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Etofenamate and anastomoses of the colon in rats

Aim: Non-steroidal anti-inflammatory drugs have been used for many years due to their analgesic and anti-inflammatory properties. They have also been used for postoperative analgesia. We aimed to study the effects of etofenamate, a non-steroidal anti-inflammatory drug, on the healing of colonic anastomoses in rats.

Materials and methods: Sprague-Dawley rats were used in this study. Resection and anastomosis were performed on the distal colon. The etofenamate group received 30 mg/kg etofenamate and the control group received 0.1 mL of 0.9% NaCl intramuscularly daily. Bursting pressures of anastomoses and hydroxyproline levels of perianastomotic tissues were determined on days 3 and 7.

Results: Four deaths were observed in each group. Mean bursting pressures (etofenamate group, day 3: 50.50 ± 7.27 mmHg, day 7: 187.37 ± 12.20 mmHg; control group, day 3: 55.13 ± 5.94 mmHg, day 7: 202.12 ± 18.64 mmHg) and mean hydroxyproline levels (etofenamate group, day 3: 2.18 ± 0.17 µg/mg tissue, day 7: 4.34 ± 0.79 µg/mg tissue; control group, day 3: 2.20 ± 0.12 µg/mg tissue, day 7: 5.07 ± 0.65 µg/mg tissue) in the etofenamate group were lower than those in the control group on both days 3 and 7. This finding was not statistically significant.

Conclusion: According to our findings, etofenamate treatment during the postoperative period does not alter the healing of colonic anastomoses in rats.

Key words: Non-steroidal anti-inflammatory drugs, etofenamate, colon, anastomosis, healing, rat

Etofenamate'in rat kolon anastomozuna etkisi

Amaç: Nonsteroid antiinflatuar ilaçlar, analjezik ve antiinflatuar olarak yıllardır kullanılmaktadırlar. Ayrıca postoperatif ağrı tedavisinde de kullanılan ilaçlardır. Çalışmamızda bir nonsteroid antiinflatuar ilaç olan etofenamate'in ratlarda kolon anastomozu iyileşmesi üzerine etkilerini araştırdık.

Yöntem ve gereçler: Çalışmada Sprague-Dawley ratlar kullanıldı. Ratların distal kolonlarında rezeksiyon yapılarak anastomoz gerçekleştirildi. Etofenamate grubu'ndaki ratlara 30 mg/kg/gün etofenamate, kontrol grubu'ndaki ratlara ise 0,1 mL/gün % 0,9 NaCl intramüsküler uygulandı. Üçüncü ve yedinci günlerde anastomozların patlama basınçları ve anastomoz etrafındaki dokuların hidroksiprolin düzeyleri ölçüldü.

Bulgular: Her iki gruptan da dörder rat kaybedildi. Ortalama patlama basınçları (etofenamate grubu, 3. gün: $50,50 \pm 7,27$, 7. gün: $187,37 \pm 12,20$, kontrol grubu, 3. gün: $55,13 \pm 5,94$, 7. gün: $202,12 \pm 18,64$ mmHg) ve ortalama hidroksiprolin düzeyleri (etofenamate grubu, 3. gün: $2,18 \pm 0,17$, 7. gün: $4,34 \pm 0,79$, kontrol grubu, 3. gün: $2,20 \pm 0,12$, 7. gün: $5,07 \pm 0,65$ µg/mg doku) etofenamate grubu'nda kontrol grubu'na göre daha düşük bulundu. Bu bulgular istatistiksel olarak anlamlı değildi.

Sonuç: Postoperatif dönemde etofenamate kullanılması ratlarda kolon anastomoz iyileşmesine olumsuz etkisi gözlenmemiştir.

Anahtar sözcükler: Nonsteroid antiinflatuar ilaçlar, etofenamate, kolon, anastomoz, iyileşme, rat

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used for many years for analgesic, anti-inflammatory, and antithrombotic purposes and have become one of the most commonly used medications. Their perisurgical use has increased (1-3). NSAIDs are generally well tolerated (1). The action of NSAIDs is

based on the inhibition of cyclo-oxygenase, which converts the arachidonic acid that is set free from the cell wall into prostaglandins, prostacyclin, and thromboxane (4).

Etofenamate is a long-acting NSAID and rapid-acting intramuscular analgesic. Etofenamate has effects on pain similar to those of fentanyl and can be used safely (5).

Resection and anastomosis of the colon are associated with a high incidence of anastomotic leakage ranging between 0% and 35% (6,7). When this serious complication occurs, morbidity and mortality rates and the hospital length of stay greatly increase after colonic surgery (8). Many factors may compromise the healing of colonic anastomoses (7,9). Contradictory reports exist concerning the role of anti-inflammatory drugs in wound healing (3).

As the perisurgical use of NSAIDs has increased we studied anastomotic healing in the colon of rats treated with etofenamate during the postoperative period. We examined the bursting pressures of anastomoses and hydroxyproline contents of perianastomotic tissues.

Materials and methods

The Ethical Committee of Fatih University Medical School approved the methodology of this experimental study.

Forty male Sprague-Dawley rats (240-280 g) were used. A biostatistician was consulted to determine the number of rats. The animals had free access to normal laboratory food (Medaş, Ankara) and tap water. They were housed at 21 °C and 12 h light-dark cycle.

On the day of surgery, anaesthesia was maintained with ketamine (Ketalar, Eczacıbaşı) 30 mg/kg, intramuscularly. After shaving, the abdomen was prepared with povidone-iodine. Surgery was performed under sterile conditions through a lower midline 2 cm abdominal laparotomy; 0.5 cm of the colon was resected 3 cm proximal to the peritoneal reflection. The mesenteric arcade was protected during the resection. Anastomosis was performed in a single layer with 9-12 interrupted inverting 6/0 polypropylene sutures (Prolone, Ethicon). The abdomen was closed with 3/0 polyglactine (Polysorb, Tyco) running sutures in 2 layers.

The rats divided into 2 groups randomly. The etofenamate group (EG) received 30 mg/kg etofenamate daily (Flexo ampul, Santa Farma) and the control group (CG) received 0.1 mL/day 0.9% NaCl intramuscular. On the third postoperative day, 8 rats from each group were randomly selected for measurement of bursting pressure and hydroxyproline level in the perianastomotic region. Anaesthesia was maintained with ketamine 30 mg/kg. The whole colon was resected. Great attention was paid not to break the adhesions of the perianastomotic region. The rats were sacrificed by cardiac puncture.

Colonic anastomoses can be measured using bursting pressure and collagen concentration (7,10). We used these 2 measures to evaluate anastomotic healing.

The proximal end of the colon was attached to an air pump supplying 1 mL of air per minute. The distal end was attached to a continuous pressure display system (Petaş KMA365B). The colon was immersed into saline. The air pump started. The pressure was recorded as "bursting pressure" when the pressure went down suddenly or bubbles were seen. The perianastomotic region was cleared after bursting. A colon segment of 1 cm (0.5 cm each side of anastomosis) was taken and rinsed with saline for hydroxyproline determination.

The samples were stored at -40 °C for subsequent analysis of hydroxyproline content of the perianastomotic region.

Determination of hydroxyproline level was performed as described by Jamall et al. (11). Hydroxyproline was released from colon tissue homogenates using acid hydrolysis. The free hydroxyproline was then oxidised by chloramine to produce a pyrrole-type compound. The addition of Ehrlich's reagent resulted in the formation of a chromophore measured at 560 nm. The absorbances were read using distilled water as reference and the results were given as µg/mg tissue.

Other rats from each group underwent the same procedure on day 7.

SPSS for Windows 10.0 was used for the statistical analysis. A Mann-Whitney U test was used to determine differences in the groups. The statistical significance level was set at $P < 0.05$.

Results

Four deaths were observed in each group. Three rats on day 3, 2 rats on day 4, and 3 rats on day 5 of surgery died because of septic complications due to anastomotic leakage. The other rats survived until the end of the study without any septic, surgical, or anaesthetic complications. There was no statistical difference in the groups in terms of mortality ($P > 0.05$).

Mean bursting pressure in the etofenamate group was lower than that in the control group on both days 3 and 7. This finding was not statistically significant (Table 1).

Mean hydroxyproline content in the etofenamate group was also lower than that in the control group on both days 3 and 7. This finding was not statistically significant either (Table 2).

Discussion

NSAIDs are a commonly used class of medication and have a broad spectrum of clinical applications including perioperative pain (12,13). Perioperative

use of NSAIDs reduces postoperative pain and opioid consumption (14). The use of these drugs continues to increase (2). They have anti-inflammatory as well as analgesic, antipyretic, and platelet inhibitory actions (15).

The pharmacologic activity of NSAIDs is attributed to inhibition of cyclooxygenase enzymes. Cyclooxygenases catalyse the first step of the synthesis of prostanoids (16).

It is reported that inhibition of cyclooxygenase significantly affects the healing of sutured surgical incisions in mouse skin (17).

Anastomotic healing requires many complex processes and factors. A number of interactions have been identified as having a profound effect on healing. Many components of the healing process are common to all tissues as anastomotic healing. Initially inflammatory response includes infiltration and capillary ingrowth; then early collagenolysis takes place. The proliferative phase is characterised by proliferation of fibroblasts, which synthesise collagen in the submucosal layer. Collagen is the major protein of the extracellular matrix and the predominant

Table 1. Mean bursting pressures of groups.

| Day | Group | Number of rats | Bursting pressure (Mean ± SD mmHg) | P value |
|-----|-------------|----------------|------------------------------------|---------|
| 3 | Control | 8 | 55.13 ± 5.94 | 0.154 |
| | Etofenamate | 8 | 50.50 ± 7.27 | |
| 7 | Control | 8 | 202.12 ± 18.64 | 0.141 |
| | Etofenamate | 8 | 187.37 ± 12.20 | |

Table 2. Mean hydroxyproline contents of groups.

| Day | Group | Number of rats | Hydroxyproline Content (Mean ± SD µg/mg tissue) | P value |
|-----|-------------|----------------|---|---------|
| 3 | Control | 8 | 2.20 ± 0.12 | 0.494 |
| | Etofenamate | 8 | 2.18 ± 0.17 | |
| 7 | Control | 8 | 5.07 ± 0.65 | 0.052 |
| | Etofenamate | 8 | 4.34 ± 0.79 | |

constituent of the final healing scar. Collagen continues to form and remodel via cross-linking during the maturative phase. This results in the formation of mature scar tissue (8,9,18,19). Cellular activities for wound healing include migration, phagocytosis, chemotaxis, adhesion, mitogenesis, and synthesis of matrix components (19).

NSAIDs delay the onset of fibroblast proliferation (20). This influences the proliferative phase of healing negatively.

Prostaglandins and thromboxane are involved in inflammatory processes, but their conversion from arachidonic acid is inhibited by NSAIDs, which may have a negative influence on the inflammation. As an adequate inflammatory reaction is essential for healing, drugs interfering with the inflammatory phase like NSAIDs probably modify anastomotic healing (3,17).

Polymorphonuclear leukocytes are the first cell type to accumulate in inflammatory reactions. It has been shown that NSAIDs can inhibit polymorphonuclear leucocyte responses such as the extracellular release of lysosomal enzymes, chemotaxis, migration, aggregation, adherence and spontaneous motility, and the production of superoxide anion (21,22). These effects of NSAIDs may be independent of their inhibition of prostaglandin synthesis (21). Cyclooxygenase inhibition increases histamine levels, which may be also inhibitory for leukocyte mobility (22). However, in the inflammatory phase of the healing process of anastomosis, neutrophils play an important role by clearing up debris, protecting from infection, and releasing growth-promoting substances (23,24). Decreased neutrophil infiltration coincides with defective anastomotic healing in rats (23).

A number of NSAIDs inhibit the expression and release of cytokines in activated human monocytes, namely IL-1 β , TNF α , and IL-6, and chemokines such as IL-8. The mechanism by which cytokine expression is inhibited by NSAIDs is not clear (15). Inhibition of these substances has a negative influence on the healing because IL-1 β and TNF α function in matrix synthesis regulation and cell recruitment and activation. TNF α also functions in neutrophil activation and angiogenesis. IL-6 functions in cell recruitment and activation (24).

NSAIDs can inhibit superoxide anion generation (25), but decreased production of oxygen-derived free radicals may negatively affect the healing of anastomoses with a higher incidence of abscess formation (18), because superoxide anions play a role in phagocytosis and antimicrobial function (24).

Angiogenesis is required in the healing phase of a colorectal anastomosis (26,27). Adequate tissue perfusion and oxygen delivery are necessary for good anastomotic healing (28). Prostaglandins may modulate fibroblast growth factor β and vascular endothelial growth factor induced angiogenesis. Cyclooxygenase inhibitors have been shown to block angiogenesis in vitro and in vivo in the rat cornea, rat air pouch model, and in mouse tumour models (17).

Some NSAIDs have a distinct inhibitory effect on protein synthesis, which may influence the healing negatively (29).

Bursting pressure of an anastomosis is a mechanical parameter of the healing of the anastomosis (6,23). Hydroxyproline content of the perianastomotic tissue is a measure of the amount of collagen (6,30). According to our findings, bursting pressures and hydroxyproline contents of etofenamate treated rats did not differ from those of the controls.

There are contradictory reports of the effects of NSAIDs on the healing of wounds (3,13,17,20). In a study by Quirinia and Viidik influences of diclofenac and indomethacin on the healing of normal and ischemic incisional wounds are investigated. They found that neither of these 2 NSAIDs had an influence on healing (13). Dvivedi et al. reported that ibuprofen and diclofenac sodium caused a reduction in the strength of wound and the weight of granuloma formed (3). Blomme et al. reported chronic daily treatment with selective cyclooxygenase-2 inhibitor or diclofenac had no significant effect on cutaneous healing (17). In another experimental study, Sousa et al. investigated the effects of diclofenac sodium on intestinal anastomotic healing and found that it had a negative effect (20).

In conclusion, NSAIDs have some properties that may have negative effects on the healing of wounds, like a negative influence on inflammation, inhibition of polymorphonuclear leucocyte responses, inhibition of cytokine release and expression, inhibition of

superoxide anion generation, blocking angiogenesis, and inhibitory effect on protein synthesis. Despite the reported effects of NSAIDs on the healing of wounds,

on the basis of the present results etofenamate treatment during the postoperative period does not alter the healing of colonic anastomoses in rats.

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