NEW METHODOLOGIES

Evaluation of a New Hemostatic Agent
Ankaferd Blood Stopper in Experimental Liver Laceration

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ABSTRACT

Introduction: Hemorrhage is a leading cause of death after trauma. It is also the major cause of operating room deaths among patients who undergo liver surgery. Various techniques and materials have been attempted to manage bleeding, but a standard method has not been defined yet. We studied the hemostatic effects of Ankaferd Blood Stopper on liver injury in comparison with regenerated oxidized cellulose. Materials and Methods: Thirty Wistar albino rats underwent partial hepatic laceration by scissors. The animals were randomized to the treatment of resected surface with either Ankaferd Blood Stopper® (ABS, n = 11) or regenerated oxidized cellulose (Surgicel®, n = 9), or were left untreated (controls, n = 10). All the animals were resuscitated with lactated Ringer’s solution at 3.3 ml/min/kg to a mean arterial pressure (MAP) of 100 mmHg. Survival time, total blood loss, resuscitation volume, and MAP were recorded for 30 min or until death. The rats that were alive at the end of 30 min were sacrificed with blood withdrawal from catheters. Results: Rats in the ABS and Surgicel groups survived significantly longer than rats in the control group (p = .0001). There were no significant differences between the ABS and the Surgicel groups in survival (p = .91). Application of ABS and Surgicel was associated with a significant reduction in blood loss compared to controls (p = .008), with no significant differences between active treatment groups (p = .74). The resuscitation volume was not different. Conclusions: ABS is as effective as Surgicel in achieving hemostasis following partial liver excision in an experimental rat model.

Keywords: Ankaferd Blood Stopper, hemorrhage, liver, hemostasis, trauma, rat

INTRODUCTION

Hemorrhage is one of the major causes of death after trauma and the leading cause of operating room deaths among patients undergoing major surgery [1]. Hemorrhage is one of the major causes of death after trauma and the leading cause of operating room deaths among patients undergoing major surgery [1]. Hepatic trauma may cause moribund hemorrhage due to hepatic laceration. The extensive vascularity and sinusoidal structure of the liver are the major factors considered responsible for diffuse bleeding [2]. Crush injuries and incisional surfaces bleed from multiple sites that are often very difficult to control by suturing and ligation. Incomplete hemostasis of injuries is the leading cause of reoperation for hemorrhage, which leads to increased morbidity and mortality [3].
Conventional hemostatic agents are expected to aid the patient’s coagulation system in the rapid development of an occlusive clot through platelet adhesion, platelet activation, and blood coagulation [4]. Some agents, such as gelatine sponge and cellulose, are ineffective in many patients with a life-threatening hemorrhage, because the hemostatic properties of these agents depend on adequate platelet and clotting factors [5]. There is a need for an absorbable hemostatic agent that can be successfully used in major solid organ injuries, which does not depend on platelet and clotting factors for its hemostatic efficacy. Ankaferd Blood Stopper\textsuperscript{©} (ABS) is a folkloric medicinal plant extract which has been used in Turkey traditionally for hemostasis. ABS has been approved in the management of dental surgery and external hemorrhage by the Ministry of Health in Turkey. Tests have proved its safety, efficacy, sterility, and nontoxicity (www.Ankaferd.com).

The present study was designed to evaluate the efficacy of a new hemostatic agent ABS in controlling hemorrhage from liver injury in comparison with Surgicel. The model was selected considering the ease of hemostatic agent application to the free-flowing hemorrhagic surface, the ability to determine the time to survival, the ease of instrumentation and no requirement for anticoagulation, and reproducibility [6].

**MATERIALS AND METHODS**

Thirty-nine male Wistar albino rats (303.2 ± 32.94 gm) were used in this study. The animals were housed in a climate-controlled facility. Food and water was available ad libitum. They were fed with pellet food produced especially for experimental animals. All the animals were maintained in Zonguldak Karaelmas University (ZKU) Experimental Animals Research Unit. The study protocol was approved by the Ethic Committee of ZKU Experimental Animals Research Unit. All the animals received humane care in accordance with the requirements of the U.S. Animal Welfare Act. The animals were allocated randomly into three groups to receive either Surgicel (n = 9), ABS (n = 11), or no treatment (controls, n = 10).

The animals were anesthetized with an intramuscular injection of 100 mg/kg ketamin (Ketalar\textsuperscript{©}, Parke Davis-Eczacıbaşı, Istanbul, Turkey). Femoral arterial and venous catheters were placed. The femoral venous line was connected to an infusion pump for fluid resuscitation and the femoral arterial line was connected to a precalibrated pressure transducer. Heart rate, mean arterial pressure (MAP), and systolic and diastolic blood pressures were recorded.

After performing a midline laparotomy, the abdominal cavity was wiped dry with cotton sponges. A portion of the liver was sharply excised by scissors (Figure 1), as described by Matsuoka et al. [7] and Holcomb et al. [8]. As a rapidly bleeding liver surface was easily visualized, no manipulation to the bleeding surface was done. The resected surface was then either treated with ABS or Surgicel, or left untreated according to randomization. Following the completion of liver injury, lactated ringer solution infusion (40°C) was initiated with...
Hemostatic Effect of Ankaferd Blood Stopper

A rate of 3.3 ml/min/kg via femoral venous line. The end point of resuscitation was a MAP of 100 mmHg. The resuscitation was reinitiated whenever the MAP decreased. The weight of excised median lobe and the ratio of the excised liver portion to pre-injury total body weight were calculated. The animals were monitored for 30 min after the liver injury or till death whichever came first. Death before 30 min was defined as no respiration and no pulse. The animals still alive after 30 min following the liver injury were sacrificed via blood withdrawal from vascular lines.

In the control group, the animals were allowed to bleed freely from the cut surfaces of the liver as the abdominal cavity was left open. In the ABS group, Ankaferd Blood Stopper® (Trend Teknoloji Ilac AS, Istanbul, Turkey) was sprayed using a syringe to the actively bleeding cut surface of the liver by one of the investigators immediately after excision of the liver portion (Figure 1). In the Surgicel group, a sufficient piece of Surgicel® (Ethicon, UK) was applied to cover the cut surface of the liver by one of the investigators immediately after excision of the liver portion (Figure 2). Blood was allowed to accumulate in the peritoneal cavity in all groups, but excess blood was collected with small cotton sponges in order to avoid spillage out of the abdominal cavity. Care was taken to collect excessive blood from the cut surface of the liver. Figure 3 shows the excised liver portion of the median lobe on postmortem rat liver specimen.

At the end of the study period, shed blood in the abdominal cavity was collected with small cotton sponges. The total blood loss was calculated as blood-soaked cotton sponges minus the weight of preweighed dry cotton sponges used for each animal. Time to death and total resuscitation volume were recorded.

Statistical Analysis

Body weight, baseline MAP, survival time, estimated blood loss, resuscitation fluid volume, and the weight of the excised liver portion were defined. Blood loss and volume of resuscitation fluid were corrected for body weight (ml/kg). Data are reported as mean ± SD. Data were examined for heterogeneity of variance and normality using the Levene test and the Kolmogorov-Smirnov test. Comparisons of the group means were analyzed using one-way variance analysis. Different groups were detected by using the Tukey post-hoc test. Actual probabilities ≤0.05 are considered statistically significant.

RESULTS

Thirty of the 39 rats used for this study had the appropriate resection of the median lobe as described above in accordance with the method used by Holcomb et al. [8]. Nine rats were excluded from the study. Eight of them excluded as the excised liver portion was too small or too large which resulted either in insignificant bleeding or rapid bleeding to death and one of them that had vascular laceration while vascular line insertion. All further analysis and comments refer to these 30 rats.

Figure 3. Postmortem rat liver specimen; separate lower section represents the excised portion of liver. Forceps point to the cut surface of the median lobe.
There were no differences among three groups in animal body weight, starting MAP, and the fraction of liver excised. The mean time to death was 7.08 ± 2.01 min, 28.46 ± 2.42 min, and 28.89 ± 2.42 min for the control group, the ABS group, and the Surgicel group, respectively. Seven rats were sacrificed after the completion of 30 min in accordance with the study design, in each the ABS group and the Surgicel group. In the ABS group three rats survived shorter than 30 min for an average of 26.3 min. On the other hand, two rats in the Surgicel group survived shorter than 30 min with a mean of 25.0 min. In the control group neither of the rats survived longer than 12 min. Time to death was significantly longer for the ABS group and the Surgicel group than for the control group (p = .0001). ABS-treated animals showed no difference compared to the Surgicel group with regard to time to death (p = .90). Application of ABS and Surgicel was associated with a significant reduction in blood loss compared to controls (8.54 ± 3.59, 7.60 ± 2.97, and 11.82 ± 1.50, respectively. p = .008, one-way ANOVA) with no significant differences between treatment groups (p = .74). There was no significant difference in the blood loss normalized for 1 kg of body weight among experimental groups treated with a hemostatic agent. The MAP in the treatment groups fell significantly from baseline levels, but to a lesser extent than the changes measured in the control group. There was a significantly greater fall in MAP in the control group compared to the values measured in the treatment groups. Resuscitation fluid volume was not different for all three groups (Table 1).

Table 1. Comparability of groups treated and treatment results in ABS group, Surgicel group, and control group

<table>
<thead>
<tr>
<th></th>
<th>Control group mean ± SD (n = 10)</th>
<th>ABS group mean ± SD (n = 11)</th>
<th>Surgicel group mean ± SD (n = 9)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>305.20 ± 37.60</td>
<td>311.09 ± 32.06</td>
<td>299.11 ± 48.69</td>
<td></td>
</tr>
<tr>
<td>Starting MAP (mmHg)</td>
<td>114.20 ± 7.77</td>
<td>112.91 ± 6.06</td>
<td>115.22 ± 8.35</td>
<td>.94</td>
</tr>
<tr>
<td>Lowest MAP (mmHg)</td>
<td>20.00 ± 4.35</td>
<td>37.45 ± 17.25</td>
<td>45.00 ± 15.84</td>
<td>.92</td>
</tr>
<tr>
<td>BW% excised b</td>
<td>0.75 ± 0.05</td>
<td>0.76 ± 0.03</td>
<td>0.77 ± 0.04</td>
<td>.02</td>
</tr>
<tr>
<td>Blood loss (mg)</td>
<td>11.82 ± 1.50</td>
<td>8.54 ± 3.59</td>
<td>7.60 ± 2.98</td>
<td>.036</td>
</tr>
<tr>
<td>Blood loss (mg/kg)</td>
<td>39.13 ± 6.46</td>
<td>28.04 ± 12.47</td>
<td>26.48 ± 12.82</td>
<td>.07</td>
</tr>
<tr>
<td>Resuscitation volume (ml)</td>
<td>7.37 ± 1.85</td>
<td>7.44 ± 3.29</td>
<td>7.52 ± 2.16</td>
<td>.99</td>
</tr>
<tr>
<td>Resuscitation volume (ml/kg)</td>
<td>24.41 ± 7.64</td>
<td>24.27 ± 11.4</td>
<td>26.03 ± 9.43</td>
<td>.99</td>
</tr>
<tr>
<td>Survival time (min)</td>
<td>7.08 ± 2.01</td>
<td>28.46 ± 2.42</td>
<td>28.89 ± 2.42</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

a MAP: Mean arterial pressure.
b BW% excised: The percentage of excised liver portion to the body weight.

The mean ABS volume to be used was 0.6 ml/rat.

DISCUSSION

Trauma is one of the most common causes of death regardless of age and the liver is one of the major sources of exsanguinating hemorrhage [9–11]. The method most frequently used to control hemorrhage in severe liver injury is packing with a number of complications [12]. Additionally, a second operation is required for the removal of gauze [13]. A potentially beneficial agent which can be used in both conventional and laparoscopic interventions for hemostasis might improve surgical complications, decrease hospital costs, and simplify surgery [14].

The liver injuries were equivalent across three study groups. This liver injury model yields a simple, easily reproducible severe liver injury in nonheparinized rats. An experimental resection model was preferred over a trauma model, because parenchymal damage in a trauma model would not be as standardized as a resection model [15]. A hepatic resection model provides an acute and serious bleeding scenario in a standardized injury.

In the case of hemorrhage, hemostasis is naturally carried out by vassal contraction, platelets, and coagulation factors [4, 16]. Hepatic resection resulted in profuse bleeding when not treated. There are a number of hemostatic methods (manual compression and
tamponade) and topical agents (oxidized regenerated cellulose, Fibrin sealants, microfibrillar collagen, and gelatine sponges) that have been used for hemostasis [4, 17–21]. Surgicel® is one of the most commonly used topical agents that are used for hemostasis [21]. It conforms well to shapes and is easily manipulated. It is pliable and can be reshaped during application [22]. An artificial hemostatic plug forms when Surgicel is applied on a severed surface [23].

ABS consists of a standardized mixture of the plants Urtica dioica 0.12 mg, Vitis vinifera 0.16 mg, Glycerhiza glabra 0.18 mg, Alpinia officinarum 0.14 mg, and Thymus vulgaris 0.1 mg, in an ampule of 2 ml (www. Ankaferd.com). ABS leads to the formation of a protein network. Goker et al. [24] showed that normal hemostatic elements were spared during hemostatic action of ABS. Protein network formation contains aggregation of blood cells, particularly erythrocytes and interactions between ABS and blood proteins, mainly fibrinogen [24]. Goker et al. [24] also showed that normal hemostatic agents were spared during formation of the protein network.

The present study was undertaken to evaluate the effectiveness of a new hemostatic agent in controlling hemorrhage from liver laceration. We also aimed to compare the hemostatic effect of ABS with the hemostatic effect of Surgicel on hemorrhage from liver injury in a standardized rat model. To our knowledge, this is the first study in English literature to investigate hemostatic effect of the ABS in an experimental rat model (Pubmed, Medline, 16.06.2008). An in vivo study evaluating the effectiveness of ABS demonstrated that ABS has an advantage of stopping hemorrhage rapidly (i.e., less than 1 s) without affecting any individual clotting factor in in vitro studies [24]. Goker et al. stated that individual clotting factors, namely factor V, VII, VIII, IX, X, XI, XIII are not affected in in vitro experimental studies. They also revealed that plasma fibrinogen, total protein, albumin, and globulin levels were decreased with the interactions of ABS [24, 25].

The main aim of the resuscitation and hemostatic processes is to keep the living being with hemorrhagic injury alive. In the present study, rats in the ABS group and in the Surgicel group survived significantly longer than rats in the control group (p = .0001, one-way ANOVA). There were no significant differences between the ABS and Surgicel groups in survival (p = .91). Application of ABS and Surgicel was associated with a significant reduction in blood loss compared to controls (p = .008), with no significant differences between active treatment groups (p = .74). The volume of infused fluid was not different for the three groups. In animal models of uncontrolled hemorrhage, early massive fluid resuscitation causes re-bleeding due to increased blood pressure, decreased vasoconstriction, dislodgement of blood clots, and dilution of clotting factors [26]. Thus, the study was designed as the animals were resuscitated using lactated ringer solution infusion (40°C) with a rate of 3.3 ml/min/kg to an end point of a MAP of 100 mmHg. The resuscitation was reinitiated whenever the MAP decreased. Rats in the control group required continuous fluid resuscitation during the study period, but neither of them survived more than 12 min.

There are many advantages of ABS. Since it is a medicinal plant extract, it does not transmit blood-borne infections and possesses no reported side effects [24]. It has a potential usage in laparoscopic procedures. One of the major advantages is that it provides a very rapid (less than 1 s) protein network formation in vitro. Clinically it has been recognized that bleeding ceases in a very short time in the ABS group. A mean ABS volume of 0.6 ml/rat is used to manage bleeding from the cut surface of liver.

Our study has several limitations. First, as the rats were sacrificed at the end of 30 min, we do not know the long-term results of hemostatic state. Further studies with large series are thus called for to evaluate the long-term effects of ABS. Second, we need to determine the effect of ABS on late-phase bleeding. Third, it is of course necessary to test these results in other more aggressive models (where the bleeding is difficult to stop) such as the swine model. Fourth, the application of ABS in the intraperitoneal cavity might suggest a potential risk of causing tissue adhesions. Further investigations are needed to clear out these aspects.

In conclusion, the results of this controlled randomized study demonstrate that ABS is as effective as Surgicel in achieving hemostasis and in reducing blood loss following partial liver excision in an experimental rat model.

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**DECLARATION OF INTEREST**

There are none.

**REFERENCES**