EXPERIMENTAL STUDY

The effects of sponges soaked with chlorhexidine gluconate and metronidazole on safety of colonic anastomosis in an experimental model of peritonitis

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ABSTRACT

BACKGROUND: The present study aims to evaluate the use of the chlorhexidine gluconate and metronidazole impregnated compresses concerning anastomosis safety in the left colonic anastomosis in the presence of peritonitis.

METHODS: This study was conducted on 21 Wistar-Albino-rats divided into three equal groups. After median laparotomy, the whole layer of the left colon was cut 2 cm over the pelvic peritoneum. The faeces were spread around the injury for fecal contamination. Then, fasia and skin were closed with 3/0 silk. After one day period, relaparatomy was performed. The abdomen was cleared isotonic sodium chloride with impregnated material before starting colonic anastomosis in the first group and then double layer colonic anastomosis was performed. In the second Group-II, abdomen was cleared with the metronidazole impregnated compresses then double layer colonic anastomosis was performed. In the group-III, abdomen was cleared with the chlorhexidine gluconate impregnated compresses then double layer colonic anastomosis was performed. Tissue hydroksiproline levels and anastomosis bursting pressures were measured and histopathologic findings on the anastomosis line were evaluated on the postoperative tenth day by performing relaparatomy.

RESULTS: The highest anastomosis bursting pressure was found in Group-III ($p<0.05$). The highest tissue hydroksiproline level was found in Group-III ($p<0.005$ Group I-III, Group II-III). When histopathologic findings were evaluated by comparing the three groups in this study, the healing of the intestine tissue score was statistically insignificant between group-II and III, for both group-II and III, healing score was statistically significant higher than Group-I ($p<0.05$ Group I–III and Group I-II).

CONCLUSION: Cleaning the abdomen before the anastomosis using antibacterial soaked material increased resection safety in the presence of peritonitis and anastomosis safety in primary anastomosis.

Keywords: Anastomosis leakage; chlorehexidin gluconate; colon anastomosis; peritonitis.

INTRODUCTION

Gastrointestinal system anastomoses are among the most common surgeries performed in general surgery clinics. Over the last several years, studies to improve colon anastomosis

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have been attracting the attention of many colorectal surgeons. Anastomosis leakages are the main cause of mortality, morbidity, and hospital stay following colon anastomosis.[1,2]

In the gastrointestinal tract, the section where anastomotic leakage most commonly occurs is the colon, and the risk increases towards the distal colon. Lack of collateral connections in arterial supply, the rich bacterial flora it includes, and the high activity of collagenase enzyme facilitate the formation of leakage after surgical interventions. Besides this, the comorbid problems of patients undergoing colorectal surgery render the colon surgery more risky.[3,4]

Vascularization of the stomach and small intestines is very good and there is less bacteria colonization. For this reason, anastomosis leakage as a result of resection and anastomosis of these organs is rare, and anastomosis reaches sufficient stability after one week. Vascularization of the esophagus and colon is lower than in the stomach and small intestine. While, on one hand, high bacterial contamination in the distal colon delays collagen synthesis, on the other hand, it causes excessive collagen lysis by improving the effects of collagenase.[5] Currently, approximately 10–20% of anastomosis leakage is seen in colorectal operations.[6] In the state of contaminated abdomen, both in elective and emergency colon surgeries, the primary anastomosis, particularly, of left colon intervention is avoided, and multistep procedures are preferred. The reason for this is the impairment of wound healing in a contaminated environment, high risk of anastomosis leakage, and the increase in mortality and morbidity. High mortality and morbidity rates in colon surgery have led researchers to various studies related to anastomotic healing.[7,8]

Metronidazole is a 5-nitroimidazole compound that was first used in 1959 for the treatment of Trichomonas vaginalis infection. It is used in combination with other antibiotics, such as aminoglycosides in amebiasis and anaerobic infections.[9] Chlorhexidine is a cationic biguanide and was first published as an antimicrobial agent in 1954. They have a strong effect on Gram-positive and negative bacteria and viruses.[10]

In this experimental study, we evaluated the effects of chlorhexidine gluconate and metronidazole on anastomosis safety in left colon anastomosis performed in a peritonitis environment.

MATERIALS AND METHODS

Preparation of the Subjects

After Local Ethics Committee approval, Wistar Albino type rats, weighing 200–250 grams, were obtained from Fırat University Experimental Research Centre (FÜDAM) and used in our study. A single surgeon performed all laparotomy and anastomosis of experiments at the laboratories of FÜDAM. All the subjects were raised with the same standard provender and urban drinking water. Food and water were withheld from subjects for 12 hours before the experiment.

Separation of the Subjects into Groups

Experimental animals were separated into randomized groups, each consisting of seven rats.

Group 1 (Control Group): Colon anastomosis was performed in this group. After performing colostomy by laparotomy to the left colon, the abdomen was washed with saline (SF) and was protected with saline absorbed sponges.

Group 2 (Metronidazole Group): Colon anastomosis was performed in this group. After laparotomy, the intra-abdomen was washed with saline after contamination and was protected with metronidazole (Flagyl®Eczacıbaşı) absorbed sponges.

Group 3 (Chlorhexidine Gluconate Group): Colon anastomosis was performed in this group. After laparotomy, the intra-abdomen was washed with saline after contamination and was protected with chlorhexidine gluconate (Klorhex solution®Drogsan) absorbed sponges.

Anesthesia and Surgical Procedure

The animals were anesthetized by applying intramuscular ketamine hydrochloride (50 mg/kg; Ketalar®Pfizer) and xylazine hydrochloride (5 mg/kg; Rompun®Bayer). After abdominal wall shaving antisepsis, laparotomy was performed with a 4 cm vertical midline incision. The left colon was cut full layer 2–3 cm above the peritoneal reflection. For the certain formation of fecal peritonitis, feces inside the lumen contaminated the wound. Then the abdomen was closed, continuously, through two layers (fascia and skin) with 3/0 silks. After one day, the abdomen was reopened under general anesthesia and peritonitis was evaluated by modifying with Mannheim Peritonitis Index. Before starting colon anastomosis, the abdomen was washed with saline. Colon anastomosis was performed while protecting with saline absorbed sponges in the 1st group of rats, with metronidazole (Flagyl®Eczacıbaşı) absorbed sponges in the 2nd group of rats, and with chlorhexidine gluconate (Klorhex solution®Drogsan) absorbed sponges in the 3rd group of rats. On the postoperative 10th day, by performing re-laparotomy, hydroxyproline levels on the anastomosis line and anastomosis burst pressures were measured, and a histopathology evaluation was performed. During the experiment, additional anesthetic agent need was supplied by 50 mg/kg ketamine hydrochloride (Ketalar®Pfizer). After anastomosis was completed, the abdomen was closed through two layers (fascia and skin) continuously with 3/0 silks.

Peritonitis Scoring: The peritonitis scores of the rats were determined by modifying the macroscopic findings of the Mannheim Peritonitis Index based on the macroscopic findings, grading of subjects for peritonitis.[11]

Care of the Subjects

The subjects were kept together at FÜDAM laboratories until the operation and until the re-laparotomy day (10th day)

at standard temperature and humidity. During this period, all rats were given a standard diet. Three rats from Group I died before the end of this period. Three new rats were added to the experiment with the same methods.

Performing Re-laparotomy and Taking Samples

On the postoperative 10th day, to evaluate anastomosis healing, the sutures were removed from the abdominal wall and the abdomen was re-opened following general anesthesia.

Measurement of Burst Pressure of Anastomosis Region

After the abdomens of the rats were evaluated by a single surgeon, blind to all groups, for complications, such as anastomosis leakage, abscess, and fistula, anastomosis burst pressures were measured in-vivo using the apparatus described below.

A catheter was advanced 3–4 cm from the anus and placed with the end at the middle of the anastomosis line. The abdomen was filled with saline. The catheter was tied 2 cm under and 2 cm above the colon anastomosis with 2/0 silk. The colon segment, where the catheter was placed, was inflated using an infusion pump with a speed of 4ml/min (Abbott LC 5000 infuor USA) with saline colored with methylene blue. Pressure values, during the inflation procedure, were monitored (Petaş KMA 375 S/N 0013 Turkey) with the aid of a pressure transducer (Abbott single transpact USA). Pressure values were monitored while the inflation process was in progress. Intra-abdominal fluid was colored with methylene blue, and the pressure was decreased suddenly. This value was recorded as burst pressure.

Taking Samples

2 cm of colon segment was resected to encompass the anastomosis line; the colon was opened throughout the lumen, washed with saline, and purified from intestinal content. One-third of this tissue was put inside flacons, including 10% formaldehyde, for histopathology evaluation. The remaining two-thirds of the tissue were wrapped in aluminum foil paper and stored at -80°C in the biochemistry laboratory.

Histopathology Evaluation

Tissue samples taken from the anastomosis region were embedded in paraffin blocks after the routine tissue follow-up process. After hematoxylin and eosin were applied to 4–5 micron tissue sections, they were examined with light microscope.

Microscopic Examination

I. Types of inflammatory cells (polymorph nucleus leucocyte – PMN - with lymphocytes and plasma cells) in the anastomosis site were graded semi-quantitatively for neovascularization and collagen fiber density as -, +, ++, +++.

II: The degree of wound healing in the anastomosis region was scored between 1 and 5 as below:

Score 1: Only fibrin-purulent exude exists.

Score 2: Granulation tissue developed in less than 25% of the anastomosis region.

Score 3: Granulation tissue developed in 25–75% of the anastomosis region.

Score 4: There is granulation tissue in more than 50% of the anastomosis region and collagen fibers in less than 25%.

Score 5: There are collagen fibers in more than 25% of the anastomosis region.

Measurement of Hydroxyproline Value

Tissue was cleaned with bi-distilled water, dried with blotting paper, and divided into small pieces during the tissue homogenization procedures. They were stored until the day of the experiment by freezing at -80°C. Hydroxyproline determination (OH-P) was performed by partially modifying the method described by Woessner. Results were given as mg/g dry tissue.

Statistical Assessment

Data obtained from the study groups were given as mean±standard deviation. Compliance with the normal distribution was evaluated with the Kolmogorov-Smirnov test. Independent samples t-test and One-way ANOVA tests were used for the evaluation of differences between the groups for parameters. $P<0.05$ values were accepted as significant.

RESULTS

Three rats in the control group died after the 1st, 2nd and 4th days of the anastomosis. Anastomosis leakage was observed when laparotomy was performed on the dead rats. To complete the number of subjects, three rats were added to the control group, and the experiment was continued. There was no mortality in the other groups.

Tissue Hydroxyproline Levels

When all the groups were considered, tissue hydroxyproline levels were observed significantly lower in the control group than in the Metronidazole and Chlorhexidine groups statistically. Also, hydroxyproline levels in the Chlorhexidine group were found statistically significantly higher than in the metronidazole group (Table 1).

Assessment of Burst Pressure Values

When all the groups were considered, anastomosis burst pressures were found statistically significantly lower in the control group than in the Metronidazole and Chlorhexidine groups. Also, anastomosis burst pressures in the Chlorhex-

Table 1. Burst pressures of the groups and hydroxyproline levels

	Control group	Metronidazole group	Chlorhexidine group	p*
Hydroxyproline (µg/mg)	1.58±0.3	2.7 ±0.4[a]	4.2±0.7[b,c]	<0.001
Burst pressure (mmHg)	173.7±22.1	207.3±14.3[d]	256.4±12.74[e,f]	<0.001

*One –way ANOVA. [a]p=0.0002; When compared with the control group. [b]p<0.0001; when compared with the control group. [c]p=0.0004; when compared with the Metronidazole group. [d]p=0.0055; when compared with the control group. [e]p<0.0001; when compared with the control group. [f]p<0.0001; when compared with the Metronidazole group.

Table 2. Histopathology assessment results of the groups

	Control	Metronidazole	Chlorhexidine
PMN (mean)	2 (1– 2)	1 (1– 2)	0 (0– 1)
Lymphocyte	2 (1– 2)	1 (1– 1)	1 (1– 2)
Eosonophyl	1 (0– 2)	1 (0– 2)	1 (0– 1)
Collagen	2 (1– 2)	2 (1– 2)	3 (2– 3)
Neovascularization	2 (1– 3)	1 (1– 2)	1 (0– 2)
Tissue healing score	3 (2– 4)	4 (3– 4)	4 (3– 5)

PMN: Polymorphonuclear monocyte.

idine group were observed statistically significantly higher than in the Metronidazole group (Table 1).

Histopathology Assessment

While there was no significant difference between the control group and the metronidazole group for tissue PMN levels, there was a significant difference between the control group and the chlorhexidine group statistically (p=0.107 and p=0.002, respectively). Also, a statistically significant difference was seen between the Chlorhexidine group and the Metronidazole group (p=0.01).

While there was a statistically significant difference between the control group and the Metronidazole group for the tissue mononuclear cell (MNH) (Lymphocyte) levels, there was not a significant difference seen between the control group and the Chlorhexidine group (p=0.023 and p=0.07, respectively). There was not a significant difference seen between the Metronidazole group and the Chlorhexidine group (p=0.06).

There was not a statistically significant difference seen between the control group and the Metronidazole and Chlorhexidine groups for tissue MNH (Eosinophil) levels (p=1 and p=0.479, respectively). There was not a significant difference seen between the Metronidazole and the Chlorhexidine groups (p=0.293).

While there was not a statistically significant difference between the control group and the Metronidazole group for tissue collagen levels, there was a significant difference between the control group and the Chlorhexidine group (p=1 and p=0.014, respectively). On the other hand, a significant difference was seen between the Metronidazole and Chlorhexidine groups (p=0.15).

There was a significant difference between the control group and the metronidazole and Chlorhexidine groups for tissue neovascularization levels (p=0.026 and p=0.029, respectively). There wasn't a significant difference seen between the Metronidazole and Chlorhexidine groups (p=0.705).

There was a statistically significant difference between the control group and the other groups for tissue wound healing (p=0.01 and p=0.07, respectively). There was not a significant difference seen between the Metronidazole and Chlorhexidine groups (p=0.335).

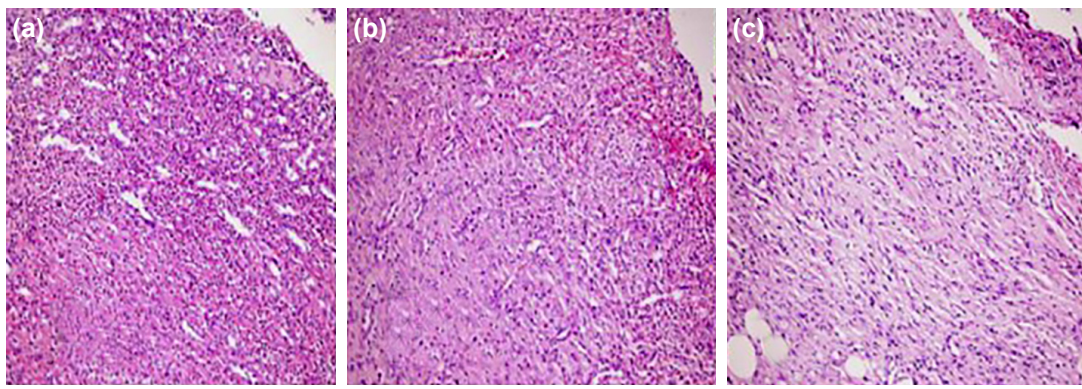

Figure 1. (a-c) Histopathology images of anastomosis line of the control group, metronidazole group and chlorhexidine groups consequently (100x magnification).

Histopathology assessment of the groups is presented in Table 2. Histopathology images of the groups are demonstrated in Figure 1.

DISCUSSION

Anastomosis leakage is one of the most critical complications of colorectal surgery since it leads to high morbidity and mortality. In recent years, the mechanism of wound healing has been better understood. The leakage rate of colon anastomosis is higher than anastomosis performed in other localizations of the gastrointestinal system. The incidence of all leakages in intestinal anastomoses is between 2–35%. Most of these are minimal leakages that were limited by the defense mechanism of the patient.[1,12]

Intra-abdominal sepsis negatively affects the healing of colon anastomosis. Because the number of organisms inside the colon lumen is more than the other parts of the gastrointestinal system, detachment risk in colon anastomoses are higher than in gastric and small intestine anastomoses. Colon resection and anastomoses, particularly the distal part of the left colon, has a high risk of anastomosis leakage and detachment. These complications lead to high morbidity and mortality. 20–80% of postoperative mortalities in patients who had colon anastomosis are due to anastomosis leakage and the complications developing due to this.[12–14] Choosing end colostomy formation (Hartmann's procedure) have been questioned by surgeons for the last years. An abscess or fecal peritonitis is not a contraindication for anastomosis.[15] Many reasons like high mortality and morbidity rate of redo surgery of Hartmann's colostomy. Primary anastomosis has better results for complication and mortality rates.[14,15]

Mechanical, biochemical and histopathology methods are used to determine the healing degree of intestinal anastomoses. Burst pressures of the intestinal segments that include the site of anastomosis are measured using a mechanical method. Tissue hydroxyproline levels, as an indicator of tissue collagen levels, are determined by biochemical methods. The healing stages of the anastomosis wound are evaluated by the histopathology method.[16] The strength of anastomosis is evaluated with burst pressure or tensile strength. While the force vector is in the longitudinal plan in tensile strength, it is stated that these measurements do not exactly reflect the pressure in organs with lumens. It is suggested that, since burst pressure measures the intraluminal pressure, this reflects pathophysiological development better in living systems.[16–19]

Jiborn[17] stated that after colon anastomosis, the burst pressure values on the 7th, 10th, 14th and 28th days are close, and it catches the burst pressure of the intact intestine group on the 7th day. Jiborn stated in their study that, in burst pressure measurements, the detachment occurs on the anastomosis line on the 4th day and outside the anastomotic line on the 7th day. In consideration of these studies, anastomotic line burst pressure measurements were performed on the 10th day of our study. We chose the burst pressure designations in our study because of the ease of application.

The negative effects of intra-abdominal infection on colon anastomosis healing have been demonstrated in clinical and experimental studies.[20] Schrock et al.[21] have reported in their clinical study that colon anastomosis performed in the presence of the infection has a high risk of anastomosis leakage. Irvin et al.[22] have demonstrated that there is a significant decrease in the amount of collagen in the anastomosis line than in the control group on the postoperative third day in the presence of intra-abdominal infection. Ersoy et al.[23] showed that application of melatonin in early sepsis increases anastomotic healing in their experimental study. In this experimental study, the treatment showed significantly higher capillary permeability, fibroblast proliferation, and collagen deposits. Ahrendt et al.[13] have demonstrated in their infection model created by cecum ligation and perforation in rats that infection leads to a decrease in burst pressure and collagen concentration. For this reason, controlling intra-abdominal sepsis is highly important for anastomosis safety.

The bactericide effect of Metronidazole is made possible by damaging the helical structure of DNA as a result of the reduction of nitro groups to anionic radicals, nitro derivations and hydroxylamine. Another effect mechanism of Metronidazole on anaerobes is the formation of free oxygen radicals in an oxygenated environment. This mechanism is particularly effective for metronidazole resistant anaerobes.[9,24] Chlorhexidine consists of acetate (diacetate), gluconate and hydrochloride salts. It is used in liquid solutions or some similar detergent preparations at 0.5 and 0.75% (In some detergent preparations is used at 2–4%). Its main target is the bacterial cytoplasmic membrane. After causing severe damage in the cytoplasmic inner membrane, precipitation and coagulation of proteins and nucleic acids occur. It also damages the outer membranes of gram negatives and cell membranes of gram-positives. Chlorhexidine also causes damage to cell membranes of yeasts and prevents the external growing of bacterial spores however does not prevent germination. The activity spectrum of chlorhexidine depends on concentration. It has a bacteriostatic effect in low concentrations on most gram-positive bacteria (for instance, 1 μgr/ml), to gram-negative bacteria (for instance 2–2.5 μgr/ml), and bacterial spores. Bactericidal effect is seen, as in yeasts, in chlorhexidine concentrations 20 μgr/ml and over.[25]. Besides its use as an antiseptic, it is reported that chlorhexidine gluconate can be used safely for the prevention of surgical area infections.[26] Also, it is used in place of hypertonic saline during hydatid cyst operations to prevent the dissemination of live protoscoleces.[27]

In our study, we examined left colon anastomosis in a peritonitis environment using different infection cleaning materi-

als and their effects on each group. For anastomosis healing parameters, the best group was the chlorhexidine group for burst pressure, tissue hydroxyproline level and histopathology improvement score. Also, better results were observed in the metronidazole group than in the control group. These results demonstrated that abdominal cleaning materials absorbed with the antibacterial solution are beneficial for anastomosis safety.

In light of the findings obtained in this study, we suggest that the use of abdominal cleaning materials absorbed by antibacterial solution increases the safety of anastomosis by reducing the harmful effects of an infected abdomen and thereby decreasing the morbidity and mortality rates in intestinal anastomoses. However, we suggest that more comprehensive experimental and clinical studies with a larger number of subjects are needed for clinical use.

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DENEYSEL ÇALIŞMA - *ÖZET*

Peritonitli ortamda klorhexidin glukonat emdirilmiş kompres ve metronidazol emdirilmiş kompres kullanımının bağırsak anastomozu üzerine etkileri

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AMAÇ: Bu çalışmanın amacı peritonitli ortamda yapılmış olan sol kolon anastomozlarının güveliğinin klorheksidin emdirilmiş kompres ve metronidazol emdirilmiş kompres kullanımı ile incelenmesidir.

GEREÇ VE YÖNTEM: Üç eşit gruba ayrılmış 21 Wistar Albino sıçan kullanılarak çalışma hazırlandı. Median laparatomiden sonra pelvik peritonun 2 cm üzerinden kolon tam kat olarak kesildi. Fekal kontaminasyon amaçlı feçes yarlanma alanının çevresine yayıldı. Ardından fasia ve cilt 3/0 ipekle kapatıldı. Bir gün sonra relaparatomi yapıldı. İlk grupta anastomoz öncesinde batın izotonik sodyum klorür emdirilmiş materyalle temizlendi ve ardından çift kat anastomoz uygulandı. Grup II'de batın metronidazol emdirilmiş materyallerle temizlendikten sonra çift kat anastomoz uygulandı. Grup III'te batın klorheksidin glukonat emdirilmiş materyal ile temizlendikten sonra çift kat anastomoz uygulandı. Ameliyat sonrası 10. günde relaparatomi yapılarak doku hidroksiprolin düzeyleri, anastomoz patlama basınçları ölçüldü ve anastomoz hattının histopatolojik bulguları incelendi.

BULGULAR: En yüksek anastomoz patlama basıncı Grup-III'te tespit edildi ($p<0.05$ Group I-III, Group II-III). En yüksek doku hidroksiprolin düzeyleri Grup III'te tespit edildi ($p<0.005$ Group I-III, Group II-III). Üç grup arasında histopatolojik bulgular değerlendrildiği zaman Grup II ile Grup III arasındaki iyileşme skorları arasında anlamlı farklılık izlenmezken, Grup I ile karşılaştırıldığında hem Grup II hem de Grup III'te iyileşme skorları istatsitiksel olarak anlamlı derecede yüksekti ($p<0.05$ Group I-III and Group I-II).

TARTIŞMA: Antibakteriyel ajan emdirilmiş materyalle anastomoz öncesinde batının temizlenmesi peritonitli ortamda rezeksiyon güvenliğinin ve primer anastomoz güvenliğini arttırmaktadır.

Anahtar sözcükler: Anastomoz kaçağı; klorheksidin glukonat; kolon anastomozu; peritonit.

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