Effects of selective and non-selective cyclooxygenase (COX) inhibitors on postoperative adhesion formation in a rat uterine horn model

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Summary

Objective: To investigate the effects of cyclooxygenase (COX) inhibitors including celecoxib, indomethacin, and nimesulide on postoperative adhesion formation. *Material and Methods:* Forty-eight female Wistar-Albino rats were randomly divided into four groups: control (saline solution), celecoxib, indomethacin, and nimesulide groups. The uterine horns of rats were traumatized with unipolar electrocautery. Drugs of each group and saline in the control group were insillated on travmatized areas of horns as intraperitoneally. After three weeks, the extent and severity of adhesions with a standardized scoring system were evaluated. *Results:* The extent and severity of postoperative adhesions were significantly reduced in nimesulide group compared with the control group. The extent but not severity of adhesions in rats given indomethacin was significantly reduced. Celecoxib showed no significant reduction in the extent and severity of adhesions. *Conclusion:* Nimesulide is more effective than the other COX inhibitors in the prevention of postoperative adhesions in rats.

Key words: Adhesion; Cyclooxygenase; Nimesulide; Celecoxib; Indomethacin.

Introduction

Postoperative pelvic adhesions cause various medical problems including infertility, chronic pelvic pain, bowel obstruction, and increase in health expenses [1, 2]. Despite modern surgical techniques, adhesion formation and reformation are still an unavoidable event in reproductive pelvic surgery. Although many adjuvants have been tested in animal models and clinical trials, intraperitoneal fluid instillates and barrier methods are used in clinical practice [3-6]. However, effective application is limited by technical difficulties, including the need for hemostasis and removal of excess peritoneal fluid [7, 8].

Adhesions are the results of the inflammatory response to tissue trauma, infection, hemorrhage, or foreign materials in the peritoneal space. This inflammatory response is due largely to the local release of eicosanoids, including prostaglandins and leukotrienes, caused by tissue trauma [9]. Non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit prostaglandin production, have been shown to decrease adhesion formation [10, 11]. Most of the available NSAIDs may inhibit both cyclooxgenase-2 (COX-2) and cyclooxygenase 1 (COX-1), showing no selectivity for either of the COX isozymes, whereas several NSAIDs express selectivity for COX-1 over COX-2. In most previous reports, the effects of nonselective and selective COX inhibitors have been investigated on postoperative adhesion formation, however, there are few studies comparing the effects of these drugs [10-14]. In this study, we compared the effects of COX inhibitors , celecoxib (a highly selective COX-2 inhibitor), indomethacin (a nonselective COX inhibitor) and nimesulide (a partially selective COX-2 inhibitor) on postoperative adhesion formation in a rat uterine horn model.

Materials and Methods

Forty-eight female Wistar albino rats at the age of 10-12 weeks, weighing 200-220 g were used. They were housed five animals to a cage, with the appropriate diet and water ad libitum. All rats were observed for several days to ascertain health before operations. All procedures were approved by and performed under the guidelines of the Animal Care and Use Committee of Cumhuriyet University.

Each rat was anesthetized with ketamine hydrochloride (40 mg/kg intravenously). Before the surgery, the abdomen was shaved and prepared with a povidone iodine solution. Using a sterile technique, a 3 cm midline vertical incision was made and both uterine horns were exposed, and then a 2 cm segment of each uterine horn was traumatized at ten spots on the antimesenteric surface using unipolar cautery. Care was taken to avoid gross bleeding from injured sites. Handling of other tissues was minimized. Rats were randomly assigned into four groups each consisting of 12 rats. Treatment groups were as follows: (i) control, saline solution only; (ii) celecoxib, (iii) indomethacin and (iv) nimesulide groups. Before the final throw of the abdominal closure, saline solution and drugs were instilled immediately after injury onto uterine horns. Dosages of drugs were determined as 0.5 mg/ml according to our previous study [13]. All treatments were given in 2 ml volumes. The incision

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was closed in a single layer, excluding the peritoneum, with a running 4-0 monofilament delayed absorbable suture. The total operative time was less than 10 min. Rats were allowed to recover for three weeks. Celecoxib was obtained from the Pharmacia Corp, Chesterfield, MO and indomethacin and nimesulide were obtained from Sigma, St Louis, MO, USA.

On postoperative day 21 animals were sacrified by cervical dislocation. The previous abdominal incisions were visually inspected for integrity. A transverse subcostal incision was made above the cephalad extent of the midline laparatomy site, and the abdominal cavity was inspected for the presence of adhesions. The extent and severity of adhesions in the operation site for each uterine horn were evaluated according to Linsky *et al.'s* criteria [15] and recorded by an investigator blinded to the treatment groups. The extent of adhesions was evaluated as follows: 0, no adhesion; 1, 25% of traumatized area; 2, 50% of traumatized area; 3, total involvement. The severity of adhesions was measured as follows: 0, no resistance to separation; 0.5, some resistance (moderate force required); 1, sharp dissection needed.

Data are expressed as mean ± SD. Analysis of the adhesion extent and severity scores was done by one-way ANOVA with a Tukey post-hoc test. Significance was assumed when the *p* value was less than 0.05.

Results

There was no mortality in the study groups. Forty-eight rats recovered without incident after operation and resumed preoperative physical activity and feeding patterns postoperatively. All animals appeared healthy and were evaluated. There were no signs of impaired wound healing or bleeding complications.

The mean ± SD extent score of adhesions in the control, celecoxib, indomethacin, and nimesulide groups was recorded as 1.58 ± 0.99, 0.83 ± 0.93, 0.50 ± 0.52, and 0,33 ± 0,65, respectively. As shown in Figure 1, the extent of adhesion scores were significantly lower in indomethacin and nimesulide groups than those of the control group. Although the celexoxib group appeared to have a lower extent of adhesions than those of the control group, the difference was not significant. No differences were found in the extent of adhesions between the celecoxib, indomethacin and nimesulide groups.

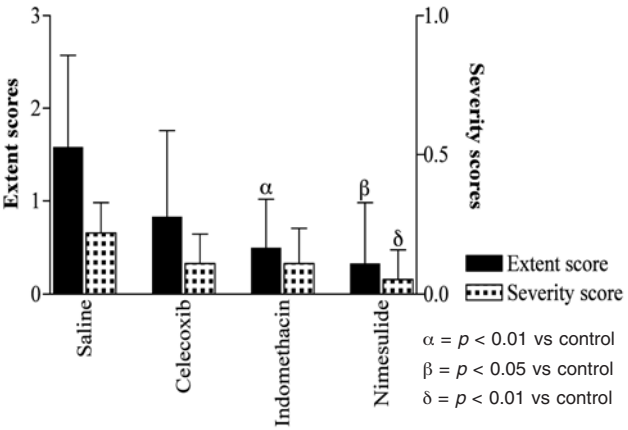

Figure 1. — The extent and severity of adhesions in the control, celecoxib, indomethacin and nimesulide group.

The mean ± SD severity score of adhesions in the study groups was as follows: severity in the control, celecoxib, indomethacin, and nimesulide groups was 0.66 ± 0.32 in control group, 0.33 ± 0.32 in celecoxib group, 0.33 ± 0.38 in the indomethacin group, and 0.16 ± 0.32 in the nimesulide group. The severity scores of adhesions was significantly lower in the nimesulide group than those of the control group. Although the severity score of adhesions was lower in the celecoxib and indomethacin groups than that of the control group, there was no statistically significant difference (Figure 1). No significant differences were found between the celecoxib, indomethacin, and nimesulide groups.

Discussion

Adhesion formation follows the sequence of tissue inflammation, fibrin deposition, and collagen formation. Inflammation occurring as an initial response to peritoneal injury is an integral part of postsurgical repair and leads to extravasation of serum and cellular elements. [16]. The site of peritoneal injury is covered predominantly by polymorphonuclear cells entangled in fibrin strands, which are soon outnumbered by macrophages. When normal fibrinolysis occurs, islands of mesothelial cells proliferate throughout the injury site and completely cover the defect within four to five days. If normal fibrinolysis is inhibited by several factors, macrophages persist and fibroblasts proliferate at this site. Within five days, the fibrin network between adherent structures is replaced by fibrous adhesions of bundles of collagen and fibroblasts. Inflammatory mediators such as prostaglandins (PGF2alfa and PGE2) might play an important role in this process of adhesion formation [17]. It has been shown that anti-inflammatory drugs that suppress prostaglandin synthesis were able to prevent adhesion formation following surgical trauma to peritoneum [10, 11, 13, 18]. The suppressive effect of anti-inflammatory drugs on prostaglandin synthesis is mediated by inhibition of cyclooxygenases. Cyclooxygenases (COXs) catalyze the conversion of arachidonic acid to prostaglandin H2, which serves as the common precursor for the synthesis of prostaglandins, prostacyclins, and thromboxanes. COXs exist in three isoforms (COX-1, COX-2 and COX-3), which exhibit similar catalytic properties but differ in terms of regulation of expression [19, 20]. The existing nonsteroidal antiinflammatory drugs (NSAIDs) differ in their relative specificities for COX-2 and COX-1. NSAIDs used traditionally, including indomethacin, ibuprofen, and flurbiprofen inhibit the activities of COX-1 and COX-2 non-selectively. In contrast, recently developed NSAIDs such as nimesulide, and celexocib are designed to inhibit COX-2 selectively.

There is evidence that both traditional NSAIDs and selective inhibitors of COX-2 may prevent adhesion formation postsurgically in animal models [12-14]. DeLeon and Greene have been reported that non-selective COX inhibitors including indomethacin and ibuprofen were effective in reducing postoperative adhesions [14, 21].

Firstly, we have previously demonstrated that nimesulide, selective COX-2 inhibitor, reduced the formation of postoperative adhesion in rat uterine horn model [13]. Greene *et al*. investigated the effects of both selective (celecoxib, rofecoxib) and non-selective COX-2 inhibitors (aspirin, indomethacine, ibuprofen) on the formation of postsurgical adhesions [14]. They found that celecoxib produced a maximal reduction in adhesion formation compared with rofecoxib and the nonselective COX-2 inhibitors. They did not investigate the effect of nimesulide in their study. In the present study, we compared the effects of nimesulide, indomethacin and celecoxib on adhesion formation. Although treatment with nimesulide and indomethacin did lead to a significant reduction in adhesion formation, celecoxib did not reduce as well as nimesulide and indomethacin. Nimesulide significantly reduced both the extent and severity of adhesions whereas indomethacin significantly reduced only the extent of adhesions. Some possible explanations may be suggested based on different results of these studies. First, there are methodologic differences between studies. In the study of Greene et al., drugs were administrated orally to male mice, while drugs were applied intraperitoneally to female rats in our study. Second, nimesulide might prevent adhesion formation through different mechanisms accompanying COX-2 inhibition. In addition, it has been suggested that nimesulide is different from both non-selective COX and selective COX-2 inhibitors [22].

The data of the present study and above-mentioned studies are consistent with the hypothesis that both COX-1 and COX-2 mediate postoperative adhesion formation. Additionally, these results suggest that other factors except COXs may also be responsible for adhesion formation. During peritoneal repair, the cellular events appear to be coordinated at least in part by cytokines that function as chemoattractants and immunostimulants. Interleukin-6 (IL-6), transforming growth factor-α, epidermal growth factor, transforming growth factor-β and interleukin-1α have been found to be adhesiogenic, whereas antibodies to IL-6, tumor necrosis factor-α (TNF-α), and interleukin-1 (IL-1) reduce postoperative adhesion formation [21, 23-26]. The anti-inflammatory properties of nimesulide may also contribute to its inhibitory effect on TNF-α production [27]. Interleukin-6 is a cytokine that is produced by macrophages as well as by activated fibroblasts. Saba et al. have determined that IL-6 had a major role in peritoneal adhesion formation and using IL-6 neutralizing antibodies preoperatively, did lead to a reduction of adhesion formation without a significant effect on wound healing. [23]. Nimesulide at therapeutic concentrations is a potent inhibitor of IL-6 production [28]. Although celecoxib has an inhibitory effect on IL-6, this effect is not higher than that of nimesulide. In the study of Bianchi *et al*. it was found that the effects of nimesulide on synovial fluid concentrations of interleukin (IL)-6 and IL-8 were more marked than for celecoxib [29]. Nimesulide also decreases histamine release from tissue mast cells and inhibits the production of platelet-activating factor by human basophils [30]. These findings may explain why the efficacy of nimesulide was better than celecoxib on adhesion formation. In conclusion, nimesulide was more effective in the prevention of postsurgical adhesions in the rat uterine horn than the other COX inhibitors. The mechanism of action of nimesulide seems to be multifactorial and not limited to the inhibition of COX-2. Further studies should be performed to substantiate these initial observations in human and animal trials.

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