulgaris*, *G. glabra*, *V. vinifera*, *A. officinarum* and *U. dioica*, each of which has some effect on haematological and vascular parameters, and cellular proliferation (Goker et al., 2008a). Traditionally, these plant extracts have been used in fixed proportions (2 ml product contains; *Urtica dioica* (dried root extract) 0.12 mg, *Vitis vinifera* (dried leaf extract) 0.16 mg, *Glycrrhiza glabra* (dried leaf extract) 0.18 mg, *Alpinia officinarum* (dried leaf extract) 0.14 mg, *Thymus vulgaris* (dried grass extract) 0.1 mg) for the control of external bleeding from the skin (Firat et al., 2009). In earlier studies, the use of each of these extracts separately failed to demonstrate a meaningful haemostatic effect. Interestingly, this combination of all five plants in ABS appears to provide a unique composition promoting tissue oxygenation as well as initializing a physiological haemostatic process without involving any individual clotting factor (Goker et al., 2008a).

Extracts from several plants, such as *Sanguisorba officinalis, Sophora japonica, Nelumbo nucifera* have been shown to have haemostatic effects to varying degrees (Liao et al., 2008). However, in comparison, ABS as a traditional Turkish herbal medicine extract seems to have more practical implications in the field of medicine, particularly for the control of gastrointestinal bleeding.

There is a growing body of evidence on the efficacy of ABS as a haemostatic agent in several clinical settings, including the control of bleeding due to gastrointestinal cancers, mediastinal bleeding, haemorrhage after retropubic radical prostatectomy and post-tonsillectomy bleeding (Dogan et al., 2008; Ibis et al., 2008; Kurt et al., 2008a; Kurt et al., 2008b; Al et al., 2009; Arslan et al., 2009; Huri et al., 2009b; Kurt et al., 2009; Ozaslan et al., 2009; Teker et al., 2009; Baykul et al., 2010; Ercetin et al., 2010; Kurt et al., 2010; Tuncer et al., 2010). With special relevance to the gastrointestinal system, heater probe coagulation, sclerotherapy, haemoclips and argon plasma photocoagulation are the conventional and well established methods for haemostasis, As an herbal extract, ABS offers an advantage due to ease of application as well as efficacy in more challenging clinical settings such as malignant gastrointestinal bleeding, where none of the conventional methods has proved effective (Kurt et al., 2010). Although results so far have been very promising, lack of concrete data on the safety of ABS remains a serious concern. Application of ABS on compromised mucosa with exposed blood vessels runs the risk of accidental intravenous leakage, and subsequently systemic embolic complications.

In this rabbit model, blood in the marginal ear vein flows to the external jugular vein via the maxillary vein, after which it reaches the right atrium via the cranial vena cava. From there, blood is pumped to the right ventricle and into the pulmonary arteries, and subsequently the pulmonary capillary bed. When the mechanism of action of the haemostatic agent is taken into consideration, it is fair to expect ABS administered through the marginal ear vein to instantaneously result in formation of an “ABS-web” which would eventually get lodged in the pulmonary arteries / pulmonary capillary bed. Evaluation of ensuing hemodynamic instability and hypoxia could not realistically be evaluated in this rabbit model as it would have required invasive catheterization and monitoring. Simply demonstrating the presence of pulmonary emboli by histopathological examination of the lungs would not have sufficed, since it was essential to demonstrate any potential effects on haemodynamic, particularly acute cor pulmonale. For this purpose, histopathological examination of the liver and spleen was performed on the assumption that elevation in right heart and subsequently caudal vena cava pressure would result in liver congestion, and thus resulting in portal hypertension, and congestion of the spleen as well. Indeed, our results confirmed the presence of such findings.

The early deaths of the two rabbits in this study, as well as the histopathological findings of the surviving animals should be a reminder about the grave consequences of systemic placement of this topical haemostatic agent into intact systemic circulation. Systemic intravascular administration of ABS into intact vascular endothelium should never be performed under any circumstance. Further experimental and clinical studies on this liquid hemostatic agent should proceed by accepting ABS in the concept of topical hemostatic agent to be applied solely to the areas of injured vascular endothelium at the bleeding source.

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