RESEARCH ARTICLE

THE PROTECTIVE EFFECT OF DICLOFENAC SODIUM AND ENOXAPARIN ON THE FORMATION OF POSTOPERATIVE INTRA-ABDOMINAL ADHESIONS AND FIBROTIC CHANGES IN THE LARGE INTESTINE WALL IN AN EXPERIMENTAL RAT MODEL

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ABSTRACT

Postoperative intra-abdominal adhesions are a frequent complication following abdominal surgeries, potentially resulting in pain, bowel obstruction, and infertility. This experimental study aims to evaluate the protective effects of diclofenac sodium and enoxaparin in reducing intra-abdominal adhesions and fibrotic changes within the large intestine wall. Using a rat model, animals were divided into three groups: a control group, a diclofenac sodium group, and an enoxaparin group. Each group underwent a standardized surgical procedure to induce adhesion formation, with postoperative administration of respective treatments in the diclofenac and enoxaparin groups. On day 14 post-surgery, animals were euthanized, and intra-abdominal adhesions were assessed histologically. Both diclofenac sodium and enoxaparin treatment groups showed a statistically significant reduction in adhesion severity and large intestine wall fibrosis compared to the control group ($p<0.05$). Enoxaparin exhibited a slightly greater efficacy in minimizing fibrotic tissue formation. The findings suggest that diclofenac sodium and enoxaparin have a protective effect against postoperative intra-abdominal adhesions and fibrosis. Enoxaparin, in particular, shows the potential as a therapeutic option for adhesion prevention in clinical settings. Further studies are recommended to optimize dosage and evaluate long-term outcomes.

Keywords: Diclofenac, enoxaparin, fibrosis, postoperative complications, rats

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INTRODUCTION

Postoperative intra-abdominal adhesions are pathological fibrous connections that form between the visceral membranes of organs or between organs and the walls of surrounding body cavities after tissue trauma and ischemia (Arung et al., 2011). Surgical techniques have continuously evolved, yet adhesions remain a common postoperative complication, developing after 50-95% of all surgeries, regardless of the anatomical location of the surgical procedure (Lauder et al., 2010). Adhesions can vary from thin layers of connective tissue to thick vascularized and innervated fibrous bridges (Diamond et al., 2011). They significantly impair the quality of life for patients, increasing the risk of repeat and additional surgical procedures, leading to higher morbidity, mortality, and treatment costs (Sikirica et al., 2011).

In the process of forming postoperative peritoneal adhesions, a crucial role is played by the local balance between fibrin production and fibrinolysis, which determines whether normal peritoneal repair occurs or adhesions form. If fibrin is not degraded within 5-7 days post-peritoneal injury, intra-abdominal adhesions develop (Duron, 2019; Choi et al., 2011; Wei et al., 2018; Hasdemir et al., 2017). This process involves numerous types of cells, cytokines, and biological processes. Local trauma may cause exudation of bleeding into the tissue, triggering an inflammatory response, while the fibrogenesis system plays an important role in adhesion formation (Arung et al., 2016). Simply put, local trauma in the peritoneum can lead to exudation of blood into the local tissue, stimulating an inflammatory response, whereby local vascular permeability increases and fluid rich in fibrinogen is secreted, which may result in the formation of a local membrane structure at the wound site (Bello-Guerrero et al., 2016). Subsequently, the inflammatory response system and the fibrogenesis system play a significant role in the further development of adhesions (Beyene et al., 2015; Fatehi et al., 2021).

There is also significant research addressing the use of non-steroidal anti-inflammatory drugs (NSAIDs) to reduce adhesions. Although the mechanism of this effect is unclear, it is believed that it results from the inhibition of cyclooxygenase enzymes 1 and 2 (COX-1 and COX-2), which reduces the production of prostaglandins and thromboxanes (Gómez and Betancourt, 2018). Based on studies, the application of sodium diclofenac has been shown to be effective in reducing granulation tissue and adhesions (Allahverdi et al., 2014; Bahadir et al., 2017).

Despite the knowledge gained, many unknowns remain about the formation of intra-abdominal adhesions. For instance, while we know that adhesions result from a complex process, the precise mechanisms of interaction among various cells and cytokines in the inflammatory response are still not fully understood. Additionally, further research is needed to understand the optimal conditions for preventing adhesion formation and the impact of various factors, such as the type of surgery and individual patient characteristics.

Moreover, while studies have shown the efficacy of heparin in reducing adhesions, the mechanisms by which heparin acts and the optimal doses for prevention remain unclear (Opitz et al., 2003). In this context, future research should focus on clarifying these unknowns to enhance strategies for the prevention and treatment of postoperative adhesions.

The aim of this study was to investigate the protective effect of sodium diclofenac and enoxaparin on the formation of postoperative intra-abdominal adhesions and fibrotic changes in the large intestine wall using an experimental rat model.

MATERIALS AND METHODS

The study was conducted at the Veterinary Faculty and the Faculty of Medicine at the University of Sarajevo as a randomized, controlled, interventional, prospective, experimental study using a rat model.

Ethics Committee Approval

Approval for conducting the experimental work was granted by the Ethics Committee of the Veterinary Faculty of the University of Sarajevo under the number 07-03-1253-4/23.

Animals

Twenty-one healthy albino rats (Wistar strain) were used in the study, housed in a vivarium under standard conditions (temperature 21°C ± 2°C; humidity 50% ± 10%; 12 hours light/12 hours dark). Food and water were available ad libitum, with a restriction on food and water intake for 12 hours prior to surgery. Each rat was marked for unique identification.

Methods

The animals were randomly assigned to two groups (n=7) as follows:

Group I (Control): Induction of adhesions

Group II: Induction of adhesions with the application of low molecular weight heparin intraperitoneally and sodium diclofenac intramuscularly 7 days post-surgery.

Surgical Procedure for Adhesion Induction

Surgeries were performed under general anesthesia and aseptic conditions. The abdominal cavity was opened via medial laparotomy. The cecum was identified, and the terminal ileum was localized. Point bleeding on the colon was induced with sterile gauze, taking care to avoid injuries. An incision of 3 mm was made on the bowel, which was closed with sutures (polypropylene 5/0).

Therapeutic Procedures

Anesthesia was induced with ketamine hydrochloride (80 mg/kg IM). The dose of low molecular weight heparin (enoxaparin) was 0.5 mg/kg/day, and sodium diclofenac was administered at 2 mg/kg/day, both intramuscularly and intraperitoneally.

Postoperative Monitoring

The rats were monitored for 2 weeks post-surgery, with the appropriate administration of low molecular weight heparin and sodium diclofenac for 7 days, according to the previously defined groups.

Procedure for Biological Material Collection and Euthanasia

The rats were euthanized on the 14th day of the experiment with ketamine hydrochloride. Samples for adhesion analysis were collected via laparotomy.

Quantitative Evaluation of Adhesions

The assessment of the number of adhesions was conducted according to the Nair Modified Scoring Adhesion System (Table 1).

Histopathological Analysis

A 2 mm thick part of the large intestine, located 5 cm distal to the ileocolic junction, was taken for the histopathological analysis. The large intestine samples were fixed in 10% buffered formalin, stained with H&E and Masson trichrome, and qualitative histopathological analysis was performed.

Table 1 Nair Modified Scoring system for adhesion classification

Adhesion grade
0 - No adhesions present
1 - Presence of a single thin adhesion: organ-organ, or organ-abdominal wall
2- Presence of a single thick adhesion: organ-organ or organ-abdominal wall
3 - Presence of two thin or thick adhesions: organ-organ or organ-abdominal wall
4 - Presence of more than two thin or thick adhesions: organ-organ or organ-abdominal wall or intestines forming a conglomerate with adhesions without adhesion to the abdominal wall

All relevant findings were documented/photographed using an iPhone 14 Pro Max (Figure 1).

Figure 1 Grade 2 adhesions according to the Nair Modified Scoring system

Statistical Data Analysis

Collected data were statistically analyzed using R 4.4.2. (R Foundation for Statistical Computing, Vienna, Austria) and Rstudio 2024.04.2 (Posit Software, PBC, Boston, Massachusetts, USA). Numerical variables are represented by arithmetic means and standard deviations, and median, interquartile range (Q1-Q3) and range (min-max). Quantitative variables were tested for normal distribution using Shapiro-Wilk test and evaluated by QQ-plot and histograms.

RESULTS

The median value of the modified Nair scoring system in the control group was 3 (IQR=2-4), while in the group treated with intraperitoneal low molecular weight heparin and intramuscular diclofenac, it was significantly lower at 1 (IQR=1-2), which was statistically significant (p=0.01).

In the group of animals that did not receive treatment, a higher average intensity of adhesions was observed. Two animals (29%) had a Nair score of 2, while three animals (43%) had two adhesions present. Additionally, two animals (29%) had more than two adhesions.

In contrast, among the animals treated with intraperitoneal low molecular weight heparin combined with intramuscular diclofenac, one (14%) animal had no adhesions, four (57%) had one thin adhesion, one (14%) had one thick adhesion, and one (14%) had two adhesions (Table 2).

Table 2 Results of the Modified Nair scale by groups

Variable	Group		p-value
	Control N = 7	LMWH (IP) + DS (IM) N = 7	
Nair Modified Scoring system			**0.0101**
Mean (±SD)	3.00 (±0.82)	1.29 (±0.95)	
Median [Q1–Q3]	3.00 [2.00–4.00]	1.00 [1.00–2.00]	
Range (Min–Max)	2.00–4.00	0.00–3.00	
Modified Nair Scoring Scale (descriptive), n (%)			0.0792
No adhesions present (0)	0 (0%)	1 (14%)	
Presence of one thin adhesion (1)	0 (0%)	4 (57%)	
Presence of one thick adhesion (2)	2 (29%)	1 (14%)	
Presence of two thin or thick adhesions (3)	3 (43%)	1 (14%)	
Presence of more than two adhesions (4)	2 (29%)	0 (0%)	
1Wilcoxon rank-sum test			
2Fisher's exact test			

LMWH, Low Molecular Weight Heparin; DS, Diclofenac Sodium

Results of the Histopathological Analysis

Group 1

The histopathological analysis of the large intestine in animals from this group revealed marked alterations of the intestinal walls (Figures 2A and 2C). The mucosa was infiltrated with mononuclear cells, particularly pronounced beneath the surface epithelium. Mild oedema of the lamina propria was also present in some areas (Figure 2C). The mononuclear infiltrate was extending from the lamina propria into the superficial parts of the submucosa, leading to reduced visibility of the muscularis mucosae. Additionally, fibrous changes in the submucosa were observed in places. The subserosa was markedly thickened due to an increased amount of fibrous connective tissue, and occasionally, due to an increase in cellular connective tissue (Figure 2A and 2E).

Group 2

In this group of animals, the large intestine walls exhibitedmild alterations and a clear stratification (Figures 2B and 2D). The mucosa was also infiltrated with mononuclear cells but without pronounced accumulation beneath the epithelium (Figure 2D). Fibrosis and infiltration in the submucosa were rare, with a clear boundary between the mucosa and submucosa. The subserosa was slightly thickened, dominantly composed of cellular connective tissue (Figure 2B and 2F).

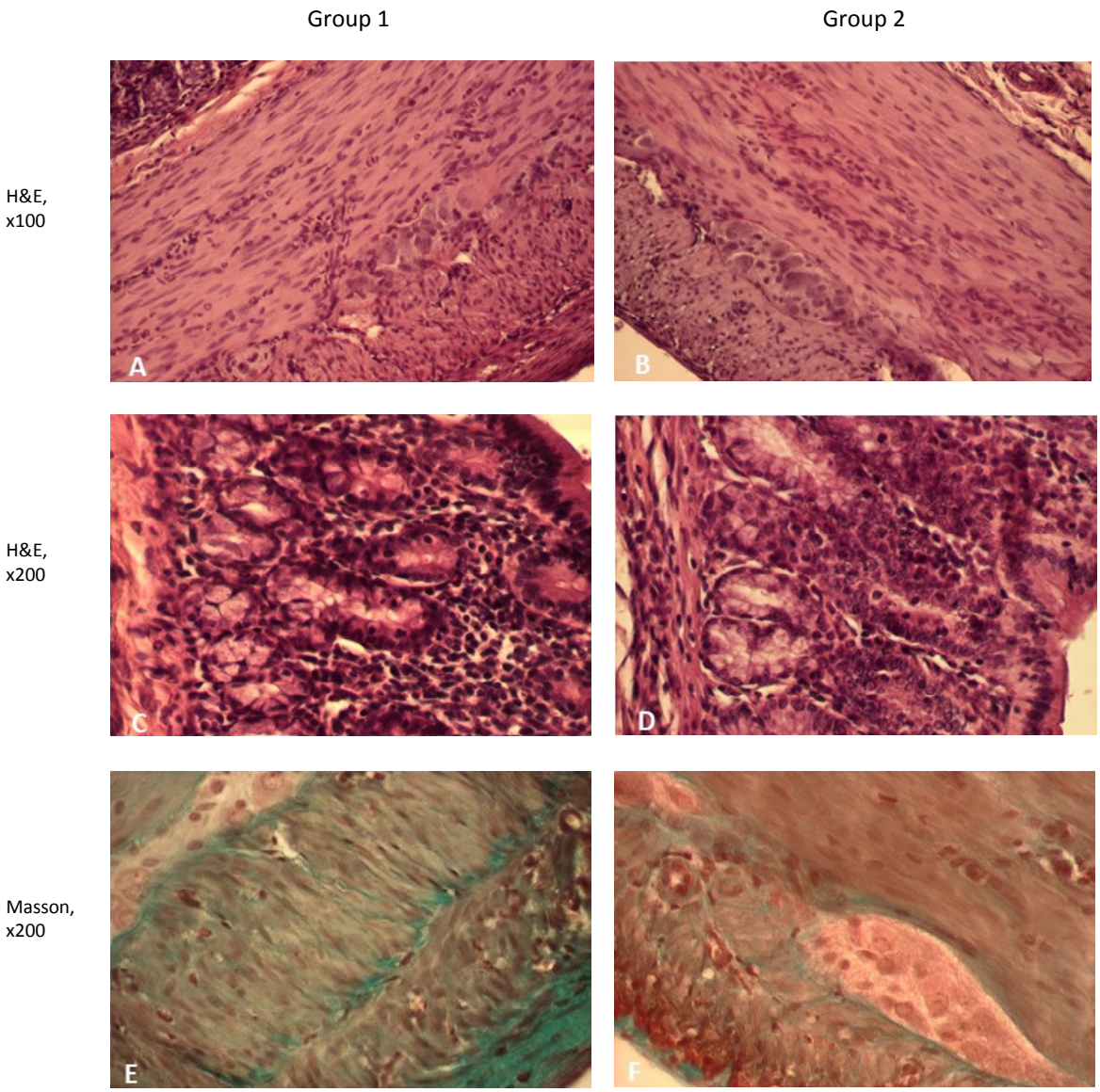

Figure 2 Representative photomicrographs of groups 1 and 2

DISCUSSION AND CONCLUSION

Abdominal postoperative adhesions represent a significant clinical problem, often causing numerous complications, readmissions, and even chronic abdominal pain. These pathological fibrous connections, which form between the membranes of visceral organs or between organs and the walls of surrounding body cavities after surgical procedures, can lead to subocclusive disturbances in patients. Literature reports that up to 80% of patients exhibit symptoms of these disturbances, further highlighting the seriousness of the issue (Arung et al. 2011; Sikirica et al., 2011; Choi et al., 2018; Wei et al., 2018).

Certain chronic conditions, such as hypertension, diabetes mellitus, and metabolic syndrome, have been shown to increase the risk of developing postoperative adhesions. These complications not only worsen the quality of life for patients, but

also significantly increase hospital treatment costs, estimated to be in the billions of dollars annually. Therefore, understanding the pathophysiology of adhesion formation and developing strategies for their prevention is essential (Vipond et al., 1990).

Many strategies, including mechanical barriers, chemicals, and pharmacological approaches, have been investigated to prevent the formation of postoperative adhesions. In our study, we focused on the effectiveness of low molecular weight heparin (enoxaparin) in preventing intra-abdominal adhesions by disrupting the coagulation cascade, thereby reducing the formation of fibrin bridges. Our results indicate a significant reduction in adhesions in the group treated with enoxaparin, which aligns with previous research suggesting that low molecular weight heparins have a prophylactic effect (Kement et al., 2011; Arikan et al. 2005; Kaptanoglu et al. 2008; Rosillo et al., 2011).

In addition to heparin, we investigated the effectiveness of sodium diclofenac, a COX-2 inhibitor, which may limit the inflammatory response of the peritoneum. Our study reveals that intramuscular administration of diclofenac resulted in reduced inflammation and fibrosis in the wall of the large intestine, supporting findings from other researchers who have reported similar effects (Girish and Pradhan, 2012; Lisete et al., 2005; Lardinois et al., 2004; Harvey et al., 2019).

What is particularly significant in this study is the synergistic effect of the combination of enoxaparin and diclofenac. Our results suggest that the application of this combination led to a statistically significant reduction in the incidence of postoperative adhesions and fibrotic changes compared to control groups. These findings support the hypothesis that inhibition of fibrinogen formation, along with limiting the inflammatory response, can effectively reduce adhesion formation.

In conclusion, our research demonstrates that low molecular weight heparin (enoxaparin) and sodium diclofenac significantly reduce the incidence of postoperative adhesions. The synergistic effect of this drug combination further contributes to the reduction of inflammation and fibrosis, suggesting that their application may effectively prevent complications following surgical procedures. These results underscore the need for further research to better understand the mechanisms of action and optimize their clinical use.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Conception: EK, EH, DK; Design: EH; Supervision: AB, DK; Materials: EK, MS; Data Collection and/or Processing: MS, EK; Analysis and/or Interpretation: ED, EH, MS; Literature Search: EK, EH, DK; Writing – Original Draft: EK; Critical Review: EH

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ZAŠTITNI EFEKAT DIKLOFENAK NATRIJUMA I ENOKSAPARINA NA FORMIRANJE POSTOPERATIVNIH INTRAABDOMINALNIH ADHEZIJA I FIBROZNIH PROMJENA U ZIDU DEBELOG CRIJEVA NA EKSPERIMENTALNOM MODELU STAHORA

SAŽETAK

Postoperativne intraabdominalne adhezije predstavljaju čestu komplikaciju abdominalnih operacija i mogu izazvati bol, opstrukciju crijeva i neplodnost. Cilj našeg eksperimentalnog istraživanja je evaluacija zaštitnih efekata diklofenak natrijuma i enoksaparina u smanjenju intraabdominalnih adhezija i fibroznih promjena koje nastaju u zidu debelog crijeva. Korištenjem modela štakora, životinje su podijeljene u tri grupe: kontrolnu, diklofenak natrijum grupu i enoksaparin grupu. Svaka grupa je podvrgnuta standardiziranoj operativnoj proceduri sa ciljem indukcije adhezija uz postoperativnu aplikaciju odgovarajuće terapije u diklofenak i enoksaparin grupi. Životinje su eutanazirane 14. dan postoperativno, a intraabdominalne adhezije su histološki analizirane. Diklofenak natrijum grupa i enoksaparin grupa su pokazale statistički signifikantno smanjenje adhezija i fibroze intestinalnog zida u usporedbi sa kontrolnom grupom. Enoksaparin je pokazao nešto bolji učinak u minimiziranju nastanka fibroznog tkiva. Rezultati pokazuju da i diklofenak natrijum i enoksaparin imaju zaštitno djelovanje kod nastanka intraabdominalnih adhezija i fibroze. Naročito enoksaparin pokazuje terapijski potencijal u prevenciji adhezija u kliničkim uvjetima. Preporučuju se dodatna istraživanja u svrhu optimiziranja doze i procjene dugoročnih rezultata.

Ključne riječi: Diklofenak, enoksaparin, fibroza, postoperativne komplikacije, stahori