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Original Research

Heparin for adhesion prevention: Comparison of three different dosages with Seprafilm in a murine model

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ABSTRACT

Background: This study aimed to assess the safety and effectiveness of different dosages of heparin for adhesion prevention by comparing with Seprafilm®, in a murine model.
Materials and methods: Seventy five Balb/c mice were randomized into five groups. Group C were reserved as controls, and 62.5 IU, 125 IU, 250 IU of heparin, and Seprafilm® were intraperitoneally applied in studied groups. The severity and locations of adhesions were assessed after the sacrification on day 14. The cause of death was investigated to evaluate the side effects of the drugs.
Results: The death of 2 subjects due to peritonitis (1 in Group C, 1 in Group H62.5) left 14 subjects in Group C and Group H62.5 ($P \geq 0.05$), and no hemorrhage related death was observed. The use of the products significantly reduced the severity score of adhesion and the number of animals, had adhesions in different locations of the abdominal cavity, when the results were compared with the control group ($P < 0.05$ for all comparisons). Higher dosages of heparin seemed to be more effective. The results in group S, groups H250 and H125 were quite similar.
Conclusions: Relatively high doses (125 IU and 250 IU) of intra-abdominal heparin may be comparable in safety and effectiveness to Seprafilm® in adhesion prevention in mice.
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1. Introduction

Postoperative intra-abdominal adhesions are accepted as a regular consequence of surgical intervention and occur after almost all abdominal procedures, ranging from 67 to 93% of all operations, thus believed to be one of the most challenging problems in current medical practice.[1] Although most of the postoperative adhesions do not cause any further complications, studies have revealed that intra-abdominal adhesions are responsible for 50–70% of hospitalizations due to small bowel obstruction. Other sequelas of adhesion related morbidity include chronic abdominal pain and female infertility.[2–5] They may also increase the risk of inadvertent visceral injuries during the entry to the abdomen; accordingly lengthen the operation time in case of a subsequent laparotomy. Finally, these complications incur considerable economic costs.[3,6]

Seprafilm® is a sterile, bioresorbable, translucent adhesion barrier composed of sodium hyaluronate and carboxymethyl cellulose and acts as a temporary bioabsorbable barrier separating apposing tissue surfaces. The physical presence of the membrane separates adhesiogenic tissue while the normal tissue repair process takes place. The effect of Seprafilm® in reducing intra-abdominal adhesions have been confirmed by experimental and clinical trials, and it has been accepted as the best choice of product being used for this purpose.[6,7]

Heparin is an anticoagulant product that has been widely used for the prevention and treatment of deep venous thrombosis. Since, it theoretically stops fibrin formation and consequent adhesiogenesis, heparin has been extensively monitored for adhesion prevention in laboratory setting.[8] Investigations have revealed that heparin significantly reduces postoperative adhesions.[9] The optimal dose of heparin to be used for the purpose of adhesion prevention is not known. The favorable dosage of the product should decrease the severity of intra-abdominal adhesions without increasing the risk of drug-related complications, particularly bleeding. Thus, we believe that different dosages deserve to be reanalyzed in order to find out the optimal dose of the drug as an anti-adhesive product besides carefully addressing the side-effects of heparin.

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Table 1
Mazuji adhesion severity scoring system.[10]

Grade	Description
0	No adhesions
1	Slight adhesions can be separated with blunt dissection
2	Moderate adhesions can be mostly separated with blunt dissection. but require sharp dissection in less than 50% of the adhered segments
3	Severe adhesions require sharp dissection in more than 50% of the adhered segments
4	Presence of serosal injury
5	Presence of full-thickness injury

Current study aims to find out the optimal heparin dose that will decrease adhesion severity without any increase in complication risk. The results are planned to be compared with the data obtained from subjects who received Seprafilm® that has been accepted the choice of product used for adhesion prevention.

2. Materials and methods

The study was approved by Kartal Education and Research Hospital Ethics and Education Planning Committee (EPK), and operations were performed at Experimental Medicine Research Center (DETAM), Istanbul University Medical School.

A total of 75 Balb/c mice were subjects of the study. Animals were fed with a standard laboratory diet, given tap water and kept in metal cages at room temperature. All procedures were performed under clean but non-sterile conditions by one surgeon (ZC). Anesthesia was achieved with intraperitoneal 30 mg/kg ketamine HCL (Ketalar®, Parke-Davis, USA) injection during operation and adjuvant ether inhalation when needed. The abdominal skin was shaved and cleansed with povidone iodine, and a 15 mm long midline laparotomy was performed. The cecum was found and exteriorized out of the abdomen. Intra-abdominal adhesions were created with a multiple abrasion model consisting of meticulous abrasion of the cecum and a 2 cm-long terminal ileum segment with strokes of a dental toothbrush. The bleeding was stopped by simple finger pressure application. Then, the subjects were randomized into five groups (15 mice in each group). Animals in groups H62.5, H125 and H250 intraperitoneally received 62.5 IU, 125 IU and 250 IU heparin (Nevparin® fla., Mustafa Nevzat Ilac San A.S, Istanbul, Turkey) in 2 cc saline. A piece (10×30 mm) of Seprafilm® (Genzyme Biosurgery, Cambridge, MA, USA) was placed over the viscera under the incision in Group S animals and saline (2 cc) was intraperitoneally applied in the Control group (Group C). Animals were marked with special ear cuts for further identification after the closure of the abdominal incision with double layer sutures. If an animal had died prior to the sacrification, an immediate autopsy was carried out by the operating surgeon in order to reveal the cause of death.

Animals were sacrified on day 14 with Phenobarbital overdose. Evaluation was done via an U-shape abdominal incision by an investigator blinded to the randomization (MO). The severity of adhesions was evaluated with a scoring system ranging from 0 to 5 developed by Majuzi (Table 1).[10] In addition, the presence/absence of adhesions was assessed in five locations: between cecum and cecum (cecum over itself), cecum and fatty tissue, cecum and small intestine, cecum and abdominal wall; and cecum and liver[11] (Fig. 1). Finally, the total number of adhesion-positive locations among the 5 investigated sites was named as 'location score' ranging between 0 and 5, and calculated for each animal. The data were compared within the groups.



Fig. 1. Grade 3 adhesions between cecum and small intestine.

Table 2
Severity of adhesions and comparisons of the results within the groups.

Grade	Group C ($n = 14$) (%)	Group H62.5 ($n = 14$) (%)	Group H125 ($n = 15$) (%)	Group H250 ($n = 15$) (%)	Group S ($n = 15$) (%)
0	0 (0)	4 (28.6)	9 (60)	9 (60)	10 (66.7)
1	2 (14.3)	5 (35.7)	2 (13.3)	4 (26.7)	3 (20)
2	4 (28.6)	3 (21.5)	3 (20)	2 (13.3)	2 (13.3)
3	5 (35.7)	0 (0)	1 (6.7)	0 (0)	0 (0)
4	1 (7.1)	2 (14.3)	0 (0)	0 (0)	0 (0)
5	2 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)

Overall $P < 0.001$. $P < 0.05$ for group 62.5 vs. group C. $P < 0.01$ for group 125. group 250 and group S vs. group C. $P \geq 0.05$ for all other comparisons.

All statistical analyses were performed with SPSS 16.0 for Windows software. In all sample analyses, means were compared with Tukey's and Student's *t*-tests and frequency distributions, with Pearson's chi-squared or Kolmogorov–Smirnov tests. $P = 0.05$ (two-sided) was considered as the limit of significance.

3. Results

The death of 2 animals (2.7 percent) (1 in Group C and 1 in Group H62.5) left 14 subjects in Group C and Group H62.5 prior to the sacrification. The autopsies revealed that peritonitis was the cause of death in both animals, and no hemorrhage related death was observed. The mortality rates were not significantly different within the groups ($P = 0.544$).

The animals in all study groups had significantly less adhesions than those in the control group ($P < 0.05$ for all comparisons), and Majuzi Adhesion Severity Scoring System revealed no statistical difference within the study groups ($P \geq 0.05$ for all comparisons) (Table 2). Further analyses were done by questioning different locations within the abdomen for presence/absence of adhesions. These analyses showed the superiority of Seprafilm® and 3 different dosages of heparin applications over the use of saline in almost all comparisons (Table 3). Finally, the location scores were significantly less in study groups compared to control group ($P < 0.05$ for all comparisons) (Table 4). However, a borderline statistical significance was observed between the comparison of the locations scores of groups S and H62.5 ($P = 0.042$). Although no statistically significant difference was seen between the 3 heparin groups, higher doses seemed to be more effective. The results in group S and groups H250 and H125 are quite similar ($P \geq 0.05$ for all comparisons).

4. Discussion

Postoperative intra-abdominal adhesions are one of the most challenging problems in current medical practice, since they may cause subsequent complications including small bowel obstruction, chronic abdominal pain and female infertility. Search has been long continued in order to find out an effective product, and experimental and clinical studies have revealed that intrabdominal use of Seprafilm® may be the best choice to prevent adhesions. A recent multicenter prospective randomized trial has showed that Seprafilm® use may reduce the risk of reoperations due to small bowel obstructions secondary to intra-abdominal adhesions.[12] However, Seprafilm® may increase the incidence of some serious complications including anastomotic leak, peritonitis, abscess or intra-abdominal sepsis.[13] Furthermore, unusual problems such as postoperative acute aseptic peritonitis have been reported after Seprafilm® application.[14] Finally, the cost of the product makes the operation and hospitalization price more expensive. Thus, search is continuing in order to find out a novel and more effective product

Table 3
Comparison of adhesions' localizations in groups.

Localization	Group C ($n = 14$) (%)	Group H62.5 ($n = 14$) (%)	Group H125 ($n = 15$) (%)	Group H250 ($n = 15$) (%)	Group S ($n = 15$) (%)	Overall P
Cecum–cecum	6(42.9)	5(35.7)	5(33.3)	1(6.7)*	1(6.7)*	**0.034**
Cecum-fatty tissue	9(64.3)	5(35.7)	2(13.3)**	6(40)	3(20)*	**0.039**
Cecum- abdominal wall	5(35.7)	1(7.1)*	1(6.7)*	1(6.7)*	1(6.7)*	**0.014**
Cecum-liver	2(14.3)	2(14.3)	0(0)	1 (6.7)	2(13.3)	0.61
Cecum-small intestine	11(78.6)	4(28.6)**	4(26.7)**	1(6.7)**	1(6.7)**	**0.001**

*$P < 0.05$ vs. group C. **$P < 0.01$ vs. group C. Significant P values are marked in bold.

with less complication risk. On the other hand, we believe that any clinical or experimental study analyzing the effectiveness and safety of a novel anti-adhesive product needs to be compared with the outcomes after Seprafilm® use.

Intra-abdominal adhesions are formed as a result of a dynamic mechanism. Peritoneal inflammation caused by a peritoneal injury leads to the formation of an inflammatory exudate which contains strands of fibrin. The fibrinogenetic mechanism after this step is very similar to that which happens during coagulation.[2,15,16] Thus, using anticoagulant products (i.e. low molecular weight heparin [LMWH]) seems to be a bright idea for adhesion prevention, and it has been shown in experimental setting that LMWH may stop or slow down the adhesiogenesis.[17,18] Heparin is another anticoagulant product and has been widely used in clinical practice for the prevention and treatment of deep venous thrombosis. It is an acidic, anionic, sulfated glycosaminoglycan with molecular weight of 6000–20000. Only a part of the molecule in commercial use contains a specific pentasaccharide sequence which is responsible for binding to antithrombin III (AT-III). This process results in conformational changes in AT-III causing a multifold increase in its fibrin inhibitory potential. This heparin-AT-III complex also causes inhibition of activated coagulation factors IX, X, XI, and XII. Heparin has also some anticoagulant actions independent from AT-III, such as Heparin Cofactor II binding to prothrombin, direct binding to coagulant factors, and release of some endogenous anticoagulant glycosaminoglycans. In addition to anticoagulant properties, heparin has also well known anti-inflammatory effects which include anticomplement activity and inhibition of histamine, serotonine and endothelin-1 release from mast cells.[19] Since, it theoretically reduces fibrin formation and inflammation, heparin has been extensively monitored for adhesion prevention in laboratory setting.[8] These investigations have revealed that heparin significantly reduces postoperative adhesions, but the high risk of bleeding limits its routine use for this purpose.[9] A recent study from our institution has showed that 500 IU heparin might be as effective as Seprafilm® in preventing postoperative adhesions in a murine model. However, in that study, mortality secondary to intra-abdominal bleeding was significantly higher in heparin group.[20] Since there may be an optimal dose which decreases the adhesion severity without increasing the risk of bleeding, we believe that different dosages deserve to be reanalyzed as an anti-adhesive product particularly addressing the side-effects heparin. Current study aims to investigate the effectiveness and safety of three different dosages of heparin (62.5 IU, 125 IU and 250 IU) and compare the outcomes with those of Seprafilm® use in a murine setting.

Current study has revealed that heparin is an effective and safe anti-adhesive material. Through the adhesion prevention perspective, our data have revealed that both 125 IU, and 250 IU of heparin use gave similar results to Seprafilm® application ($P > 0.05$ for all comparisons). Although Seprafilm® was better than the lowest dose of heparin use (62.5 IU) in a single comparison, the statistical difference was borderline ($P = 0.042$), and was not repeated in other calculations. Most importantly, all three different dosages of heparin application gave better results than the control group in almost all comparisons. Although no statistical difference was observed between three different dosages of heparin use, the anti-adhesive effect seems to be increasing by higher doses. Thus, we believe that all three dosages of heparin may effectively reduce the intensity of postoperative intra-abdominal adhesions as well as Seprafilm®.

Drug use generally produces numerous results, but one of them is the primary goal of treatment, which is called as the desired effect. The undesired (adverse) effects may appear with different dosages, and the gap between minimum efficient concentrations of desired and adverse effects is named as therapeutic window, which reflects the concentration range that provides efficacy without causing undesired toxicity.[21] While the amount of drug is being increased, the intensity of desired effect generally escalates by an optimum dose, but stops at a level and does not rise even by more dosages. However, the severity of the side-effects continues to rise and sometimes even death may happen. The desired effect of heparin in the current and similar studies is adhesion prevention, and the main undesired toxicities are bleeding and related mortality. In a recent study from our institution has shown that 500 IU of heparin may effectively reduce intra-abdominal adhesions, but cause bleeding and consequent mortality.[20] In the current study, lower dosages of 62.5 IU, 125 IU and 250 IU were used, and our results denied any drug-related complications. However, although current data showed that mentioned dosages of heparin are safe and do not cause bleeding, we believe that further experimental studies are necessitated to evaluate the safety of heparin, since 500 IU of heparin only 2, 4 and 8 times more of that used in the current study may cause severe bleeding and statistically significant more mortality in our previous study.[21] In other words, the therapeutic window of heparin for adhesion prevention is still unknown, and deserves to be reanalyzed.

A final question to be answered is the appropriate analysis of the current data for a possible application of the heparin use in human beings for intra-abdominal adhesion prevention. Heparin seems to be a promising anti-adhesive product, which has been proven in different animal studies; but, in our opinion, further experimental analyses are necessitated before planning a clinical

Table 4
Location scores[a] and comparison of the data within the groups.

Number of Location	Group C ($n = 14$) (%)	Group H62.5 ($n = 14$) (%)	Group H125 ($n = 15$) (%)	Group H250 ($n = 15$) (%)	Group S ($n = 15$) (%)
0	0(0)	4(28.6)	8(53.3)	9(60)	9(60)
1	1(7.1)	5(35.7)	1(6.7)	3(20)	4(26.7)
2	6(42.8)	4(28.6)	4(26.7)	2(13.3)	1(6.7)
3	4(28.6)	0(0)	1(6.7)	1(6.7)	1(6.7)
4	3(21.4)	1(7.1)	0(0)	0(0)	0(0)
5	0(0)	0(0)	1(6.7)	0(0)	0(0)

Overall $P < 0.001$. $P < 0.01$ *for* all groups vs. group C. $P < 0.05$ for Group H62.5 vs. group S. $P \geq 0.05$ for all other comparisons.

[a] Location score was the total number of adhesion-positive locations among the 5 investigated sites: between cecum and cecum (cecum over itself). cecum and fatty tissue. cecum and small intestine. cecum and abdominal wall; cecum and liver.

trial because of some major concerns: First, the gap between the minimum anti-adhesive and lethal doses is quite small and the accurate therapeutic window is still unclear; thus the optimal dosage should be investigated whether or not lower doses of heparin is as effective as the dosages used in the current study. Secondly, the calculation of optimal dose for humans may be difficult since the capacities of abdominal cavity in mice and human are different. Thus, bigger animals such as dogs or pigs may be preferred during further studies. On the other hand, as LMWH has taken place of heparin in the prophylaxis of deep venous thrombosis since it does not require a close monitoring, it may be a good idea to compare this product with different dosages of heparin in the future studies.

5. Conclusions

Our study has shown that heparin is an effective and safe product for adhesion prevention in a murine model. All three dosages are as effective as Seprafilm® for adhesion prevention, but higher doses of use seem to be more effective. Further experimental studies are necessary to determine the optimal dosage of intra-abdominal heparin use for humans in the perspectives of safety and effectiveness.

Ethical approval

The study was approved by Kartal Education and Research Hospital Ethics and Education Planning Committee (EPK), and operations were performed at Experimental Medicine Research Center (DETAM), Istanbul University Medical School.

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There were not any sources of funding for our research.

Contributions of authors

Metin Kement, M.D.: Conception and design, analysis and interpretation of data, drafting the article. Zafer Censur, M.D.: Conception and design, acquisition of data. Mustafa Oncel, M.D.: Conception and design, revising of article. Mehmet E Buyukokuroglu, M.D.: Revising of article. Fazli C Gezen, M.D.: Analysis and interpretation of data.

Conflicts of interest

There were not any conflicts of interest.

References

1. Risberg B. Adhesions preventive strategies. *Eur J Surg Suppl* 1997;**577**:32–9.
2. Ellis H. The magnitude of adhesion related problems. *Ann Chir Gynaecol* 1998;**1**:9–11.
3. Ray NF, Denton WG, Thamer M, Hernderson SC, Perry S. Abdominal adhesiolysis: inpatient care and expenditures in the U.S. in 1994. *J Am Coll Surg* 1998;(186):1–9.
4. Hammoud A, Gago LA, Diamond MP. Adhesions in patients with chronic pelvic pain: a role for adhesiolysis? *Fertil Steril* 2004;**82**:1483–91.
5. Peters AA, Trimbos-Kemper GC, Admiraal C, Trimbos JB, Hermans J. A randomized clinical trial on the benefit of adhesiolysis in patients with intraperitoneal adhesions and chronic pelvic pain. *Br J Obstet Gynaecol* 1992;**99**:59–62.
6. Ray NF, Larsen JW, Stillman RJ, Jacobs RJ. Economic impact of hospitalizations for lower abdominal adhesiolysis in the United States in 1988. *Surg Gynecol Obstet* 1993;**176**:271–7.
7. Oncel M, Remzi FH, Senagore AJ, Connor JT, Fazio VW. Comparison of a novel liquid (Adcon-P) and a sodium hyaluronate and carboxymethylcellulose membrane (Seprafilm) in postsurgical adhesion formation in a murine model. *Dis Colon Rectum* 2003;**46**:187–91.
8. Gandhi NS, Mancera RL. The structure of glycosaminoglycans and their interactions with proteins. *Chem Biol Drug Des* 2008;**72**:455–82.
9. Kutlay J, Ozer Y, Isik B, Kargici H. Comparative effectiveness of several agents for preventing postoperative adhesions. *World J Surg* 2004;**28**:662–5.
10. Mazuji MK, Calambaheti K, Pover B. Prevention of adhesion with polivinil pyrrolidone. *Arch Surg* 1964;**89**:1011–5.
11. 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.
12. Fazio VW, Cohen Z, Fleshman JW, van Goor H, Bauer JJ, Wolff BG, et al. Reduction in adhesive small-bowel obstruction by Seprafilm adhesion barrier after intestinal resection. *Dis Colon Rectum* 2006;**49**:1–11.
13. Beck DE, Cohen Z, Fleshman JW, Kaufman HS, van Goor H, Wolff BG. Adhesion Study Group Steering Committee. A prospective, randomized, multicenter, controlled study of the safety of Seprafilm adhesion barrier in abdominopelvic surgery of the intestine. *Dis Colon Rectum* 2003;**46**:1310–9.
14. Remzi FH, Oncel M, Church JM, Senagore AJ, Delaney CP, Fazio VW. An unusual complication after hyaluronatebased bioresorbable membrane (Seprafilm) application. *Am Surg* 2003;**69**:356–7.
15. Holmdahl L. Making and covering of surgical footprints. *Lancet* 1999;**53**:1456–7.
16. Thompson JN, Whawell SA. Pathogenesis and prevention of adhesion formation. *Br J Surg* 1995;**82**:3–5.
17. Turkcapar AG, Ozarslan C, Erdem E, Bumin C, Erverdi N, Kutlay J. The effectiveness of low molecular weight heparin on adhesion formation in experimental rat model. *Int Surg* 1995;**80**:92–4.
18. Arikan S, Adas G, Barut G, Toklu AS, Kocakusak A, Uzun H, et al. An evaluation of low molecular weight heparin and hyperbaric oxygen treatment in the prevention of intra-abdominal adhesions and wound healing. *Am J Surg* 2005;**189**:155–60.
19. Erke HP, Fjellner B, Hagermark O. Inhibition of complement dependent experimental inflammation in human skin by different heparin fractions. *Int J Immunopharmacol* 1986;**8**:277–86.
20. Bahadir I, Oncel M, Kement M, Sahip Y. Intra-abdominal use of taurolidine or heparin as alternative products to an antiadhesive barrier (Seprafilm) in adhesion prevention: an experimental study on mice. *Dis Colon Rectum* 2007;**50**:2209–14.
21. Buxton ILO. Pharmacokinetics and Pharmacodynamics. In: Brunton LL, Lazo JS, Parker KL, editors. *Goodman and Gilman's the Pharmacological Basis of Therapeutics*. New York: McGraw-Hill; 2006. p. 1–39.