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Original Article

The effects of non-steroid anti-inflammatory drugs on healing of colonic anastomosis in rats

Ersoy Esen[a], Ilker Sucullu[a*], Huseyin Sinan[b], Zafer Küçükodacı[c], Ali Ilker Filiz[a], Ergun Yucel[a], Mehmet Levhi Akın[a]

[a]*Gulhane Military Medical Academy, Haydarpasa Training Hospital, Department of General Surgery, Istanbul, Turkey*

[b]*Gulhane Military Medical Academy, Department of Emergency Medicine, Ankara, Turkey*

[c]*Gulhane Military Medical Academy, Haydarpasa Training Hospital, Department of Pathology, Istanbul, Turkey*

Abstract. NSAIDs have dual roles of providing analgesia and promoting healing. This study investigated the effects of 3 different NSAIDs on healing of colonic anastomosis in rats. The aim is to provide an evidence-based rationale for choice of postoperative analgesic agent in surgical patients who undergo this procedure. Forty-eight Sprague-Dawley conventional rats were separated into 4 groups of 12 rats, with one group as control, and the other groups receiving tenoxicam, metamizole, or diclofenac, respectively. Each group was subdivided into 2 subgroups according to sacrifice on postoperative day 3 or 7. Anastomotic healing was assessed by 3 parameters: (1) physical evaluation (bursting pressure), (2) histological evaluation (fibrotic index), and (3) biochemical evaluation (tissue hydroxyproline levels). Diclofenac had a negative effect on bursting pressures. Tenoxicam significantly increased the fibrotic index at day 7. Metamizole increased tissue hydroxyproline levels at day 3, but there was no significant difference between control and NSAID groups at day 7. Each drug showed benefits and drawbacks. Because tenoxicam promoted fibrosis, and metamizole increased collagen synthesis, with both agents showing few disadvantages, these 2 agents are recommended above diclofenac for promoting healing.

Key words: NSAID, Colonic anastomoses, rats

1. Introduction

Failure of anastomotic healing, ranging between 0% and 35% (1), is a serious complication of large bowel surgery, which increases morbidity and mortality rates significantly (2). Many factors have been shown to have a profound effect on wound healing and gastrointestinal healing (3,4,5).

The inflammatory reaction is essential for healing and includes infiltration, capillary ingrowths, migration, phagocytosis and chemotaxis, following which early collagenolysis takes place (5). Success at healing of colonic anastomosis is closely related to collagen migration toward the anastomosis (6,7). The bowel wall derives its integrity and mechanical strength largely from collagen; this structural protein is concentrated mainly in the bowel wall's submucosal layer (8). It is generally accepted that collagen levels fluctuate during the healing process as a result of degradation and synthesis near the wound area (9). In the first days of operation, the collagen concentration around the anastomosis decreases (10), reflecting a net loss of collagen. In a later phase, newly synthesized collagen gradually becomes of major importance in restoring preoperative strength (11). Limitations of collagenolysis may benefit intestinal strength during the first postoperative period (12).

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to relieve postoperative pain. The action of NSAIDs is based on the inhibition of cyclooxygenase (13). Cyclooxygenase converts arachidonic acid into prostaglandins, prostacyclin, and thromboxane. These substances are mediators in the inflammatory process (5). Because of the cyclooxygenase inhibition, anti-inflammatory drugs reduce the initial inflammatory response

[*]Correspondence : Assistant Professor Ilker SUCULLU,MD, Gulhane Military Medical Academy, Haydarpasa Training Hospital, Department of General Surgery, Istanbul, Turkey
Phone: + 902165422806, +905057169133
Fax: + 902164494480
e-mail : suculluilker@yahoo.com

Table 1. Study groups

Group	NSAID	Dose
1	Control	1 ml/day 0.9% NaCl
2	Tenoxicam (Oksamen® flakon, Mustafa Nevzat, Istanbul)	0.3 mg/day, single dose
3	Metamizole (Nova® ampule, MSB ilaç Fabrikası, Ankara)	30 mg/kg/day, at 2 equal doses
4	Diclofenac sodium (Voltaren® ampule, Novartis, Istanbul)	4 mg/kg/day, at 2 equal doses

Table 2. Criteria for evaluating fibrosis at the anastomosis

Scale	Criteria
0	No fibrosis
1	Presence of too-thin fiber, sparse, irregular collagen
2	Presence of irregular but denser, thick fiber collagen
3	Presence of regular and thick fiber, dense collagen

which is essential for anastomotic healing, and may interfere with wound healing (3,5).

The aim of our study was to investigate the effects of the 3 most commonly used NSAIDs on the healing of colon anastomosis in the rat.

2. Materials and methods

2.1. Animals

Forty-eight Sprague-Dawley conventional rats weighing between 200 and 300 g were used. During the study, 6 rats died and were replaced by new rats. The rats were kept in cages at a constant temperature of 20±2°C, exposed to 12-hour light/dark cycles, and fed with regular rat chow and tap water from drinking bottle. Our experimental protocol was designed according to the *Principles of Laboratory Animal Care* and ethical standards for animal use, and approved by the local ethical committee of animal use.

2.2. Experimental protocol

The rats were separated into 4 groups of 12 rats. While one group served as control, the other groups received tenoxicam, metamizole, or diclofenac (Table 1). Each group was subdivided into 2 subgroups according to being sacrificed at postoperative day 3 or day 7.

2.3. Operative procedure

Following overnight fasting, anesthesia for all animals was induced using ketamine HCl (Ketalar®, Eczacibasi, Istanbul) 100 mg/kg + Xylazine (Rompun® ampule, Bayer) 10 mg/kg, intraperitoneally. After shaving, the abdomen was prepared with povidone-iodine. This procedure was followed by laparotomy with a 4-cm midline incision. The colon was divided at 3 cm proximal to the peritoneal reflection. An inverting one-layer end-to-end anastomosis was constructed using 5/0 monofilament polypropylene suture (Prolene®, Ethicon LTD, Edinburgh, UK), and the abdomen was closed. The rats were then resuscitated with 5 ml of normal saline solution administered subcutaneously. All rats received the same resuscitation load. After surgery, the rats were fed with regular rat chow and tap water from drinking bottle starting from postoperative day 1.

After performance of the colonic anastomoses, the rats received physiological saline solution (Neofleks® serum, Turktipsan AS, Ankara), plus one of the NSAIDs (tenoxicam, metamizole, or diclofenac) beginning from the time of operation. Deaths during the study period occurred as follows: 1 rat in the tenoxicam group, 2 rats in the metamizole group, and 3 rats in the diclofenac group. Anastomosis leakage was determined as the cause of all deaths at necropsy. At postoperative days 3 and 7, the rats were reoperated in the same manner and the anastomoses were evaluated physically, histopathologically, and biochemically. The rats were euthanasied via intraperitoneal high-dose

Table 3. Results of the study groups (mean ± SD)

		Day 3	*p*	Day 7	*p*
Bursting pressure (mmHg)	Tenoxicam	25.83 ± 7.3	0.57	122.5 ± 26.2	0.2
	Metamizole	25.3 ± 10.6	0.74	122.7 ± 13.7	0.23
	Diclofenac	21.83 ± 3.8	0.81	99.8 ± 11.6	0.01*
	Control	22.3 ± 3.8	-	137.5 ± 18.9	-
Fibrotic index	Tenoxicam	1 ± 0.81	0.36	3 ± 0.51	0.04*
	Metamizole	1 ± 0.4	0.36	2 ± 0.51	0.57
	Diclofenac	1.5 ± 0.54	0.22	2 ± 0.51	0.99
	Control	1 ± 0.4	-	2 ± 0.4	-
Hydroxyproline levels (µg/100 mg)	Tenoxicam	38.17 ± 32.8	0.87	75.3 ± 30.3	0.05
	Metamizole	71.8 ± 25.7	0.03*	102.3 ± 17.9	0.81
	Diclofenac	53.7 ± 24.9	0.26	87.8 ± 19.5	0.17
	Control	36±22.6	-	100 ± 26.36	-

* Statistically significant

tiopental sodium (Pentotal®, IE Ulagay, Istanbul).

2.4. Assessment of the anastomoses

Anastomotic healing was assessed by 3 parameters: (1) physical evaluation (bursting pressure), (2) histopathological evaluation (fibrotic index), and (3) biochemical evaluation (tissue hydroxyproline levels).

Bursting Pressure Measurements: Measurements were done in vivo, during which intestinal blood flow was maintained. Preserving the anastomotic line, the colon was resected at 2 cm distal to the anastomotic line. The proximal side of the colon was ligated 1 cm above the anastomosis. A catheter was inserted from the resected distal end of the colon, and this end was ligated over the catheter with silk stitch. Methylene blue added to normal saline solution was infused via this catheter at a rate of 6 ml/min. One end of a triple tap was attached to the pressure measurement tool, while the other end was attached to the colonic segment in which the bursting pressure would be measured. Leakage of blue fluid from the bursting point and prompt pressure descent were recorded.

Histopathological and Biochemical Evaluations: After the measurement of bursting pressure, a 2 cm-long colonic segment, 1 cm on each side of the anastomosis line, was resected and prepared for histopathological examination and hydroxyproline measurements. The resected segment was opened parallel to its long axis. Two separate tissue samples 5 mm wide and 10 mm long, including the anastomotic line, were obtained. One of these samples was fixed by 10% formalin for histopathological evaluation, whereas the other was kept in deep-freeze (at -71ºC) for hydroxyproline measurements. For histopathological examination, paraffin blocks of tissue samples were prepared, and 6 µm-thick sections were dyed with Masson's trichrome and hematoxylin-eozin. Results were analyzed by a single pathologist in a double blind fashion by light microscopy. Fibrosis at the anastomosis was evaluated according to the criteria shown in Table 2. Hydroxyproline levels were measured by the G. Keseva Reddy method. Tissue homogenate was read by spectrophotometry following hydrolysis.

2.5. Statistical analysis

SPSS for Windows 11.0 software was used for statistical analysis. The chi-square test was used for statistical comparisons, and Fisher's exact test was used for inappropriate data (4 or less frequency at cells). The Mann-Whitney U-test and Kruskal-Wallis analysis were used to determine differences among groups. Results were considered significant at $p < 0.05$.

3. Results

Physical evaluation (bursting pressure): There was no significant difference between control and NSAID groups at postoperative day 3. At

postoperative day 7, the mean bursting pressure was significantly lower only in the diclofenac group compared with the other groups (p = 0.01).

Histological evaluation (fibrotic index): There was no significant difference between control and NSAID groups at postoperative day 3. At postoperative day 7, the mean fibrotic index in the tenoxicam group was significantly higher than that in the control group (p = 0.04).

Biochemical evaluation (hydroxyproline levels): While the mean hydroxyproline level at postoperative day 3 was significantly higher in the metamizole group than in the control group (p = 0.03), there was no significant difference between control and NSAID groups at postoperative day 7. The results are shown in Table 3.

4. Discussion

In our study, we found that metamizole had a positive effect on hydroxyproline levels at postoperative day 3, while neither tenoxicam nor diclofenac had any effect. On the other hand, diclofenac had a negative effect on bursting pressures and tenoxicam had a positive effect on fibrosis at postoperative day 7, while metamizole had no effect.

Oxlund et al. (14) reported that the loss of collagen in the early postoperative phase causes weakness in colonic stretching power. Fibroblasts start to synthesize collagen on the second day of healing. Production of collagen changes to consumption at postoperative days 3 through 5. At the end of postoperative day 7, the level of collagen reaches normalcy (15). The increase of collagen at the anastomotic area is a sign of anastomotic healing (16,17). It has been microscopically determined that polymorphonuclear leukocytes are the dominant cells for the first 24 hours, and they are responsible for post-anastomotic collagen degradation (10).

The action of NSAIDs is based on the inhibition of cyclooxygenase (13). Cyclooxygenase converts arachidonic acid into prostaglandins, prostacyclin, and thromboxane. NSAIDs inhibit the secretion of lysosomal enzymes, migration, aggregation, adhesion, spontaneous movement and secretion of superoxide anions of polymorphonuclear leukocytes (18,19). These effects change according to the inhibition capacity of prostaglandin synthesis. The decrease of leukocyte proliferation at the inflammation site caused by NSAIDs has been shown. The inhibition of the vasodilatation effect of prostaglandin E_2 by NSAIDs prevents leukocyte migration to the inflammation site (19).

Another important factor in anastomotic healing is angiogenesis, which is regulated by the cyclooxygenase-2 enzyme. Anastomotic healing can be impaired by the inhibition of angiogenesis with NSAIDs (1,20,21). The cause of deaths of rats in all groups could be anastomotic leakage, which induced by using the NSAIDs. Mastboom et al. (12) reported also similar findings in their study.

In comparing the anastomotic bursting pressures, our results detected no differences among the groups at postoperative day 3. But at postoperative day 7, the mean bursting pressure in the diclofenac group was significantly lower than the mean bursting pressures of the control and other groups ($p = 0.01$). Inan et al. (5) studied the effects of diclofenac on anastomotic bursting pressure and tissue hydroxyproline levels, and reported that diclofenac decreased the anastomotic bursting pressures at the 3rd and 7th postoperative days. De Sousa et al. (22) investigated the effects of diclofenac on small intestine anastomotic healing in rats and found that diclofenac had a negative effect on intestinal healing as shown by an increased rate of anastomotic dehiscence, delayed acute inflammatory response, and decreased tensile strength of the anastomoses. Some properties of diclofenac may thus impair anastomotic healing.

There was no significant difference among groups in histopathologic evaluation at postoperative day 3, but the mean fibrosis score of the tenoxicam group was significantly higher than that of the other groups at postoperative day 7 ($p = 0.01$). De Sousa et al. (22) reported that diclofenac histopathologically reduces the accumulation of inflammatory cells and the formation of fibrin at postoperative day 3. These results shows that NSAIDs do not affect fibrosis formation negatively during the early postoperative period, but paradoxically, tenoxicam causes an increase in fibrosis formation in the late period of healing. However, this property of tenoxicam is not helpful with respect to bursting pressure and collagen synthesis.

In our study, the mean hydroxyproline level in the metamizole group at postoperative day 3 was higher than that of the control group; this difference was statistically significant ($p = 0.03$). There was no statistically significant difference among groups in the mean hydroxyproline levels at postoperative day 7. Mastboom et al. (12) studied piroxicam and ibuprofen, and Brennan et al. (23) studied flurbiprofen, and found that these

drugs significantly increased the hydroxyproline concentration in the early postoperative period following experimental colonic anastomosis. Similarly, de Sousa et al. (22) reported that diclofenac increased hydroxyproline levels after intestinal anastomosis. Conversely, Inan et al. (5) reported that diclofenac decreased the hydroxyproline concentration around the perianastomotic area. Uzunköy et al. (24) determined that tenoxicam had no significant effect on hydroxyproline levels. Dissimilar results among various studies show that different NSAIDs can affect collagen production and consumption in different ways. The high mean hydroxyproline level at the early postoperative period in the metamizole group may be explained by the lower anti-inflammatory effect of this agent.

5. Conclusion

In summary, among our 3 study agents of tenoxicam, metamizole, and diclofenac, we observed diclofenac to have the greatest *negative* effect on anastomotic healing. The positive effects of tenoxicam on histopathological fibrosis could not be shown in all healing periods. Metamizole, which was seen to increase collagen synthesis, and tenoxicam, which increased fibrosis in the later healing period, were observed as safer agents.

Pain is the most common complaint of surgery patients, and pain control is crucial to postoperative recovery. How fortunate that a class of drugs exists that provides both analgesic and healing benefits. Our results suggest that in rats, tenoxicam and metamizole are more effective than diclofenac at satisfying this dual role. This is a start toward creating an evidence-based rationale for choosing the most effective NSAID after colonic anastomotic surgery. Further studies will more clearly sort out the nuances of each agent, translating into optimal post-surgical care of our patients.

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References

1. Hendriks T, Mastboom WJ. Healing of experimental intestinal anastomoses. Dis Colon Rectum 1990; 33: 891-901.
2. Buchs NC, Gervaz P, Secic M, et al. Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. Int J Colorectal Dis 2007; 23: 265-270.
3. Kiyama T, Onda M, Tokunaga et al. Effect of matrix metalloproteinase inhibition on colonic anastomotic healing in rats. J Gastrointest Surg 2001; 5: 303-311.
4. Sucullu I, Sinan H, Filiz AI, et al. The effects of hyperbaric oxygen therapy on colonic anastomosis in rats with peritonitis. J Investigative Surg 2008; 21: 195-200.
5. Inan A, Koca C, Sen M. Effects of diclofenac sodium on bursting pressures of anastomoses and hydroxyproline contents of perianastomotic tissues in a laboratory study. Int J Surg 2006; 4: 222-227.
6. Chowcat NL, Savage FJ, Lewin MR, et al. Direct measurement of collagenase in colonic anastomosis. Br J Surg 1990; 77: 1284-1287.
7. Savage FJ, Lacombe DL, Hembry RM, et al. Effect of colonic obstruction on the distribution of matrix metalloproteinases during anastomotic healing. Br J Surg 1998; 85: 72-75.
8. Gabella G. The cross-ply arrangement of collagen fibres in the submucosa of the mammalian small intestine. Cell Tissue Res 1987; 248: 491-497.
9. Hunt TK, Hawley PR, Hale J, et al. Colon repair: the collagenous equilibrium. In Hunt TK, ed. Wound Healing and Wound Infection. New York: Appleton-Century-Crofts, 1980, pp 153-159.
10. Hendriks T, Vereecken TH, Hesp WL, et al. Loss of collagen from experimental intestinal anastomoses: early events. Exp Mol Pathol 1985; 42: 411-418.
11. Peacock EE, van Winkle W. The biochemistry and the environment of wounds and their relation to wound strength. In: Peacock EE, van Winkle W, eds. Wound Repair, 2nd ed. Philadelphia: WB Saunders, 1976, pp 145-203.
12. Mastboom WJB, Hendriks T, van Elteren P, et al. The influence of NSAIDs onexperimental intestinal anastomoses. Dis Colon Rectum 1991;34:236-243.
13. Beck A, Krischak G, Sorg T, et al. Influence of diclofenac (group of nonsteroidal anti-inflammatory drugs) on fracture healing. Arch Orthop Trauma Surg 2003; 123: 327-332.
14. Oxlund H, Cristensen H, Seyer-Hensen M, Andreassen TT. Collagen deposition and mechanical strength of colon anastomoses and skin incisional wounds of rats. J Surg Res 1996; 66: 25-30.
15. Jiborn H, Abonen J, Zederfeldt B. Healing of experimental colonic anastomoses. III. Collagen metabolism in the colon after left colon resection. Amer J Surg 1980; 139: 398-405.
16. Brasken P, Renvall S, Sandberg M. Fibronectin and collagen gene expression in healing experimental colonic anastomoses. Br J Surg 1991; 78: 1048-1052.
17. Martens MF, Hendriks T. Postoperative changes in collagen synthesis in intestinal anastomoses of the rat: differences between small and large bowel. Gut 1991; 32: 1482-1487.
18. Angelis-Stoforidis P, Vajda FJ, Christophidis N. Effects of non-steroidal anti-inflammatory drugs (NSAIDs) on human polymorphonuclear leucocyte function in buffer and plasma. Clin Exp Rheumatol 1998; 16: 703-708.
19. Hirasawa N, Ohuchi K, Watanabe M, et al. Mechanism of the inhibitory action of cyclooxygenase inhibitors on leukocyte infiltration:

involvement of endogenous histamine. Eur J Pharmacol 1987; 144: 267-275.

20. Blomme EAG, Chinn KS, Hardy MM, et al. Selective cyclooxygenase-2 inhibition does not affect the healing of cutaneous full-thickness incisional wounds in SKH-1 mice. Br J Dermatol 2003; 148: 211-223.

21. Hendriks JM, Hubens G, Wuyts FL, et al. Experimental study of intraperitoneal suramin on the healing of colonic anastomoses. Br J Surg 1999; 86: 1171-1175.

22. De Sousa JB, Soares EG, Aprilli F. Effects of diclofenac sodium on intestinal anastomotic healing. Experimental study on the small intestine of rats. Dis Colon Rectum 1991; 34: 613-617.

23. Brennan SS, Foster ME, Morgan A, et al. Prostaglandins in colonic anastomotic healing. Dis Colon Rectum 1984; 27: 723-725.

24. Uzunköy A, Akıncı OF, Coşkun A, et al. Effects of antiadhesive agents on the healing of intestinal anastomosis. Dis Colon Rectum 2000; 43: 370-375.