Invivo Hemostatic Effect of Ankaferd Blood Stopper in Rat Major Renal Trauma Model: Controlled Trial of Novel Hemostatic Agent

Cankon GERMIYANOGLU[1], Emre HURI[1], Turgay AKGUL[1], Ali AYYILDIZ[1], Huseyin USTUN[2]

[1] Ankara Training and Research Hospital, 2. Urology Department
[2] Ankara Training and Research Hospital, Pathology Department, Ankara, TURKEY

ABSTRACT

Ankaferd Bloodstopper (ABS) which is a medicinal product has been approved in the control of hemorrhage externally, dental surgery bleedings in Turkey to control the bleeding in renal trauma model was evaluated. Twelve Wistar rats were divided into two groups. Group I (GI),control, Group II (GII), study group. One cm2 tissue was resected. ABS solution was applied to resected area in study group. Standard suture was applied to control group. Bleeding time, number of ABS, live condition were evaluated. Histopathologic evaluations were completed. Mean time of bleeding control was 3.2 (2.4-3.6) min in GII, no difference with GI ($p > 0.05$). In GII, active hemostasis was provided. Mean number of ABS gout was 6.0 (5-8). Glomerular necrosis was detected with higher rate in GI compared with GII. Erythrocyte aggregation was confirmed in GII. Calcification was formed significantly in GI compared GII ($p < 0.05$). ABS could be an effective agent to stop active major bleeding in renal trauma model.

Keywords: Ankaferd blood stopper, Hemorrhage, Hemostasis

ÖZET

Rat Major Renal Travma Modelinde Ankaferd Kan Durdurucunun in Vivo Etkisi: Yeni Hemostatik Ajanın Kontrollü Çalışması

Ülkemizde eksternal ve dental kanamaların kullanımında onay almış tıbbi ürün olan Ankaferd Bloodstopper'ın renal travma modelindeki etkinliği araştırıldı. Oniki Wistar rat çalışmaya alınarak iki gruba ayrıldı. Grup I control, Grup II çalışma grubu olarak belirlendi. 1 cm2 doku rezeke edildi. Çalışma grubunda ABS solüsyonu rezeke edilen Alana uygulandı. Kontrol grubuna ise standart suture uygulandı. Kanama süresi, ABS uygulama sayısı, yaşam devamlılığı değerlendirildi. Histopatolojik değerlendirme yapıldı. Ortalama kanama süresi GII'de 3.2 (2.4-3.6) dak. Idi GII ile anlamlı fark yoktu ($p>0.05$). GII'de aktif hemostaz sağlandı. Ortalama ABS sayısı 6.0 (5-8) idi. Glomerüler nekroz GI'de GII'ye gore daha yüksek oranda saptandı. Eritrosit agregasyonu GII'de saptandı. Kalsifikasyon GI'de anlamlı olarak fazlaydı ($p<0.05$). ABS aktif mahör renal travma modelindeki kanamalarda etkili olabilir.

Anahtar Kelimeler: Ankaferd kan durdurucu, Kanama, Hemostaz

INTRODUCTION

Our previous study regarding the use of Ankaferd Bloodstopper® (ABS) in partial nephrectomy model indicated that ABS provides active hemostasis in experimental partial nephrectomy model with and without warm ischemia[1] However, ABS has not been licensed for human use during the kidney or the other urogenital surgical procedures yet as a haemostatic agent. We postulated that ABS might have a potential role in the treatment of major renal injuries, especially those that could be difficult to repair with conventional techniques. ABS is a unique folkloric medicinal plant extract, which has historically been used in Turkish traditional medicine. Mixture of five plants were included to ABS, comprises a standardized mixture of the plants 5 mg Thymus vulgaris, 9 mg Glycyrrhiza glabra, 8 mg. Vitis vinifera, 7 mg Alpinia officinarum and 6 mg Urtica dioica in 100 ml Ankaferd solution and each of them has some effect on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cell mediators.[2] For that, we created a complex renal injury model to assess the haemostatic efficacy of ABS and compared with conventional suture technique. Additionally, the effect of ABS on renal parenchyma was evaluated via histopathologic analyze.

MATERIAL AND METHODS

Animal Preparation. A total of 12 Wistar rat weighing 1 to 3 gram were studied at our institutional review board approved protocol. Trained personnel and our regular follow-up provided intensive care for the animals throughout the study period. Healthy animals were selected for this study, and food was withheld for 24 hours prior to surgery. All rats received prophylactic single dose broad-spectrum antibiotics. Anesthesia was induced with cetamin intramusculary.

Study Design: Twelve rats were divided into two groups. Major renal injury was provided with incision of lower pole of kidney including collecting duct system sharply, excision of this part of kidney suddenly. Demonstration of major bleeding from injured area was obligated. All rats underwent this procedure. Following the major renal injury, in group I (GI) of control group, bleeding was controlled with conventional suture technique without surgical bolster. In group II (GII), Ankaferd was applied onto the injured area directly with compressive fashion at least three minutes as a sutureless group (Figure 1). Afterward, if the bleeding had not being stopped, application of ABS was continued second or more time. At first month, following the sacrification of 12 rats, right nephrectomy was performed to evaluate histopathologic results. Kidneys were fixed in formalin solution. Gross specimens and histological sections were evaluated in a blind fashion by one pathologist (Hacettepe University) stained with haemotoxylen-eozine (H&E).

Operative technique and renal injury: A midline incision was made in the abdomen after sterile prep and draping. The right kidney was completely mobilized (Figure 2). Right kidney lower pole segment

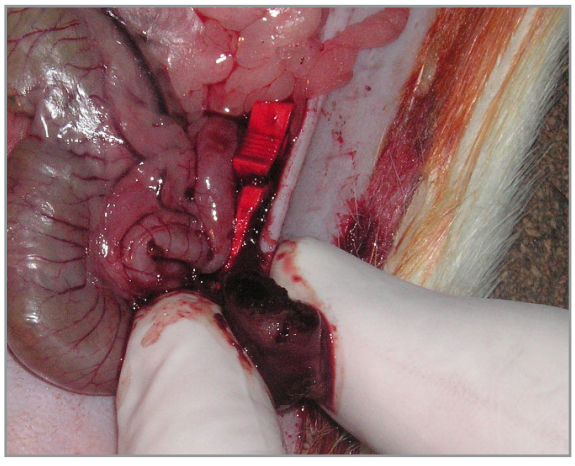

Figure 1. The application of ABS onto the traumatic renal surface and compression of tissue approximate three minutes

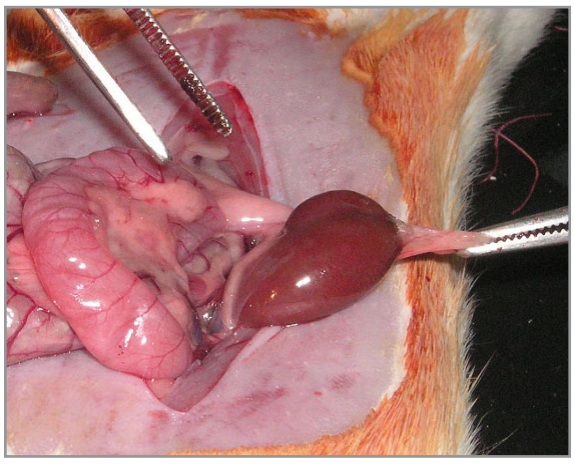

Figure 2. Mobilized right kidney, ready to cut

Table 1. The findings of detailed histopathologic evaluation of the groups with new scoring system

Histopathologic Parameters	Group I (n= 6)	Group II (n= 6)
Absence of Giant Cell Reaction	3 (50%)	4 (66.7%)
Absence of Glomerular Necrosis	0 (0.0%)	5 (83.3%)
Absence of acute inflamation	3 (50%)	5 (83.3%)
Absence of Calcification	0 (0.0%)	5 (83.3%)
Absence of fibrosis	3 (50%)	6 (100%)
Absence of adhesions	4 (66.7%)	6 (100%)
Absence of tubular tiroidization	6 (100%)	6 (100%)
Absence of fibroblast activation	2 (33.3%)	6 (100%)
Absence of fistula	6 (100%)	6 (100%)
Presence of eritrocyst aggregation	1 (16.7%)	6 (100%)
Presence of microvascular proliferation	4 (66.7%)	6 (100%)
Presence of cyderopahge	6 (100%)	6 (100%)

were incised with surgeon's knife (15 no.) The depth of the renal laceration was approximately 2 cm. Renal arterial occlusion was not applied during the surgical procedure. The surgeon with assistant immediately began one of two techniques, randomly determined, reparative procedures. After the procedure, sponges were used to collect the all visible clothes and blood. And then kidney was replaced in the renal fossa. Drains were not used.

Haemostatic techniques: As a conventional therapy, bimanual compression was maintained by the surgical assistant to the amputated renal margin throughout the duration of suture reconstruction. Segmental vessels and collecting system were repaired with absorbable sutures. Sponges or Surgicel® were not used. As an alternative to conventional method, injectable form of Ankaferd solution (2cc) was applied to the amputated renal margin slowly until the bleeding had stopped. Electrocautery was not used.

Parameters of study: Reconstruction time (RT) and ABS application number were recorded. Time to hemostasis was defined as time from initial renal injury to complete hemostasis (RT). Weight and measures of renal specimens were determined. Live condition was observed at the end of the first month. At the sacrification, urine extravasation, adherence to the adjacent organs, infection in renal operated margin was evaluated. Pathologic specimens were evaluated with emphasis on presence or absence of giant cell reaction, intestinal metaplasia, acute inflammation, foreign material reaction, fibrosis, adhesions, necrosis, fistula, erythrocyte aggregation, microvascular proliferation, fibroblastic activation, cyderopahge, glomerular necrosis, and calcification. We scored the pathologic parameters with our new accepted scoring system.[1] Therefore, we compared the groups according to the most important pathologic features (Table 1).

Statistical Analysis: The results were analyzed with SPSS 12.0/Windows (SPSS Inc., Chicago, IL). Fisher's exact test and Mann-Whitney U tests were used for evaluating significance among the groups.

RESULTS

Major renal injury with surgeon's knife was completed in 12 rats. Active bleeding was demonstrated for each kidney. The rats used for this experimental study had similar morphometric characteristics in body and kidney shape (1.5 x 1 x 3 cm). The resected lower pole kidney tissues were also similar in size and shape, approximate 1 cm^2. All animals survived during the operation and postoperative period.

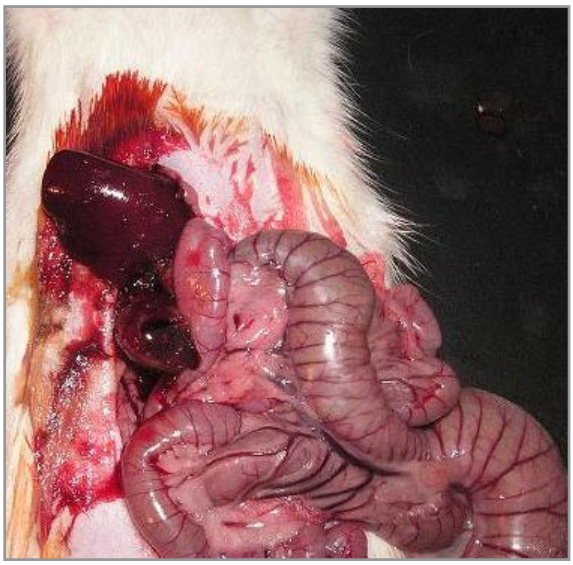

Figure 3. Ankaferd induced protein network, macroscopic view

Peroperative findings: Hemostasis was attained intraoperatively in all control and experimental animals. All ABS experimental animals received completely sutureless renal treatment. The blood loss could not being evaluated objectively due to the small kidney size and resected tissue. There were no significant intraoperative complications. Mean RT and time to hemostasis was 3.7 (2.2-4.2) minute, 3.2 (2.8-3.6) minute. in GI, GII respectively with no difference was detected ($p > 0.05$). Mean number of ABS gout was 6.0 (5-8) in GII. In ABS group, the formation of aggregate (protein network) was observed macroscopically onto the resected area following the active hemostasis (Figure 3).

Sacrification and postsacrification data: No clinically significant perinephric hematomas or urinomas were noted in any treatment group on macroscopic view. In GI, half of the group had a significant adherence to the adjacent (Figure 4A) tissue while in GII, they were all in a good shape especially nearly to resected area, however, gelatinous, redness and wealthy tissue were observed in a macroviews at transected kidney (Figure 4B).

Histopathologic findings: As a scoring system which was written by our group in previous study (1), we assessed the specimens due to this scoring system. Glomerular necrosis and tissue calcification were highly demonstrated in GI compared with GII ($p < 0.05$) (Figure 5). Erythrocyte aggregation was confirmed significantly higher in ABS group (Figure 6). Giant cell reaction, acute inflammation, fibrosis, adhesion, tiroidization, fibroblast activation, microvascular proliferation were not statistically different among groups ($p > 0.05$) (Table 1)

DISCUSSION

Patients with minor renal trauma (GI, II) may often be observed without surgical intervention.[3,4] However, the renal deep trauma like our experimental model may generally need surgical treatment to stop the bleeding and prevent the mortal end. Incre-

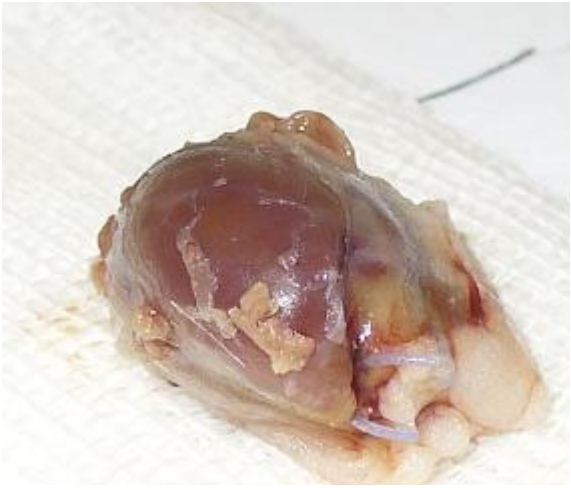

Figure 4A. Fibrotic, adherence to the adjacent tissue in conventional suture group

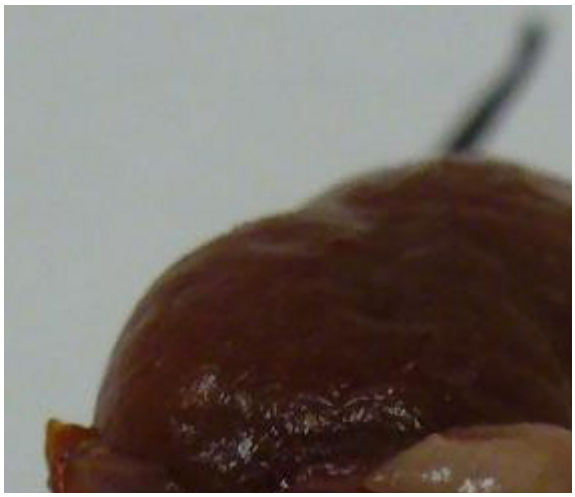

Figure 4B. Wealthy, reddy area of trauma surface of renal tissue in ABS group

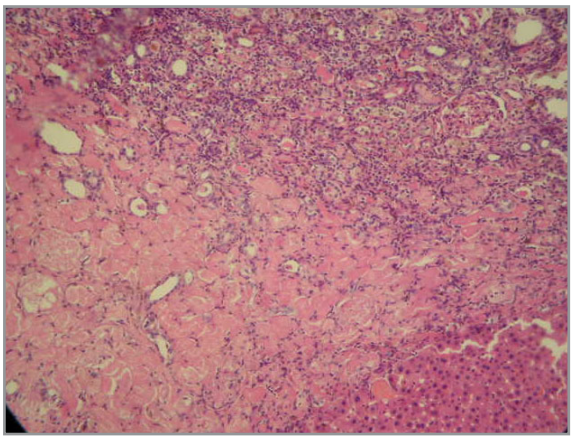

Figure 5. Glomerular necrosis in area of resected section in renal trauma group, H&E, reduced from X20

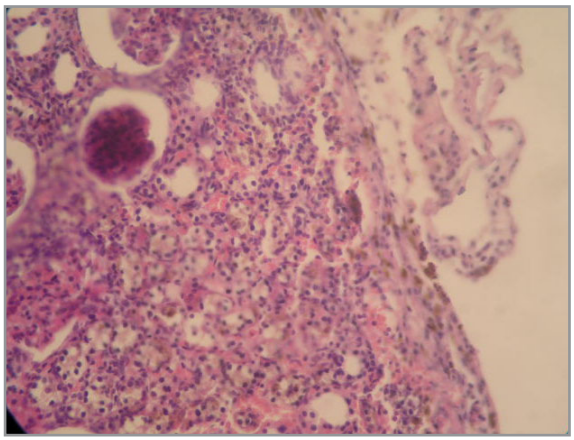

Figure 6. Erythrocyte aggregation in ABS group, H&E reduced from X40

ased risk of hemorrhage in penetrating renal trauma concludes the unfortunate nephrectomy. The nephrectomy rates in this type of renal trauma is reported up to 50%.[3,5,6] For this reason, the popularity of haemostatic agents in renal trauma for stop the bleeding has been increasing nowadays.

Various type of renal trauma model was created in the literature.[7,8,9] In experimental models, the aim is providing a major bleeding in renal parenchyma and collecting duct as in our model. Additionally, we cut a lower pole renal tissue following the penetrating trauma to evaluate histopathologic effect of ABS. However, application of only laceration to the renal tissue may not provide to observe the efficacy of ABS as a haemostatic agent because surgical access to bleeding segmental vessels deep within the kidney is often problematic.[7]

Ankaferd Bloodstopper a medicinal product, has been approved by Ministry of Health, in the management of external hemorrhage and dental surgery bleedings in Turkey based on safety and efficacy reports indicating its sterility and nontoxicity (www.ankaferd.com)[1], however, includes a standardized mixture of five plants that having hematological and vascular actions.[10-13] It comprises a standardized mixture of the plants Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum and Urtica dioica. The main mechanism of action for ABS is the formation of an encapsulated protein network that provides focal points for vital erythrocyte aggregation.[2] ABS has also been used in a case with upper gastrointestinal bleeding effectively.[14] In another one, therapeutic potential for the management of hemorrhage in open heart surgery was confirmed.[15] In our study, ABS has been used to control bleeding, by the way of that; the efficacy and responsibility of the new haemostatic agent have been assessed.

The various type of haemostatic agents were tried to aspect the efficacy in renal trauma model. Morey et al. showed that the absorbable fibrin adhesive bandage appears to decrease blood loss and the time to hemostatis significantly compared with conventional techniques of renal renal reconstruction in porcine renal stab wound model.[16] In our study, no significant difference in time to hemostasis was detected among the groups. However, the effective hemostasis was provided in ABS group. Hick et al demonstrated the efficacy of gelatin matrix in porcine renal complex injuries[8], however, the histopathologic effects of these agents were not discussed in the literature as well. We stressed the importance of microscopic evaluation of renal trauma tissue following the conventional or ABS therapy. Actually, the results of histopathologic evaluation were similar to our previous study regarding the partial nephrectomy.[1] Presence of erythrocyte aggregation and absence of glomerular necrosis and calcification in ABS group showed the reliability of this product on renal tissue application. Additionally, it was shown by Cipil et. al, ABS had in vivo hemostatic actions that might provide a therapeutic potential for the management of patients with deficient primary hemostasis in clinical medicine, however, the

other article had also been confirmed the hemostatic acitivity of ABS via topically on rectal ulcer bleeding.[17,18]

In this study, we aimed the evaluation of reliability and efficacy of ABS in renal trauma model. It may be concluded that the goals were achieved by our team with demonstrating the efficacy and biocompatibility of ABS. The small size of vessels in rat kidney can be thought as a restrictive factor throughout the evaluation of hemostasis in kidney.

CONCLUSION

ABS significantly provided the hemostasis in major renal trauma model in rats. Demonstrating the erythrocyte aggregation in our ABS groups is compatible with the basic mechanism of action for ABS that appears to be the formation of protein network providing focal points for erythrocyte aggregation. Preclinical and clinical studies are recommended to evaluate exact efficacy of ABS in renal trauma with uncontrollable bleeding.

REFERENCES

1. Huri E, Akgül T, Ayyıldız A, et al. Haemostatic role of the folkloric medicinal plant extract Ankaferd Blood stopper® in rat partial nephrectomy model: Controlled experimental trial. J Urol 181:2349-2354, 2009.
2. Goker H, Haznedaroglu IC, Ercetin S, et al. Haemostatic actions of the folkloric medicinal plant extract Ankaferd BloodStopper. J Int Med Res 36: 163-170 2008.
3. Armenakas NA, Ducket CP, McAninch JW. Indications for nonoperative management of renal stab wounds. J Urol 161: 768- 771, 1999.
4. Eastham JA, Wilson TG, Ahlering TE. Urological evaluation and management of renal-proximity stab wounds. J Urol 150: 1771- 1773, 1993.
5. Cavalcanti AG, Filho DR, Javaroni V, et al. Incidence of urinary tract lesions in 715 surgical explorations due to abdominal gunshot wound. J Urol suppl 163: 3, abstract 13, 2000.
6. McAninch JW, Carroll PR, Armenakas NA, et al. Renal gunshot wounds: methods of salvage and reconstruction. J Trauma 35: 279- 283, 1993.
7. Griffith BC, Morey AF, Rozanski TA, et al. Central renal stab wounds:treatment with augmented fibrin sealant in a porcine model. J Urol 171: 445-447, 2004.
8. Hick AJ, Morey AF, Haris RA, et al. Gelatin matrix treatment of complex renal injuries in a porcine model. J Urol 173: 180-1804, 2005.
9. Pursifull NF, Morris MS, Haris RA, et al. Damage control management of experimental grade 5 renal injuries:further evaluation of Floseal gelatin matrix. J Trauma 60: 346-350, 2006.
10. Matsuda H, Ando S, Morikawa T, et al. Inhibitors from the rhizomes of Alpinia officinarum on production of nitric oxide in lipopolysaccharide-activated macrophages and the structural requirements of diarrylheptanoids for activity. Bioorg Med Chem 14: 138-142, 2006.
11. Lee SJ, Umano K, Shibamato, et al. Identification of volatile components in basil (Ocimum basilicum L.) and thyme leaves (Thymus vulgaris L.) and their antioxidant properties. Food Chem 91: 131-137, 2007.
12. Testai L, Chericoni S, Calderone V, et al. Cardiovascular effects of Urtica dioica L.(Urticaceae) roots extracts: in vitro and invivo pharmacological studies. J Ethophramacol 81: 105-109, 2002.
13. Sheela ML, Ramakrishna MK, Salimath BP. Angiogenic and proliferative effects of the cytokine VEGF in Ehrlich ascites tumor cells is inhibited by Glycyrrhiza glabra. Int Immunopharmacol 6: 494-498, 2006.
14. Kurt M, Disibeyaz S, Akdoğan M, et al. Endoscopic application of Ankaferd Bloodstopper as a novel experimental treatment modality for upper gastrointestinal bleeding: A case report. Am J Gastroenterol 103: 2156-2158, 2008.
15. Dogan OF, Ozyurda U, Uymaz UK, et al. New anticoagulant agent for CABD surgery. Eur J Clin Invest 38: 341, 2008.
16. Morey AF, Anema JG, Haris R, et al. Treatment of renal stab wounds with absorbable fibrin adhesive bandage in a porcine model. J Urol 165: 955-958, 2001.
17. 17. Cipil H, Kosar A, Kaya A, et al. In vivo hemostatic effect of the medicinal plant extract Ankaferd Bloodstopper in rats pretreated with Warfarin. Clin Appl Thromb Hemost 15: 270-276, 2009.
18. Ibis M, Kurt M, Onal IK, et al. Successful management of bleeding due to solitary rectal ulcer via topical application of Ankaferd blood stopper. Altern Complement Med 14: 1073-4, 2008.

Correspondence

Dr. Emre HURİ

Hilmi Barlas Arinnapark Sitesi 1

808. Sokak No: 2/A/24

Çayyolu, Ankara / TURKEY

Tel: (+90.312) 240 04 68

Fax: (+90.312) 485 36 76

E-mail: dremrehuri@yahoo.com

emrehuri@gmail.com