EXPERIMENTAL STUDY

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The efficacy of the hemostatic agent Ankaferd blood stopper on end-to-end unilateral sleeve fish-mouth anastomosis

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Objective: The aim of this study was to examine if the application of Ankaferd Blood Stopper (ABS) reduces the number of sutures and therefore reduces anastomosis completion time in a unilateral end-to-end sleeve fish-mouth anastomosis model.

Methods: Femoral artery end-to-end unilateral fish-mouth anastomosis models were created from the right and left femoral arteries of 14 male Wistar albino rats (weight: 250 to 300 grams) and divided into 2 equal groups. Rats in Group A received ABS and Group B was the control group. Rats were further divided into equal 2 subgroups, and anastomoses of rats in Group 1A and 1B were explored on the 7th day and on the 14th day in Group 2A and 2B. The groups were compared for anastomosis completion time, macroscopic and microscopic patency, existence of microaneurysm and inflammatory response.

Results: In the ABS group (1A and 2A), mean anastomosis completion time was 13:00±1.50 minutes, and 18:56±2.5 minutes in the control groups (1B and 2B). This difference was statistically significant (p=0.001).

Conclusion: Ankaferd Blood Stopper may be used to reduce the number of sutures and shorten the completion time of artery-to-artery anastomosis of arteries with small diameter and low blood flow rate.

Key words: Anastomosis time; Ankaferd Blood Stopper; fish-mouth sleeve anastomosis.

Reconstructive microsurgery involves current techniques such as free tissue transfer, replantation and supermicrosurgery. In microsurgical anastomosis, a simple interrupted suture technique is considered to be the traditional method. As well as being relatively time-consuming, traditional methods present certain problems, including increased reaction to foreign bodies and vascular thrombosis.

In our study, we examined the efficacy of the hemostatic agent Ankaferd Blood Stopper (ABS) in unilateral end-to-end sleeve fish-mouth anastomosis and compared our findings with traditional methods.

Materials and methods

This study was assessed and approved by the Bağcılar Training and Research Hospital Experimental Animal

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Trials Ethics Committee in 2010 (Ref. no: 2010/314) and performed in the Experimental Animal Laboratory of the same hospital.

Fourteen male Wistar albino rats weighing between 250 to 300 grams were used in the study. Rats were separated into 2 equal groups of 7 rats and 14 anastomoses. Groups were further divided into two subgroups. In Groups 1A (n=7) and 2A (n=7), right femoral artery end-to-end sleeve fish-mouth anastomosis was performed. Groups 1B (n=7) and 2B (n=7) were the control groups and left femoral artery end-to-end anastomosis with simple interrupted sutures was performed. Anastomoses in Group 1 were explored on Day 7 and on Day 14 in Group 2.

Rats were anesthetized using intraperitoneal ketamine hydrochloride at a dose of 90 mg/kg. In all groups, the femoral artery was cut and an approximating clamp was placed.

In Groups 1A and 2A, the distal part of the femoral artery was prepared as a fish-mouth with 2 vertical incisions and the proximal part of the artery was placed in the distal artery in an end-to-end sleeve fashion. Anastomosis was performed by placing 2 sutures at 12 o'clock and 6 o'clock, using 10.0 nylon sutures (Figs. 1 and 2). Upon completion of the anastomosis, the distal clamp was removed to allow for partial vascular refilling in the medium. The distal clamp was reapplied and ABS was sprayed generously on the sleeve anastomosis (Fig. 1). After 10 to 15 seconds, first the distal clamp, then the proximal clamp was opened. Leakage and patency control was observed along the anastomosis line (Fig. 1). Duration of anastomosis performed was recorded using a chronometer.

In Groups 1B and 2B, the same procedure was performed on the left femoral artery without application of ABS. Adventitial simple interrupted sutures in the required number (4 to 6) were made on the anterior and posterior walls of the anastomosis in order to terminate anastomosis leakage.

Anastomosis time, patency ratio and microaneurysm formation were recorded. Anastomosis time was defined as the time interval between the placement and the removal of the approximator clamps after completion of macrodissection and microdissection. Vessel patencies were evaluated using Acland's milking test, immediately after the anastomosis and prior to histological sampling on Days 7 and 14.[1] Formation of microaneurysm was checked under microscope before histological sampling on Days 7 and 14.

Groups were re-operated on Days 7 (Group 1) and 14 (Group 2) and anastomosis areas were explored. Samples were stained using hematoxylin-eosin stain. All cross-sections were assessed in terms of endothelial integrity, tissue necrosis, existence of sutures, existence of inflammatory cells (lymphocytes, PMNLs), inversion and changes of the adventitia. Reaction developed in the adventitia against foreign body, lymphocytes, PMNL, histiocytes and vessel proliferation were evaluated. Luminal obliteration and microvascular proliferation were examined. Endothelial proliferation was evaluated as follows; Grade 0: One to two endothelial cells, Grade 1: Three to four endothelial cells, Grade 2: Five to six

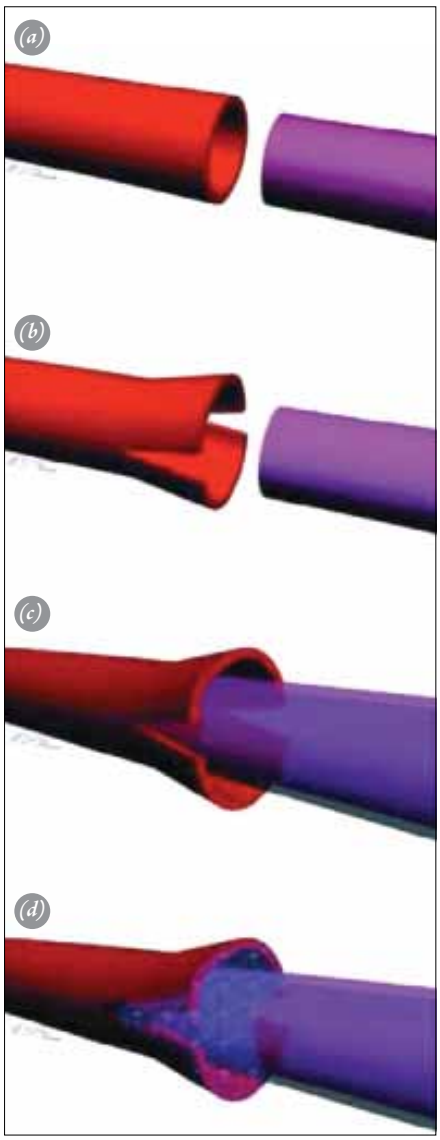

Fig. 1. **(a-d)** Schematic view of the phases of end-to-end unilateral sleeve fish-mouth anastomosis using Ankaferd Blood Stopper. The proximal vessel is stained in purple and distal vessel in red. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

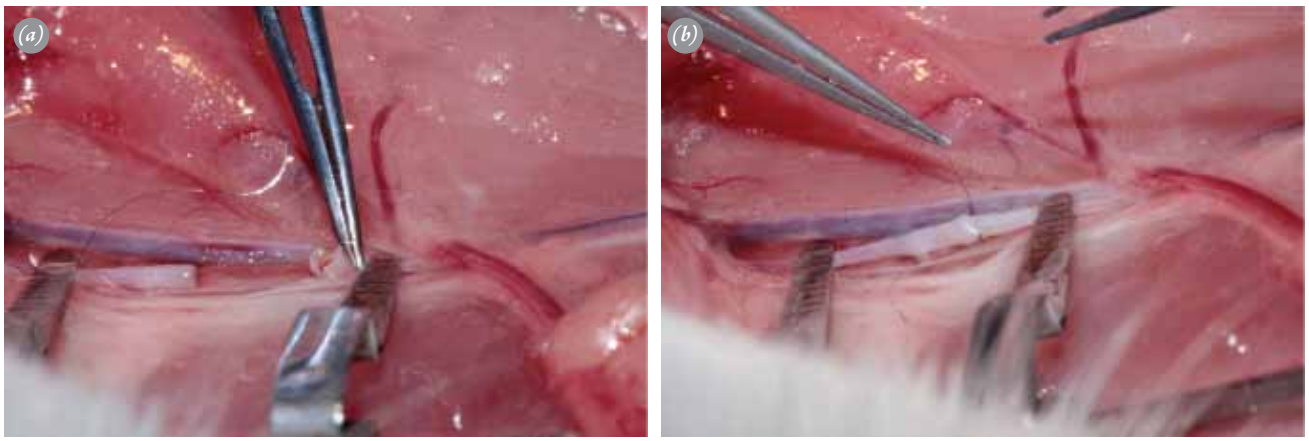

Fig. 2. Macroscopic views of **(a)** creating a fish-mouth anastomosis and **(b)** end-to-end sleeve anastomosis after two sutures were placed in 180° locations. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

endothelial cells, and Grade 3: Seven or more endothelial cells. Tissue necrosis was defined as; Grade 1: 1/3 of the wall, Grade 2: 2/3 of the wall, and Grade 3: more than 2/3 of the wall. Inflammatory cells (lymphocytes, PMNL, histiocytes) and vessel proliferation were evaluated semi-quantitatively to as mild (+), moderate (++), and severe (+++) (Fig. 3).

Data were analyzed using the Mann-Whitney U-test, chi-square test and Fisher's exact test on SPSS 19.0 (SPSS Inc., Chicago, IL, USA) software. Statistical significance was set at $p<0.05$.

Results

Mean anastomosis time was significantly lower in the experimental group (13:00±1.50 minutes) than in the control group (18:56±2.5 minutes) ($p<0.001$) (Table 1).

On Days 7 and 14, rat anastomoses were re-explored to determine the anastomosis integrity and existence of microaneurysm. Anastomoses were evaluated using Acland's 'discharge and refill' technique and 'uploading' technique.[1] No significant difference was observed between the control and test groups in terms of patency ratios ($p>0.05$) (Table 1).

Test and control groups displayed similar histological findings on Day 7. Foreign object reaction continued in both groups on Day 14, while inflammatory response (foreign body reaction, vessel proliferation, lymphocytes, histiocytes, PMNL) was less in the experimental group than the control group (Fig. 3) (Table 2).

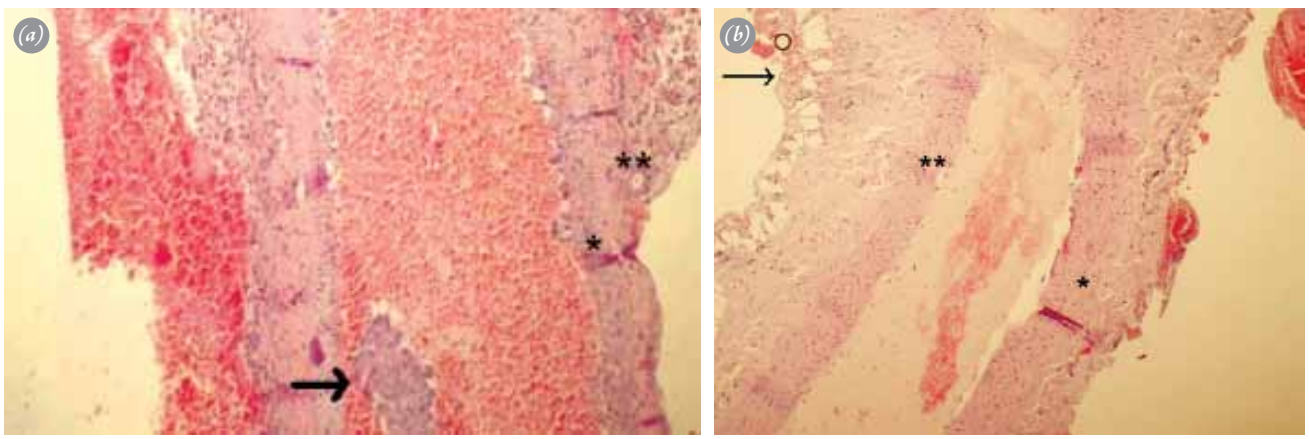

Fig. 3. **(a)** Histopathological image from the ABS group on Day 7. Arrow denotes the adventitial flap partially obliterating the lumen. Single asterisk denotes the suture reaction. Double asterisk denotes the lymphocyte style inflammatory reaction (HE, x100). **(b)** Histopathological image from the ABS group on Day 14. Grade 3 endothelial hyperplasia is seen whereas necrosis in the vessel wall is not observed. Although particularly histiocytes dominate the periadventitial inflammatory response to the suture, it is of lymphocyte style (shown by the arrow). Single asterisk denotes the suture line. Double asterisk denotes the decreased amount of endothelial proliferation on Day 14 (HE, x100). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

Table 1. Anastomosis completion time, patency and microaneurysm data for all groups on Days 0, 7 and 14.

	ABS group		Control group	
Anastomosis completion time	13:00±1.50 min. (range: 10:13-16:25 min.)		18:56±2.5 min. (range: 16:13-22:30 min.)	
	Group 1A	Group 2A	Group 1B	Group 2B
Patency				
Day 0	100%	100%	100%	100%
Day 7	86% (6/7)	–	100%	–
Day 14	–	100%	–	100%
Microaneurysm				
Day 7	None	–	None	–
Day 14	–	None	–	None

Table 2. Histologic findings of the test and control groups.

	Endothelial continuity	Endothelial hyperplasia				Necrosis				Inflammatory response		
		G_0	G_1	G_2	G_3	G_0	G_1	G_2	G_3			
ABS group day 7	+	–	1	–	6	4	1	1	1	6*	2	7†
Control group oay 7	+	1	2	1	3	2	2	2	1	5*	3	7†
ABS group day 14	+	6	1	–	–	6	1	–	–	3*	–	7†
Control group day 14	+	4	1	1	1	4	1	–	2	5*	2	7†

*: Although the inflammatory response in the intimal layer is infrequent PMNL weighted, it was observed to be weaker compared to inflammatory changes due to foreign object reaction observed in the adventitia. †: Although adventitial inflammatory response is observed in all groups, it was assessed in detail in Group 2A and Group 2B.

Discussion

Microvascular anastomosis is an essential part of a successful free tissue transplant or organ replantation. Many methods have been defined for microvascular anastomosis, with end-to-end anastomosis using simple, interrupted, full-ply sutures considered the standard technique.[2] Due to the standardization of this method, establishment of universal rules and current success rates of up to 98%, this method remains the gold standard.[3,4] However, despite advantages provided to surgeons by conventional methods, certain disadvantages created by unsolved problems of multiple sutures, extended surgery time, luminal constriction and consequential foreign body reaction exist.[5-10]

Multifactorial effects such as internal dynamics of the artery subject to anastomosis, surgical experience, trauma, infection and existence of radiation prevent the 100% patency success of the anastomosis.[11,12] Today, anastomosis techniques used as an alternative to conventional methods include different suture methods (end-to-side anastomosis, changing the concavity of the artery inlet, continuous suture methods, sleeve anastomosis), mechanical devices (coupler clip stents), tissue adhesives (cyanoacrylates, fibrin adhesive) and laser.[13]

Ankaferd Blood Stopper is a hemostatic agent mixed at various ratios from 5 herbal extracts; Thymus vulgaris (0.05 mg/ml), Glycyrrhiza glabra (0.07 mg/ml), Vitis vinifera (0.08 mg/ml), Alpinia officinarum (0.07 mg/ml) and Urtica dioica (0.06 mg/ml).[14] ABS initiates the coagulation process without interrupting the coagulation factors through the primary and secondary hemostatic system by inducing erythrocytes and Protein A.[15,16] Huri et al. reported that ABS shortened the partial nephrectomy time and decreased warm ischemia period in a rat model.[17] Iynen et al.[16] and Teker et al.[18] demonstrated that ABS decreased blood loss, facilitated hemostasis and decreased the total knot count in children undergoing adenoidectomy and tonsillectomy. In vitro studies performed by Çipil et al. reviewed its effects on human umbilical vein endothelial cells and reported that ABS effectively initiated the coagulation cascade and created a protein complex within the vessel.[19]

In this study, ABS was used at the completion of end-to-end sleeve fashion fish-mouth anastomosis. It was hypothesized that ABS reduces the number of microvascular sutures required by triggering the coagulation

cascade. The ABS used in this study was not a fibrin adhesive. For this reason, alternative anastomosis methods given in the literature were modified to be suitable for ABS use. This study is a pioneer study that examines the effects of ABS on end-to-end fish-mouth sleeve anastomosis. Previous studies have combined fibrin adhesives for use with standard sleeve and fish-mouth anastomosis techniques.[20,21] The efficacy of ABS revealed similar results with fibrin adhesive products.[20] The end-to-end unilateral sleeve fish-mouth anastomosis technique used in this study was chosen in order to supply blood in the medium so that the ABS is maximally effective. Anastomosis times of the control group were significantly longer than those of the experimental group ($p<0.001$). Fibrin adhesives act by forming fibrin polymers, whereas ABS has to create complexes with proteins in the blood in order to be effective. This protein complex is structurally weaker than fibrin polymers. Padubidri et al. reported that fewer sutures and the atraumatic technique are required to prevent formation of microaneurysm.[22] Although microaneurysm was not observed in our study, we think that this parameter should be examined in vessels of different diameter. In studies with ABS, endothelialization was completed within 1 week due to less number of sutures requiring shorter surgery duration not exceeding hypoxic limit time. In addition, the need for a fewer number of stiches leads to reduced intraluminal foreign objects and consequential minimization of inflammatory response and shortened clamp time.

Due to the weaker resistance of the fibrin plug created by ABS than that of fibrin adhesives, we did not use vein-to-vein and artery-to-vein anastomoses with wider luminal diameter in our study. It was statistically determined that end-to-end anastomosis with ABS was completed with significant speed in terms of anastomosis time compared with the conventional technique. On the other hand, the method was not statistically different from the traditional method in terms of vessel clearance ratio and aneurism formation. In anastomoses needing revision, the segments of the vessel used in the anastomosis must be resected due to the effects of ABS on the vessel wall. The technique used in this study does not require separate training or equipment and is easy to apply. ABS induces a very rapid formation of a protein network within the plasma and serum by affecting the fibrinogen-erythrocyte agglutination relationship. Individual clotting factors, namely factors 5, 7, 8 and 9, 10, 11, are not affected by ABS during the consecutive measurements. Therefore, ABS is not designed for intravascular applications due its thrombogenic effects. Cytotoxicity analysis of this folkloric medicinal plant extract was carried out by the Hacettepe University Department of Pharmacology and it has been found to be non-cytotoxic. Anastomosis with ABS is not intended for vessels that have a gap between the two vessels when they are approximated due to possible intravascular leakage.

The ABS used in our study is an easily available commercial product and is safe in terms of disease contamination thanks to its herbal content. Using the same box of ABS both for multiple anastomoses in the same patient and in anastomoses performed in different patients at different times ensures a very low cost per anastomosis. We believe that this agent will be of great benefit in situations in which a surgeon needs to perform multiple anastomoses in a short period of time. Clinical studies using this agent are planned for the future.

In conclusion, ABS may be an alternative to fibrin adhesives due to its reduction of the number of sutures and consequent shortened operation time in the anastomosis of arteries with small diameter and low blood flow rate such as the digital artery. Although the efficacy of ABS in vein-to-vein and artery-to-vein anastomoses is worth investigating, the efficacy will be better for artery-to-artery anastomoses and warrants further experimental and clinical trials for routine use for this indication.

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Conflicts of Interest: No conflicts declared.

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