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Effects of heparin and prednisolone on postoperative intra-abdominal adhesions in Wistar rats

Wistar cinsi ratlarda heparin ve prednizolonun postoperatif intra-abdominal adezyonlara etkisi

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Abstract

Aim: Postoperative intra-abdominal adhesions are one of the complications surgeons want to avoid. It was aimed to compare the effects of physiological saline, heparin, Ringer's lactate, and prednisolone, which have been commonly used for surgical adhesions, with repeated lavage on postoperative adhesions.

Methods: Upon the approval of the Ethics Committee of Fırat University, Faculty of Medicine, 35 Wistar Albino (female) rats were randomized into 5 groups (Heparin BID, Heparin QD, Prednisolone QD, Prednisolone BID, and Control) of 7 rats, and adhesion was created with peritoneal injury model in all rats. The differences in the adhesions created between groups were compared using the macroscopic adhesion scoring system of Linsky.

Results: No significant differences were found between physiological saline-heparin and Ringer's lactate-prednisolone groups in terms of adhesion scores (P=0.06). There was a difference in adhesion severity on the left only in the groups receiving heparin QD and prednisolone QD (P=0.04).

Conclusion: We demonstrated that none of the agents (Physiological saline, Heparin, Ringer's lactate, and prednisolone) we used in our study model reduced adhesions. It is thought that no difference was detected because of the adhesion model we established. Advanced technology-based studies are still needed to prevent postoperative intra-abdominal adhesions that are globally observed to reduce a huge economic burden and decrease mortality and morbidity.

Keywords: Animal models, Heparin, Postoperative intra-abdominal adhesions

Öz

Amaç: Cerrahi sonrası postoperatif batın içi yapışıklıklar halen cerrahların karşılaşmak istemediği bir sorun olarak karşımıza çıkmaya devam etmektedir. Serum fizyolojik, Heparin, Ringer laktat ve prednizolonun mükerrer lavaj ile postoperatif adezyonlar üzerine etkisini karşılaştırmaktır.

Yöntemler: Fırat Üniversitesi Tıp Fakültesi Etik Kurulu'nun onayı alındıktan sonra 7'şerli 5 grup (Heparin günde 2 defa, Heparin günde 1 defa, Prednizolon günde 1 defa, Prednizolon günde 2 defa ve Kontrol) halinde toplam 35 adet Wistar Albino tipi (dişi) rat kullanılmış, tüm ratlarda peritoneal hasar paterni ile adezyon oluşturulmuştur. Her grupta oluşan adezyonlar Linsky'nin kullandığı adezyonları skorlayan, makroskopik adezyon skorlama yöntemiyle gruplar arası farklılıklar kıyaslanmıştır.

Bulgular: Serum fizyolojik-heparin ve Ringer laktat-prednizolon gruplarının karşılaştırılmasında adezyon skoru açısından istatistiksel fark bulunmamıştır (P=0,06). Sadece Heparin günde bir defa ve prednizolon günde bir defa alan gruplarda solda adezyon şiddeti bakımından istatistiksel farklılık bulunmuştur (P=0,04).

Sonuç: Çalışma modelimize göre kullandığımız Serum fizyolojik, Heparin, Ringer laktat ve prednizolonun adezyonları azaltmadığını kanıtladık. Fark olmamasının nedeninin, oluşturduğumuz adezyon modelinden kaynaklandığı düşünülebilir. Tüm dünyada mortalite ve morbiditeyi arttıran postoperatif batın içi adezyonlarını önlemede ileri teknolojik tabanlı çalışmalara ihtiyaç duyulmaktadır.

Anahtar kelimeler: Hayvan modeli, Heparin, Postoperatif intra-abdominal adezyon

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Introduction

Postoperative peritoneal adhesions remain one of the most important causes of mortality and morbidity, resulting in many complications, such as chronic pelvic pain, infertility, and ileus [1]. Despite many novel approaches and surgical developments, there is still no effective method to prevent intra-abdominal adhesions after surgery [2]. In the literature, the intra-abdominal adhesions account for the most crucial factor in the development of postoperative ileus as well as the most frequent reason for referral to the hospital after gynecological or ovarian surgery [3-5].

It is accepted by many clinicians that unexplained female infertility and chronic pelvic pain in women are secondary to intra-abdominal adhesions [6]. One of the most common issues that surgeons still struggle globally is the serious complications that may occur after intra-abdominal adhesions [7]. For this reason, varied materials that might prevent adhesions have been used in many experimental studies. In the literature, many organic and synthetic components have been used to prevent adhesion formation, and numerous studies using the latest technologies are still ongoing. The objective of this study is to investigate the effect of repeated peritoneal lavage on adhesions using the combinations of heparin and physiological saline and prednisolone and Ringer's lactate after abdominal pelvic surgery.

Materials and methods

This study was performed at the Laboratory of Fırat University Experimental Research Center (FÜTDAM) and the Medical Genetics Laboratory of Fırat University. Ethics committee approval of our study was obtained in 2012 with the decision number 76 from Fırat University Experimental Research Center. Thirty-five 12- to 14-week old Wistar Albino adult female rats with regular cycles weighing between 190-220 grams were used in our study. To maintain their regular biological rhythms, they were kept in cages with 5 rats per each at an ambient temperature between 21-23 °C for 12 hours (08.00-22.00) under artificial light and 12 hours in the dark. Animals were fed using standard pellet and city water [8]. Oral feeding was discontinued 18 hours before the experiment, allowing the animals only to drink water. They were anesthetized with chloral hydrate at a dose of 400 mg/kg intraperitoneally. The abdomen was cleaned with a 10% povidone-iodine solution before the surgery. Thirty-five rats were randomly and prospectively divided into 5 groups of 7 rats and placed in the supine position on the operation table. Their abdomens were opened with a midline incision.

The groups established as per the study design and their characteristics are listed below:

Control group (n=7): Laparotomy was performed, all were peritoneally injured, then closed.

Heparin QD group (n=7): In this group, laparotomy was performed to create peritoneal injury, a drain was placed, and abdomens were closed. Peritoneal lavage with 5 ml physiological saline containing 250 IU heparin/ml was performed once a day for 3 days through the drain.

Heparin BID group (n=7): In this group, laparotomy was performed to create peritoneal injury, a drain was placed, and abdomens were closed. Peritoneal lavage with 5 ml physiological saline containing 250 IU heparin/ml was performed twice a day for 3 days through the drain.

Prednisolone QD group: (n=7) In this group, laparotomy was performed to create peritoneal injury, a drain was placed, and abdomens were closed. Peritoneal lavage with 5 ml mixture of 1 vial prednisolone and 1 L Ringer's lactate was performed once a day for 3 days through the drain.

Prednisolone BID group: (n=7) In this group, laparotomy was performed to create peritoneal injury, a drain was placed, and abdomens were closed. Peritoneal lavage with 5 ml mixture of 1 vial prednisolone and 1 L Ringer's lactate was performed twice a day for 3 days through the drain.

The procedure including laparotomy

The animals were anesthetized with chloral hydrate at a dose of 400 mg/kg administered intraperitoneally. The abdomen was cleaned with a 10% povidone-iodine solution before sterile surgical intervention. Thirty-five rats were randomly and prospectively divided into 5 groups of 7 rats. The rats were placed on the operation table in supine position and laparotomy was performed through a 3 cm-long midline incision. A 2x2cm section of the parietal peritoneum was removed from the right side and it was sutured with 3/0 silk. A 2 cm midline parallel straight incision was made on the left side, which was sutured with 3/0 silk. Drains were placed, and the layers of the abdomen and the skin were sutured with 3/0 silk. Rats, which were monitored for blood pressure, heart rate, and fever, were kept in cages of 5 rats per each until the end of the experiment. Regular peritoneal lavage was performed through the drain using 5 ml physiological saline containing Heparin 250IU/ml once a day in the Heparin QD group and 5 ml twice a day in the Heparin BID group for 3 days. Regular peritoneal lavage was performed using a 5 ml mixture of 1 vial prednisolone and 1 L Ringer's lactate once a day in the Prednisolone QD group, and twice a day in the Prednisolone BID group. After 14 days, a relaparotomy was performed to evaluate the adhesions. Powder-free gloves and surgical instruments were used during the operation. The 2x2 cm flap was removed from the right side. Then, repeated peritoneal lavage was performed for 3 days through this drain (Figures 1-6). After surgery, the animals were placed in their cages in separate groups and followed up for 14 days under standard feeding and living conditions.

After 14 days, the animals were anesthetized with chloral hydrate at a dose of 400 mg/kg administered intraperitoneally, and their abdomens were reopened with a midline incision. All adhesions in the abdomen were examined and recorded (Figure 7). The adhesions were scored macroscopically, as shown in Tables 1 and 2, using the scoring system of intra-abdominal adhesions [9].

Statistical analysis

The difference between groups were analyzed with Kruskal Wallis, Mann-Whitney U, and post-hoc Tuckey tests using the SPSS version 15.0 program. Statistical significance was set at $P<0.05$.

Figure 1: Midline incision

Figure 2: Creation of peritoneal injury

Figure 3: Removal of a 2x2 cm flap from the peritoneum

Figure 4: Suturing the removed flap with 3/0 silk

Figure 5: 2 cm straight incision on the left side and suturing with silk 3/0

Figure 6: Placement of drain and closing abdomen.

Figure 7: Midline incision and evaluation of adhesions

Table 1: Macroscopic evaluation of adhesion sizes (Linsky et al. [9])

Score	Adhesion size
0	No adhesion
1	25% of the traumatized area
2	25-50% of the traumatized area
3	50-100% of the traumatized area

Table 2: Macroscopic evaluation of adhesion severity (Linsky et al. [9])

Score	Adhesion severity
0	No resistance to separation
0.5	Some resistance
1	Sharp dissection needed

Results

The mean values of adhesion sizes and severities in the groups that received heparin or prednisolone once and twice daily, along with the control group is presented in Table 3. The comparison of four groups showed a difference in the severity of adhesions on the left side (P=0.328). The sizes of adhesions were similar between the right and left sides and the severity was comparable on the right side among groups (P=0.06, Kruskal Wallis Test). In post-hoc analysis, the severity of left side adhesions was different among the groups in which heparin and prednisolone were used once daily (P=0.08). The comparison of the remaining groups among themselves showed similar results (P=0.06, post-hoc Tukey test).

Table 3: Comparative results of the groups

	Control group	Heparin QD	Heparin BID	Prednisolone QD	Prednisolone BID	*P*-value
Adhesion size, right	2.4	1.6	1.1	2.3	1.4	0.06
Adhesion size, left	2.9	2.7	2.4	2	2.6	0.3
Adhesion severity, right	0.9	0.7	0.4	0.7	0.4	0.08
Adhesion severity left	0.9	1	0.9	0.6*	0.8	0.04

* Groups receiving heparin once daily and Prednisolone once daily were different in terms of adhesion severity on the left.

Discussion

The reason for performing peritoneal lavage repeatedly for 3 days through the drain placed in the abdominal cavity and comparing the fluid barriers was that we thought it could be more effective. Various models, such as the injured uterus horn model, peritoneal injury model, ileal transection model, colonic anastomosis model, bacterial peritonitis model, and clamping model were previously established to create peritoneal adhesions. The peritoneal injury model of our study was chosen as it was close to the surgical procedures performed in clinical practice [9].

The postoperative intra-abdominal adhesions remain to be an issue in general surgery and gynecology since they lead to an elevated level of morbidity and mortality [10]. In the literature, postoperative peritoneal adhesions are among the most common causes of ileus. Today, studies using advanced nanotechnology products and different agents to prevent intra-abdominal adhesions are ongoing at a fast pace and a further step is taken each day to obtain a curative solution [11]. To prevent intra-abdominal adhesions and keep them at the minimum level, many approaches, such as usage of anti-inflammatory agents, antibiotics, fibrinolytic agents, chemical and physical barriers, have been tried [12].

In our study, we compared physiological saline, heparin, Ringer's lactate, and prednisolone, all which have been used against peritoneal adhesions. These agents were chosen because they were safe, simple, cheap, easily accessible, and they each have a different mode of action [13]. We comparatively analyzed the effectiveness of the anticoagulant effects of heparin, reduction of local inflammatory response with prednisolone, removal of fibrin buildup with physiological saline, and its use with fluid barriers and repeated lavage.

In our study, there was no difference in terms of adhesion scores between physiological saline-heparin and Ringer's lactate-prednisolone groups according to the results of Mann-Whitney U test. There was a difference in adhesion severity on the left only in the groups receiving heparin and prednisolone once a day. It is thought that no difference was detected because of the adhesion model we established. There was no difference between these groups in terms of adhesion intensity and size. In conclusion, we found that none of the agents we used in our study model reduced adhesions.

Some basic surgical principles have been widely accepted clinically in the prevention of postoperative intra-abdominal adhesions. These can be listed as gentle handling of the tissues, continuous irrigation, using biologically compliant suture materials, avoiding unnecessary peritoneal dissection, and leaving the peritoneum open [14-16].

However, the fact that the solution to this problem with known pathophysiology remains unknown causes all surgeons to encounter more difficult cases each passing day.

Intra-abdominal adhesions resulting from previous operations are an important problem as they cause reoperations, increase morbidity, mortality, and healthcare expenses. Investigations are ongoing to solve this problem, but despite many positive results, there is no consensus on an absolute method. There are promising studies on biodegradable hyaluronic acid derivatives in the literature [17].

In the current literature, methods such as blockage of immunological factors and mode of actions (tumor necrotizing factor), administration of high dose botulinum toxin, pure olive oil, and platelet-rich plasma have been proven successful in preventing postoperative adhesions by promising studies [18-21].

Limitations

The biggest factor limiting our study is the low sample size and the small number of agents we used.

Conclusion

Since the number of subjects in our study is insufficient, larger, well-planned studies on the effects of different dosages of agents we used for the prevention of adhesions are needed. We believe that further, technology-based research may shed light on this issue.

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