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Utility of the Enoxaparin and Diclofenac in Prevention of Postoperative Abdominal Adhesions. Experimental Study in Murine Model

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Abstract

Postoperative abdominal adhesions constitute a condition that occurs in more than 90% of patients undergoing abdominal surgery, and cause major morbidity as intestinal obstruction, secondary infertility and chronic abdominal pain. The purpose of this study was to assess the usefulness of enoxaparin as inhibitor of the coagulation cascade and diclofenac as a non-steroidal anti-inflammatory drug to reduce inflammation, and thus the formation of abdominal adhesions. We used 90 Sprague Dawley rats divided into nine treatment groups of 10 animals each. Surgical adhesions were induced using a controlled brushing of the caecum with sterile cotton gauze and followed by a 3 mm long incision in ileum, repairing with suture. According with the experimental group, Enoxaparine and diclofenac were applied locally or systemic alone or in combination, while all experimental groups were compared against a suitable control group through a midline incision and terminal euthanasia. By far, the greater degree of formation of abdominal adhesions occurs in the non-treated control group I ($p<0.05$). In contrast, groups III (enoxaparin SC), IV (diclofenac IP) and VIII (diclofenac IP and enoxaparin SC) induced a lower degree of postoperative abdominal adhesions ($p<0.01$), and the combination of enoxaparin SC and diclofenac (IP) showed be better that when administered individually ($p<0.05$). Results demonstrated that the combined subcutaneous enoxaparin and intraperitoneal diclofenac reduced the formation of postoperative abdominal adhesions significantly.

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Introduction

Abdominal adhesions are vascularized and innervated connective tissue bridges, which form between the peritoneum, bowel and abdominal wall [1]. The intra-abdominal surgery adhesions can cause surgical re intervention, chronic abdominal pain, intestinal obstruction and infertility in women [2-4] has been described that up to 93% of patients who have had one or more previous surgery developed abdominal adhesions [5] 16 and 15% to 20% of female infertility due to post-surgical adhesions [4]. Postoperative Abdominal Adhesions (AAP) result from tissue damage either by incisions, cautery, suture or foreign objects that assaulted the parietal and visceral peritoneum, and this reacts forming scar tissue from the fibrin [6,7]. Fibrin is the end result of the coagulation cascade and it formed deposits, while there is a proliferation of fibroblasts with the objective of forming an Extra Cellular Matrix (ECM) and collagen, which favors the formation of adhesions. COX-2 is an enzyme that regulates the processes of angiogenesis on the development of post-surgical adhesions and inflammatory. The presence of tissue hypoxia and/or adhesion fibroblasts, COX-2 increases its expression [8]. We can summarize that the postoperative abdominal adhesions, are due to stimulation of the coagulation cascade and the imbalance between the fibrinogenesis and fibrinolysis in favor of the former associated with tissue hypoxia secondary to damage mesothelial, to their own response to the increase in the population of adhesion fibroblasts which inhibit the degradation of the extracellular matrix and inflammation. Strategies in search of decrease (AAP) postoperative peritoneal adhesion formation, based on some specific points in the adhesions path physiological origin have been numerous [3,9]. NSAIDs not drugs (NSAIDs) work by inhibiting the COX-1 and COX-2 avoiding the synthesis of prostaglandins and thromboxanes [2]. COX-2 inhibitors NSAIDs have been pharmacological agents that have shown to prevent post-surgical adhesions in various animal models, however, does not exist as such, significant evidence of the specific use of diclofenac(COX-1 and COX-2 inhibitor), medication use and low-cost [10-12]. Heparin is a sulfated glycosaminoglycan acid that stops the formation of fibrin. In a study with rats, was that the ministration of heparin at different doses is effective and safe (62.5 UI, 125 IU and

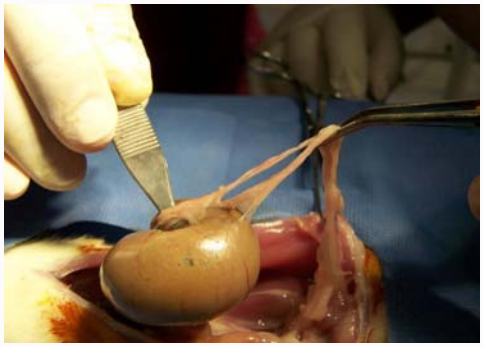

Figure 1a: Grade 1 adhesion between viscera and viscera.

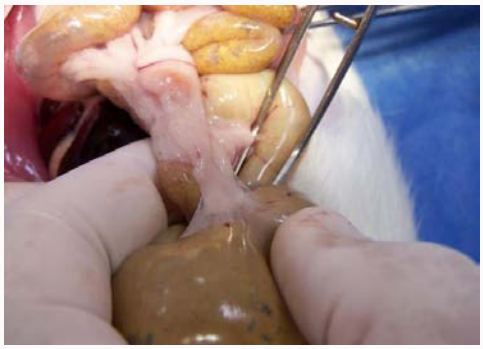

Figure 1b: Grade 4 more than two adhesion bands and intestine forming a lump.

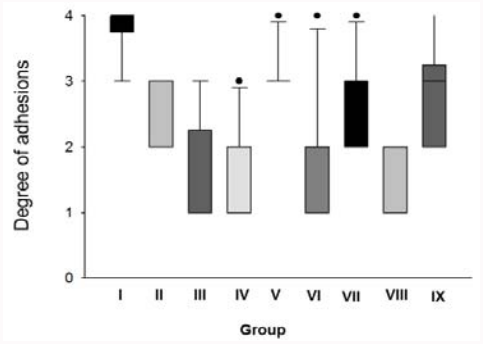

Figure 2: Postoperative Abdominal Adhesions (PAA) with different drug therapies.

250 IU) in combination with carboxy methylcellulose, having better results with the highest dose of heparin [13]. The optimal dose for even braking adhesion formation is unknown, so it is necessary to identify the effective dose to prevent the formation of adhesions without increasing the risk of bleeding. In animal models, has been that Heparin Low Molecular Weight (LMWH) decreases the formation of adhesions, given both intraperitoneal and subcutaneous, but is not managed yet and not recommended dose [14,15]. The present work intends to find a mechanism that is effective, simple to implement and low cost in the prevention of the formation of postoperative abdominal adhesions through the reduction of inflammatory responses and coagulation with Diclofenac and the enoxaparin.

Materials and Methods

All experimental manipulations were performed according to the Mexican Guidelines set forth in the NOM-062-ZOO-1999 and were approve by our local IACUC and the Ethics and Research Commission of the Facultad Mexicana de Medicina, Universidad La Salle, A.C, and Mexico.

Animals

Ninety male Sprague Dawley rats, weighing 400-450 g. were used for this study. All animals were housed singly in conventional conditions under an environmentally controlled animal facility (temperature 21°C ± 2; relative humidity 55% ± 10%; and 12:12 hr dark/light cycle), and were fed ad libitum with Lab Diet 5008® and fresh water. Fasting for 12 hours prior surgery was established and all animals were sedated before surgical preparation using 3 mg/kg of Azaperone (Sural®) Preventative analgesia was implemented by injecting Buprenorphine (0.05 mg/kg IM; Brospina®, Pisa) IM, and anesthesia was induced with Tiletamine-zolazepam (3 mg/kg IP; Zoletil®). All surgical procedures were carried out in a surgical suite under sterile conditions using prior abdominal disinfection with iodopovidone 10%.

Surgical model for the induction of adherences

All animals were selected randomly to establish nine groups treated as indicated in (Table 1), with 10 animals per group. A mid-line incision was done over the muscular and peritoneal layer to expose the abdominal cavity; once the caecum was located it was subjected to repeated moderate brushing using a sterile cotton gauze without injuring the adjacent intestinal loops, until punctuate bleeding appeared (10-12 ± 1 strokes). The abrasion was always executed by the same surgeon, followed by a 3 mm long incision located 5 cm distant to the ileocolic junction and closed with interrupted sutures 5/0 Polypropylene (Atramat®), placed equidistantly over the defect. The midline incision was closed in all groups using a continuous suture layer with 3/0 Polypropylene (Atramat®), while the skin was closed with 3/0 Atramat® Nylon.

Therapeutic procedure

The enoxaparin dose was determined based on the following criterion: if the average prophylactic dose for a 70-kg human is 20 mg to 40 mg, i.e. 0.28 to 0.56 mg/kg; we opted to use the maximum level to facilitate dosing, defined as 0.5 mg/kg/day. Consequently, diclofenac was set at a dose of 2 mg/kg /day IM.

Clinical assessment of adherences

In order to assess adherence formation all rats were euthanized with CO_2 and subjected to laparotomy, from the xiphoid process to the pubis. All findings were photographically documented using a Kodak Easy Share digital camera (10X). Blind assessment of the adherences was executed by two surgeons 14 days after surgery using the scale of Nair, modified by Guzmán-Valdivia (Figure 1 and Table 2).

Statistical analysis

Statistical analyses were performed using the software package Graph Pad Instat 3.1. The data is grouped as mean ± deviation standard. Means were compared by ANOVA. The non-parametric Kruskal-Wallis test was employed to define differences among the groups, and depending on the results the multiple comparison test of Dunn was applied to determine if significant differences existed among the treated groups. For the comparison among two independent groups the Mann-Whitney U Test was used. Results are expressed with a confidence interval of 95%. Probability values below 0.05 were considered statistically significant. In order to determine

Table 1: Arrangement and Treatment of Nine Experimental Groups. IP: Intraperitoneal, SC: Subcutaneous.

Experimental Group	Procedure
I (control)	Induction of adhesions
II	Induction of adhesions + Enoxaparine IP
III	Induction of adhesions + Enoxaparine SC OD/ 7 days
IV	Induction of adhesions + Diclofenac IP
V	Induction of adhesions + Diclofenac IM OD/7 days
VI	Induction of adhesions + Enoxaparine IP y Diclofenac IP
VII	Induction of adhesions + Enoxaparin IP + Diclofenac IM OD/7 days
VIII	Induction of adhesions + Diclofenac IP + Enoxaparine SC OD/ 7 days
IX	Induction of adhesions + Enoxaparine SC OD/7 days + Diclofenac IM OD/7 days

Table 2: Nair Modified Scoring Adhesion System.

Degree of Adhesions
0 = Not present
1= Single filmy thickness band: viscera-viscera or viscera-abdominal wall
2 = Single dense thickness band: viscera-viscera or viscera-abdominal wall
3 = Double, filmy or dense thickness band: viscera-viscera or viscera-abdominal wall
4 = More than two adhesion bands: viscera-viscera or viscera-abdominal Wall or intestine
Forming a lump without adhering to abdominal wall.

Nair SK, Bhat IK, Aurora AL. Role of proteolytic enzyme in the prevention of postoperative intraperitoneal adhesions. Arch Surg. 1974 108(6):849-53

the inter observer concordance used the Kappa Coefficient (κ).

Results

A total of 90 rats were sacrificed on postoperative day 14 to evaluate macroscopically adhesion formation. They were compared the experimental groups with the control group and between groups with treatment. During the investigation, died 5 animal experiments, which were replaced, all they underwent autopsy. The causes of death were: two cases with anesthetic complications, and evisceration, intra-abdominal and abdominal sepsis, hemorrhage, a case of each. The results reveal that the control group significantly formed greater degree of adhesions in comparison to groups with treatment (Table 3). The comparison between the groups with treatment (Figure 2) shows that groups III (SC enoxaparin), Group IV (IP diclofenac), Group VI (IP enoxaparin and IP diclofenac) and the Group VIII (SC enoxaparin and IP diclofenac) demonstrated a lower degree of postoperative abdominal adhesions. (Table 4) summarizes the comparison among the averages of each group exhibiting less adhesion formation, hence observing:

- Subcutaneous administration of enoxaparin was superior that the intraperitoneal route.
- Intraperitoneal administration of diclofenac was superior to the intramuscular route.
- There was no difference between subcutaneous applications of enoxaparin versus the intraperitoneal diclofenac route.
- The expected synergistic action of the combination of subcutaneous enoxaparin and intraperitoneal diclofenac failed; therefore, it was not superior to the independent administration of each drug tested.
- The kappa coefficient (k) was 0.81 (very good), which means that the evaluation of adhesions between observers is the same.

Discussion

Abdominal postoperative adhesions are a significant clinical problem; they can be the cause of multiple surgeries and escalate with repetitive formation constituting a greater risk of frequent hospital readmissions for surgical treatment, including intestinal resections, while standing as an important cause of infertility in woman and chronic abdominal pain. Such events have a significant economic impact for the patient and the institutional health care systems [1-3]. According to the pathophysiology of the PPA formation, factors involved include the activation of the inflammation process of the coagulation cascade to form fibrin bridges, under a poor process of fibrinolysis, amid a tissular hypoxic environment concomitant to prior damage to the mesothelium or peritoneum. In short, it can then be asserted that these events concomitantly led to a diminished activity of the activator of plasminogen, to cytological and biochemical alterations of the extracellular matrix being formed, an increase of fibrogenesis and angiogenesis and a reduction of apoptosis [16,17]. Numerous strategies have been attempted to prevent the formation of PPA, including mechanical barriers, chemicals and various pharmacologic drugs with no definite results for humans. In this work, we attempted to validate the efficacy of a LMWH (enoxaparin) to prevent the formation of PPA, by interfering with the coagulation cascade to form fibrin bridges, hence differentiating its efficacy among its systemic and local administration. In addition, we aimed to determine the effectiveness of a commonly used NSAID (diclofenac), a COX-2 inhibitor, and its ability to limit the peritoneal inflammatory response and differentiate it´s effects either when administered systemically or locally. Finally, we wanted to find out if the suggested synergistic combination of both drugs had any beneficial effect to limit the formation of PPA. On the other hand, as reported for Opitz [18], Watson [19] and Jansen [20] in their studies for the prevention of PPA with heparin, they resulted most controversial given that different results were observed, when in their experiences failed to show human efficacy; in contrast, the research of Kement [21] and Sharifi [16] found a decrease in the formation of postoperative adhesions associated to the use of a physical barrier of carboxymethyl cellulose and acid hyaluronic. Under the concept that by diminishing the formation of fibrin or limiting its activation during the coagulation cascade, is then that various experiments

Table 3: Results of Adhesion Scores of Each Group and Statistical Significance to Control Group I.

Fibrous Adhesion Scores			
Group	**Minimum- Maximum**	**Mean ± SD**	**P***
I	3 - 4	3.9 ± 0.3162	
II	2 - 3	2.7 ± 0.4830	P<0.01
III	1 - 3	1.6 ± 0.8433	P<0.01
IV	1 - 3	1.7 ± 0.6749	P<0.01
V	3 - 4	3.1 ± 0.3162	P<0.05
VI	1 - 4	1.8 ± 0.9189	P<0.01
VII	2 - 4	2.4 ± 0.6992	P<0.01
VIII	1 - 2	1.6 ± 0.5164	P<0.01
IX	4-Feb	2.9 ± 0.7379	P<0.01

*Statistical analysis comparing Mean adhesion scores from treated animals to control animals, noting that the control group has the greatest degree of adhesion formation.

Table 4: Comparison of two Independent Samples Using the U Mann-Whitney Non-parametric Test.

Group Comparison	p	Interpretation According to Route of Administration
II *vs*. III	P=0.001*	SC Enoxaparine > tan IP
IV *vs*. V	P=0.006*	IP Diclofenac > tan IM
III *vs*. IV	P=0.637 ns	SC Enoxaparine = tan IM
VIII *vs*. III	P=0.078*	SC Enoxaparine + IIP Diclofenac > tan SC Enoxaparine
VIII *vs*. IV	P=0.084*	SC Enoxaparine + IP Diclofenac > IP Diclofenac

have been devised and executed, using heparins of low molecular weight, predominantly Nadroparin by the intraperitoneal route [22] and enoxaparin, using a twofold dose with respect of the estimated prophylactic dose, as was reported by Arikan, et al. [23] who employed 1 mg/kg, injected subcutaneously for 7 days.

In our experiment, we used enoxaparin at a dose of 0.5 mg/kg for being assessed as having prophylactic effects and being less risky to induce internal bleeding. In this case, the drug was applied both, intraperitoneal (locally) and subcutaneously (systemic) for seven days, finding its best prophylactic effect against the formation of PAA when injected systemically (subcutaneously). In the search of new treatments to decrease adhesion formation by inhibiting the inflammatory process, through the use of Non Steroidal Anti-Inflammatory Drugs (NSAIDs), specifically COX-2 inhibitors, as seen in the cases of Rofecoxib [24] administered by the oral route and Celecoxib compared by Greene AK, et al. [25] against other anti-inflammatory drugs such as aspirin, naproxen, ibuprofen, indomethacin and Rofecoxib (oral), he concluded that Celecoxib resulted the best anti-inflammatory drug able to reduce the formation of PAA. In our study we wanted to assess the efficacy of diclofenac, a commonly used anti-inflammatory COX-1 and COX-2 inhibitor, easy to acquire and inexpensive, which according to our knowledge it has never been studied before in any experimental animal model. In this case, diclofenac was applied using the therapeutic dose according to animal-weight, and further injected locally and systemically, finding its best effect when used by the intraperitoneal route and administered as a single dose per day. Although there are studies combining physical barriers primarily based on sodium hyaluronate and methylcellulose with LMWH [26], there is not prior experience reporting the combined effects of NSAIDs and LMWH, expecting that the synergistic action of both drugs would led to a decrease in the formation of PAA by inhibiting two different pathways: fibrin formation, when the coagulation cascade is activated, and limiting the active factors of inflammation. In our experiment, we found that the combination of systemic Enoxaparin with diclofenac intraperitoneal have a synergist action, demonstrating lower incidence of postoperative adhesions that applying the drugs alone. With these results we are performing the experiment in a porcine model using the same drugs on routes of greater efficiency, trying to assess the results in an animal model with more complex and more like the human inflammatory response and measure the response of inflammation and fibrosis by microscopic evaluation and biochemical markers.

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