The Hemeostatic Efficacy of ANKAFERD after Excision of Full Thickness Burns: A Comparative Experimental Study in Rats

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Abstract

Objective: In order to evaluate the effects of Ankaferd blood stopper (ABS) on post-excisional burn wound bleeding via monitoring early blood hemoglobin level changes and to make a comparison with a standart topical treatment, a controlled experimental study was conducted. Design and Interventions: A contact burn model of full-thickness injury with 30% TBSA was used. Following the burn injury, excisions of burned areas were done at postburn 48th hour. 24 male Spraque Dawley rats were divided into control, adrenaline, ABS-solution and ABS-pad treatment groups. In control group no treatment was done for bleeding, but the other three groups had topical treatments. Samples were taken at the begining of the study, just before and 1 hour after the burn wound excisions for measurement of haemoglobine (Hb) levels and additionally the external bleeding amounts were measured by weighing the topical pads. Measurements and main results: Baseline Hb levels of control, adrenaline, ABS-solution and ABS-pad treatment groups were 15.06 ± 0.83, 15.82 ± 0.83, 16.23 ± 1.14 and 15.16 ± 1.46 respectively. At the 48th hour of postburn injury, the Hb levels of these groups were 13.82 ± 0.58, 13.68 ± 1.26, 13.79 ± 0.90 and 13.57 ± 0.89. Mean blood loss amounts (ml) after burn wound excisions in groups were 1.44 ± 0.26, 0.65 ± 0.07, 0.53 ± 0.08 and 0.44 ± 0.09. Conclusions: ABS was found to be as effective as topical adrenaline on reducing excisional bleeding in the experimental burn injury model.

Keywords: Ankaferd Blood Stopper, Burn Surgery, Bleeding

1. Introduction

Bleeding is a major problem during early excision of burned skin [1]. Reducing blood loss during burn surgery is important for decreasing patient morbidity from hemodynamic derangement and minimizing the risk of transfusion-associated infections [2]. Different techniques have been used to reduce the intraoperative blood loss in burn surgery including warm saline-soaked pads, topical solutions of thrombin with or without adrenaline, topical vasoconstructors, compressive dressings or tourniquets, and vasopressin continuous intravenous infusion [3,4].

Ankaferd blood stopper (ABS) is a herbal extract which has been used historically as hemostatic agent in Traditional Turkish medicine. It comprises of standardized mixture of herbs Thymus vulgaris, Glycyrrhiza glabra, vitis vinifera, alpinia officinarum and urtica dioica. Exposure to ABS results in a very rapid formation of network within the plasma and serum without interfering with any individual clotting factor [5]. ABS seems to be effective in bleeding control in different tissue injuries.

We tested the hypothesis that ABS could be useful agent for decreasing blood loss after post-burn wound excision in a randomized, controlled experimental study. Change in blood hemoglobin levels was used as a primary outcome as well as actual blood loss, as it has been shown that changes in hemoglobin levels is strictly correlates with physiologic parameters of hemorrhage [6].

2. Methods

24 young adult male spraque-dawley rats (mean weight
200 ± 20 gram) obtained from Inonu University Animal Research Center were used in the study. The animals were kept in a room at a constant temperature of 21°C 12 h dark and 12 h light cycle and fed standard pellet chow and water, which were available ad libitum. The study protocol (Figure 1) was approved by Institutional Ethical Committe (approval no: 2009-25). The experimental project was furtherly supported by Inonu University Scientific Research Foundatition (project number, 2010/38). All the rats were allowed free to take water and ad libitum for post-injury days. Three rats were housed in each cage during postburn management. The animals were divided randomly into four groups as control, adrenaline, ABS solution, and ABS pad.

Prior to burn injury, the animals were anesthesized with ketamine hydrochloride and xylazine hydrochloride. Dorsum of each rat was shaved with an electrical clipper and then the area was burned in order to obtain 30% TBSA of full-thickness burn injury.

A comb burn model of full-thickness injury with intervening unburned interspaces was used. The method depended on contact burn injury using a metal brass thomb heated in boiling water. The dimensions of the brass comb were 20 × 20 × 60 mm, with four 10 × 20 mm rectangular prongs separated by three 5-mm-wide grooves. The brass comb was preheated in boiling water (100°C) for 5 minutes and applied without pressure on one side of the back for a period of 3 minutes [7].

2.1. Resuscitation Fluid Formulation and Its Estimation

Following to the burning insult, serum physiologic was subcutaneously given to each rat’s dorsal neck region at a rate of “4 × kg × burn percentage” for fluid resuscitation. The amount of resuscitation fluid used for each rat was:

\[ 4 \times 0, 20 \text{ kg} \times 30 = 24 \pm 2 \text{ cc} \]

for the first day.

Body surface area is difficult to measure in small laboratory animals. It is therefore estimated as surface area (cm²) = 10 x Mass (g 0.66). The prefactor “10” is empirically adjusted. The prefactor for rats is 9.1. Therefore to find the surface area of a rat, plug its mass into the equation 9.1 × gram 0.66. So “a 300 gram rat” would have a surface area of 392.6 cm² [8,9]. In our model 87 cm² would corresponds to 30% of TBSA, as our average rat weight was 200 gr. The preheated brass comb was applied to the dorsum in order to obtain a mixed contact burn area of 87 cm² in repeating sessions.

At the 48 th hour of postburn, the burned areas were excised with using surgical blade no.10 under ketamine (Alfamine, Alfasan, Nederland) and Xylazine Hcl (Alfazyne 2%, Alfasan, Nederland) anesthesia. Ketamine and xylazine was given to the rats at dose of 50 mg/kg and 5 mg/kg intraperitoneally in each session. No electrocautery was used during burn wound excision. The topical treatments were started just after the completition of surgical debridement in all groups, which were epi-nephrine or Ankaferd soaked gauze and ankaferd pads or simply serum physiologic soaked gauzes application to the wounds following to debridements. In adrenaline group, the required amount of epinephrine solution (Adrenalin 0.25 mg/ml, Biofarma, Turkey) was diluted 1:10 with saline (concentration 0.025 mg/ml). Bleeding or oozing amounts were also macroscopically recorded, photographed and measured in each group (Figure 2).

![Figure 1. Experimental protocol. *Burn injury on rats; **Burn wound excision time. Black arrows: timing of obtaining blood samples for hemoglobin measurements, zero time, just before burn wound excision and 1 hour later at the second postburn day. 1d; First day, 2d; second day](image)

2.2. Amount of Bleeding Estimation

The amount of bleeding was measured by means of a soaked gauze. The blood was collected on soaked gauze, which was weighed before and after the procedure on a 0.1 g accurate scale. The difference in the weight of the gauze before and after the procedure indicated the amount of bleeding. The application time over the bleeding area varied between 45 minutes and 1 hour.
2.3. Measurement of Hemoglobin Concentration in Whole Blood and Blood Sampling

Hemoglobin levels were measured preoperatively, at the second day of postburn and 1h after completion of the operation. For measurement of hemoglobin concentration in whole blood, samples of 0.5 cc. were taken from hearts of each rat into containers. Lower hemoglobin concentrations were found in heart blood than in the tail blood of small rats (less than 100 g body weight). It has been shown that there was no significant difference in the haemoglobin values of blood taken from the tail or from the heart in large rats [10].

The blood samples were send rapidly for hemoglobin analysis in each session. After obtaining the third and the last blood samples, all the rats were sacrificed.

The levels of hemoglobin in blood were estimated by using the cyanomethemoglobin method described by Drabkin in a spectrophotometer [11]. Drabkin’s solution on mixing with whole blood converts Hemoglobin to Cyanomethemoglobin. The absorbance of Cyanomethemoglobin is proportional to the Hemoglobin concentration. Drabkin’s solution contains potassium ferricyanide, potassium cyanide and sodium bicarbonate. This method is simple, rapid and reliable and measures all types of hemoglobin except sulfohemoglobin.

2.4. Statistical Analysis

The data were presented as mean ± standart deviance. A software system of SPSS 11.0 version was used statistical analysis. ANOVA and posthoc Tukey test were used for multiple comparisons between the groups. Wilcoxon Signed Rank test was used for comparision of repeated measures of blood hemoglobin levels. A P value less than 0.05 was accepted as significant.

3. Findings

Hb levels measured separately in three sessions is shown in Table 1. Hb levels at the begining of study did not differ between the groups (p > 0.05). Hb levels obtained after two days of burn injury did also not show any difference between the groups, but significantly decreased in comparision of previous values in all groups (Wilcoxon signed rank test, p < 0.05). Hb levels obtained 1 hour following to burn excisions from control group was significantly lower than three other groups (p < 0.05). There was no difference between the groups of A, ABS-S, and ABS-P with respect to Hb levels obtained at third session (p > 0.05).

There were significantly higher amounts of blood loss in group C than other three groups (p > 0.05). A, ABS-S and ABS-P groups showed no difference with respect to blood loss amounts (p > 0.05).

4. Results and Discussion

Topical hemostatic products containing thrombin are commonly used in burn surgery to facilitate focal hemostasis and graft adherence. But using these agents sometimes may encounter with unexpected clinical complications. One example to this type of clinical problem has been reported by Foster et al. They found coagulopathy due to factor V deficiency as evidenced by excessive bleeding and abnormal coagulation parameters following to repeated applications epineprine solution containing bovine thrombin in a severe burn patient with 75% TBSA [12].

A restrictive blood transfusion strategy and the use of hemostatic agents may decrease morbidity and mortality in trauma and burn patients [13]. Kahalley was the first to report diminished intraoperative blood loss with subcutaneous injection of a saline-vasopressor solution under donor sites and debrided areas without severe systemic side reactions [14]. This clinical observation was further supported with others especially for children with burn [15,16]. Minimal absorption of adrenaline without systemic effects using a tumescent technique has been described. This technique was later modified and found to be effective in reducing the intraoperative and total blood transfusion requirements in thermally injured patients [17]. Anthony et al. studied the effects of adrenaline on hemodynamics and markers of tissue perfusion in burned patient with comparing non-burned controls included mostly adult age group of patients [18]. They found increased heart rate, elevated adrenaline serum levels up to 6h, and hiperlactatemia lasting up to 4 h fol-
lowing to topical adrenaline application. Although the use of topical adrenaline still seems to be a safe way to be used in burned patients, its potential dangerous side effects do not make it an ideal haemostatic agent.

Newer products are under investigation for finding the most ideal haemostatic agent. In vitro studies evaluating the haemostatic effects of ABS have shown that its addition to plasma and normal serum has resulted in formation of an encapsulating protein mesh that acts as a matrix within which erythrocyte aggregation occurs in a very fast way (less than a second) [19]. Several animal studies of different experimental models including incisional or excisional traumas have revealed its efficacy in stopping the bleeding in vivo [20,21].

There are many prospective, retrospective or and case-control series studies in which ABS was effective to stop neoplastic gastrointestinal bleedings, epistaxis, bleeding after knife tonsillectomies, active cutaneous- subcutaneous incisional bleedings and bleedings where endoscopic variceal ligation failed [22-24]. Clinical reports are also present of successful ABS usage via endoscope or oral and rectal routes in gastrointestinal system haemorrhages in where optimal bleeding control could not be achieved in spite of endoscopic adrenaline injection and argon plasma coagulation [25,26].

We chose to have 30% TBSA in the model, so as to have a prominent Hb decline without causing fatal serious morbidity. Other reasons for choosing this model are following: First, actual burn injuries seen in clinical practice are mixed, i.e. some body areas with full-thickness and some with uninjured. So the model reflects much actual burn clinical scenario. Second, it is cheaper and relatively safe for investigators [27]. Fatherly, over the course of next few days after injury most of these ischemic interspace progresses to full-thickness necrosis. The model has also been shown that gross visual appearance is good indicator of whether necrosis is truly present as evidenced histopathologically 7 days after injury. Resuscitation fluid was given subcutaneously instead of giving intraperitoneally, because it has been shown that subcutaneous fluid administration as means of rehydration is effective in other species including humans [8]. It has also been shown that prophylactic fluid administered mice via subcutaneous route have improved survival times after carcinogen treatment [9]. Burn wound excision was done at 48th hour of postburn injury, so that a closer resembling of early excision in a clinical picture as early tangential excision of deep burns is a widely accepted treatment modality. The decrease of Hb levels seen in control group after burn wound excision was not observed in adrenaline and ABS treatment groups (p < 0.05).

ABS would be an advantage in traditional theraphies on several aspects. It has antimicrobial and antifungal effects which was demonstrated by several in vitro studies [28,29]. A novel finding of which ABS was found to be inhibitory for MRSA, Acinetobacter, E. Coli and Pseudomonas species growth was reported by Sarbas et al recently. This is very important, as most of these organisms are responsible for burn wound sepsis [30].

Regarding to the effects of ABS on wound healing, literature is scarce. Isler et al. reported ABS decreased the inflammation and necrosis process and increased the new bone formation in early bone healing period without causing any foreign body reaction [31]. ABS application time was only 1 hour in our study. We did not see any adverse effects during the observation and could not make any conclusion on wound healing. In an observational clinical study, Ozaslan et al. revealed that endoscopic application of ABS as a primary therapy in patients with bleeding due to chronic radiation proctitis was found to be effective in healing radiation induced ulcers [32]. The authors had also confirmed their finding with another case report [33]. For now, the effects of ABS on wound healing seem to be limited by the observation of a decreasing effect on microvessel density measurements in the tissue exposed to ABS, which suggest the presence of secondary more sustained mechanism of hemostasis besides the initial protein network [34].

Exposure to ABS in a certain area provides physiological haeostasis process together with tissue oxygenation without calling out any individual coagulation factor. This unique mechanism advantage provides advantage to ABS compared with others. Bleeding models in animals pretreated with aspirin, warfarin or heparin revealed that ABS had effectively reduced the duration and amount of bleeding [35,36]. Another most striking effect of ABS is its in vitro positive effects over mesenchymal stem cell proliferation as shown by Kilic et al. [37]. This would be another potential advantage of this natural substance over traditional therapies, because a severe regenerative process is needed during recovery phase of massive burn injury.

5. Conclusion

Burned patients have a consumption coagulopathy which, in combination with haemodilution during operation results in a clinically significant deficiency of coagulation factors II, VII and X [38]. Any hemostatic agent that does not interfere with coagulation factors would take the advantage over other haemostatically active products. The levels of coagulation factors II, VII, IX, X, XI, and XIII were not affected by ABS [39]. Furthermore, ABS as an adjunctive drug has been shown to treat a challenging upper gastrointestinal bleeding case with a low
platelet count [40]. Fibrinogen appears not to be a necessary component for formation of the protein network of ABS, as it was found to be effective in a patient with afibrinogenemia [41]. Overt disseminated intravascular coagulation in which hypofibrinogenemia occurs due to cosurnption coagulopathy seem to occur in critically ill burn patients [42,43]. So the use of ABS in severe burn patients with third degree injury may be useful adjunctive measure to prevent fulminant course.

The experimental study supports the use of ABS in achieving hemostasis in intraoperative managent of blood loss at burn wound excision site and but further studies are needed to clearify the effects of ABS on other parameters in burn management.

6. References


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