International Journal of Surgery Open 24 (2020) 156–160

Contents lists available at ScienceDirect

International Journal of Surgery Open

journal homepage: www.elsevier.com/locate/ijso

Research Paper

Effect of pimecrolimus on postoperative peritoneal adhesions in rat: An experimental study

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ARTICLE INFO

Article history:
Received 15 January 2020
Received in revised form
17 May 2020
Accepted 20 May 2020
Available online 29 May 2020

Keywords:
Adhesion bands
Laparotomy
Pimecrolimus
Tensile strength

ABSTRACT

Introduction: Postoperative peritoneal adhesions (PPAs) is a common complication of abdominal surgeries causing significant morbidity and mortality. The inflammatory response to damaged peritoneal tissue is a speculated culprit. The aim of this study is to investigate the protective effect of pimecrolimus, an anti-inflammatory and immunomodulator agent, in formation of PPAs in rats.

Methods: Complied with the Animal Research Reporting In Vivo Experiments (ARRIVE) Guidelines Checklist, 50 Albino rats underwent laparotomy and were allocated into 5 groups. In groups 1 to 3, topical pimecrolimus (25, 50 and 100% concentration, respectively) was applies on a scratched area of peritoneum. In group 4, only topical Eucerin was used and group 5, was the control group. On postoperative days 7 and 28, five rats from each group were randomly selected and the tensile strength and adhesiveness of intraabdominal fibrotic bundles were assessed.

Results: There was no significant difference in tensile strength and adhesiveness, between the groups on postoperative day 7. On postoperative day 28, however, the tensile strength was significantly lower in pimecrolimus groups than in Eucerin (<0.001) and control (<0.001) groups. Groups with higher concentrations of pimecrolimus (group 2 and 3) developed significantly less adhesions than group 1, in which lower pimecrolimus concentration was used.

Conclusion: Administration of topical pimecrolimus decrease adhesions and their tensile strength on postoperative day 28 in rats.

1. Introduction

Postsurgical peritoneal adhesions (PPAs) can develop in 67–03% of laparotomies and about 97% of gynecological surgeries [1,2]. These are pathological fibrous bands that can bind to viscera and abdominal wall. PPAs can cause gastrointestinal obstruction and infertility in women [3]. The use of anti-inflammatory drugs, anticoagulants, fibrinolytics, mechanical devices to separate intestinal loops and omental patch has been proposed to prevent the development of PPAs [4–8]. Laparoscopic approaches have been shown to reduce the incidence of adhesions [9–11]. Other effective agents include tissue plasminogen activator (t-PA), gonadotropin releasing hormone (GnrH), immunomodulators such as transforming growth factor-beta (TGF-β), interlukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, calcium channel blockers, fibrinolysin and immune suppressors [12–15]. These act either by decreasing fibroblastic activity or by modulating fibrinolysis pathways. Despite various measures, PPAs are still a common problem.

Pimecrolimus, an immune suppressive agent with direct action on T-cells, is available in ointment form and is used topically without systemic absorption [16,17]. An interesting advantage of

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https://doi.org/10.1016/j.ijso.2020.05.009

pimecrolimus is its significant anti-inflammatory and immune modulatory activity with low systemic immunosuppressive potential. The mechanism of action of pimecrolimus is the blockage of T-cell activation via inhibiting the protein phosphatase calcineurin, preventing calcineurin from dephosphorylating the nuclear factor of activated T cells (NF-AT), a transcription factor, which in turn leads to the blockage of signal transduction pathways in T cells and inhibition of the synthesis of inflammatory cytokines, specifically Th1- and Th2-type cytokines. Pimecrolimus has also been shown to prevent the release of cytokines and pro-inflammatory mediators from mast cells 16, 17. This study was conducted to evaluate the effect of topical pimecrolimus on tensile strength and adhesiveness of postoperative peritoneal adhesions in rats.

2. Methods

After approval of the ethics committee, this study was performed according to the ARRIVE statement [18] in the Animal Research Center of kashan medical university Fifty male albino rats weighing 250–300 g with mean age of 11 weeks were enrolled. The animals were kept in air-conditioned colony rooms and fed with standard rat chow diet, water and libitum. The animals were housed at the Center for Laboratory Animal Care and were acclimatized for one week before the experiment. After adaptation, they were randomly assigned to five different groups of equal numbers. The only exclusion criterion was the death of the rat during the study. All of the animals were fasted for 12 h before surgery.

2.1. Procedure

General anesthesia was induced by 60 mg/kg intramuscular injection of ketamine and 4 mg/kg xylazine. We did not administer any antibiotics. After hair removal, the abdomen was cleaned with 1% antiseptic povidone-iodine solution and a 4 cm midline laparotomy incision was made in the supine position. On the right side and anterior to the cecum, with approximately 1 cm distance from the midline incision, a 2 × 2 centimeter area of the peritoneum was scratched by 20 knocks of dry sterile surgical gauze to induce serosal petechiae (Fig. 8). The terminal ileum and cecum also were scraped with the same technique. Hemorrhage was induced in all cases and finally, the two intra-abdominal traumatized surfaces were brought in contact. In three groups (1, 2 and 3) 2 mL of 25, 50 and 100% topical pimecrolimus was applied immediately after scraping on the surfaces. In group 4, 2 mL of eucerin cream was applied and for group 5, the control group, no intervention was used.

At the end of the procedure, the cecum was repositioned in the abdominal cavity, the midline incision was closed with absorbable 5/0 polyglactin (Vicryl) continuous sutures for the fascia and non-absorbable 5/0 nylon interrupted sutures for skin closure. All rats were observed closely for development of surgical complications. On the 7th and 28th postoperative day, 5 rats were randomly selected from each group. Repeated laparotomy was performed with a 3 cm incision, parallel but far from the first mid line incision, the anterior abdominal wall was reopened, the fascia was elevated gently and adhesive bands were evaluated using the method described by Linsky et al. [19]. The severity of adhesions was determined grossly by trying to separate the adhesions using the following classification:

Grade 1: no adhesions are seen (Fig. 1)
Grade 2: release needs fine blunt dissection (Fig. 2)
Grade 3: release needs blunt dissection (Fig. 3)
Grade 4: adhesion bands with neovascularization, requiring sharp dissection (Fig. 4)

Fig. 1. No adhesion present.

Fig. 2. Adhesion bands, needs fine blunt dissection, 51–100 g required to dissect bands.

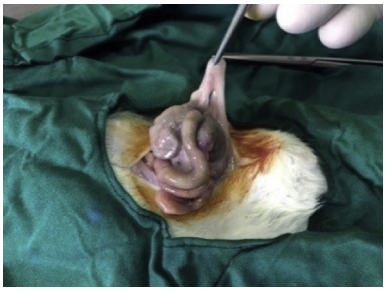

Fig. 3. Adhesion bands, needs coarse blunt dissection.

Fig. 4. Adhesion bands with neovascularization, needs course and sharp dissection.

The tensile strength of the strongest adhesion was measured with a tensiometer and classified as follows:

Grade 0: no tension required (Fig. 1)
Grade 1: 0–50 g required to dissect the adhesion bands attaching viscera to abdominal wall (Fig. 5)
Grade 2: 51–100 g required to dissect the adhesion bands attaching viscera to abdominal wall (Fig. 2)
Grade 3: 101–150 g required to dissect the adhesion bands attaching viscera to abdominal wall (Fig. 6)
Grade 4: 151–200 g required to dissect the adhesion bands attaching viscera to abdominal wall (Fig. 7)

Adhesion grade and tensile strength were evaluated and recorded separately by two blinded observers.

Data analysis: was performed using IBM SPSS Statistics 16. The means and standard deviations were calculated and data normality exam was performed. For comparing the data, ANOVA and Tukey post hoc test were used. A p-value of less than 0.05 was considered as significant.

3. Results

All animals tolerated the procedure well. No signs of infection were observed in the incision site throughout the study period. Three rats died due to unknown cause, on day 2, 6 and 23 and were excluded from the study. Adhesiveness and tensile strength were significantly less in groups 1, 2 and 3 on the 28th day in comparison to the eucerin and control group. No difference was seen on the 7th day. Comparing the different concentrations of pimecrolimus, lower adhesion scores and less tensile strength was observed with higher concentrations on day 28 (Table 1, Digram 1 and 2). The Kolmogorov-Smirnov test (K–S test) showed normal distribution of tensile strength and adhesiveness for all 5 groups.

4. Discussion

There are many experimental models for engendering peritoneal adhesions such as damaged uterine horn model, peritoneal damage model and bacterial peritonitis model [19,20,21]. We choose the scraping model for this study, because direct mechanical intestinal wall damage from gauze scraping mimics abdominal surgery damage.

Many efforts have been made to prevent post-surgical peritoneal adhesions, but an effective protocol has not yet been developed. However, there are some recommendations; careful tissue handling, minimizing surgical trauma, using laparoscopic surgical techniques, avoiding excessive desiccation and ischemia, minimizing the use of electro cautery, elimination of foreign bodies (starch and talc) and optimization of hemostasis [22,23]. As mentioned previously, some preventive agents are under investigation, including anti-inflammatory drugs, which decrease peritoneal fibrosis via preventing fibroblast production. Other examples are non-steroidal anti-inflammatory drugs (NSAID), corticosteroids, fibrinolytics, and immune suppressors. Anti-inflammatory and immune modulatory agents have been shown to reduce the extent of PPAs in animal models [24]. Pimecrolimus is also an immune suppressive agent with direct action on T-cells and has significant anti-inflammatory activity and immune modulatory capabilities with low systemic immunosuppressive potential [16,17]. We observed a significant decrease in adhesion formation in rat via applying topical pimecrolimus, especially with higher concentrations of 50% and 100%. Similar studies showed decreased adhesion formation with intraperitoneal applying of colchicine, antibiotics, nitric oxide, extract of green tea, Allium Sativum (garlic) oil and methylprednisolone [25–31]. Also, intra-abdominal administration of noxythiolin was effective in decreasing the intensity of adhesions in rats. Noxythiolin, reduced both the total and the mean number of adhesions and their mean length of attachment. The anti-adhesive effect of noxythiolin may be due to its anticoagulant, cytotoxic or antibacterial properties [32–36].

To our knowledge, there is no similar study evaluating the effect of pimecrolimus on peritoneal adhesion formation. This topical cream is an FDA-approved second line treatment for atopic dermatitis and has no systemic toxicity. The effect of pimecrolimus is probable not only due to its function as a mechanical barrier, but also for the anti-inflammatory effect that decreases adhesion formation, as less adhesions were observed in comparison to eucerin and also with higher concentrations of pimecrolimus.

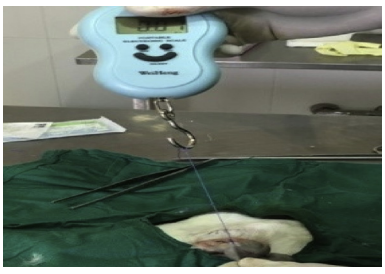

Fig. 5. Grade 1: 0–50 g required to dissect bands from viscera to abdominal wall.

Fig. 6. Grade 3: 101–150 g required to dissect bands from viscera to abdominal wall.

Fig. 7. Grade 4: 151–200 g required to dissect bands from viscera to abdominal wall.

Fig. 8. *Scraped* by 20 knocks of dry gauze to create serous petechia and hemorrhage.

Table 1
Tensile strength and adhesiveness on the 7th and 28th postoperative day.

time	Variable	Group 1	Group 2	Group 3	Eucerin	Control	p-value
7th day	Tensile strength (grade)	1.8 ± 0.4	1.7 ± 0.5	2 ± 0.7	1.8 ± 0.4	2.2 ± 0.5	0.64
	Adhesiveness (grade)	1.8 ± 0.8	1.7 ± 0.5	1.6 ± 0.5	1.8 ± 0.8	2.0 ± 0.8	0.95
28th day	Tensile strength	2.4 ± 0.5	1.2 ± 0.8	1.0 ± 0.8	2.8 ± 0.8	3.6 ± 0.5	<0.001
	Adhesiveness	1.4 ± 0.5	1.2 ± 0.8	0.7 ± 0.5	2.0 ± 0.7	2.8 ± 0.4	<0.001

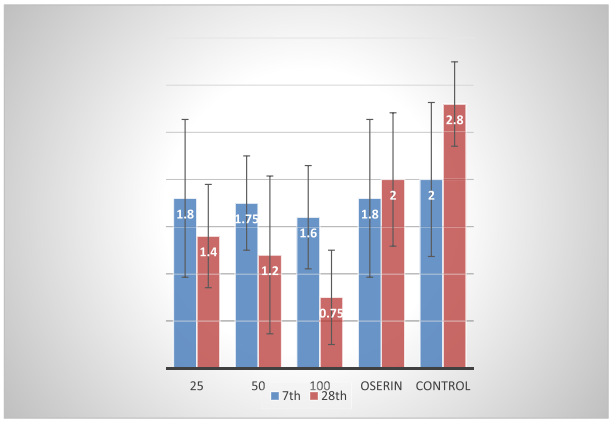

Diagram 1. Adhesiveness on 7th and 28th day.

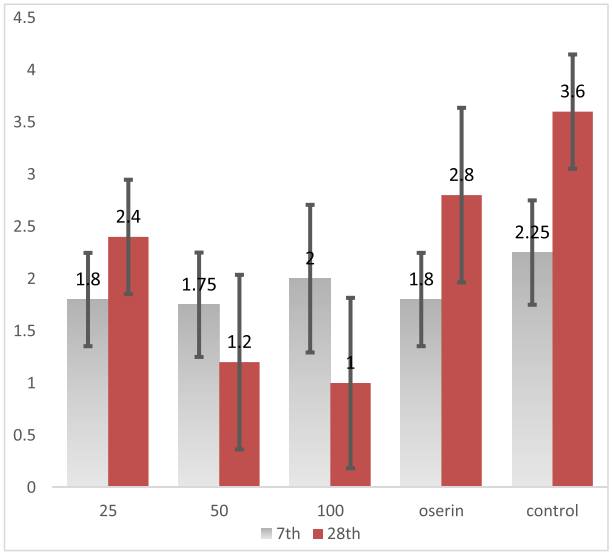

Diagram 2. Tensile strength on 7th and 28th day.

5. Conclusion

Applying topical pimecrolimus decreases postoperative peritoneal adhesions in albino rats. As this topical cream has no systemic adverse effects, it might evolve as an effective agent in decreasing postoperative adhesions. Further investigations is needed to confirm the efficacy and the optimal dosing of pimecrolimus.

5.1. Limitations

This study was carried out on a small number of rats and the follow-up period was short. Future research should be done over a longer period and with larger sample size.

Ethical approval

Approval by the kashan University of Medical Sciences (kaums) Ethics Committee and supported by Animal Research Laboratory.

Funding

There is no source of funding other than the authors.

Author contribution

Adoulhossein Davoodabadi MD, Hamed Azadi Hossein Akbari, Hamidreza Banafsheh, Mahdi Norroddini and Abbas Hajian MD are principal investigators. This study was the general Medicine thesis was done by Hamed Azadi MD under supervision of Abdoulhossein Davoodabadi.

Abdoulhossein Davoodabadi also participated in drafting of the manuscript. All authors read and approved the manuscript.

Conflict of interest statement

The authors declare no conflicts of interest.

Guarantor

Abdoulhossein Davoodabadi.

Research registration number

Name of the registry:
Unique Identifying number or registration ID:
Hyperlink to the registration (must be publicly accessible).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijso.2020.05.009.

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