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Cohort Study

Different doses of enoxaparin in the prevention of postoperative abdominal adhesions. Experimental study

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ABSTRACT

Background: Postoperative abdominal adhesions (PAAs) are present in more than 90% of patients undergoing abdominal surgery. They are a cause of chronic pain, hospitalizations, multiple surgeries, and infertility in women of reproductive age. The participation of three processes have been recognized: coagulation, fibrinolysis, and inflammation. The usefulness of subcutaneous enoxaparin in their prevention has been established. The objective is to establish the safest and most efficient dose for PAA prevention by testing five different doses of subcutaneous enoxaparin (0.25, 0.5, 1, 1.5, and 2 mg/kg/day) given in one dose/day for seven days.
Material and methods: Fifty Sprague-Dawley rats were studied, 10 in each group. Adhesions were induced through controlled rubbing of the cecum and suturing of an incision in the terminal ileum. Two independent observers recorded the degree of adhesion formation at 14 days and histologically studied the adhesions.
Statistical analysis: ANOVA compared group averages. The nonparametric Kruskal-Wallis test was used to identify group differences.
Results: The 0.5 mg/kg/day group had greater formation of adhesions ($p < 0.001$). There was no significant difference between the 1.5 and 2 mg/kg/day groups, though the latter group had an incidence of 27.2% of bleeding in the abdominal cavity. The degree of adhesions in the histological sections coincided with the macroscopic findings. The interobserver agreement was kappa = 0.88 (very good).
Conclusion: The safe and effective dose of subcutaneous enoxaparin to prevent PAA formation was 0.5–1.5 mg/kg/day for seven days.

1. Introduction

Postoperative abdominal adhesions (PAAs) are the result of the repair process of the parietal and visceral peritoneum that cuts have damaged, burns, the presence of a foreign body, or manipulation, and they form bridges of vascularized and innervated connective tissue between the abdominal viscera or between viscera and wall [1,2]. In clinical practice, PAAs are mainly related to the organs treated, the type and time of surgical intervention, the suture material used, the degree of contamination of the peritoneal cavity, the amount of bleeding, and the use of drains; its general incidence is up to 93% of patients undergoing surgical intervention in the abdominal cavity [3,4].

Among the recognized clinical repercussions of PAAs are surgical reinterventions for intestinal obstruction and its complications, chronic abdominal pain that requires treatment, and even hospitalization in some cases, and they cause 15–20% of infertility cases in women [5]. It is a problem that the surgeon has to face and surely wants to avoid.

The processes of PAA formation are well studied, and the evidence shows that PAA formation is mainly related to the activation of three interrelated biological processes: 1) the coagulation cascade, 2) fibrinolysis, and 3) the inflammation process, initiated by the lesion of the mesothelial cells of the peritoneum. These lead to the activation of fibrin formation by adhesion fibroblasts and the decrease in fibrinolysis in the fibrin-rich extracellular matrix, as well as to a hypoxic environment, favoring the maturation of fibrin and collagen through inflammatory cell activities, the activation and inactivation of signaling molecules, vasoactive substances, interleukins, and growth factors, among others [6–8].

The methods to eliminate or reduce the formation of PAA involve intervention in one of the three processes described above or the

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E-mail addresses: gilberto.guzmanvaldivia@lasalle.mx (G. Guzmán-Valdivia Gómez), eduardo.tena@lasalle.mx (E. Tena-Betancourt), angulo.t.monica@gmail.com (M. Angulo Trejo).

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implantation of physical barriers with substances that limit any of these processes. Enoxaparin is a low molecular weight derivative of heparin; it acts as an anticoagulant, restricting the coagulation cascade process.

In abdominal surgery, a prophylactic dose of low-molecular-weight heparin is administered to patients at risk of venous thromboembolic disease, so the initiation of this in the preoperative period may be beneficial in reducing the formation of PAA without the risk of bleeding. as seen in these patients [9].

Recently, our group reported our experience in preventing PAA formation with the administration of subcutaneous enoxaparin at a prophylactic dose for seven days in a murine model. We reproduced the results in a porcine model with a porcine model good results [10,11]. This study intended to compare different doses of enoxaparin to find the most effective.

2. Material and methods

The study's hypothesis is to verify that enoxaparin reduces the formation of postoperative abdominal adhesions and the main objective is to establish the efficient dose in this reduction without risk of bleeding.

The Research Committee approved the research project and Subcommittee on Research and Care of Laboratory Animals of the Mexican School of Medicine of Universidad La Salle, Mexico, under the Official Mexican Standard 062-ZOO-1999 and the NIH (USA) Guide for the Care and Use of Experimental Animals. Registration number: CEI-2020-3.

The work has been reported in accordance with the ARRIVE guidelines (Animals in Research: Reporting In Vivo Experiments) [12].

3. Animals

A total of 54 female Sprague-Dawley rats weighing 400–450 gr were used. All animals were housed individually under conventional conditions in an environmentally controlled animal facility (temperature 21 °C ± 2; relative humidity 55% ± 10%; and 12:12 h dark/light cycle) and were fed ad libitum with the Lab Diet 5008® and freshwater. A fast of 12 h was enforced before surgery, and all animals were sedated before surgical preparation with 3 mg/kg azaperone (Sural®).

Anesthesia was induced with tiletamine-zolazepam (3 mg/kg IP; Zoletil®, Virbac Laboratories, Carros, France). All surgical procedures were performed in an operating room under sterile conditions by prior abdominal disinfection with 10% iodopovidone.

3.1. Surgical model for the induction of abdominal adhesions

The animals were randomly selected to establish five groups of 10. The group's assignment in the different stages of the experiment (during the assignment, the realization of the investigation, the evaluation of results, and the data analysis) was in charge of ET, head of the Animal Farm. The groups were treated with different doses of postoperative subcutaneous heparin as indicated in Table 1; comparison does not require a control group. The approach to the abdominal cavity was through an average incision 3 cm in length. The cecum was located through traction of the abdominal wall and was grabbed with non-toothed dissecting forceps for exposure. The cecum was subjected to repeated brushing with sterile gauze until the appearance of petechiae on the wall (10–12 ± repetitions), always performed by the same surgeon (ETB), followed by an incision in the terminal ileum 3 cm from the ileocolic junction that was closed with a continuous suture with 5/0 polypropylene (Atramat®). The aponeurotic muscle wall was closed with a continuous suture with Vicryl 3/0 (Atramat®, PGA), while the skin was closed with Nylon Atramat® 3/0 with separate stitches.

Table 1
Distribution and treatment of the five experimental groups.

Experimental group	Procedure + dose of enoxaparin
I	Adhesion induction +0.25 mg/kg/day for 7 days
II	Adhesion induction +0.5 mg/kg/day for 7 days
III	Adhesion induction +1 mg/kg/day for 7 days
IV	Adhesion induction +1.5 mg/kg/day for 7 days
V	Adhesion induction +2 mg/kg/day for 7 days

3.2. Treatment

The dose of enoxaparin was determined based on the following criterion: If the average prophylactic dose for a human being of 70 kg was 20–40 mg, that is, from 0.28 to 0.56 mg/kg, we opted to use 0.5 mg/kg/day as an average dose. The corresponding doses of 0.25, 0.5, 1, 1.5, and 2 mg/kg/day subcutaneously for seven days were calculated, starting in the immediate postoperative period.

3.3. Animal care, monitoring, and clinical evaluation of adhesions

After surgery, the laboratory animals returned to the same environmental conditions, humidity, and feeding, described in the Animals section.

All rats were sacrificed with CO2 to evaluate adhesion formation and underwent laparotomy on day 14 postoperatively, with an incision from the xiphoid to the pubis. All findings were photographically documented with a Kodak Easy Share (10X) digital camera. The blind assessment of adhesions was performed by two independent surgeons using the Nair scale modified by Guzmán-Valdiviaxx (Table 2) and by a pathologist according to the Yilmazxx histological classification (Table 3).

3.4. Statistical analysis

The statistical analysis was performed using the GraphPad Instat 3.1 package (GraphPad, San Diego, CA, USA). The data were grouped into mean ± standard deviation. ANOVA compared the averages. The nonparametric Kruskal-Wallis test was used to define group differences. The results are expressed with a confidence interval of 95%, and P values less than 0.05 were considered statistically significant. To determine the agreement between the independent evaluating surgeons, the kappa coefficient was used.

4. Results

A total of 54 rats were included in the experiment. Three rats died postoperatively, one from group I on day one and two from group V on days 3 and 4. Necropsy was performed on all three, and no alteration was found in the rat's abdominal cavity from group 1, so its death was deemed due to anesthetic toxicity. For the rats from group V, the finding was bleeding in the abdominal cavity in both cases. The three animals were replaced to continue the study with ten rats in each of the five groups, of which one rat in group V died due to bleeding in the abdominal cavity on day 3, which was also replaced. Three deaths from hemorrhage in 13 animals correspond to 23%. There were no dehiscences or infectious processes.

The comparison between the groups is shown in Fig. 1. The results

Table 2
Modified Nair adhesion evaluation system.

Degree of adhesions
0 = Not present
1 = Single thin and transparent band: viscera–viscera or viscera–abdominal wall
2 = Single dense band: viscera–viscera or viscera–abdominal wall
3 = Two bands, thin or thick: viscera–viscera or viscera–abdominal wall
4 = More than two bands: viscera–viscera or viscera–abdominal wall or the intestine forming a lump without adhering to the abdominal wall.

Source: Nair SK, Bhat IK, Aurora AL. Role of proteolytic enzyme in the prevention of postoperative intraperitoneal adhesions. Arch Surg. 1974; 108 (6):849–853.

Table 3
Yilmaz histological classification.

Histological grade of fibrosis
0 = No fibrosis
1 = Thin clusters of fibrosis
2 = Wide areas of fibrosis with reduced vascularization
3 = Fibrotic areas formed by thick collagen bands.

Source: Yilmaz HG et al. Micronized purified flavonoid fraction may prevent formation of intraperitoneal adhesions in rats. Fertl Steril. 2005; 84(suppl 2):1083–1088.

show that the dose of 0.25 mg/kg/day was insufficient to prevent PAA formation and that there was no significant difference between the doses of 1–2 mg/kg/day. However, postoperative bleeding occurred in 27.27% of group V. Fig. 2 shows examples of different degrees of adhesion. Table 4 shows the microscopic findings, which coincided with what was observed macroscopically.

The kappa coefficient of interobserver agreement was 0.88 (very good), showing the validity of the observation according to the macroscopic classification.

5. Discussion

PAAs continue to be a cause of long-term morbidity, even requiring hospitalizations for the management of abdominal pain and intestinal obstruction symptoms that might need surgical treatment resulting in the formation of new adhesions, not to mention the importance of PAA prevention for the reduction of infertility in women of reproductive age, up to 20% of which PAA causes cases [3,5]. Prevention of the formation of PAA will help contain costs for not only the patient but also the clinic and/or health care institution.

PAAs begin to form when the parietal and visceral peritoneum are damaged by incision, by intense or long-lasting manipulation, or by burns, as well as by the presence of blood and foreign bodies such as sutures and drains. Surgery on pelvic organs such as the uterus, adnexa, and the colon or rectum is more likely to generate adhesions [13]. PAAs are also more likely in obese, diabetic, hypertensive patients and patients with lipid metabolism disorders [14]. During the repair of the peritoneum by its mesothelial cells, the activation of vasoactive substances comes into play, as do cytokines that promote the processes of inflammation and coagulation, the activation of fibrinogenesis, the limitation of fibrinolysis, and the anti-inflammatory process in a hypoxic environment where adhesion fibroblasts are activated, causing excessive deposition of fibrin and mature collagen as well as supporting connective tissue [6–8].

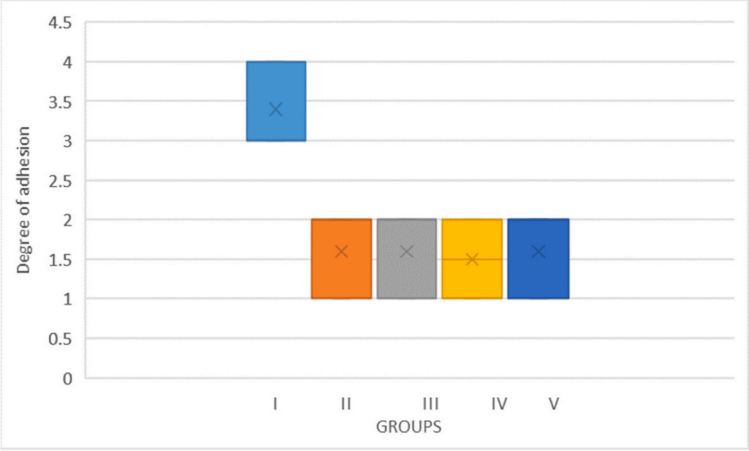
Strategies have been proposed to limit damage to the peritoneum. These include refined and minimally invasive surgical techniques, with less use of electrocautery, limited use of drains, and the use of inert sutures, as well as medications or agents that reduce the inflammatory response, act on the coagulation cascade, or favor fibrinolysis or even reduce the contact of the organs with the damaged peritoneum through physicochemical barriers [2,15,16]. In the various published studies, mainly in the experimental field and in animal models, the agents that act in adhesion formation have shown their usefulness but have not managed to have an objective impact on humans. Of the strategies most studied in humans, mainly in pelvic surgery, there are physicochemical barriers with diverse and inconclusive results due to their frequent and/or universal use.

Among the agents that act to limit the coagulation cascade and aim to decrease fibrin's formation as a final product is heparin. It has had discordant effects because there is no consensus on the dose, administration route, and administration time [17–19]. It also brings the possibility of bleeding, which Dr. Almamar reduced by administering it in the peritoneal cavity in the form of an aerosol [20]. The use of low-molecular-weight heparin has also been used with good results. Arikan gave subcutaneous enoxaparin at a 1 mg/kg/day dose for seven days with encouraging and safe results [21].

Our research group has focused on studying the prevention of PAA with low-molecular-weight heparin (enoxaparin) in a murine model [10] and being able to scale it to a porcine model with good results in the prevention of adhesions at doses of 0.5 mg/kg/day for seven days [11]. The reason for using only females in this model was that these have the largest population in our animal facility service.

The objective of the present experiment was to find the most efficient and safe dose. Our findings show that 0.25 mg/kg/day was insufficient, 0.5 to 1.5 is safe and efficient, and 2 mg/kg/day presented a risk of bleeding of up to 27%. These data agree with the work of Arikan [19]: A dose of 1 mg/kg/day for seven days is safe and decreases the formation of PAA. The dose of 2 mg/kg/day for seven days resulted in a 23% death rate from abdominal bleeding.

The analysis of these results could suggest the context that there are patients who have undergone surgery and have received a previous low molecular weight heparin as prophylaxis for venous thromboembolic disease, and that adhesions are observed in subsequent surgeries. However, we believe it is important that these observations should be supported by methodological observations carried out by monitoring patients with specific information on their history, comorbidities, and



The dose of 0.25 mg / kg / day is inefficient in preventing the formation of AAP, while the doses of 0.5 to 2 mg / kg decrease its formation.

Fig. 1. Postoperative abdominal adhesions (PAAs) under different doses of enoxaparin (see data in Table 4).

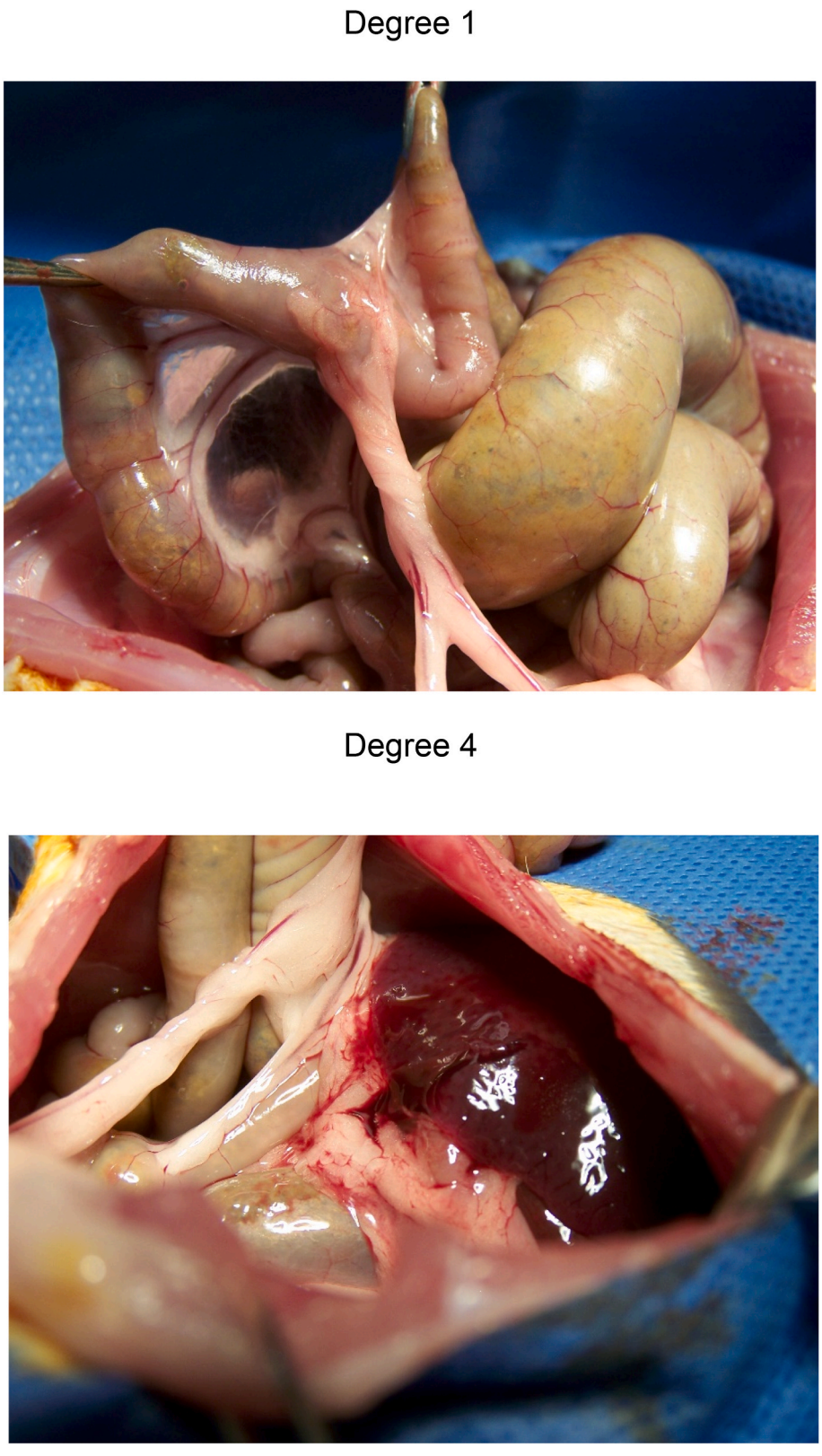


Fig. 2. Representative images of adhesions in the experiment.

characteristics of their intervention.

6. Conclusion

The safe and effective dose of subcutaneous enoxaparin to prevent PAA formation is 0.5–1.5 mg/kg/day for seven days.

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Table 4
Macroscopic and histological degrees of adhesion formation and statistical significance compared to group I.

Group	Degree of adhesion		p[a]	Degree of histological adhesion		p[a]
	Min - Max	Mean ± SD		Min - Max	Mean ± SD	
I	3–4	3.4 ± 0.51		2–3	2.75 ± 0.46	
II	1–2	1.6 ± 0.51	p < 0.01	1–2	1.25 ± 0.70	p < 0.01
III	1–2	1.6 ± 0.51	p < 0.01	1–2	1.4 ± 0.51	p < 0.01
IV	1–1.5	1.5 ± 0.52	p < 0.01	1–2	1.4 ± 0.54	p < 0.01
V	1–2	1.6 ± 0.51	p < 0.01	1–2	1.62 ± 0.51	p < 0.01

[a] Statistical analysis comparing the mean adhesion scores to those of group 1, as this group had the highest degree of adhesion formation.

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Provenance and peer review

Not commissioned, externally peer-reviewed.

Data statement

The authors declare the availability of the data of the present investigation.

Ethical approval

The research project was approved by the Research Committee and Subcommittee on Research and Care of Laboratory Animals of the Mexican School of Medicine of Universidad La Salle, Mexico with number CIE-2019-3.

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Author contributions

GGVT. Experimental design, bibliographic review, inductor surgeon of adhesion, monitoring of the postoperative evolution and reoperation of laboratory animal, results analysis, manuscript edition.

ETB. Surgical assistance, monitoring of the postoperative evolution, postoperative evolution, manuscript edition.

MAT. Bibliographic review, adherence evaluator, manuscript edition.

Registration of research studies

1. Name of the registry:
2. Unique Identifying number or registration ID:
3. Hyperlink to your specific registration (must be publicly accessible and will be checked):

Guarantor

Dr. Gilberto Guzmán-Valdivia Gómez.
Dr. Eduardo Tena-Betancourt.

Consent

Does not apply.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2021.103132.

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