Protective Value of a Folkloric Medicinal Plant Extract Against Mortality and Hemorrhage in a Life-threatening Renal Trauma Model

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OBJECTIVES To compare the efficacy of a folkloric medicinal plant extract (Ankaferd Blood Stopper [ABS]) with that of oxidized cellulose (Surgicel) in a life-threatening renal injury model. ABS is a mixture of 5 plants that has historically been used in Turkish traditional medicine. It has been approved by the Ministry of Health to manage external hemorrhage and dental surgery bleeding in Turkey.

METHODS Twenty-two Wistar albino rats underwent partial nephrectomy after intravenous heparin anticoagulation (2000 U/kg). The cut surface received 1 of 3 therapies, namely no treatment, Surgicel (Johnson & Johnson, New Brunswick, NJ) or ABS (Trend Teknoloji İlaç A.Ş, Istanbul, Turkey). Blood pressure was continually monitored. Survival time, total blood loss, and mean arterial pressure were recorded for 60 minutes or until death. Rats that were alive (mean arterial pressure ≥20 mm Hg) at the end of 60 minutes were sacrificed with blood withdrawal with the help of catheters.

RESULTS All animals that received no treatment died within 60 minutes of follow-up. One of 7 in the Surgicel group, and 5 of 7 animals in the ABS group, survived. Mean survival times for the Surgicel and ABS groups were 42.7 and 53.4 minutes, respectively. Rats in the ABS and Surgicel groups survived significantly longer than rats in the control group (P <.05). There were no significant differences between the ABS and the Surgicel groups in survival (P =.128).

CONCLUSIONS ABS is as effective as Surgicel in achieving hemostasis and lengthening survival time following partial nephrectomy in an experimental rat model. UROLOGY 75: 1515.e9–1515.e14, 2010. © 2010 Elsevier Inc.

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 Although, 80%-90% of renal trauma cases may be managed conservatively, hemorrhage from this parenchymal organ may be life-threatening, especially in the presence of coagulopathies.2 Due to limited efficacy of conventional hemostatic techniques (such as pressure, ligature, cautery), various topical hemostatic agents were studied to achieve renal parenchymal hemostasis during nephron-sparing open/laparoscopic renal surgery and renal trauma.3–6

Topical hemostatic agents can be divided into 2 groups-those that act directly in the coagulation cascade (active) and those that act passively through contact activation and promotion of platelet aggregation. Passive agents are not highly effective if clotting mechanisms in a trauma patient are impaired. In 2001, Tuthill et al7 compared the hemostatic efficacy between a passive (gelfoam + thrombin) and an active (fibrin sealant) hemostatic agent in a renal hemorrhage model in heparinized rats, and demonstrated better results with liquid fibrin sealant. An important issue for fibrin glues and devices is that these products must be prepared in advance and is less suitable for use in such situations where bleeding occurs unexpectedly.8 Surgeons should also be trained in the technical use of these hemostatic agents.

To date, fibrin or matrix tissue sealants and synthetic glues like oxidized cellulose (Surgicel) were found to be the most effective topical hemostatic agents yet developed.9 Unfortunately, there is still little knowledge of whether they are suitable for a coagulopathic trauma patient, with multiple bleeding sites, or if hemostasis achieved stands for a durable condition.9 So, there is a need for an effective, easily applicable, and safe topical agent that can be successfully used in major solid organ injuries, which does not depend on platelet and clotting factors for its hemostatic efficacy.

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The excised part was removed and weighed. The injured kidney was resected in guillotine fashion with a single stroke of an amputating knife just inferior to the renal pelvis and hilum. Preweighed cotton sponges were placed in the abdominal cavity to collect any blood. The lower third of the left kidney was fully dissected free of the kidney, allowing complete kidney mobilization. Any blood loss occurred up to this point was collected with small cotton sponges. The total blood loss was calculated as the weight of preweighed dry cotton sponges used for each animal. Time to death was recorded. All renal manipulations, resections, experimental product applications, and bleeding evaluation were held by the same surgeon (H.T.).

Ankaferd Blood Stopper (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. Tests have proved its safety, efficacy, sterility, and nontoxicity (http://www.ankaferd.com). To the best of our knowledge, present study is the first, comparing hemostatic efficacy of ABS with a commonly used hemostatic agent Surgicel, in an experimental model of life-threatening renal injury.

**MATERIAL AND METHODS**

**Animals**

All animals were maintained in compliance with the Principles of Care and Use of Laboratory Animals prepared by the National Institutes of Health, revised 1985. The Institutional Animal Care and Use Committee at our institution approved all procedures. Twenty-two adult male Wistar albino rats weighing 300-440 g were used in this study. The animals were housed in a climate-controlled (24°C ± 1°C) facility on a 12-hour light–12-hour dark cycle. Food and water was available ad libitum. They were fed with pelleted food produced especially for experimental animals. All the animals received humane care in accordance with the requirements of the US Animal Welfare Act. The rats in all groups were anesthetized with an intramuscular injection of 100 mg kg⁻¹ ketamine HCl (50 mg mL⁻¹, Ketalar, Parke-Davis, Eczacıbaşı, Istanbul, Turkey) for all surgical procedures and did not regain consciousness. Providone-iodine solution was used to prepare the surgical site.

**Experimental Procedure**

After anesthesia induction a midline laparotomy incision was made. Using sterile techniques, a cannula was then inserted into the right femoral vein for heparin infusion and the right femoral artery was cannulated to allow invasive arterial pressure monitoring. The arterial pressure and heart rate of the anesthetized rats were monitored and recorded continuously using a monitoring system (Petas KMA 800; Petas, Turkey). Body temperature was measured using a rectal probe and maintained at 37°C ± 0.5°C with an external heat lamp. The abdominal cavity was wiped dry with cotton sponges. After cannulation of femoral artery and vein, dissection was carried down to the left kidney. Gerota’s fascia was carefully dissected free of the kidney, allowing complete kidney mobilization. Any blood loss occurred up to this point was blotted with preweighed cotton sponge and recorded as pre-treatment blood loss. Fluid resuscitation was not administered in any animal. But, normal saline (0.9% saline) was infused to the amputated renal margin using a syringe (Johnson, New Brunswick, NJ) was placed to the whole cut renal surface (Fig. 1). In ABS group, 1 mL of the injectable form of ABS (Trend Teknoloji İlac AS, Istanbul, Turkey) was applied to the amputated renal margin using a syringe (Fig. 2). No premixing is required for this product. No manual pressure was administered in any group. Blood was allowed to accumulate in the peritoneal cavity in all groups, but excess blood was collected with small cotton sponges to avoid spillage out of the abdominal cavity. Care was taken to collect excessive blood away from cut surface of the kidney. 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 was recorded. All renal manipulations, resections, experimental product applications, and bleeding evaluation were held by the same surgeon (H.T.).
Treatment groups (with controls with no significant differences between with a significant reduction in net blood loss compared
survival time (ence compared with the Surgicel group with regard to (and the Surgicel group than for the control group
Survival time was significantly longer for the ABS group
than 15 minutes except 1 that survived 39 minutes. In
37 minute). In contrast, 6 rats in Surgicel group survived
decreased below 20 mm Hg in 25 and 49 minutes (mean:
utes in accordance with the study design, in the ABS as
animals were sacrificed after the completion of 60 min-
utes in accordance with the study design, in the ABS as
in addition, the percentage of excised kidney portion to the total body weight was not different in all groups (Table 1). No rats died before renal injury. Mean pretreatment blood loss and fluid replacement with saline infusion was 0.9 g.
The mean time to death was 14.25 ± 10.45 minutes 42.71 ± 11.54 minutes, and 53.43 ± 13.11 minutes for the control, Surgicel, and ABS groups, respectively. Six animals were sacrificed after the completion of 60 minutes in accordance with the study design, in the ABS as well as the Surgicel group. In ABS group, MAP in 2 rats decreased below 20 mm Hg in 25 and 49 minutes (mean: 37 minute). In contrast, 6 rats in Surgicel group survived for less than 60 minutes, with a mean of 39 minutes. In the control group, neither of the rats survived longer than 15 minutes except 1 that survived 39 minutes. Survival time was significantly longer for the ABS group and the Surgicel group than for the control group (P < .05) (Table 1). ABS-treated rats showed no difference compared with the Surgicel group with regard to survival time (P = .128).
Application of ABS and Surgicel was not associated with a significant reduction in net blood loss compared with controls with no significant differences between treatment groups (P = .383). In addition, there was no significant difference in the blood loss normalized for 1 kg of body weight among study and control groups. However, during the initial 30-minute period, mean blood loss was significantly lower in the ABS group when compared with control (Table 1).
Following partial nephrectomy, the changes in MAP during 60 minutes continuous monitoring period can be seen in Fig. 3. The fall in MAP in the ABS group was significantly lower than the fall in MAP in the control group (P = .021) (Fig. 3). However, it was not the case for the Surgicel group (P = .694). Although, the P value was close to significant values (<.05), there were no statistically significant differences, when final MAP were compared between the experimental groups (P = .097). Treatment with ABS significantly increased survival, when compared with controls (P = .007). Unfortunately, treatment with Surgicel had no effect on survival (P = .467). The P value was 0.051, when the effect of treatments amount of survival of the animals was analyzed (Power analysis of our study revealed a power of >99% with α of 0.05 (two-tailed), when the major outcome was accepted as mean survival time in each groups. When the study was powered for total survival at 60 minutes, a power of 96.6% with α of 0.05 was observed. The power of observed difference in total survival at 60 minutes for the experimental groups (ABS and Surgicel) was 73.5% with α of 0.05).

RESULTS
A total of 22 open left lower pole partial nephrectomies were performed. There were no differences among 3 groups in animal body weight, initial MAP, the fraction of kidney excised, and mean weight of resected kidney specimens. In addition, the percentage of excised kidney portion to the total body weight was not different in all groups (Table 1). No rats died before renal injury. Mean pretreatment blood loss and fluid replacement with saline infusion was 0.9 g.

Statistical Analysis
The SPSS 11.5 software package was used in the analysis of the data. All descriptive data are presented as the mean ± standard deviation. Body weight, baseline MAP, survival time, estimated blood loss, and the weight of the excised kidney portion were defined. Blood loss was corrected for body weight (mL/kg). Comparisons of the group means were analyzed using Mann–Whitney U test and chi-square analysis. P < .05 was defined as the level of statistical significance.

COMMENT
Topical hemostatic agents have an important role during many surgical operations. Because the kidney receives a fifth of the cardiac output every minute, any trauma or surgical procedure related to this organ may result in significant hemorrhage depending on the extent of the injury to the parenchyma. In different animal models, various techniques with different topical hemostatic agents and with disparate doses/applications have been identified.4-7,9 So, assessment and comparison of relative potencies of certain hemostatic agents are difficult, solely with experimental studies.

Our primary aim in the present study was to create a consistent magnitude of injury in all animals and groups as well as to produce a lethal parenchymal organ injury by inducing a highly coagulopathic state. As evidenced in control group, amputation of the lower third of a kidney tissue following administration of a dose of 2000 U/kg heparin, was sufficient. In contrast to some other rat hemorrhage models, a stable core body temperature during the whole process was maintained.11-13 Presently, we know that coagulation cascade more or less depends on body temperature. Tuthill et al18 observed that rats tend to clot more efficiently if they became hypothermic from the anesthesia and were prone to aberrant deaths if overheated. However, a recent study interpreted that local hemostatic agents were not effective enough to keep the hemostasis when the coagulation status of the hypothermic animal was impaired.9 In anyway, regula-
formation of body temperature seems to be an important parameter to the reproducibility of a model.

In the case of hemorrhage, hemostasis is naturally carried out by vassal contraction, platelets, and coagulation factors. There are a number of topical agents (oxidized regenerated cellulose, fibrin sealants, microfibrillar collagen, and gelatin sponges) that have been used for hemostasis. Surgicel is one of the most commonly used topical agents in clinical practice, and was preferred as the comparator in our study. It conforms well to shapes used topical agents in clinical practice, and was preferred for hemostasis.

ABS consists of a standardized mixture of the plants *Urtica dioica* 0.12 mg, *Vitis vinifera* 0.16 mg, *Glycyrrhiza glabra* 0.18 mg, *Alpinia officinarum* 0.14 mg, and *Thymus vulgaris* 0.1 mg, in an ampoule of 2 mL (http://www.ankaferd.com). The basic ABS mechanism of action is the formation of an encapsulated protein network that provides focal points for vital erythrocyte aggregation. The experimental study by Huri et al confirmed this aggregation with the absence of glomerular necrosis and calcification in renal tissue near the aggregate. Protein network formation contains aggregation of blood cells, particularly erythrocytes and interactions between ABS and blood proteins, mainly fibrinogen.

* Mann–Whitney U test.
† Statistically significant P values.
‡ Chi Square analysis.

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

<table>
<thead>
<tr>
<th></th>
<th>Control Group, mean ± SD (n = 8)</th>
<th>Surgical Group, mean ± SD (n = 7)</th>
<th>ABS Group, mean ± SD (n = 7)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>392.4 ± 34.3</td>
<td>357.3 ± 26.4</td>
<td>368.4 ± 21.4</td>
<td>.189 .232 .902</td>
</tr>
<tr>
<td>Excised kidney (mg)</td>
<td>437.5 ± 5.1</td>
<td>414.3 ± 3.78</td>
<td>428.6 ± 7.55</td>
<td>.463 .955 .620</td>
</tr>
<tr>
<td>TRW% excited</td>
<td>33.3 ± 3.4</td>
<td>34.1 ± 4.4</td>
<td>32.4 ± 3.3</td>
<td>.867 .694 .535</td>
</tr>
<tr>
<td>BW% excited</td>
<td>0.1 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.11 ± 0.02</td>
<td>.779 .694 .805</td>
</tr>
<tr>
<td>Initial MAP (mmHg)</td>
<td>125.12 ± 8.79</td>
<td>130.00 ± 10.04</td>
<td>129.71 ± 5.49</td>
<td>.536 .281 .902</td>
</tr>
<tr>
<td>Final MAP (mmHg)</td>
<td>0</td>
<td>9.8 ± 26.07</td>
<td>33.28 ± 31.25</td>
<td>.694 .021† .097</td>
</tr>
<tr>
<td>Net blood loss-30 min (mg)</td>
<td>6.10 ± 1.02</td>
<td>4.78 ± 2.24</td>
<td>3.62 ± 1.77</td>
<td>.232 .014† .383</td>
</tr>
<tr>
<td>Net blood loss-30 min (mg/kg)</td>
<td>15.69 ± 3.24</td>
<td>13.53 ± 6.51</td>
<td>9.90 ± 5.12</td>
<td>.613 .021† .456</td>
</tr>
<tr>
<td>Net Blood Loss-60 min (mg)</td>
<td>6.41 ± 1.32</td>
<td>6.45 ± 1.29</td>
<td>5.54 ± 1.49</td>
<td>.955 .397 .383</td>
</tr>
<tr>
<td>Net blood loss-60 min (mg/kg)</td>
<td>16.42 ± 3.46</td>
<td>18.13 ± 3.63</td>
<td>15.04 ± 4.11</td>
<td>.397 .694 .097</td>
</tr>
<tr>
<td>Survival time (minutes)</td>
<td>14.25 ± 10.45</td>
<td>42.71 ± 11.54</td>
<td>53.43 ± 13.11</td>
<td>.002† .001† .128</td>
</tr>
<tr>
<td>Survival at 60 min/total (n) (%)</td>
<td>0/8 (0%)</td>
<td>1/7 (14.3%)</td>
<td>5/7 (71.4%)</td>
<td>.467† .007† .051†</td>
</tr>
</tbody>
</table>

Figure 3. Mean arterial pressure changes over time in rat renal hemorrhage model: Control, ABS, and Surgicel groups. * P < .05, Control vs Surgical group, Mann–Whitney U test. † P < .05, Control vs ABS Group, Mann–Whitney U test.
ABS is commercially available in 2-mL ampoules, which cost about $40 each.

Our hypothesis in current study was that ABS would delay life-threatening renal hemorrhage in a lower pole partial nephrectomy model, with a potency at least equivalent to Surgicel, which has been known and accepted worldwide as an effective hemostatic agent. Final MAP 1 hour after the injury was 10 mm Hg for the Surgicel group, whereas it was 32 mm Hg for the ABS group. Although there was no statistical significance (P = .09), treatment with ABS maintained the hemodynamic stability better than treatment with Surgicel. In the ABS group, 5 of 7 animals (71.4%) survived, although only 1 (14.3%) rat survived in the Surgicel group at the end of the study period. This was a clinically significant, but a statistically insignificant difference (P = .051). Unfortunately, application of either ABS or Surgicel to the cut surface was not associated with a significant reduction in blood loss compared with controls. Clinically, after clamp removal, in control group, we observed profuse bleeding that resulted in dramatic decreases in MAP values and death in approximately 15 minutes. For the experimental groups, the bleeding was slower, rather spread over time. In the initial 30-minute period of trauma, mean blood loss was significantly lower in the ABS group, when compared with control (Table 1). But, for better macroscopic and clinical evaluation, similar trial should be planned in a larger animal model such as swine or sheep.

Recently, we evaluated and compared efficacy of both products in an experimental liver laceration model, and we found out that net blood loss was significantly lower in the ABS and Surgicel groups when compared with controls. Similarly, mean survival times were significantly higher in experimental groups. Nevertheless, no difference was observed between treatment with ABS and Surgicel in terms of survival and blood loss.

An advantage of both ABS and Surgicel is that, they are easily applicable and does not require technically challenging methods. During a partial nephrectomy or renal trauma surgery, for rapid control of bleeding, this feature offers advantage over conventional surgical techniques including suturing as well as fibrin sealants.

The limitations of our study include the unknown influence that ketamine HCl may have on hemodynamic variables. Second, as the rats were sacrificed at the end of 60 minutes, we do not know the long-term results of hemostatic state. Late-phase bleeding is an important clinical issue and can even be fatal. Third, in clinical setting, the coagulopathy of the trauma patient is complicated and multifactorial. The inhibition of the coagulation system by heparin only is not sufficient in simulating a true trauma case.

CONCLUSIONS
The results of this controlled randomized study demonstrate that ABS is as effective as Surgicel in achieving hemostasis and lengthening survival time following life-threatening renal injury in an experimental rat model. In urological surgical armamentarium, identification of an ideal topical hemostatic agent would especially be useful in partial nephrectomy surgeries by decreasing or eliminating warm ischemia time and, providing rapid hemorrhage control. We cannot say both for ABS and Surgicel that, they are the ideal agents. Surgicel has been used for more than 45 years and proved its efficacy and safety. For ABS, we think that additional preclinical and clinical trials, which are especially focused on safety, may give more conclusive data.

References


