



Comparison of Anastomosis Safety in Colonic Dissections Using Scalpel, Scissors and Cautery in Rats: Experimental Study

Sıçanlarda Bistüri, Makas ve Koter Kullanılarak Kolon Diseksiyonlarında Anastomoz Güvenliğinin Karşılaştırılması: Deneysel Çalışma

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Abstract

Objective: Postoperative anastomotic leakage is still an issue in modern surgery. Re-hospitalization due to postoperative anastomosis leakage prolongs hospital stay and re-operations increase the cost. There is still no consent on how to dissect the intestines. The objective of the present study is to analyze the safety of colonic anastomoses after dissections using scalpel, scissors and cautery in rats.

Method: There were 4 groups of 32 Wistar Hannover adult rodents. Each group consisted of 8 animals: group 1: sham, group 2: scalpel, group 3: scissors, group 4: cautery. Anastomosis was done over a single layer. Bursting pressure (BP) was measured at day 7. Tissue and blood samples were taken for the evaluation of biochemical and histopathological parameters.

Results: Statistically significant disparity was seen among the sham, scalpel, scissors and cautery groups regarding the mean BP average, mean hydroxyproline levels and fibrosis distributions.

Conclusion: Cautery is the best choice for hemostasis; however, when considering tissue healing, scalpel and the scissors were found to be safest alternatives.

Keywords: Anastomotic leak, colonic dissection, hydroxyproline, rats, tissue healing

Öz

Amaç: Postoperatif anastomoz kaçağı modern cerrahide hala bir sorundur. Postoperatif anastomoz kaçağı nedeniyle yeniden hastaneye yatış hastanede kalış süresini uzatır ve yeni ameliyatlara maliyeti artırır. Bağırsakların nasıl diseke edileceğine dair hala bir fikir birliği yoktur. Bu çalışmanın amacı, sıçanlarda bistüri, makas ve koter kullanarak diseksiyon sonrası kolon anastomozlarının güvenliğini karşılaştırmaktır.

Yöntem: Otuz iki yetişkin Wistar Hannover sıçanı rastgele olarak her biri 8 hayvan içeren 4 gruba ayrıldı: grup 1: sham grubu, grup 2: bistüri grubu, grup 3: makas grubu, grup 4: koter grubu. Anastomoz tek tek sütürler ile tek kat üzerinden yapıldı. Patlama basıncı 7. günde ölçüldü. Biyokimyasal ve histopatolojik parametrelerin değerlendirilmesi için doku ve kan örnekleri alındı.

Bulgular: Sham, bistüri, makas ve koter grupları arasında patlama basıncı ortalaması, ortalama hidroksiprolin seviyeleri ve fibroz dağılımları açısından istatistiksel olarak anlamlı bir fark tespit edildi.

Sonuç: Hemostaz için koter en iyi seçimdir, ancak doku iyileşmesi düşünüldüğünde, neşter ve makas en güvenli alternatifler olarak bulunmuştur.

Anahtar kelimeler: Anastomoz kaçağı, doku iyileşmesi, hidroksiprolin, kolon diseksiyonu, sıçan



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Introduction

Postoperative anastomotic leakage is seen in the intestinal and colonic operations at the rates of 1% and 0.5%-30%, respectively (1). 1% of the patients who were previously known to have an intestinal anastomosis are re-hospitalized due to postoperative anastomosis leakage and in addition, their hospital stay is prolonged. Secondary interventions to such patients are the leading problems of gastrointestinal surgery due to their difficulties. In a study involving 6,174 patients, Hammond et al. (2) reported that hospital stay after colonic anastomosis increased by 7.3 days and the average cost was \$24,129.

The objective of the present study is to analyze the safety of colonic anastomoses after dissections using scalpel, scissors and cautery in rats.

Materials and Methods

Experimental Design

In this study, 32 adult Wistar Hannover rats (İstanbul Bağcilar Experimental Research and Skills Development Center, BADABEM) with an average weight of 300-500 grams were used. All animals were kept at room temperature of 22 °C for 12 hours in a dark/light cycle. Animals in all groups were fed freely with rat feed containing 21% protein. Fresh drinking water was given daily. All subjects were kept in separate cages in groups. The study was approved by the Local Ethics Committee of University of Health Sciences Turkey, Bağcilar Training and Research Hospital (project no: 2013-28). Patient consent form was not required due to the nature of the study.

Study Groups

Rats were randomly divided into 4 groups, each containing 8 animals:

Group 1, sham group,

Group 2, scalpel group, large intestine transection with scalpel,

Group 3, scissors group, large intestine transection group with scissors,

Group 4, cautery group, large intestine transection group with cautery.

Operative Procedure

The rats were anesthetized with ketamine hydrochloride (50 mg/kg, Ketalar; Farke-Davis, Istanbul, Turkey) and xylazine (10 mg/kg, Rompun; Bayer, Istanbul, Turkey) anesthesia,

asepsis with 10% povidone iodine solution, and following the provision of antisepsis, a 3 cm incision was made in the abdominal median line under sterile conditions.

In Group 1, the descending colon was manipulated. The abdomen was closed without anastomosis.

In Group 2, the descending colon was cut with a scalpel 2 cm proximal to the peritoneal reflection in the scalpel group and anastomosed end-to-end over a single layer with 5.0 poli (glicolid-co-lactid) (Pegelak, Doğusan, Istanbul Turkey) suture.

In Group 3, the scissors group, the descending colon was cut with scissors 2 cm proximal to the peritoneal reflection and end-to-end anastomosis with 5.0 poli (glicolid-co-lactid) suture over a single layer was performed.

In Group 4, the cautery group, the descending colon was cut with cautery 2 cm proximal to the peritoneal reflection and end-to-end anastomosis with 5.0 poli (glicolid-co-lactid) suture over a single layer was done.

All procedures were performed by the same surgeon. 10 mg/1 mL paracetamol was administered intraperitoneally for analgesia before the incision was closed. Abdominal incisions were closed in two layers with 3.0 interrupted silk sutures (Doğusan, Istanbul, Turkey). Feeding was started immediately after the operation. On day seven, all animals were sacrificed. None of the rats experienced significant weight loss between the initial surgery and sacrifice.

Bursting Pressure Measurement

The anastomosis line was resected as an anterior block, and the 2 cm distal and 2 cm proximal of the line were clamped. 0.9% NaCl stained with methylene blue was infused into the intestine at a rate of 2 mL/min. Intra-segment pressure was monitored. The pressure value at which the leak was seen in the anastomosis line was recorded as BP.

Hydroxyproline Analysis

The samples for hydroxyproline (H) levels were weighed, cut into small pieces, and homogenized in a phosphate buffer to yield a 20% homogenate. Aliquots of the homogenate were added to an equal volume of 6 N hydrochloric acid, and hydrolyzed in Teflon-capped vials at 102 °C for 16 hours. The H content of the tissue hydroxylates was determined spectrophotometrically by using the standard addition method developed by Kivirikko et al. (3) (Hypopronosticon, Kit lot/ch. B:E 92401, Organon Teknika., Boxtel, Holland). Results were expressed in milligrams, such as in H/100 mg (wet weight).

Histological Analysis

The degree of fibrosis of hematoxylin-eosin-stained preparations was evaluated under a light microscope using a scale adjusted for the severity of fibrosis. (0: minimal, 1: slight, 2: moderate, 3: severe).

Sacrifice and Necropsy

No deaths related to peritonitis or any complications of anastomosis leakage developed. At autopsy, none of the rats had any ileus or evidence of anastomosis insufficiency (feces within the abdominal cavity or abscess).

All groups were sacrificed with high dose ketamine anesthesia on postoperative 7th day and blood samples of all animals were taken by intracardiac puncture. Colonic anastomosis lines of all animals were removed, BP values of anastomosis line were measured.

Anastomosis lines were resected unblock 1 cm distal and proximal. The anastomosis line was divided by mesenteric borders. One segment was frozen in liquid nitrogen and stored at -80 °C for H analysis, the other segment was placed in formaldehyde solution for histopathological examination.

Statistical Analysis

Statistical analysis was performed by NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA). Descriptive statistical methods (mean, standard deviation, median, interquartile range) were used in the evaluation of the data, as well as Dunn's multiple

comparison test in the Kruskal-Wallis test subgroup comparisons, Mann-Whitney U test in the comparison of the paired groups, and chi-square test in the comparison of the qualitative data. Results were evaluated at $p < 0.05$ level of significance.

Results

There was a statistically significant difference among the mean sham, scalpel, scissors and cautery groups ($p = 0.0001$) in BP average. The mean of the sham group was significantly higher than the mean of the scalpel, scissors and cautery groups ($p = 0.001$) (Table 1). No statistically significant difference was found between scalpel cautery groups ($p = 0.748$) (Table 2).

There was a statistically significant difference among the mean H levels of Sham, Scalpel, Scissors and Cautery groups ($p = 0.041$). The mean H of the sham group was found to be significantly higher than the mean of scalpel, scissors and cautery groups ($p = 0.049$, $p = 0.021$). No significant difference was observed between scalpel and scissors groups and also between scalpel and cautery groups ($p > 0.05$) (Table 2).

There was a statistically significant difference among fibrosis distributions of groups ($p = 0.0001$). The presence of minimal fibrosis was significantly higher in the Sham group than in the Scalpel, Scissors and Cautery groups.

When the scalpel, scissors and cautery groups were compared, no significant difference was found in the distribution of fibrosis (Table 3).

Table 1. Bursting pressure and tissue hydroxyproline levels

		Pressure (mm/Hg)	Hydroxyproline (./100 mg)
Sham group	Median (IQR)	290 (286.25-300)	7657.97 (1715.49-11764.28)
Scalpel group	Median (IQR)	10 (0-148.5)	725.2 (114.545-2385.8)
Scissors group	Median (IQR)	120 (66.25-166.25)	1304.32 (1141.3-2478.66)
Cautery group	Median (IQR)	50 (2.5-68.75)	656.68 (368.51-774.09)
	p	0.0001	0.041

IQR: Interquartile range, $p < 0.05$ level of significance

Table 2. Dunn's multiple comparison test of groups

Dunn's Multiple Comparison test	Pressure (p)	Hydroxyproline (p)
Sham group/Scalpel group	0.001	0.021
Sham group/Scissors group	0.001	0.049
Sham group/Cautery group	0.001	0.044
Scalpel group/Scissors group	0.040	0.600
Scalpel group/Cautery group	0.748	0.916
Scissors group/Cautery group	0.035	0.012

$p < 0.05$ level of significance

Table 3. Histopathological evaluation of each group

Fibrosis	Sham		Scalpel		Scissors		Cautery		p
Minimal	8	100.00%	0	0.00%	0	0.00%	0	0.00%	0.0001
Mild	0	0.00%	1	12.50%	0	0.00%	2	25.00%	
Intermediate	0	0.00%	5	62.50%	3	37.50%	4	50.00%	
Severe	0	0.00%	2	25.00%	5	62.50%	2	25.00%	

Discussion

To our knowledge, there is elevated mortality and morbidity rates in case of anastomotic leak or dissociation occur (4). Aforementioned complications occur more frequently in large bowel anastomosis operations. (13%-69%) (5). Risk factors for anastomotic leakage are smoking, chronic heart disease, diabetes and obesity (6-8).

Different techniques, materials and agents have been developed throughout years whereas no solution has been overcome yet (9). Some authors covered the anastomosis with different type of meshes but problem is still existing (10,11). Golden standard method for dissecting tissue is scalpel. This way of dissection is simple and low cost; however, it prolonged surgery time due to lack of hemostasis. It has some disadvantages like the lack of hemostasis which prolongs surgery time and risk of unintended injuries (12).

In the past decade, the invention of electrocautery has solved several defects of scalpel (13). Heat energy is used to denature proteins to ensure hemostasis (14), but electrocautery damages the tissue by heat energy (15).

Scalpel cuts are preferred over electrocauter in some studies in which they have fewer infection in wounds with an increase in wound durability (16,17). However, in some studies, no difference for infection rate in wounds is shown for electrocautery and scalpel (18).

Collagen filaments and filament improvement with the submucosal layer are related to the strength of the anastomosis (19). Recovery pursues the sequential states of tissue renovation mostly constructed by growth factors and cytokines (20). Fibrin and fibronectin template pro forma secures and attaches two ends of bowel at the hemostatic phase. Since day 3, temporary matrix evolves with fibroblasts, macrophages and new blood vessels making new granulation tissue (21). Fibroblasts mainly produce collagen (22). On the day 6 and 7, maximum peak of collagen production emerges, and this is correlated with breaking strength (23). Anastomotic repair and inhibition of submucosal fibers of collagen degradation promote angiogenesis and granulation

texture deposition, including non-overlapping, and the acceleration of epithelization improved through different mechanisms (24).

Tissue H levels and BP are indirect indicators of anastomotic healing. To determine the level of H is a decent way for assessing the quantify of collagen texture (25,26).

Beginning at about 5 days, it approaches the normal level while the H condensation reduces by 40% and rises from normal level on the 10-14th day (27).

BP is an indication for the healing process of an anastomosis (28). Because of larger numbers of microorganisms, minimal arteriole nurture, and greater feces quantity, leak in left sided colon anastomosis risk is greater than the other parts (29).

Quality and quantity of newly synthesized collagen target to bring tissue strength to pre-anastomotic levels (30,31). The measurement of H is a decent parameter of anastomotic healing (32). BP measurement is robustness test of the anastomosis (33).

Conclusion

Leakage in left-sided colon anastomosis is still a serious complication. The etiology of colonic anastomotic leakage remains unclear. Minimal damage to colonic tissue when dissecting will cause the most acceptable outcomes. Cautery is best for hemostasis but scalpel and scissors dissection have the better results of tissue healing. More research is needed to introduce which way is the best for intestinal healing and to prevent complications.

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Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Committee of University of Health Sciences Turkey, Bağcılar Training and Research Hospital (project no: 2013-28).

Informed Consent: Patient consent form was not required due to the nature of the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.Y., O.B.G., C.E., K.Ö., A.B., Design: H.Y., O.B.G., A.Ç., F.Ç., H.U., E.Y., Data Collection or Processing: C.E., E.Y., K.Ö., A.K.A., H.U., A.B., H.Y., Analysis or Interpretation: C.E., F.Ç., A.B., O.B.G., A.Ç., H.U., A.K.A., K.Ö., Writing: H.Y., A.Ç., F.Ç., A.K.A., E.Y.

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