Hemostatic Efficacy of Ankaferd Blood Stopper® in a Swine Bleeding Model

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Abstract
Objective: The purpose of this study was to show the hemostatic effect of spray, solution and tampon forms of Ankaferd Blood Stopper® (ABS), a unique medicinal plant extract historically used as a hemostatic agent in Turkish folklore medicine, in a porcine bleeding model.

Materials and Methods: Two 1-year-old pigs were used as bleeding models for superficial and deep skin lacerations, grade II liver and spleen injuries, grade II saphenous vein injury and grade IV saphenous artery injury. Spray, solution or tampon forms of ABS were applied after continuing bleeding was confirmed. The primary outcome was time to hemostasis. Volume of blood loss was not measured. The pigs were euthanized at the end of the experiment.

Results: Spray or direct application of ABS solution resulted in instant control of bleeding in superficial and deep skin lacerations as well as puncture wounds of the liver. A 40-second application of ABS tampon was sufficient to stop bleeding of skin lacerations, while 1.5- and 3.5-min applications were used to control hemorrhage from the saphenous vein and artery, respectively. No rebleeding was observed once hemostasis was achieved. However, repeated applications of ABS solution and tampon were only temporarily effective in the hemostasis of spleen injury.

Conclusions: The data showed that ABS was an effective hemostatic agent for superficial and deep skin lacerations and minor/moderate trauma injuries in a porcine bleeding model.

Introduction

Approximately half of deaths due to trauma are attributable to exsanguination [1]. After securing the airway and establishing adequate ventilation, control of hemorrhage is the second most important step in trauma surgery [2]. Diverse pharmacological agents are being developed and used to control the bleeding, exerting their effect by vasoconstriction, forming a scaffold for coagulation and inducing clot formation.

Ankaferd Blood Stopper® (ABS) is a herbal extract obtained from 5 different plants: Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum and Urtica dioica. For centuries, it has had a historical role in traditional Turkish medicine as a topical hemostatic...
agent, usually for skin wounds [3]. ABS has been shown to promote the formation of an encapsulated protein mesh which acts as an anchor for erythrocyte aggregation, without significantly interfering with individual coagulation factors [3]. The use of ABS as a modern medicinal product has only recently gained momentum, and in Turkey ministerial approval was obtained for the management of dermal and postdental surgery bleeding, after its safety and efficacy as a topical agent have been shown.

The aim of this study was to evaluate the hemostatic efficacy of 3 different forms of ABS (spray, solution and tampon) in superficial and deep skin lacerations, severed artery and vein, as well as blunt trauma to the liver and spleen in swine.

**Materials and Methods**

Two 1-year-old pigs, weighing 9–9.5 kg, were used in this experimental study. Both pigs were housed in metal cages with a wire netting bottom and maintained at a temperature of 23°C (±5°C). The animals were allowed free access to solid diet and tap water. The pigs were allowed to roam freely for an hour in a small garden twice daily.

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.

**Anesthesia and Preparation**

Anesthesia was induced by intramuscular injection of a combination of xylazine hydrochloride (1.1 mg/kg, Xylazine Bio 2%, Biveta, Czech Republic) and ketamine hydrochloride (15 mg/kg, Ketamidor, Richter Pharma AG, Wels, Austria), and maintained by inhalation of isoflurane (Abbott, Italy) and oxygen. The pigs were placed in a dorsal recumbent position. The skin was shaved and prepared for aseptic surgery. At the end of the study, euthanasia was performed on both pigs using 500 mg intravenous Pentothal.

**Experimental Design**

Pig 1 was used as a bleeding model for superficial and deep abdominal lacerations, as well as spleen and liver injuries. Superficial abdominal lacerations consisted of two longitudinal incisions, 0.6–0.8 cm in depth, 5 cm on either side of the linea alba. About 1–2 ml of ABS solution was then applied topically over the first of the two incisions. For the second incision a 2.5 × 7 cm ABS tampon was applied with initial gentle pressure for 40 s. For liver and spleen injuries an upper midline laparotomy incision was made through the skin and the underlying subcutaneous and muscle tissue to expose the transverse fascia. ABS spray was immediately applied to control the ensuing bleeding from this incision. The liver was externalized, and a sharp-ended probe was inserted 1.5 cm into the left lobe. A second puncture wound was created 3 cm apart from the first. ABS spray was applied on the first incision, while 2–3 ml of ABS solution was applied topically and into the second puncture wound. The spleen was externalized, and 2 incisions (2 cm in length and 0.5 cm in depth) were made 2 cm apart. In anticipation of the profuse nature of splenic bleeding, all 3 forms of ABS (spray, solution and tampon) were used sequentially until hemostasis was achieved. All incisions were observed for at least 1 min or until continuous bleeding was confirmed. Both liver and spleen injuries were grade II according to the American Association for the Surgery of Trauma (AAST) injury scaling and scoring system [4].

Pig 2 was used as a bleeding model for a severed saphenous vein (SV; grade II, AAST) and saphenous artery (SA; grade IV, AAST) [5]. A longitudinal incision was made in the right thigh exposing the SV and SA. First the SV was completely severed with a scalpel, bleeding was confirmed (for 14 s) and a 20 × 20 cm ABS tampon was applied with firm pressure for a period of 1 min 30 s. For the SA, the same procedure was repeated for a period of 3 min 30 s.

No sutures or other pharmacological agents were used to aid hemostasis. The primary outcome was time to hemostasis. The volume of blood loss was not measured.

**Results**

Overall, ABS spray, solution and tampon forms were successful in achieving and maintaining hemostasis in all bleeding models, except for the spleen injury, which resulted in temporary hemostasis after repeated efforts combining solution and tampon applications (table 1). Details of results of each bleeding model are presented below.

**Skin Lacerations**

Application of ABS resulted in instant cessation of bleeding for all skin incisions. Rebleeding was not observed from either site during the ensuing 25-min period. In addition, the spray form of ABS was successful in achieving sustained hemostasis for the upper midline laparotomy incision.

**Grade II Liver Injury**

ABS spray managed to instantly control the bleeding from the first puncture wound of the liver (fig. 1a, b). Application of ABS solution to the second site also resulted in instant cessation of bleeding (fig. 1c). Hemostasis was sustained throughout the ensuing observation period of 5 min for both puncture wounds.
Grade II Spleen Injury

Bleeding from the splenic incisions proved to be more difficult to control. After two incisions had been made (fig. 2a), initial application of ABS spray on the first incision failed to stop the bleeding. Subsequent topical applications of ABS solution did not have a sustained hemostatic effect. Bleeding from the second incision persisted despite treatment with ABS tampon. This prompted application of another ABS tampon (5 × 7.5 cm) to cover both incisions for another 75 s (fig. 2b) which was again insufficient to control the bleeding. Finally, both incisions were once again treated topically with 2 ml of ABS solution each, and this was repeated 20 s later. Although hemostasis was achieved in both incisions after this (fig. 2c), it only lasted for 3 min before bleeding recommenced.

**Table 1.** Hemostatic outcome of ABS spray, solution and tampon forms in different bleeding models

<table>
<thead>
<tr>
<th>Incision/injury (AAST grade)</th>
<th>Spray</th>
<th>Solution</th>
<th>Tampon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial skin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Deep skin</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Liver, grade II</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Spleen, grade II</td>
<td>–</td>
<td>+/–</td>
<td>+/–</td>
</tr>
<tr>
<td>SA, grade IV</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>SV, grade II</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Hemostasis achieved and no rebleeding observed; – = hemostasis not achieved; ND = not determined; +/- = temporary hemostasis lasting for 3 min was achieved after repeated efforts combining solution and tampon applications.

**SV (Grade II) and SA (Grade IV) Injuries**

The ABS tampon was successful in controlling bleeding from both the severed SV (fig. 3a–c) and SA (fig. 3d–f). Rebleeding did not occur in the following 3 min.

Ankaferd Blood Stopper® in a Swine Bleeding Model

**Fig. 1.** Application of ABS for bleeding control in penetrating liver injuries in the porcine model. a A sharp-ended probe was inserted twice into the liver 3 cm apart. Bleeding control in the first site (b) after application of ABS spray and the second site (c) after application of ABS solution.

**Fig. 2.** Application of ABS for bleeding control in penetrating spleen injuries in the porcine model. a Two incisions were made in the porcine spleen. b The splenic lesions were covered by an ABS tampon (5 × 7.5 cm). c Hemostasis was achieved after both application of ABS tampon and solution.
Discussion

We have shown that ABS solution, in spray form or applied directly to the site of injury, was instantaneously effective on superficial and deep skin lacerations as well as puncture wounds of the liver. Similarly, ABS tampon was effective on superficial and deep skin lacerations within 40 s of application. In addition, ABS tampon was determined to be effective in the control of hemorrhage from a severed SV and SA when applied for a few minutes with gentle pressure. However, application of ABS solution or tampon or the combined use of both was not sufficient for sustained hemostasis in the spleen.

ABS is a herbal extract traditionally used in skin cuts and lacerations. Its mechanism of action involves formation of a protein network that acts as focal points for erythrocyte aggregation. Since it does not depend on coagulation factors, ABS may potentially be used in patients with acquired or inherited coagulopathies [3]. Tissue adhesives such as cyanoacrylates have been increasingly used in the repair of skin lacerations, and their cosmetic results are comparable to standard wound closure techniques such as sutures, staples and adhesive strips [6]. The wound-healing properties of ABS were not examined in this study. On the basis of the hemostatic effect of ABS in skin lacerations, the value of ABS in cosmetic wound healing should be considered in future investigations.

In addition, ABS may be a useful product in the management of bleeding during surgical procedures. However, extensive safety studies should be performed showing complication-free survival before clinical studies can be conducted. Currently, collagen microfibers, oxidized cellulose, thrombin, purified gelatin and fibrin sealants are being used as topical agents to obtain hemostasis during elective surgeries [7–10]. These topical agents are not easily available. Some of them have limited efficacy and some others, e.g. fibrin glue, have a disadvantage in their industrial production, since human blood is used as its source.

Fig. 3. The SV (a) and SA (d) were cut with scissors, resulting in bleeding (b and e). ABS tampons were applied on both locations, and hemostasis was achieved (c and f).
[9, 10]. Biological materials also have the risk of infectious contamination. For these reasons there is a need for new effective topical agents. Since biological agents are expensive and carry the risk of transmission of infectious agents, some research had a focus on plants. A few plant extracts with a proven topical hemostatic effect have been reported [11–13]. The ideal topical hemostatic agent should be easy to use (even on the battlefield), effective within minutes, in both arterial and venous bleeding, and not toxic and anaphylactic [14]. Its effect should be prolonged as well. The current experiment and our experience with dental surgery show that ABS has all these properties. It is economical and does not require a special medium for conservation with a long shelf life either.

In vitro data on the anti-infectivity of ABS [15] and preliminary successful applications in mediastinal and gastrointestinal bleedings [16, 17] represented novel clues for ABS activity. Therefore, bleedings other than the approved labeled indications of ABS had become subject of its utilization for the control of hemorrhage based on the failure of classical blood-stopping methods, including a wide variety of clinical bleedings in distinct disease states.

In cases of trauma involving major arteries and veins or internal organs, it is crucial to control the hemorrhage in a timely manner. There are several products being developed to be used in prehospital settings after combat trauma or civilian casualties [18]. Dry fibrin sealant dressing (including fibrinogen, thrombin, factor VIII and Ca\(^{2+}\)), rapid deployment hemostat (poly-N-acetyl glucosamine, Rapid Deployment Hemostat), chitosan dressing (deacetylated poly-N-acetyl glucosamine) and Quik Clot (granular zeolite) are among the best studied hemostatic agents for prehospital control of trauma [19, 20]. ABS tampon may be a candidate for use in this setting, pending its ability to reduce blood loss and increase survival in more complicated trauma models involving coagulopathy and more complex injuries.

**Conclusion**

Our preliminary study showed that ABS is a potent hemostatic agent for superficial and deep skin lacerations and minor moderate trauma injuries in the porcine bleeding model. Further studies are needed to determine the full scope of ABS in the control of hemorrhage during surgeries and prehospital management of trauma injuries.

**References**