The potential effects of Ankaferd blood stopper and fibrin sealent on sleeve gastrectomy staple-line healing: An experimental study

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Abstract. Staple-line bleeding and leakage is a life-threatening complication in obese patients following laparoscopic sleeve gastrectomy. The aim of this study was to examine the potential effects of Ankaferd blood stopper (ABS) and Fibrin Sealant (FS; Tisseel®) on sleeve gastrectomy staple-line healing in an experimental animal model. A total of 30 Wistar albino female rats were divided into three groups and were subjected to sleeve gastrectomy with linear stapling. Group A (control group) had nothing administered, Group B was administered FS on the staple-line, and Group C was administered ABS on the staple-line following sleeve gastrectomy. After sacrifice on postoperative day 5, anastomotic burst pressure, tissue hydroxyproline levels and histopathological parameters were measured. The results revealed that group C had the highest mean bursting pressure level. However, the values of this parameter were not found to differ significantly between the groups ($P>0.05$). Group B and C had a similar hydroxyproline levels but increased compared with group A ($P<0.001$). Histopathological parameters were similar between the groups, except macrophage scores in group C. In the present experimental study, ABS was demonstrated to improve gastric-sleeved staple-line healing compared with FS. ABS may be used as a novel reinforcement agent in bariatric surgery.

Introduction

Laparoscopic sleeve gastrectomy (LSG) is a popular bariatric procedure in obese patients for weight loss and improves obesity-related comorbidities such as arterial hypertension, diabetes mellitus, obstructive sleep apnea and dyslipidemia (1,2). The major complications of LSG are leak and staple-line bleeding despite technical developments and surgical improvements in bariatric surgery (3,4). The incidence of leak after LSG and staple-line hemorrhage has been reported to be 0-8 and 0-8.7% respectively (5,6). In order to prevent these major complications, several synthetic or biological reinforcement materials, such as fibrin sealant and/ or gelatin matrix agents, Seamguard, clips and sutures has been used following LSG (4,7,8). However, there are controversial studies regarding the best method for staple-line reinforcement or its necessity in the current literature (4,8,9).

Fibrin sealant (FS; Tisseel) is an agent that provides hemostasis, sealing and adhesion and it has a wide area of use in various surgical procedures (10,11). In their study, Coşkun H and Yardımcı (4) suggested that FS is a reliable and useful agent to reinforce the staple-line and may prevent twists in the sleeved stomach.

Ankaferd blood stopper® (ABS) is a previously developed topical hemostatic agent containing a standardized mixture of the plants *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica* (12). Through its effects on the endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cell mediators; ABS is shown to have hemostatic and regenerative proliferation effects in *in vivo* and *in vitro* studies (13,14). The topical use of ABS has been approved by the Turkish Ministry of Health for the management of postsurgical bleeding (12).

In this experimental study, the aim was to compare the effects of ABS and FS in terms of bursting pressure measurement, hydroxyproline and histological examination in resected sleeve gastrectomy specimens of rats. To the best of our knowledge, this is the first study investigating the potential effects of ABS as well as FS on staple line healing in an experimental study.

Materials and methods

This animal study was performed after the approval of Akdeniz University Experimental Animal Studies Local Ethical Committee (Ethical committee no: B.30.2.AKD.0.05.07.00/120). A total of 30 adult female Wistar Albino rats weighing between 200 and 250 g were obtained from the Akdeniz University Department

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of Experimental Animals Care and Production Unit (Konyaaltı/Antalya, Turkey). The rats were kept in standard colony cages (15x25x40 cm) under controlled conditions including temperature (18˚C), light (12-h light-dark cycle) and humidity (50-55%). The rats were fed with the standard rat chow and tap water *ad libitum* during the experimental procedure except 12 h fasting period before the surgery.

After a 12-h fast, subjects were anesthetized using an intraperitoneal injection of 10 mg/kg xylazine (Bayer AG, Leverkusen, Germany) and 50 mg/kg of ketamine hydrochloride (Eczacibasi Holding A.S., Istanbul, Turkey). All surgeries were performed by a single surgeon blind to the subjects' grouping. After cleaning the skin with 10% povidone iodine (Central Laboratory, Istanbul, Turkey), a 3.5-4 cm midline laparotomy was made to access the abdominal cavity. The stomach was identified and the gastrosplenic ligament was ligated with 6/0 polyglycolic sutures and divided. The great omentum was ligated with 6/0 polyglycolic sutures and divided down to the level of pylorus. A part of the pylorus was left intact by starting the excision of the stomach 3-5 mm above the level of the pylorus, in order to maintain the free passage of the food to the duodenum. Stitches were placed defining the incision line, which contained the gastric fundus. The stapler (two rows, 3.5 mm height-1.5 mm when closed; Echelon Flex powered vasculer stapler; Ethicon, Endo-Surgery, Inc., Cincinnati, OH, USA) was positioned on that line and the great curvature along with the gastric fundus was removed, leading to a drastic reduction of the gastric volume of ~70-80% (Sleeve gastrectomy model; Fig. 1). An orogastric tube was not used during the excision of the stomach.

There were 3 experimental groups studied: Group A (control group)-only sleeve gastrectomy was performed (n=10); Group B-was administered a 4 ml box FS on staple-line after sleeve gastrectomy by spray method (n=10); and Group C-was administered 0.2 cc of ABS topically using an insulin injector on staple-line after sleeve gastrectomy (n=10). The abdominal wall was closed with running 3/0 polyglycolic suture and the skin was sewed with a 4/0 running intracutaneus suture.

Measurement of staple-line bursting pressure. On postoperative day 5, the abdomen was reopened and the sleeved stomach was located. Thereafter, total gastrectomy was made in all subjects. A 8-French catheter was fixed with 2/0 silk suture through in pylorus which was connected to a metilen perfusion pump (Samtronic ST670 infusion pump; Samtronic, São Paolo, Brazil). An intracet was also fixed with 2/0 silk suture through the esophagus that is connected to a monitor (Datex-Ohmeda, Compact Anesthesia Monitor; GE Healthcare, Chicago, IL, USA) by a pressure transducer (Transpac IV; Abbbott Laboratories, IL, USA). In order to measure bursting pressure, 6 ml/min of methylene blue was infused into the gastric lumen through the catheter, which is inserted into the pylorus using the perfusion pump and was monitored. The pressure level at the time of fluid leakage or a sudden pressure drop occurred was recorded. After the bursting pressure had been measured, the staple line segment was resected and divided into two equal parts passing through the anastomosis. One part was put in 10% formaldehyde solution for 12 h and maintained at 4˚C for histopathological examination and the other was frozen at -80˚C for measurement of hydroxyproline levels.

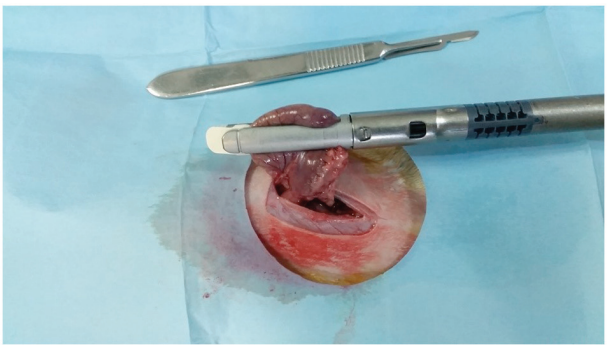

Figure 1. Sleeve gastrectomy model.

Hydroxyproline analysis. Tissue samples were weighed, cut into small pieces and homogenized in a PBS (pH 7.4) with a sonicator for 10 min at 20˚C and 35 kHz (Bandelin Electronic, GmbH & Co. KG., Berlin, Germany) for hydroxyproline levels determination. The homogenates were immediately centrifuged at 1,008 x g for 20 min, at 4˚C and supernatants were collected. The supernatants were kept on ice. Supernatants were used for the measurement of hydroxyproline levels on the same day. Hydroxyproline levels were measured spectrophotometrically (Multiscan Spectrum; Thermo Labsystems, Santa Rosa, CA, USA) using a SunRed Rat Hydroxyproline ELISA kit (Shanghai SunRed Biological Technology Co., Ltd., Shanghai, China; cat. no. 201-11-0512). The hydroxyproline values were expressed as microgram per gram of tissue (μg/g).

Histopathological examination. Formalin-fixed tissue samples were embedded in paraffin and 4.5 μm sections were cut. Replicate sections were either stained with hematoxylin and eosin for the evaluation of morphological features under a light microscope (BX51; Olympus Corporation, Tokyo, Japan) by a single pathologist blind to the sections' grouping. Tissues taken from the perianastomotic section were histopathologically examined for scoring of mucosal ischemia, anastomotic wound healing, granulation tissue formation and histological changes in local inflammatory response according to the criteria described by Biert *et al* (15) and modified by Verhofstad *et al* (16).

Statistical analysis. Descriptive statistics (mean ± standard deviation) were determined and the results were used to produce charts in which the 95% confidence interval error bars indicate the means. The results of the analysis of the bursting pressure and the tissue hydroxyproline level were analyzed using the Shapiro-Wilks. Nonparametric measurements and comparisons of the three groups in terms of pathology were performed using the Kruskal-Wallis test. Comparisons of the two groups in terms of tissue hydroxyproline levels, bursting pressure and pathology were made using the Mann-Whitney U test with Bonferroni correction. IBM SPSS Statistics ver. 22.0 PASW 22 (IBM Corp., Chicago, IL, USA), was used to

determine the statistical significance of the results. P<0.05 was considered to indicate a statistically significant difference.

Results

There was no mortality because of anesthesia and surgical operations in any group during the study period. Bursts had occurred from staple-line anastomosis in all subjects. The fluid leakage site was the gastric fundus in all rats. Although the mean bursting pressure value was increased in group C compared with in groups A and B, no statistically significant differences was found among the group values (P=0.754; Table I). Additionally there was no significant statistically difference of binary comparison of groups (Table II).

The results of the hydroxyproline levels among all groups are summarized in Tables III and IV. Analysis of the mean tissue hydroxyproline levels revealed statistically significant differences between groups A and B and between groups A and C (P<0.001 and P<0.001, respectively). Group C had the highest mean level however there was no statistically significant differences between group B and C (P>0.05). Based on these data, it was concluded that administration of ABS and FS has significant effect on the sleeved stapled-line in terms of tissue hydroxyproline levels.

The results of histopathological examination scores of the groups are given in Table V. Analysis of the groups in terms of necrosis, polymorphonuclear leukocyte, lymphocyte intensity, edema, mucosal epithelial damage and submucosal-mucosal bridging scores revealed the absence of any statistically significant differences among the groups. Group A had the highest macrophage score among all groups and there was statistically significant differences between groups (P=0.002). In the binary comparison of the macrophage score between groups, there was statistically significant differences between group A and C (P=0.002; Table VI).

Discussion

In this experimental study, the effects of FS and ABS on staple-line bursting pressure measurement, hydroxyproline level and histopathological examination in a sleeve gastrectomy model have been evaluated. It has been demonstrated that ABS and FS had better results on bursting pressure levels and tissue hydroxyproline levels in sleeved staple-line of rats. However, both ABS and FS had no effect at histopathological examinations of all groups. To the best of our knowledge, there is no study investigating the effects of FS and ABS in an experimental model of sleeve gastrectomy.

Staple-line leak and bleeding are the most common complications of LSG associated with significant morbidity and mortality (17). The hematoma formation and/or postoperative bleeding at the sleeved staple-line of the stomach can interfere with wound healing and could lead to a leak (18). In order to reduce these major complications of LSG, different surgical techniques and stapler line reinforcement materials have been defined and used. Although a number of studies have been carried on this topic, there is no enough evidence to support the necessity of reinforcement and/or hemostatic agents in LSG (9,19).

Table I. Staple-line bursting pressure measurements.

Group	Mean ± SD (mmHg)	P-value[a]
Group A (Control)	7±5.98	0.754
Group B (FS)	10±13.35	
Group C (ABS)	23.9±33.32	

Data were calculated using a Kruskal Wallis test. [a]P-value between the three groups. FS, fibrin sealent; ABS, Ankaferd blood stopper; SD, standard deviation.

Table II. Binary comparison of Staple-line bursting pressure levels among all groups.

Measurements	P[a]	P[b]	P[c]
Tissue bursting pressure (mmHg)	0.909	0.061	0.074

Data were calculated using a Mann Whitney U test. [a]P-value, group A vs. group B; [b]P-value between groups A vs. C; [c]P-value, group B vs. group C. Group A, control group; group B, fibrin sealent group; group C, Ankaferd blood stopper group.

Table III. Tissue hydroxyproline levels (μg/g wet tissue).

Group	Mean ± SD	P-value[a]
Group A (Control)	1.919±0.366	P<0.001
Group B (FS)	5.639±1.031	
Group C (ABS)	5.89±0.87	

Data were calculated using a Kruskal Wallis test.[a]P-value between the three groups. FS, fibrin sealent; ABS, Ankaferd blood stopper; SD, standard deviation.

Table IV. Binary comparison of the tissue hydroxyproline levels among the study groups.

Measurements	P[a]	P[b]	P[c]
Hydroxyproline/wet tissue weight (μg/g)	<0.001	<0.001	>0.05

Data were calculated using a Mann Whitney U test. [a]P-value, group A vs. group B; [b]P-value between groups A vs. C; [c]P-value, group B vs. group C. Group A, control group; group B, fibrin sealent group; group C, Ankaferd blood stopper group.

ABS has been shown to have hemostatic and regenerative proliferation effects in *in vivo* and *in vitro* studies (13,14). In addition, ABS has also been reported to have antimicrobial, antifungal, wound healing-enhancing effects and antiseptic properties in a small number of animal studies and case reports (13,14). It has been known that collagen

Table V. Results of histopathologic examination scores.

Variable	Group A (mean ± SD)	Group B (mean ± SD)	Group C (mean ± SD)	P[a]
Necrosis	1.1±0.88	0.5±0.53	0.4±0.52	0.109
PMNL	1.6±0.7	1.3±0.48	1.6±0.84	0.350
Lymphocyte	1.6±0.70	1.9±0.57	1.6±0.84	0.413
Macrophage	1.5±0.71	1.1±0.57	0.3±0.48	0.002
Edema	1.2±0.63	0.9±0.57	0.8±0.42	0.254
Mucosal epithelium	1.3±0.95	1.2±0.79	1.2±0.79	0.894
Submucosal-mucosal	1.3±0.82	1.0±0.67	1.1±0.74	0.602

Data were calculated using Kruskal Wallis analysis.[a]P-value between the three groups. Group A, control group; Group B, fibrin sealent group; Group C, Ankaferd blood stopper group; SD, standard deviation.

Table VI. Binary comparison of the histopathologic examination scores of the groups.

Variable	P[a]	P[b]	P[c]
Necrosis	0.104	0.061	0.661
PMNL	0.159	0.531	0.276
Lymphocyte	0.251	0.866	0.249
Macrophage	0.131	0.002	0.006
Edema	0.264	0.111	0.689
Mucosal epitelium	0.681	0.681	0.999
Submucosal-mucosal	0.328	0.516	0.737

Data were calculated using a Mann Whitney U test. [a]P-value, group A vs. group B; [b]P-value between groups A vs. C; [c]P-value, group B vs. group C. Group A, control group; group B, fibrin sealent group; group C, Ankaferd blood stopper group; PMNL, polymorphonuclear lymphocytes.

production is the main indicator of the healing process. Hydroxyproline is a measure of collagen content that has a positive correlation with the anastomose strength (20). In their study, Gulden *et al* (20) investigated the effects of ABS on colon anastomosis in an experimental study. The authors found that the levels of tissue hydroxyproline, an important indicator of anastomosis healing, were statistically significantly higher in ABS administered group (20). In the present study it was seen that ABS increased the bursting pressure on the stapler-line. Similar to Gulden *et al* study, it was demonstrated that topical use of ABS had improved tissue hydroxyproline levels on sleeved staple-line. In contrast to the Gulden *et al* study, although higher bursting pressure levels were observed in ABS administered group, there was no significant difference between the groups. The present study found that histopathological parameters were similar between the groups, except macrophage scores. The lowest macrophage score was found in group C. Previous studies reported that the higher macrophage score is correlated with poor wound healing (15,16). As a result of this the present study concluded that ABS may improve wound healing. Since the rats were scarified on postoperative day 5, further analysis with other markers of histopathological examination could provide different results in the same setting.

In the present study, apart from ABS and the control group, the FS group was formed as a third group. FS is one of the most commonly used reinforcement agent for the staple line reinforcement in bariatric surgery, although it is efficacy is under debate in studies (4,9). For this reason, the present experimental study was designed to investigate the compatible efficiencies of ABS and FS. It was demonstrated that staple-line burst pressure and tissue hydroxyproline levels were increased in the ABS group compared with the FS group. The histopatological scores were similar also in the FS group compared with other groups. Based on these data, it was concluded that ABS may be an alternative to FS as a reinforcement agent in bariatric surgery.

The present study has several limitations. First of all, the present study was an animal study carrying all bias associated with the differences in animal and human metabolisms. Secondly, in this experimental study, a formal sleeve gastrectomy was not performed in rats as in humans. Third, only hydroxyproline levels were evaluated as a separate biochemical parameter and no immuno-histochemical study was conducted. In addition a light microscope was used for the evaluation of histopathological features. Further histopathological analyses under electron microscopy could give different results. As a result of these limitations, results similar to the present study may not be obtained in humans.

In conclusion, it was first demonstrated that ABS as well as FS improved staple line healing in terms of burst pressure and at tissue hydroxyproline level in an experimental study. Although there was no statistically significant difference between effects of ABS and FS on staple-line healing, it was suggested that ABS can be used with similar efficacy instead of FS as a novel reinforcement agent in bariatric surgery. The results of the present study should be supported by further experimental and clinical studies.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

AS conceived and designed the present study; TB conceived/designed the current study, collected data and wrote the article. BM acquired, analyzed and interpreted the data. TO made critical revisions to the manuscript and conceived/designed the current study. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present animal study was performed after the approval of Akdeniz University Experimental Animal Studies Local Ethical Committee (Ethical committee no: B.30.2.AKD.0.05.07.00/120).

Patient consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no competing interests.

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