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Surgical and histopathological effects of topical Ankaferd® hemostat on major arterial vessel injury related to elevated intra-arterial blood pressure

Topikal Ankaferd® hemostatın büyük arter yaralanmalarında artmış arter içi basınç ile ilişkili cerrahi ve histopatolojik etkileri

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Abstract

Objective: The aim of this study was to assess the surgical and histopathological hemostatic effects of topical Ankaferd blood stopper (ABS) on major arterial vessel injury related to elevated intra-arterial blood pressure in an experimental rabbit model.
Materials and Methods: The study included 14 New Zealand rabbits. ABS was used to treat femoral artery puncture on 1 side in each animal and the other untreated side served as the control. Likewise, for abdominal aortic puncture, only 50% of the aortic injuries received topical liquid ABS and the others did not (control). The experiment was performed under conditions of normal arterial blood pressure and was repeated with a 50% increase in blood pressure. Histopathological analysis was performed in all of the studied animals.
Results: Mean bleeding time in the control femoral arteries was 105.0±18.3 s, versus 51.4±9.8 s ($p<0.05$) in those treated with ABS. Mean blood loss from the punctured control femoral arteries was 5.0±1.5 mg and 1.6±0.4 mg from those treated with ABS ($p<0.05$). Histopathological examination of the damaged arterial structures showed that ABS induced red blood cell aggregates.
Conclusion: ABS administered to experimental major arterial vessel injury reduced both bleeding time and blood loss under conditions of normal and elevated intra-arterial blood pressure. ABS-induced erythroid aggregation was prominent at the vascular tissue level. These findings will inform the design of future experimental and clinical studies on the anti-bleeding and vascular repairing effects of the novel hemostatic agent ABS. *(Turk J Hematol 2011; 28: 206-12)*
Key words: Bleeding, cardiovascular, surgery, hemostasis

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Özet

Amaç: Bu çalışmanın amacı büyük arter yaralanmalarında arter içi basınç artışı ile paralel olarak uygulanan topikal Ankaferd Blood Stopper (ABS)'ın cerrahi ve histopatolojik hemostatik etkilerini deneysel bir tavşan modelinde değerlendirmektir.
Yöntemler ve Gereçler: Çalışma grubunu ondört Yeni Zelanda tavşanı oluşturmuştur. Hayvanların bir ekstremitesinde oluşturulan femoral arter hasarında ABS uygulanırken karşı ekstremite kontrol olarak kullanılmıştır. Benzer biçimde, abdominal aort hasarı oluşturulan hayvanlarda, hayvanların yarısında topikal ABS uygulanırken diğer grup kontrol olarak çalışmaya alınmıştır. Büyük arter kanamaları normal arteriyel basınç altında oluşturulmuşken, çalışma intra-arteriyel basınç %50 arttırılarak tekrar edilmiştir. Histopatholojik incelemeler çalışılan tüm hayvanlarda gerçekleştirilmiştir.
Bulgular: Hasar görmüş femoral arterden gelişen ortalama 'kanama zamanı' ABS olmadan 105.0±18.3 sn. iken topikal ABS uygulamasıyla 51.4±9.8 saniyeye düşürülmüştür ($p<0.05$). Hasar görmüş femoral arterden gelişen ortalama 'kanama miktarı' ABS olmadan 5.0±1.5 mg iken topikal ABS uygulamasıyla 1.6±0.4 mg'a gerilemiştir ($p<0.05$). Abdominal aorta kanama modelinde ise ortalama 'kanama zamanı' ve ortalama 'kanama miktarı' kan basıncı yükseltildiğinde bile ABS kullanımıyla azalmasına karşın kontrol grupları ile farklılık istatistiksel anlamlılık düzeylerine erişmemiştir. Hasar görmüş arteriyel yapıların histopatolojik incelemelerinde ABS uygulaması ile ilişkili kırmızı küre aggregatları belirgin biçimde gözlenmiştir.
Sonuç: Topikal Ankaferd Hemostat uygulaması deneysel büyük arter modelinde "kanama zamanı" ve "kanama miktarı" değerlerini normal ve yüksek arter-içi basınç durumlarında aşağıya çekmiştir. ABS-bağımlı eritroid aggregasyon vasküler doku düzeyinde belirgin olarak gösterilmiştir. Gözlemler bu yeni hemostatik ajan'ın kanamayı engelleyici ve damar onarımına zemin hazırlayan etkilerinin gelecek deneysel ve klinik çalışmalarla ortaya konulması için temel teşkil etmektedir.
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Anahtar kelimeler: Ankaferd, kanama, kardiyovasküler cerrahi, hemostaz

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Introduction

Ankaferd blood stopper (ABS) is an herbal extract [1] that has been historically used as a hemostatic agent in traditional Turkish medicine [2] for centuries. The herbal medicine is comprised of a standardized mixture of *Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum*, and *Urtica dioica* [3]. Each of these herbs affect endothelium, blood cells, angiogenesis, cell proliferation, vascular dynamics, and cellular mediators. *G. glabra* inhibits angiogenesis, and decreases vascular endothelial growth factor production and cytokine-induced neovascularization. *T. vulgaris* has been shown to exhibit varying levels of antioxidant activity, which may help prevent *in vivo* oxidative damage, such as lipid peroxidation associated with atherosclerosis. *V. vinifera* has an anti-atherosclerotic effect. *A. officinarum* inhibits nitric oxide production in lipopolysaccharide-activated mouse peritoneal macrophages. *U. dioica* produces hypotensive responses via a vasorelaxation effect mediated by the release of endothelial nitric oxide and the opening of potassium channels, and via negative inotropic action [3]. The basic mechanism of action of ABS is the formation of an encapsulated protein network representing focal points for vital erythrocyte aggregation [1].

ABS was shown to effectively manage external bleeding in numerous clinical settings [4-13]. ABS-induced formation of the protein network with vital erythroid aggregation encompasses the entire physiological hemostatic process. The ABS-induced hemostatic network includes important distinct components. Vital erythroid aggregation occurs in spectrin and ankyrin receptors on the surface of red blood cells. These proteins and the required ATP bioenergy are included in the ABS protein library. ABS also upregulates the GATA/FOG transcription system, which affects erythroid functions. Urotensin II is also an essential component of ABS and represents the link between injured vascular endothelium, adhesive proteins, and active erythroid cells [14-16].

The aim of the present study was to assess the surgical and histopathological hemostatic effects of topical ABS on major arterial vessel injury related to elevated intra-arterial blood pressure in an experimental rabbit model. As there are some anecdotal reports on the use of ABS in in different surgical interventions [17-21], elucidation of the surgical and histopathological effects of ABS will inform future research on this novel hemostatic agent.

Materials and Methods

Study population

The study included 14 male white New Zealand rabbits obtained from the Refik Saydam Institute. The animals were kept in a room at a constant optimal temperature (20-22°C) under 12 h of darkness and 12 h of sun light. All of the animal experiments were carried out in accordance with the European Community Council Directive of 24 November 1986 and were approved by the Gazi University Animal Experiments Ethics Committee (G.Ü.ET-09.045).

Study design and experiments

The animals underwent surgery to generate major arterial bleeding under normal blood pressure conditions (set-up 1) and a 50% increase in arterial blood pressure (set-up 2) induced via concurrent dopamine infusion (5-10 μg·kg/min) [22]. Set-up 1 included 8 rabbits and set-up 2 included 6 rabbits. Liquid ABS was topically applied to the femoral artery puncture in 1 extremity in each animal (femoral artery 1), but not to the other extremity, which served as the control (femoral artery 2). Abdominal aortic injury was treated likewise; 50% of the aortic injuries were treated with 1 mL of liquid ABS (abdominal aorta 1) and the others were untreated and served as the control (abdominal aorta 2). Histopathological analysis was performed in all the animals.

All of the rabbits underwent surgery under proper general anesthesia without intubation. An arterial line was placed for monitorization via the ear artery and infusion vein. A 22G cannula was used for catheterization. Anesthesia was provided via intramuscular injection of ketamine hydrochloride 50 mg/kg and xylazine hydrochloride 5 mg/kg. All of the rabbits underwent femoral artery and abdominal aorta surgery. After administration of anesthesia, both femoral arteries were explored using a sterile surgical technique, and separated from the femoral vein and femoral nerve. The femoral arteries were rounded with tapes and a 21G injector needle was inserted once at 90° into the arteries to induce major bleeding [23]. After bleeding began we immediately applied compression with gasses and calculated the bleeding time and blood loss by measuring the weight of the gasses before and after decompression. After the first bleeding stopped, major bleeding was induced in the other extremity using the same needle. After the second bleeding started we immediately applied 1 mL of liquid ABS and compression, and again measured the bleeding time and blood loss, as described above. After the bleeding from both femoral arteries stopped, we made a paramedian incision to explore the abdominal aorta and rounded it with tapes just below the renal arteries. Major bleeding was induced using the same method as described above. After the experiment we obtained vessel samples (from 0.5 cm below and above the needle hole, just below the renal arteries for the abdominal aorta and femoral arteries) for pathological examination. When the experiment was completed the rabbits were sacrificed for histopathological analyses via IM pentamine injection. Systolic and diastolic oscillations in arterial blood pressure were monitored from the left carotid artery using a BIOPAC MP30 recorder (BIOPAC System, Inc., California, USA).

We analyzed the hemoglobin, hematocrit, and INR measurements before and after the procedure, and calculated blood loss and bleeding time as well. Pathological specimens were preserved in formaldehyde. Histopathological assessment of the tissue samples was performed using standard histological techniques, including formalin fixation, dehydration, embedding in paraffin blocks, obtaining serial transverse sections (4 μm), and hematoxylin-eosin staining. All histopathological investigations were carried out by the same pathologist using a light microscope.

Statistical analysis

All values are presented as mean±SEM. Comparison of findings between the treatment and control femoral arteries and abdominal aortas were statistically analyzed using the Mann-Whitney U test. Alterations in the parameters over time were analyzed using the Wilcoxon test. A p value <0.05 was considered statistically significant.

Results

Mean hemoglobin value before the procedure was 12.2±0.7 g/dL, versus 11.8±0.6 g/dL after the procedure ($p>0.05$). Mean INR was 1.0±0.3 before the procedure and 0.8±0.1 after the procedure ($p>0.05$). According to these measurements, ABS

did not significantly affect the basic coagulation parameters we studied.

In the set-up 1 animals (normal blood pressure) mean bleeding time from femoral artery 2 (control) was 105.0±18.3 s, versus 51.4±9.8 s from femoral artery 1 (ABS treatment) ($p<0.05$) (Figure 1). Mean blood loss from femoral artery 2 was 5.0±1.5 mg and 1.6±0.4 mg from femoral artery 1 ($p<0.05$) (Figure 2). Mean bleeding time from abdominal aorta 1 (control) was 243.8±48.5 s, versus 205.0±94.6 s in abdominal aorta 1 (ABS treatment) ($p>0.05$) (Figure 1). Mean blood loss was 6.6±2.6 mg from abdominal aorta 2 and 3.7±0.1 mg from abdominal aorta 1 ($p>0.05$) (Figure 2).

In the set-up 2 animals (elevated blood pressure) we intended to increase the blood pressure by 50% using dopamine infusion, and then performed the same procedure as in set-up 1 animals. We obtained an arterial line from the ear artery, and measured blood pressure before and after dopamine infusion. Mean blood pressure before the procedure was 83.3±6.5 mmHg, versus 137.5±11.6 mmHg after starting dopamine infusion ($p<0.05$). Mean bleeding time from femoral artery 2 was 150.8±21.6 s, versus 139±23.5 s from femoral artery 1 ($p>0.05$) (Figure 3). Mean blood loss was 7.1±2.9 mg from femoral artery 2 and 3.1±0.7 mg from femoral artery 1 ($p>0.05$) (Figure 4). Mean bleeding time from abdominal aorta 2 was 306.7±208 s, versus 206.7±17.6 s from abdominal aorta 1 ($p>0.05$) (Figure 3). Mean blood loss was 3.4±1.5 mg and 1.8±1.2 mg from abdominal aorta 2 and 1, respectively ($p>0.05$) (Figure 4).

Histopathological examination of the injured vessels showed that the vessel lumens were enriched with erythrocyte aggregates following ABS administration (Figure 5). In contrast, control vessels had fewer erythrocytes, without aggregation (Figure 6).

Discussion

The present study aimed to determine if ABS could be successfully used to control major arterial bleeding from the femoral artery and abdominal aorta, particularly in the presence of elevated systemic arterial blood pressure. In cardiovascular surgery major bleeding is an important complication that leads to morbidity and mortality. Bleeding sometimes cannot be controlled with current con-

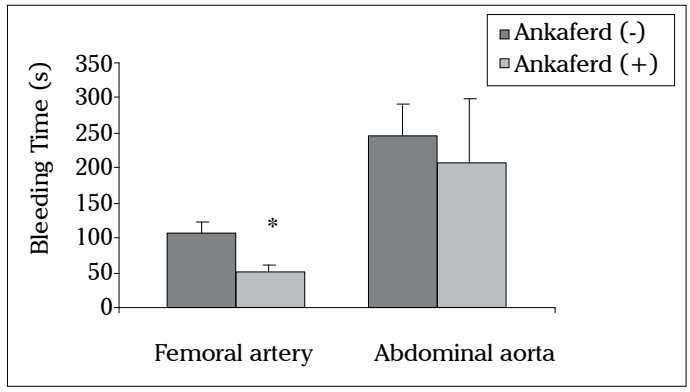

Figure 1. Femoral artery and abdominal aorta bleeding time without dopamine infusion (normal blood pressure) (*$p<0.05$)

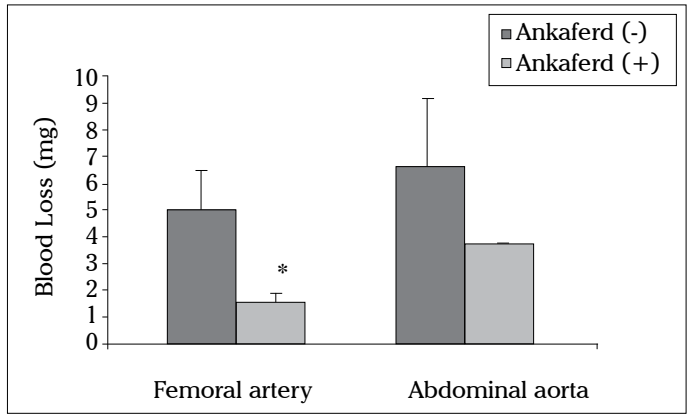

Figure 2. Femoral artery and abdominal aorta blood loss without dopamine infusion (normal blood pressure) (*$p<0.05$).

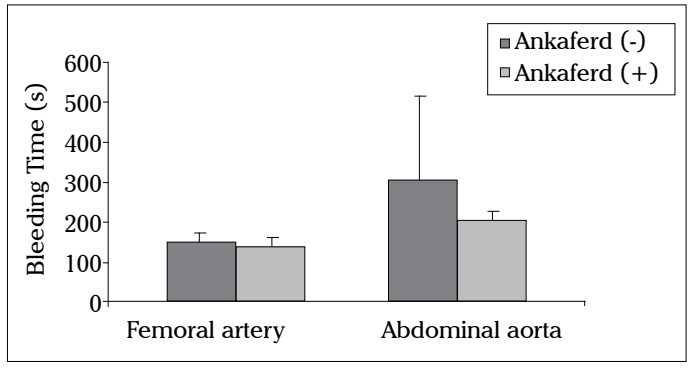

Figure 3. Femoral artery and abdominal aorta bleeding time with dopamine infusion (elevated blood pressure)

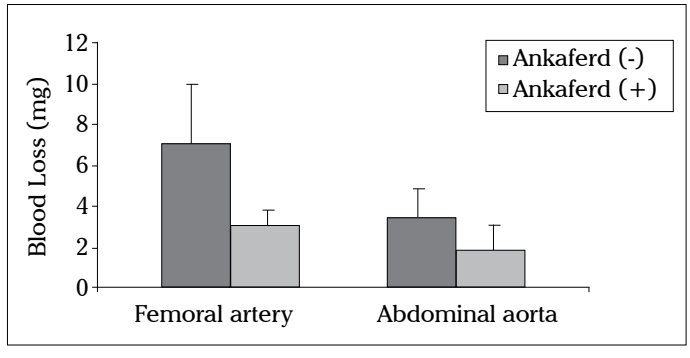

Figure 4. Femoral artery and abdominal aorta blood loss with dopamine infusion (elevated blood pressure)

Figure 5. ABS-induced erythrocyte aggregates in the vessel lumen (H&E, 100×)

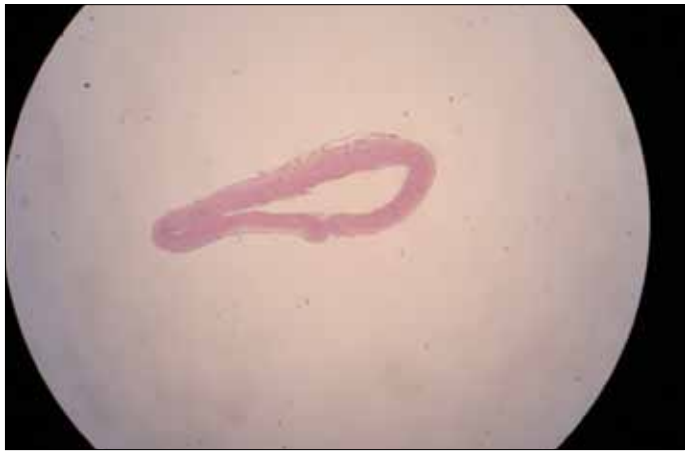

Figure 6. Fewer erythrocytes next to the wall in a vessel lumen not administered ABS (H&E, 100×)

ventional methods, even during re-operations, when the focal surgical bleeding point cannot be found and/or bleeding is massive, originating from all of the surrounding tissues. The findings of the present study represent a starting point in the search for the clinical anti-hemorrhagic effects of the novel liquid hemostatic agent, ABS.

In the present study bleeding time from the injured major arteries was shorter and blood loss was lower following the use of ABS. Better essential hemostatic parameters were observed following administration of ABS, even in the presence of elevated arterial blood pressure. Three ABS phase III studies [4,10,12] on vascular port insertion bleeding, anterior epistaxis, and post-tonsillectomy hemorrhage have led to its approval as a hemostatic agent in Turkey. Use of ABS to control bleeding in gastrointestinal disorders [8, 24-27] and mediastinal bleeding [5,17] shed further light on its hemostatic efficacy. As such, the anti-hemorrhagic efficacy and safety of ABS was determined based on a wide variety of findings designed in different ways of clinical and experimental studies

ABS has the diverse dynamic reversible actions of an EPCR and PAI-1 inside vascular endothelial cells in an HUVEC model. Immediate enhanced expression of pro-hemostatic PAI-1 and down-regulation of anti-coagulant EPCR upon exposure to ABS are compatible with the sudden anti-hemorrhagic efficacy previously shown in experimental and clinical settings. The topical hemostatic efficacy of ABS has been previously tested in animals with normal and defective hemostasis [28,29]. Experimental studies have set the preclinical stage for the development of this hemostatic product. The short-term hematological and biochemical safety of oral systemic administration of ABS to rabbits have been reported. Acute mucosal toxicity, hematotoxicity, hepatotoxicity, nephrotoxicity, and biochemical toxicity were not observed during the short-term follow-up of the animals [30]. Those preclinical results represent a starting point in the search for any possible systemic confounding effects of ABS when topically applied to internal surfaces. Use of ABS as a hemostatic agent for external hemorrhages and dental treatment in humans constitutes the first reports of ABS's safety and efficacy in humans. A phase I, double-blind randomized cross-over placebo-controlled clinical study with a 5-d wash-out period between the cross-over periods, which was conducted with healthy volunteers, reported that ABS was safe [6]. Physiological cell-based coagulation could be clinically managed via topical ABS application, so as to prevent and treat bleeding associated with many distinct clinicopathological states.

ABS also has pleiotropic cellular action [31], acting on anti-infective [32-34], wound healing [35-37], vascular dynamics [38], and apoptotic processes [15,39,40]. Histopathological examination of the damaged arterial structures in the present study showed that ABS induced red blood cell aggregates, supporting the hypothesis that ABS-induced formation of the protein network with vital erythroid aggregation encompasses the entire physiological hemostatic process.

In conclusion, topical application of ABS in an experimental major arterial vessel injury model reduced bleeding time and blood loss under normal and elevated intra-arterial blood pressure con-

ditions. ABS-induced erythroid aggregation was prominent at the vascular tissue level. The present study's findings will inform the design of future experimental and clinical studies on the anti-bleeding and vascular repairing effects of this novel hemostatic agent.

Conflict of interest statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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