



Effect of mesalamine on healing in experimental colon anastomosis: A randomised experimental study

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ABSTRACT

Objective: We aimed to investigate the effect of mesalamine on healing of experimental colon anastomosis model.

Material/Methods: Forty adult male Wistar albino rats were performed segmentary colonic resection and end-to-end anastomosis. Animals were randomly divided into four groups: group I, anastomosis group, received no treatment (GI, $n = 8$); group II, anastomosis + oral mesalamine group (100 mg/kg/day); group III, anastomosis + rectal mesalamine (2 mL) group, (GIII, $n = 8$); group IV, anastomosis + oral mesalamine + rectal mesalamine (GIV, $n = 8$) group. A sham group ($n = 8$) was constituted and was performed laparotomy. Bursting pressure, hydroxyproline levels and histopathological characteristics of the anastomosis were analyzed.

Results: Although it was not statistically significant, there was an increase in the burst pressure of the mesalamine group. When hydroxyproline measurements were compared there were statistically significant difference between the non-treated colon and all groups. There were significant differences between GI and GIII–GIV, GII and GIV. The differences between group I and II and group II and III were not statistically significant.

When we compared the median amount of the histopathological changes, we found significant difference between the anastomosis and the mesalamine groups ($P < 0.05$). But when mesalamine groups were compared with each other we did not observe a significant difference. **Conclusion:** Mesalamine had positive effects which were not statistically significant on bursting pressure and statistically different significant effects on hydroxyproline (HP) levels based on the way of administration and statistically significant positive effects on histopathologic anastomotic healing in experimental anastomosis model

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1. Introduction

Colonic resection and subsequent anastomosis due to various reasons are among the most widely performed operations in

the field of general surgery. Although these operations are generally safe, leakage of the anastomosis is a serious complication and may even result in death.^{1,2} Factors reported to effect healing and integrity of the intestinal anastomosis are

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blood supply, anastomotic technique and meticulous procedure, colonic bacteria, inflammation, age, nutritional status, associated disease, and drugs.³ Inadequate blood supply, bacterial count of colon, inflammation, technical errors, age, nutritional status, associated diseases, and drugs such as chemotherapeutics, octreotide, suramine, irradiation, and dexamethasone impair the wound healing process.^{3–6}

5-Aminosalicylic acid (5-ASA), sulfasalazine and mesalamine, have been used for over 50 years, but the mechanisms of action of 5-ASA have remained elusive. 5-ASA compounds are capable of multiple effects that may protect the colon from an inflammation-mediated damage. 5-ASA has been demonstrated to scavenge free radicals directly,^{7,8} inhibit leukotriene production,⁹ inhibit the chemotactic response to leukotriene B₄ (LTB₄),¹⁰ and inhibit cellular release of interleukin-1 (IL-1) in cultured mucosal biopsy specimens from ulcerative colitis patients.¹¹ Direct application of mesalamine via enema or suppository is also effective in patients with distal colitis. The aim of topical treatment is to provide maximal mucosal anti-inflammatory effect and to decrease drug related side effects by minimal systemic effect. Rectal usage of mesalamine is effective along the distal colon to level of splenic flexura.¹²

To date the effects of mesalamine on healing of colonic anastomoses have not been evaluated. This study investigated the effects of mesalamine on the healing of experimental colon anastomoses.

2. Materials and methods

Forty adult male Wistar albino rats, weighing between 260 and 290 g were used. The animals were acclimatized for 1 week to our laboratory conditions prior to experimental manipulation. They had free access to standard laboratory chow and water *ad libitum*. The protocol of this study and animal experimental procedures were approved by the Ethical committee of Mustafa Kemal University School of Veterinary.

All of the animals were fasted overnight before surgery. Anesthesia was achieved with intra-peritoneal injection of 10 mg/kg xylazine (Rompun; Bayer, Istanbul, Turkey) and 50 mg/kg ketamine hydrochloride (Ketalar; Parke-Davis, Istanbul, Turkey). The surgical procedures were performed using clean and sterile instruments. Ten percentage of povidone-iodine solution was used for the disinfection of the skin. After placing sterile drapes, a 3 cm midline incision was made. Upon entering the abdominal cavity, the sigmoid colon was identified and the colon divided exactly 3 cm from the peritoneal reflection while preserving the vascular arcades. This resected specimen was preserved at –20 °C for later determination of the HP concentration in normal colon tissue. The free ends of the colon inside the abdomen were anastomosed with a single layer of interrupted inverting 6/0 polypropylene sutures (Prolene; Ethicon, Scotland, United Kingdom) placed 1 mm apart. The fascia and skin layers were closed separately with running 4/0 silk sutures (Mersilk; Ethicon). To prevent dehydration, 10 mL of 0.9% NaCl was administered subcutaneously during the operation. All operative procedures were performed by the same investigator. Each animal was given free access to chow and water the morning after the procedure.

The animals were randomly assigned into four groups: group I, anastomosis, received no treatment (GI, *n* = 8); group II, anastomosis + oral mesalamine (100 mg/kg/day, Salofalk; Ali Raif, Istanbul, Turkey); group III, anastomosis + rectal mesalamine (Salofalk; Ali Raif, Istanbul, Turkey), 2 mL once a day via enema, (GIII, *n* = 8); group IV, anastomosis + oral mesalamine + rectal mesalamine (GIV, *n* = 8). Rats were killed by intracardiac puncture while under anesthesia at seventh postoperative day. Researchers were all blinded to the randomization of the study.

To assess the mechanical strength of the anastomosis, one end of the excised segment was sealed with a suture. The free end was then catheterized with a polyurethane tube (2 mm outer diameter), and a stay suture was tied circumferentially, incorporating both tissue and tube to prevent air leakage. The external end of the tube was connected to an infusion pump and a mercury manometer by way of a Y-shaped adapter. The colon segment was then placed in a saline-filled container, and air was pumped into the tube at a rate of 5 mL/min. The blood pressure reading at the instant the pressure decreased suddenly (caused by bursting of the anastomosis), or when bubbles were seen, was recorded as the “bursting pressure.”

After measuring BP, a 5 mm-wide ring of tissue, including the anastomosis, was removed. Half of this removed tissue was wrapped in aluminum foil and preserved at –20 °C for later measurement of HP amount at the anastomosis site. The other half was stored in 10% formaldehyde for later assessment of histopathologic features. When brought to room temperature, samples dry weights were recorded and, successively, the amount of hydroxyproline was determined as described previously.¹³ Absorbance was read by a Shimadzu spectrophotometer (UV-120 – 02; Kyoto, Japan), and the collagen concentration was expressed as micrograms of hydroxyproline per gram of dry weight tissue. For histopathological assessment the tissues that were fixed in 10% formaldehyde were stained with hematoxylin and eosin and were evaluated at ×20 to ×200 magnification under the light microscope. The healing parameters and inflammatory changes, granulocytic cell infiltration, mononuclear cell infiltration, fibroblastic cell infiltration, necrosis, exudate, and capillary formation were assessed semiquantitatively by assigning a score of 0–3 to each tissue specimen. Peritonitis was scored as being present or absent.²

2.1. Statistical analysis

The results were expressed as the mean ± SEM. Differences among the groups were evaluated using one-way analysis of variance (ANOVA), and multiple comparisons between the groups were performed with a post-hoc test (Tukey's HSD test). Differences were considered statistically significant when *P* < 0.05. Data were analyzed by a statistical software (SPSS for Windows 11.5; SPSS, Chicago, IL, USA).

3. Results

In the GI anastomosis group, one rat died on the fifth day of the study due to peritonitis caused by leakage from the anastomosis. The mean bursting pressures are shown in Table 1.

Table 1 – Bursting pressure measurements of the anastomoses

Groups	GI	GII	GIII	GIV
Bp (mmHg)	148.8 ± 21.6	153.3 ± 18.8	156.8 ± 18.3	162.3 ± 13.6

Although it was not statistically significant, there was an increase in the burst pressures of the mesalamine group.

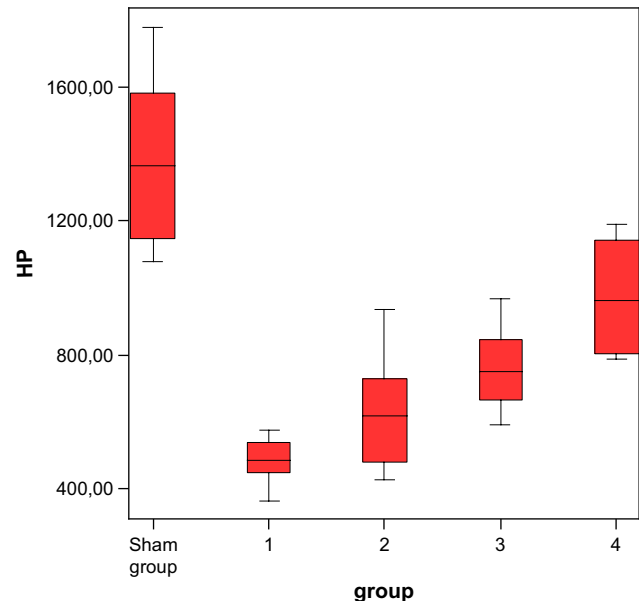
When hydroxyproline measurements were compared as in Table 2, there were statistically significant difference between the sham and all groups. There were significant differences between GI (anastomosis) and GIII–GIV (0.025 versus 0.00, respectively), GII and GIV (0.03). The differences between group I and II and group II and III were not statistically significant Fig. 1.

Inflammatory changes such as mononuclear cell infiltration, fibroblastic cell infiltration, epithelisation and capillary formation were evaluated as favourable changes while granulocytic cell infiltration, necrosis and exudate formation were unfavourable. When we compared the median amount of the histopathologic changes we found significant difference between the anastomosis and the mesalamine groups ($P < 0.05$). But when mesalamine groups were compared with each other we did not observe a significant difference (Table 3).

4. Discussion

Anastomotic healing is effected by the degree of primary inflammatory response; the rate of mucosal reepithelization; the amount, strength, and maturation rate of new collagen; and collagenolysis in the initial 3 days of the postanastomotic period. Strength of an anastomosis is based on the collagen fibers and their maturation at the submucosa.^{3,14} Synthesis of collagen is maximized in 5–7 days by proliferation of collagen-producing local fibroblasts.¹⁵

When an anastomosis is constructed in the gastrointestinal tract, an inflammation occurs as a response to traumatic injury and foreign material such as sutures.^{16,17} This inflammation is a normal constituent of wound healing. If it is exaggerated, however, wound healing is delayed because of increased collagenolysis; this is why anastomotic healing is delayed in the presence of intra-abdominal infection.^{16–18} The anastomotic area is already inflamed, and the endothelium in the perianastomotic area is already activated during the early phase of wound healing.^{17,19} As a result of proinflammatory and chemoattractant properties of the anastomosis, activated circulating PMN leukocytes secondary to sepsis-induced injury may accumulate easily in the perianastomotic area, increase the inflammatory reaction, and delay healing. Together with the proteolytic enzymes, oxygen-free radicals derived from activated PMN leukocytes and circulating

**Fig. 1 – Hydroxyproline measurements according to groups.**

xanthine oxidase may increase collagenolysis in the perianastomotic area, which delays wound healing.²⁰ In our study we evaluated the histopathologic differences between groups and we found more histologic findings favoring healing in the mesalamine groups in contrast to anastomosis group. When we compare the mesalamine groups with each other we did not observe a significant difference.

The precise mechanism of action of mesalamine is not clear, but is likely due to a combination of anti-inflammatory properties. Mesalamine has been shown to block the production of interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α). Sulfasalazine has also been found to inhibit binding of TNF- α to its receptor, thereby preventing signaling of subsequent inflammatory responses.^{12,21,22} Mesalamine is a potent inhibitor of the cyclooxygenase pathway, inhibiting the production of prostaglandin E2 in inflamed intestinal specimens. Its efficacy as an anti-inflammatory agent is also thought to be due to effects on leukotrienes metabolism. Mesalamine is also one of the most potent known free radical scavengers and antioxidants.^{9–11} Many of the effects of 5-ASA may also be explained by inhibition of activation of nuclear factor- κ B (NF- κ B), a central transcription regulatory factor involved in mediating the initiation and perpetuation of inflammatory processes. Activated NF- κ B has been detected in macrophages and epithelial cells in inflamed mucosa from Crohn's disease and ulcerative colitis. Mesalamine has been demonstrated to inhibit TNF- α stimulated NF- κ B activation, NF- κ B nuclear translocation, and degradation of inhibitory κ B α (I κ B α).^{12,23} Mesalamine might also have some other subcellular mechanisms of action such as changing levels of mucosal and

Table 2 – Hydroxyproline measurements

Groups	Sham group	GI	GII	GIII	GIV
µg/g tissue	1381.6 ± 254.7	485.0 ± 68.4	626.7 ± 170.2	780.0 ± 128.9	974.1 ± 186.9

Table 3 – Histopathologic characteristics of the groups

	GI	GII	GIII	GIV
Granulocytic cell	2.87 ± 0.35	1.50 ± 0.53	1.13 ± 0.83	0.88 ± 0.46
Mononuclear cell infiltration	1.00 ± 0.53	2.38 ± 0.52	2.50 ± 0.53	2.63 ± 0.52
Fibroblastic cell infiltration	1.25 ± 0.46	2.50 ± 0.76	2.38 ± 0.52	2.63 ± 0.52
Epithelisation	0.75 ± 0.70	2.13 ± 0.83	2.38 ± 0.52	2.50 ± 0.75
Necrosis	1.25 ± 0.46	0.25 ± 0.46	0.25 ± 0.46	0.13 ± 0.35
Exudate	1.38 ± 0.52	0.38 ± 0.52	0.25 ± 0.46	0.13 ± 0.35
Capillary formation	1.5 ± 0.53	2.25 ± 0.46	2.38 ± 0.52	2.75 ± 0.46
Microscopic peritonit	0.63 ± 0.52	0.13 ± 0.35	0.13 ± 0.35	0.00

submucosal matrix metalloproteinases (MMP). It has been shown that disturbances of the extracellular matrix play a role in the pathogenesis of anastomotic leakage and that significantly higher expressions of MMP occurred in the bowel wall of the leakage groups in studies.²⁴ Whether mesalamine showed its anti-inflammatory effect via changing levels of MMP is a potential future direction of this study.

Collagen is important during all phases of wound healing and is critical for the return of tissue integrity and strength.²⁵ In this study, we determined the HP content in the anastomotic tissue by means of a parameter of collagen synthesis and wound healing and observed that HP levels were increased significantly in the mesalamine group when compared with the anastomosis group. The difference was more significant in the rectal + oral and only rectal mesalamine group in contrast to oral mesalamine groups. In the present study no preoperative bowel preparation was given to the groups. It has been postulated that differences in bowel preparation may contribute to different results. On the other hand, experimental data have suggested that bowel preparation did not affect anastomotic collagen contents.²⁶ In the present study the groups were not sufficient to investigate the effect of different treatment techniques on the anti-inflammatory response. Future studies with larger number of groups should be planned to search these possible effects. After surgery, early anastomotic strength depends on the sutures' holding capacity because at 4 days after operation normal anastomoses are filled with loosely packed, unorganized collagen fibrils. Therefore, early bowel leak cannot be associated with insufficient collagen metabolism but must relate to a mechanical anastomotic failure, bowel ischemia, or failure to obtain a mucosal seal.²⁷ Between the fifth and seventh days after surgery, collagen synthesis peaks, and the wound strength depends mainly on these newly formed, organized collagen fibers.²⁸ For this reason the seventh postoperative day was chosen to evaluate the anastomotic wound healing in our model.

Another limitation of the current study is that we investigated the effect of mesalamine on the bursting pressure not on tensile strength. Tensile strength is an important determinant of anastomotic strength in contrast to the bursting pressure but our reason in choosing the bursting pressure is that tensile strength can reflect healing less accurately.²⁹

5. Conclusion

In conclusion, we found that mesalamine had positive effects which were not statistically significant on bursting pressure

and statistically different significant effects on HP levels based on the way of administration and statistically significant positive effects on histopathologic anastomotic healing in experimental anastomosis model.

Conflict of interest

The authors have no conflict of interest.

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Ethical approval

Ethical committee of Mustafa Kemal University of Veterinary.

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