

Ankaferd Blood Stopper Is More Effective Than Adrenaline Plus Lidocaine and Gelatin Foam in the Treatment of Epistaxis in Rabbits

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ABSTRACT

BACKGROUND: Epistaxis is an important emergency that can sometimes be life threatening without effective intervention. Persistent and recurrent bleeding can lead to aspiration, hypotension, hypoxia, or even severe and mortal cardiovascular complications. Providing prompt hemostasis is important, and the hemostatic method used must be easily and locally applicable, efficient, and inexpensive.

OBJECTIVE: The aim of this study was to assess the hemostatic efficacy of Ankaferd Blood Stopper (ABS) in an experimental epistaxis model and to determine the histopathologic alterations with topical ABS application.

METHODS: Twenty-eight New Zealand rabbits were evaluated in 4 study groups. Topical ABS, gelatin foam (GF), adrenalin + lidocaine (AL), and serum physiologic as negative control (C) were applied to the animals for controlling epistaxis. The bleeding was generated with a standard mucosal incision in all groups. Cotton pieces soaked with ABS, AL, C, and GF were applied to the nasal bleeding area. Time of hemostasis was recorded. Tissue samples were obtained after hemostasis for histopathologic examination. The samples were stained with hematoxylin and eosin (HE) and phosphotungstic acid hematoxylin (PTAH) and were examined under a light microscope. In this experimental study, the observers were blind to ABS, AL, and C but not to GF, because of its solid nature.

RESULTS: Median durations required for hemostasis in ABS, AL, GF, and C groups were recorded as 30, 90, 90, and 210 seconds, respectively. The time until termination of bleeding in the ABS group was significantly shorter than that in the AL, GF, and C groups ($P = 0.002$, $P = 0.002$, and $P = 0.001$, respectively). On histopathologic evaluation, after staining with HE, minimal fibrin at the incision

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edges and a few extravasated erythrocytes were observed in the C, AL, and GF groups. In the ABS group, a dark amorphous material surrounded by fibrin, filling the space between the edges of incisions, was noticed. Fibrin was determined in the C, GF, and AL groups with PTAH stain and in the positive control group. In the ABS group, it was observed that the amorphous substance surrounded by fibrin seen in the HE sections was not stained with PTAH.

CONCLUSIONS: Topical nasal ABS application controlled epistaxis faster than C, GF, and AL in this animal bleeding model. The bleeding model used here might fail to replicate the type of injury that would be likely to result in life-threatening bleeding in humans, which should be considered a limitation of the present study. The histopathologic findings in the nasal incision area suggest that ABS might affect global hemostasis by inducing a unique protein network formation, potentially representing a different mechanism of action among conventional antihemorrhagic applications. (*Curr Ther Res Clin Exp.* 2011;72:185–194) © 2011 Elsevier HS Journals, Inc. All rights reserved.

KEY WORDS: Ankaferd Blood Stopper, epinephrine, epistaxis, gelatin foam, lidocaine, rabbit.

INTRODUCTION

Epistaxis, bleeding from the nose, is an important emergency that may rarely be life threatening without effective intervention. Almost every physician has encountered epistaxis at least once in the outpatient clinic or emergency unit.¹ Approximately 60% of the population have nosebleeds at least once in their lifespan, and some of them require medical intervention.² However, in a minority of the patients, persistent and recurrent bleeding may lead to aspiration, hypotension, hypoxia, and even severe and mortal cardiovascular complications (eg, myocardial infarction).³ Several methods or techniques have been described to control epistaxis. Therapeutic methods can be classified under 2 headings: medical and surgical. Medical options to stop bleeding include cauterization with electrocautery or silver nitrate, gauze pad, absorbable hemostatic sponges, cotton impregnated with vasoconstrictor agents, and balloon methods. Surgical interventions include embolization, cryosurgery, septoplasty, and arterial ligation. The aim of all these therapeutic methods is to cease bleeding and diminish the rate of complications and treatment cost. Providing prompt hemostasis is important for patient comfort, length of hospital stay, prevention of complications, and cost of treatment. The hemostatic method being used must be easily and locally applicable, easily available, highly efficient, and inexpensive. Elucidation of the Ankaferd Blood Stopper (ABS; Ankaferd Medical Products Inc, Istanbul, Turkey) as a topical hemostatic agent in the setting of epistaxis is important for better clinical management of this challenging surgical emergency.

ABS is a novel topical hemostatic agent, which is indicated in surgical procedures when conventional control of bleeding by ligation or conventional procedure is ineffective. The mechanism of hemostatic effect of ABS is the formation of an encapsulated protein network representing focal points for vital erythrocyte aggre-

gation. The use of ABS in experimental studies,^{4,5} gastrointestinal bleedings,^{6–11} urologic surgery,^{5,12} tonsillectomy,¹³ and acute anterior epistaxis¹⁴ have been reported in the literature. According to these studies, ABS seems to be effective as a topical hemostatic agent.

In some conditions, it may take extensive time to control bleeding with known hemostatic agents. There are some reported cases in published literature about this situation and the results after ABS application.^{8,9} Despite these reports, there is no research in the literature comparing hemostatic efficacy and bleeding control time of ABS with other known hemostatic agents. Comparative histopathologic findings may help elucidate the hemostatic activity of this new agent. The aim of this study was to assess the hemostatic efficacy of ABS in an experimental epistaxis model and to determine the histopathologic alterations associated with topical ABS application.

METHODS

This study included 28 male, white, 20- to 42-week old (mean, 31 weeks) New Zealand rabbits weighing between 3000 and 5000 g (mean, 3850 g). They were fed rabbit growing pellets obtained from Korkutelim Feed Company (Antalya, Turkey) and fresh tap water. Room light was adjusted to 12-hour periods of dark and light; room temperature was set at 22°C (1°C), and humidity was set at 45%. The rabbits were divided into 4 groups: the ABS group, the gelatin foam (GF) group (GELITA-SPON, Gelita Medical, Amsterdam, the Netherlands), the 0.0125 mg/mL adrenaline plus 20 mg/mL lidocaine (AL) group (Jetokain Ampul, Adeka Inc, Samsun, Turkey), and the serum physiologic group as negative control (C), each consisting of 7 rabbits. Because AL and GF are commonly used hemostatic agents, they were used as the other treatment groups.

SURGICAL PROCEDURE

In all groups, 80 mg/kg of ketamine hydrochloride (Alfamine 10%; Alfasan Inc, Woerden, Netherlands) and 2 mg/kg of xylazine hydrochloride (Alfazyne 2%; Alfasan Inc) were intramuscularly administered as anesthesia. Each rabbit was given anesthesia only once under the same conditions. After administration of anesthetic agent, a mucosal incision using a blade, approximately 1 cm in length, was made on the anterior part of the septum in the right nasal passage of each rabbit to achieve bleeding.

INTERVENTIONS

ABS, AL, and C were put into injectors covered with black bands, each numbered randomly. Because of GF's lack of fluid, study of the GF group could not be blinded. Then, 1 × 1 cm cotton pieces with 0.5 mL of ABS, AL, or C and 1 × 1 cm of GF were applied to the bleeding area. During these double-blind processes, the investigators who performed the experiments and collected data were unaware of the agent used in the cotton, except for GF because of its solid nature. The bleeding area was inspected by removing the material every 30 seconds until bleeding stopped. Intervals for termination of bleeding were recorded.

HISTOPATHOLOGIC EXAMINATIONS

For the evaluation of histopathologic changes in the incision area, biopsy specimens of 0.5 cm, including the incision site, were collected 10 minutes after the termination of bleeding. The specimens were fixed in standard 10% formaldehyde solution and kept for histopathologic examination. During pathologic examination, 2 sections in 5- μm thickness were cut. One of them was stained with hematoxylin and eosin (HE) and the other was stained with phosphotungstic acid hematoxylin (PTAH). They were both examined under a light microscope. The investigator who performed the pathologic examinations of specimens was unaware of which specimen received which agent.

STATISTICAL ANALYSES

SPSS for Windows 13 software (SPSS Inc, Chicago, Illinois) was used for statistical analysis. Measurable variables were presented as median with ranges (minimum and maximum values). As a result of the Shapiro-Wilk normality test, the measurable variables were not normally distributed ($P < 0.05$). Therefore, Kruskal-Wallis variance analysis was used for the comparison of all groups and Mann-Whitney U test with Bonferroni test was used to compare paired groups. A P value < 0.05 was accepted as statistically significant.

Inonu University Ethics Committee for Experimental Animals (Research Protocol No: 2009-12) gave approval for the performance of the study.

RESULTS

In this experimental epistaxis model on rabbits, the time intervals for termination of bleeding were measured as 30 (range, 30–60), 90 (range, 60–150), 90 (range, 60–180), and 210 seconds (range, 180–300) in the rabbits that received cotton pieces soaked with ABS, AL, GF, and C, respectively. The period for termination of bleeding in the ABS group was significantly shorter than the AL ($P = 0.002$), GF ($P = 0.002$), and C ($P = 0.001$) groups. When compared separately, the periods for termination of bleeding in the AL and GF groups were significantly shorter than that of the C group ($P = 0.002$). There was no significant difference found between the periods for termination of bleeding in the AL and GF groups ($P = 0.789$).

On histopathologic evaluation, stained with HE, similar histomorphologic patterns were observed at incision edges in the groups that received C, AL, and GF. Minimal fibrin at incision edges and a few extravasated erythrocytes were observed (Figure 1). In the ABS group, a dark amorphous material surrounded by fibrin, filling the space between the edges of the incisions, was seen (Figure 2).

PTAH staining was applied to identify whether the fibrin-like amorphous substance at the incision line of all groups was fibrin. Sections prepared from fibrin without stain were used as positive controls. Fibrin was determined in the C, GF, and AL groups with PTAH stain and in the positive control group (Figure 3). In the ABS group, ABS formed a protein network; it was observed that the amorphous substance surrounded by fibrin seen on the HE sections was not stained with PTAH (Figure 4).

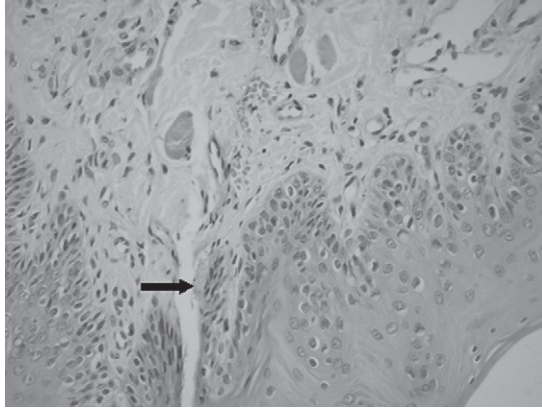


Figure 1. Minimal fibrin at incision edges in adrenalin + lidocaine, gelatin foam, and negative control groups (arrow) (hematoxylin and eosin \times 400).

DISCUSSION

Epistaxis may be an extremely difficult situation and potentially life threatening because of major bleeding.¹⁵ To control nosebleeding, various medical or surgical therapeutic protocols are implemented based on the type, severity, and cause of bleeding.¹⁶ Some of the medical methods used to control bleeding include cotton impregnated with AL or GF.³

Although there are articles mentioning the use of AL-absorbed cotton ribbons for local anesthesia and vasoconstriction in epistaxis,³ no study comparing locally applied AL soaked into cotton with other agents was found in the literature. The comparative

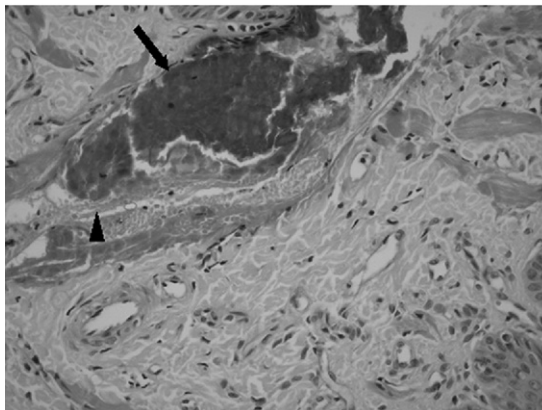


Figure 2. Dark amorphous substance (arrow) surrounded by fibrin at incision line (arrow-head) in ABS group (hematoxylin and eosin \times 400).

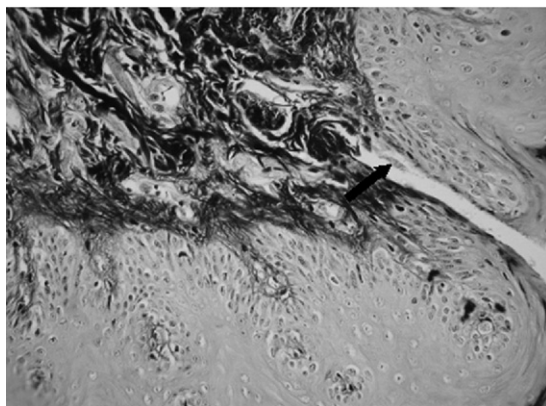


Figure 3. Fibrin at incision lines in adrenalin + lidocaine, gelatin foam, and negative control groups (arrow) (phosphotungstic acid hematoxylin \times 400).

studies about AL are related to its local injection. It was reported that 1% lidocaine + 1:100000 adrenalin was injected for vasoconstriction in persistent nosebleeding.¹⁵ Wormald et al¹⁷ reported that bleeding decreased by injection of lidocaine 2% + adrenalin 1:80.000 in endoscopic sinus surgery. Çiftçi et al¹⁸ reported results of patients who underwent dacryocystorhinostomy with general or local anesthesia. They observed epistaxis in 12 of the 182 patients under general anesthesia and 2 of 298 patients under local anesthesia with implementation of 2% of lidocaine + 1:100000 adrenalin combination. They also reported some adverse effects of adrenalin, including tachycardia, arrhythmia, and hypertension in the local anesthesia group.

GF is an absorbable hemostatic agent produced from the gelatin in animal skin.¹⁹ It adheres to the bleeding site, activates platelets, and triggers aggregation.²⁰ Ross-

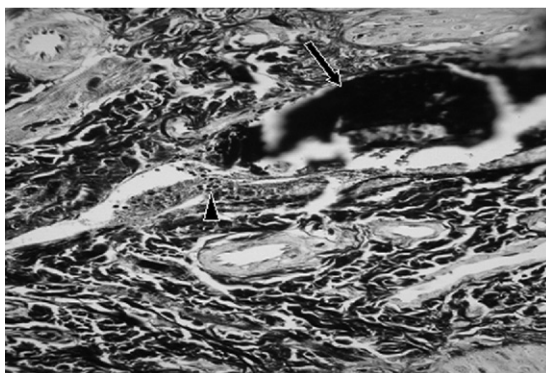


Figure 4. Amorphous substance (arrow) surrounded by fibrin (arrowhead) in Ankaferd Blood Stopper group (phosphotungstic acid hematoxylin \times 400).

mann et al²¹ applied pressure to wound sites with oxidized cellulose, GF, and gauze pads to control bleeding in 30 patients. They reported that duration of hemostasis was considerably shorter in the groups that received GF and oxidized cellulose compared with the control group. However, they reported that bleeding recurred in 40% of the groups of oxidized cellulose and controls, whereas bleeding did not recur in the GF group.²¹ Petersen et al²² reported that they placed oxidized regenerated cellulose and GF into the alveolus of extracted teeth, without any difference between 2 groups in terms of bleeding.

ABS-induced formation of a protein network with vital erythroid aggregation covers the entire physiologic hemostatic process.^{23–25} Mainly, there are distinct important components of the ABS-induced hemostatic network. Vital erythroid aggregation takes place with the spectrin and ankrin receptors on the surface of red blood cells. Those proteins and the required adenosine triphosphate bioenergy are included in the ABS protein library. ABS also upregulates the GATA/FOG transcription system affecting erythroid functions. Urotensin II is also an essential component of ABS and represents the link between injured vascular endothelium, adhesive proteins, and active erythroid cells.^{23,25,26} These concepts have been developed via Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometer (MALDI-TOF) proteomic molecular analyses, cytometric arrays, transcription analysis, and SEM ultrastructural examinations as well as numerous investigations interacting with *in vitro* and *in vivo* research settings.^{23,25,26} Three clinical ABS Phase III studies in humans performed in vascular port insertion bleedings, anterior epistaxis, and post-tonsillectomy hemorrhages proved its use as a hemostatic agent.^{13,14,27} Therefore, ABS could effectively be used both in individuals with normal hemostatic parameters and in patients with deficient primary hemostasis and/or secondary hemostasis. *In vitro* data on the antibacterial and wound healing profile of ABS as well as a bleeding control in human gastrointestinal disorders^{8–10} and human mediastinal bleedings²⁷ sheds further light on these critical issues.

Al et al²⁷ investigated the efficacy of ABS to control cutaneous and subcutaneous hemorrhages that resulted from vascular access in 69 patients with malignancy. This study compared the duration of hemostasis in 37 patients who received an ABS-absorbed tampon at the bleeding sites with 32 patients who received a dry gauze tampon. They reported the mean termination time of bleeding as 32.97 (29.9) seconds in the ABS group and 123.75 (47.5) seconds in the second group. They reported that bleeding recurred 24% and 50%, respectively. Teker et al¹³ evaluated 47 patients who underwent cryogenic tonsillectomy, who received ABS for hemorrhaging at the right tonsillar bed; suture ligation technique was used at the left side. They reported that hemostasis duration was significantly lower in the ABS site than in the sutured site. ABS was also successful in stopping gastrointestinal and nose-bleeding in a case in which conventional methods did not work.⁹ These findings, obtained from human studies, represented the initial clues for clinical approval of ABS as a hemostatic agent in Turkey.

In rabbits, physiologic mean blood clotting time is 210 seconds (range, 60–360).²⁸ In the present study, hemorrhage stopped in the ABS, AL, GF, and C groups

at 30 (range, 30–60), 90 (range, 60–150), 90 (range, 60–180), and 210 (range, 180–300) seconds, respectively. These results showed that ABS was more effective for bleeding control than GF and AL. These findings, obtained from experimental studies, will be used in novel trials intended to compare the tolerability and efficacy of ABS among other hemostatic agents in the future.

The groups that received C, AL, and GF showed similar histomorphologic features at the incision edges when stained with HE (Figure). On these materials, there were minimal fibrin and a few extravasated erythrocytes at the incision sites. In the ABS group, an acellular, dark eosinophilic, amorphous material surrounded with fibrin filling the gap between the incision edges was seen (Figure 2). The dark amorphous material seen in the ABS group was unique compared with the other groups because it was related to the unique ABS-induced hemostatic network. Protein agglutination with numerous red blood cells comprised the dark amorphous material observed in the ABS group. This finding requires further clinicopathologic correlations regarding the efficacy and hemostatic mechanism of ABS.

PTAH is used to recognize fibrin, glial fibrils, and muscle tissue. Fibrin tissue is seen as fibrils, in blue, with PTAH.²⁹ In this study, the slides were dyed with PTAH stain to identify whether the fibrin-like amorphous substance seen at the incision sites of all the groups was fibrin. The presence of fibrin was determined in the C, AL, and GF groups with PTAH staining (Figure 3). In the ABS group, the amorphous substance seen in HE sections was not dyed with PTAH (Figure 4). These findings supported Goker et al's hypotheses,³⁰ which indicated that ABS stops bleeding through protein network formation and erythrocyte aggregation.

CONCLUSIONS

ABS was found to be more effective than AL and GF as an epistaxis treatment. In our study, ABS decreased bleeding duration from 210 (range, 180–300) to 30 (range, 30–60) seconds. According to the histopathologic findings, ABS might stop bleeding by means of protein network formation. This agent has the potential to be used in many hemorrhagic cases. In serious cases of epistaxis, there are underlying structural and/or hematologic pathologies that predispose patients to severe nasal bleeding. In these cases, management can be considered a potential emergency. However, it is not clear whether the bleeding model used here replicates the type of injury that would be likely to result in life-threatening bleeding in humans. This was a significant issue that affected the interpretation of these results and should be considered as a limitation of the present study. Further investigations are needed to elucidate the place of ABS hemostatic effects on distinct tissues in various *in vivo* trauma models, including the model of bleeding diathesis.

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Dr. Kelles was responsible for the literature search, data collection, and writing of the manuscript. Dr. Kalcioğlu was responsible for the study design, literature search, data interpretation, writing, and figure creation. Dr. Samdancı was responsible for the data interpretation, figure creation, and pathological evaluation. Dr. Selimoğlu was responsible for the study design and critical evaluation of the article. Dr. İraz was responsible for the figure creation, data collection, and pharmacological evaluation. Dr. Mıman was responsible for the study design, critical evaluation of the article Ibrahim C Haznedaroğlu; Data interpretation, writing, and critical evaluation of the article.

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