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Original Article

Effects of peritoneal exposure to povidone iodine, heparin and saline in post surgical adhesion in rats

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Abstract

BACKGROUND: Adhesions are common consequences of abdominal operations and they can cause significant complications such as bowel obstruction, infertility and abdominal pain. A wide variety of adhesion-reducing substances have been evaluated but we are still far from the ideal adhesion preventing agent. In this study, we decided to evaluate the effects of peritoneal exposure to betadine and heparin in post-surgical adhesions in rats.

METHODS: A total of 39 male Wistar-Albino rats weighting 200-250 grams were randomly assigned to three groups. Anesthesia was performed using intramuscular ketamine and xylazine. After a midline laparotomy, enterotomy and repair, bowels were irrigated by saline 0.9%, heparinized saline and betadine solution. Finally, adhesions were evaluated and scored after 20 days.

RESULTS: All groups were almost similar in mean adhesion score in the site of enterotomy but mean total abdominal adhesion score in betadine group was significantly higher than those of other groups. According to pathological studies, peritoneal inflammation was more severe in the betadine group but there wasn't any statistically significant difference between frequency of foreign body reaction and wound healing stage among three groups. Surprisingly, anastomosis leakage was significantly more common in heparin group.

CONCLUSIONS: To date, the most effective means of limiting adhesions is a meticulous surgical technique although intraperitoneal irrigation with heparin seems to reduce adhesions but further studies should be done to evaluate the effects of local administration of heparin on anastomosis leakage.

KEYWORDS: Surgery, abdomen, adhesion, saline, heparin, betadine.

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Adhesions are common and undesirable consequences of abdominal operations [1] although they are a normal response of body to the tissue damage fallowing a surgical trauma. Following laparotomy, there is a greater than 5% lifetime incidence of small-bowel obstruction caused by adhesions. [2] Infertility and chronic abdominal pain are some other serious complications due to adhesion bands. [3] Also, they increase the risk, duration and complication rate of subsequent surgeries. [2-6] Nearly 50-75% of intestinal obstructions are caused by adhesion bonds. [7,8] These reports showed the major role of postoperative adhesions in postoperative compilations that involve patients, surgeons and the health system. So, it would be very important to identify surgical interventions with a high risk of adhesion-related complications and to find a strategy for adhesion prevention. [9,10] A wide

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variety of adhesion-reducing substances have been evaluated but we are still far from the ideal adhesion preventing agent. In this study, we have evaluated the effects of peritoneal exposure to povidone iodine and heparin in post-surgical adhesions in rats to find out the advantages or disadvantages of peritoneal irrigation with these substances compared to the common method (saline irrigation) in relation to adhesion formation.

Methods

In this experimental double-blind randomized controlled animal model trial, a total of 39 male Wistar-Albino rats weighting 200-250 grams were enrolled and randomly assigned to three study groups. Anesthesia was performed using intramuscular ketamine hydrochloride (4 mg/100g) and xylazine (1 mg /100g) mixing together. The abdomen was shaved, prepared and draped and then, abdominal cavity was entered via a 4 cm vertical midline incision. A 5 mm longitudinal enterotomy was performed in proximal jejunum. Transverse closure was done using 5-0 silk sutures. Manipulation of other tissues was minimized. Finally, bowels and peritoneal cavity were irrigated by normal saline 0.9% in the first group, by heparinized saline that contained 1000 unit heparin in 10 ml in the second group and by povidone iodine solution that contained 1 ml betadine 10% in 10 ml saline in the third group. The abdomen was closed in one layer by silk sutures. A mixture of iodine and chloramphenicol was sprayed to the laparotomy site as an antibiotic agent and especially due to its bitter taste, it inhibited rats to chew the sutures and prevent evisceration. Labeling the study groups was done by an assistant via cutting a part of either right or left ear. The surgeon was blind to group markers. Animals were allowed to feed ad libitum after operation but 10 ml dextrose/saline solution was also injected to the neck skin fold as a nutritional support during post-operative period. After 20 days, animals were sacrificed by chloroform inhalation and a long laparotomy was done through a paramedian incision and adhesion formation was evaluated in the enterotomy site and in total peritoneal space. Type, tenacity and extent of adhesions were scored using adhesion measurement score described by Fielder and Ordonez [11,12] (table 1). Then, histopathologic studies were done on tissue specimens and degree of inflammation in peritoneum, foreign body reaction and wound healing were assessed. Statistical analysis was performed by SPSS version 11.5 using Kruskal–Wallis one-way analysis and Mann–Whitney U tests.

Table 1. Adhesion measurement score described by Fiedler and Ordonez.

Score	Type	Tenacity	Extent (%)
0	No adhesions	-	-
1	Filmy adhesions	Easily fall apart	1–25
2	Firm adhesions	Require traction	26–50
3	Require sharp dissection to be separated	Require sharp dissection	51–75
4	More	More	76–100

*Total adhesion scores were the sum of type, tenacity and extent scores of lesions.

Results

Ten deaths were observed that all happened during the first 3 days of study. Three deaths occurred due to apnea at the time of anesthesia induction and other cases were died due to early post-operative complications including 3 cases of delayed apnea or respiratory failure and 4 cases of early leakage and peritonitis (one in saline, one in betadine and two in heparin group). After final relaparotomy, we also observed two cases of anastomosis gross leakage with interloop abscess that were both in the heparin group (figure 1). So, gross anastomosis leakage in the form of peritonitis or

intra-abdominal abscess was significantly more common in the heparin group (30.8% compared to 7.7%; P = 0.001). These 6 cases of anastomosis leakage were excluded to evaluate the direct effects of peritoneal exposure to different agents on adhesion formation. Comparison of mean adhesion score in the site of enterotomy didn't show any significant difference but adhesion score of total abdominal cavity in betadine group was significantly higher and in heparin group was significantly lower compared to other groups (table 2). The degree of peritonitis was classified into 4 stages (any, mild, moderate and severe inflammation) according to deposition of inflammatory cells in histopathological studies. Peritoneal inflammation was more severe in the Betadine group (P = 0.025; table 3).

Table 2. Comparing adhesion scores in the site of enterotomy among three groups.

Study groups	Score of adhesion in the site of enterotomy Mean± Standard deviation	Score of adhesion in the site of enterotomy Mean± Standard deviation
First group(saline)	5.3±1.78	2.7±1.80
Second group(Heparin)	5.2±1.48	1.0±1.06
Third group(Betadine)	6.5±1.43	4.8±2.17
Kruskal Wallis test P value	P=0.167	P=0.001

Foreign body reaction was reported in 7, 6 and 8 cases in the saline, heparin and betadine groups, respectively (P = 0.67). Healing stage was also evaluated in the site of enterotomy. Granulation tissue with deep fibrosis in an ulcerative and inflamed mucosa was observed in almost all cases. Delayed repair wasn't reported in any case.

Table 3. Comparing the severity of inflammatory response in peritoneum among three groups.

Severity of peritonitis	First group (Saline) N (%)	Second group (Heparin) N (%)	Third group (Betadine) N (%)
Any inflammation	1 (11.1%)	4 (50.0%)	-
Mild inflammation	5 (55.6%)	2 (25.0%)	1 (10%)
Moderate inflammation	1 (11.1%)	1 (12.5%)	3 (30.0%)
Severe inflammation	2 (22.2%)	1 (12.5%)	6 (60%)
Sum	9 (100.0%)	8 (100.0%)	10 (100.0%)

Discussion

The occurrence of intraperitoneal adhesions after abdominal surgery is a well-known clinical problem. Postoperative peritoneal adhesions are major causes of intestinal obstructions. Postmortem evaluation of patients with prior surgical interventions has demonstrated adhesions in 67% of patients. [1] Ellis [13] reported that the incidence of adhesions approaches 100% in patients with prior abdominal operations and/or prior intra-abdominal infection. Intestinal obstruction, which is the most life-threatening adhesion-related complication, has a reported mortality rate of up to 15%. [4-6] It is well established that any trauma to the peritoneum, for example, mechanical, physical, chemical or infective, causes a response on the peritoneal and serosal surfaces with the subsequent adhesion formation. [14] Although sophisticated minimally invasive and laparoscopic techniques are used in most surgical procedures, iatrogenic surgical trauma cannot be avoided. Therefore, for prevention of adhesion formation, adjuvant treatment is necessary. The pathogenesis of intra-abdominal adhesion formation is a complex process consisting of several factors that control inflammation, cellular proliferation and migration, collagen and matrix synthesis, and interactions between various cell types, blood and matrix compo-

nents. [15] A large number of therapeutic modalities have been studied clinically and in animal models in an attempt to decrease the frequency and severity of adhesion formation after peritoneal injury. The proposed mechanisms by which they may reduce adhesion formation are: reduced initial inflammatory response, inhibiting exudates coagulation, enhancing fibrinolysis, mechanically separating fibrin covered surfaces and inhibition of fibroblastic proliferation. Probably the most effective method to reduce adhesions is to diminish surgical trauma via careful surgical technique. A wide variety of adhesion-reducing substances have been evaluated in animal models, yet we are still far from the ideal adhesion preventing agent. In the best scenario, we can only reduce the rate of adhesion formation. Some materials have produced good results; for example, one study showed that intrauterine application of auto-cross linked hyaluronic acid decreases intrauterine adhesions. [16] Nonetheless, many of these agents are expensive and not readily available. Despite this significant advance in adhesion reduction with tissue barriers [17,18], it is unclear whether physical barrier can provide protection in areas other than the site of application. A physical barrier alone applied to one area, may not completely eliminate adhesion formation. Systemically-administered drugs, which can reduce adhesions in all parts of the abdomen, will obviously provide better results. In this study we evaluated the role of three accessible objects that were reported to have a role in adhesion formation according to previous studies. The first one was saline irrigation of peritoneal cavity. The results of other studies indicated that irrigation with solutions such as Ringer's lactate or saline may enhance formation of postoperative adhesions [19]. The second material was heparin. According to Parker's study, heparin decreased the formation of adhesions in ponies after experimentally induced intestinal ischemia [20]. Administration of LMWH (Low molecular weight heparin) reported to be more effective according to Kutlay's study [21] but, it isn't accessible in many operating rooms. As bleeding may be seen after administration of intravenous heparin, we decided to use intraperitoneal heparin. Fukasawa also suggested that local intraperitoneal administration of low-dose heparin throughout the immediate postoperative interval period may result in adhesion-free healing. [22] In dose-dependent fashion and in the absence of any cofactor, heparin will inhibit both enzyme-initiated aggregation of fibrinogen-coated beads and dissociates preformed aggregates of fibrin-coated beads. The third one was povidone iodine (betadine). Povidone-iodine/PVP solution significantly reduced the number of adhesions and mean length of attachment of each adhesion in another study by Gilmore et al. [23] We currently use PVP in several circumstances during different surgeries. In our study, 39 male Wistar-Albino rats were randomly assigned to three groups of thirteen rats. Peritoneal cavity was exposed to saline, heparin and betadine. Relaparotomy was done after 20 days and peritoneum was evaluated for adhesion and scoring. Zhou in a similar study in animal models, evaluated adhesion formation in his series, 14 days after the first surgical intervention. [18] In Oncel's studies, animals were killed on postoperative day 21. [24,25] The mortality rate was 25.6% in our study, which was mostly due to apnea and anesthesia complications. There wasn't any significant difference in mean adhesion scores in the site of enterotomy among three study groups (P = 0.167) but, mean adhesion score of total abdominal cavity in betadine group was significantly higher than those of others (P = 0.001). While saline and heparin were almost statistically similar, the gross total result in heparin group was better. According to pathological studies, peritoneal inflammation was more severe in the betadine group but, there wasn't any statistically significant difference between frequency of foreign body reaction among the three groups (P = 0.675). Since foreign body reaction and enterotomy site adhesions were similar in all three groups and because there was an extra inflammation in peritoneum, far away from surgical trauma in betadine group, it may be concluded that betadine cytotoxicity

causes diffuse irritation in peritoneal cavity. Healing stage was almost similar in all cases; so delayed repair wasn't reported in any case. Gilmore also reported that povidone-iodine irrigation of the rat colon before and after anastomosis did not interfere with healing or inhibit peritoneal adhesion formation. [23] Another finding in our study was significantly more anastomosis leakage in heparin group that may be explained by inhibiting effects of heparin on clot formation that is the primary stage of wound healing. A limitation in our study was the small number of cases and we suggest further studies with larger populations to evaluate the effects of local administration of heparin on anastomosis leakage.

Figure 1. Interloop abscess that was observed in two rats in the heparin group.

Conclusions

To date the most effective means of limiting the adhesions is through a meticulous surgical technique, which includes the gentle handling of the bowel to reduce serosal trauma, avoidance of unnecessary dissections, exclusion of foreign materials from the peritoneal cavity, adequate irrigation and removal of infection and ischemic debris. Intraperitoneal irrigation with heparin seems to reduce adhesions but further studies should be done to evaluate the effects of local administration of heparin on anastomosis leakage.

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References

1. Menzies D. **Postoperative adhesions: their treatment and relevance in clinical practice.** *Ann R Coll Surg Engl* 1993; 75**:** 147-153.
2. Wang E, Ashley S, Zinner M. **Small intestine. In: Brunicardi C, Billiar T, Dunn D, Hunter J.** *Schwartz's Principles of surgery.* 8th ed. Philadelphia: McGraw Hill; 2005. P.1030-31.
3. Post-operative adhesions. **SynthMed, Inc; 2003.** Avallable at: *http://www.synthemed.com/post-op_adhesion.htm.*
4. van der Krabben AA, Dijkstra FR, Nieuwenhuijzen M, Reijnen MM, Schaapveld M,Van Goor H. **Morbidity and mortality of inadvertent enterotomy during adhesiotomy**. *Br J Surg* 2000; 87: 467-71.
5. Beck DE, Ferguson MA, Opelka FG, Fleshman JW, Gervaz P, Wexner SD. **Effect of previous surgery on abdominal opening time.** *Dis Colon Rectum* 2000; 43**:** 1749-1753.
6. Coleman MG, McLain AD, Moran BJ. **Impact of previous surgery on time taken for incision and division of adhesions during laparotomy.** *Dis Colon Rectum* 2000; 43**:** 1297-1299.
7. Ray NF, Denton WG, Thamer M, Henderson SC, Perry S. **Abdominal adhesiolysis: inpatient care and expenditures in the United States in 1994.** *J Am Coll Surg* 1998; 186**:** 1-9.
8. Monk BJ, Berman ML, Montz FJ. **Adhesions after extensive gynecologic surgery: clinical significance, etiology, and prevention.** *Am J Obstet Gynecol* 1994; 170**:** 1396-1403.
9. Ellis H, Moran BJ, Thompson JN, Parker MC, Wilson MS, Menzies D *et al*. **Adhesion-related hospital readmissions after abdominal and pelvic surgery: a retrospective cohort study.** *Lancet* 1999; 353**:** 1476-1480.

10. Lower AM, Hawthorn RJ, Ellis H, O'Brien F, Buchan S, Crowe AM. **The impact of adhesions on hospital readmissions over ten years after 8849 open gynaecological operations: an assessment from the Surgical and Clinical Adhesions Research Study.** *BJOG* 2000; 107**:** 855-862.
11. Fielder EP, Guzick DS, Guido R, Kanbour-Shakir A, Krasnow JS. **Adhesion formation from release of dermoid contents in the peritoneal cavity and effect of copious lavage: a prospective, randomized, blinded, controlled study in a rabbit model.** *Fertil Steril* 1996; 65**:** 852-859.
12. Ordonez JL, Dominguez J, Evrard V, Koninckx PR. **The effect of training and duration of surgery on adhesion formation in the rabbit model.** *Hum Reprod* 1997; 12**:** 2654-2657.
13. Ellis H. **The magnitude of adhesion related problems.** *Ann Chir Gynaecol* 1998; 87**:** 9-11.
14. Wilson MS, Ellis H, Menzies D, Moran BJ, Parker MC, Thompson JN. **A review of the management of small bowel obstruction. Members of the Surgical and Clinical Adhesions Research Study (SCAR).** *Ann R Coll Surg Engl* 1999; 81**:** 320-328.
15. Chiang SC, Cheng CH, Moulton KS, Kasznica JM, Moulton SL. **TNP-470 inhibits intraabdominal adhesion formation.** *J Pediatr Surg* 2000; 35**:** 189-196.
16. Holmdahl L, Risberg B, Beck DE, Burns JW, Chegini N, diZerega GS *et al*. **Adhesions: pathogenesis and prevention-panel discussion and summary.** *Eur J Surg Suppl* 1997; 56-62.
17. Mueller PO, Hay WP, Harmon B, Amoroso L. **Evaluation of a bioresorbable hyaluronate-carboxymethylcellulose membrane for prevention of experimentally induced abdominal adhesions in horses.** *Vet Surg* 2000; 29**:** 48-53.
18. Zhou J, Elson C, Lee TD. **Reduction in postoperative adhesion formation and re-formation after an abdominal operation with the use of N, O - carboxymethyl chitosan.** *Surgery* 2004; 135**:** 307-312.
19. Yaacobi Y, Goldberg EP, Habal MB. **Effect of Ringer's lactate irrigation on the formation of postoperative abdominal adhesions.** *J Invest Surg* 1991; 4**:** 31-36.
20. Parker JE, Fibini SL, Car BD, Erb HN. **Prevention of intraabdominal adhesions in ponies by low-dose heparin therapy.** *Vet Surg* 1987; 16**:** 459-462.
21. Kutlay J, Ozer Y, Isik B, Kargici H. **Comparative effectiveness of several agents for preventing postoperative adhesions.** World J Surg 2004; 28: 662-5.
22. Fukasawa M, Girgis W, diZerega GS. **Inhibition of postsurgical adhesions in a standardized rabbit model: II. Intraperitoneal treatment with heparin.** *Int J Fertil* 1991; 36**:** 296-301.
23. Gilmore OJ, Reid C. **Prevention of intraperitoneal adhesions: a comparison of noxythiolin and a new povidone-iodine/PVP solution.** *Br J Surg* 1979; 66**:** 197-199.
24. Oncel M, Remzi FH, Senagore AJ, Connor JT, Fazio VW. **Liquid antiadhesive product (Adcon-p) prevents postoperative adhesions within the intra-abdominal organs in a rat model.** *Int J Colorectal Dis* 2003; 18**:** 514-517.
25. Oncel M, Remzi FH, Connor J, Fazio VW. **Comparison of cecal abrasion and multiple-abrasion models in generating intra-abdominal adhesions for animal studies.** *Tech Coloproctol* 2005; 9**:** 29-33.