Original Article

Effects of Streptokinase and Normal Saline on the Incidence of Intra-abdominal Adhesion 1 Week and 1 Month after Laparotomy in Rats

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Abstract

Background: Intra-abdominal adhesions after surgery are usually in the form of bands and can annoy the patient throughout life causing repeated surgical procedures. Therefore, any action to prevent adhesions after surgery can increase longevity and quality of life. For this aim, this study investigates the effect of streptokinase and normal saline on the 7th day and 1 month after laparotomy. **Materials and Methods:** Experimental study was conducted on thirty healthy male Wistar rats weighing 200–250 g with age of 3 months divided into three groups of 10. Group I: No treatment, Group II: Received normal saline, and Group III: Received normal saline and streptokinase at the same time. One week and 1 month after laparotomy, the frequency of the presence or absence of adhesion bands was performed by a person who was unaware of the sample grouping. The collected information was analyzed with the SPSS software (version 16; SPSS Inc., Chicago, IL, USA). **Results:** Adhesion frequency was found to be 20% on the 7th day (early) and 1 month after laparotomy (late) for Group 1, and it was 40% on early and late for Group II, while 0% on the early and late for Group III. Hence, in the group receiving streptokinase, no early or late adhesion was observed; therefore, it had a significant role in the prevention of intra-abdominal adhesions ($P < 0.05$). However, adhesions in the group receiving normal saline had no remarkable difference with the group receiving no drug ($P > 0.05$). **Conclusion:** According to the results of our study, we believe that streptokinase could be a good antiadhesive agent considering its effectiveness.

Keywords: *Intra-abdominal adhesion, normal saline, streptokinase*

Introduction

Abdominal and pelvic adhesions are defined as pathologic bonds between peritoneal or pelvic cavities surfaces that formed during the scarring of peritoneal surface defects.[1]

Damages on peritoneal surfaces, ischemic areas, intestinal fistulas, infection, and foreign bodies (suture, power, etc.,) play an important role in the intra-abdominal adhesions formation.[2]

The risk factors including rough manipulation of tissues during surgery, excessive devascularization, former adhesions blunt dissection, serosal tissue drying, infections such as peritonitis, peritoneal endometriosis, and free intra-abdominal blood clots have been related to adhesion formation.[3]

Approximately, in 90% of the patients who underwent laparotomy, intraperitoneal adhesions would be developed, and retreatment is needed in 5% to 20% of cases.[4]

Prior abdominal surgery induced intra-abdominal adhesion account of up to 75% of cases of small bowel obstruction.[5]

Many studies stated that fibrin deposition occurs as a consequence of peritoneal trauma; fibrin may then become fibrous, giving origin to a permanent adhesion. Several substances have been used in an attempt to prevent the formation of these adhesions; particularly those that interfere with the coagulation system are interesting among them.[6-8]

To prevent the formation of adhesion bands following surgery, numerous materials have been studied, some of which are glucocorticoids, heparin, dextran 70, normal saline, antibiotics, promethazine, antihistamines, prostaglandin synthesis inhibitor, Lactated Ringer's solution, calcium channel inhibitors,[5] streptokinase as a fibrinolytic agent,[9] rofecoxib as the inhibitor of cyclooxygenase,[10] methyl blue,[11] and octreotide.[12]

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Thus, according to several studies, several intraoperative factors and drugs including fibrinolytic agents, anticoagulants, anti-inflammatory drugs, and antibiotics are effective in preventing the formation of adhesion bands.[13-17]

Therefore, in this study, it was tried to stimulate the cohesive bands by applying talc powder (as a foreign object) and then washing with normal saline and adding streptokinase to investigate the effects of the washing and streptokinase in the formation of adhesion bands. What distinguishes this study compared with previous studies is in that the adhesion bands in this study were followed-up on the 7th day and a late time (1 month).

Materials and Methods

Animals

This was an experimental study. Initially, thirty healthy male Wistar rats weighing 200–250 g with age of 3 months were selected and then they were randomly divided into three groups of 10. The study protocol was approved by Ethical Committee (code: 185265). All animals were treated in accordance to the principles of laboratory animal care.

- Group I: The control group that received no drug
- Group II: Received normal saline group
- Group III: Received normal saline + streptokinase group

The rats had not undergone previous surgery or any medical interventions. They were also in the same condition and standard in terms of nutrition, light (12 h light, 12 h dark), and temperature (23°C ± 2°C).

Surgery

Adhesion lesions were performed under anesthesia. Standard surgical procedures were performed in the same way in all the samples by one person. All three groups of rats were anesthetized using a combination of two drugs: acepromazin 0.5 mg/kg (Alfasan Co., Netherlands) and ketamine 5 mg/kg (Alfasan Co., Netherlands). After anesthesia, the samples in the three groups were put on the surgery table lying on their back, and then their abdomen skin was sterilized using betadine 10% solution, and the hair was shaved well to prepare the skin for the operation incision. Then, under sterile conditions, a two cm incision was made on the midline of the abdomen. In Group I, no drug was used. To stimulate the formation of adhesion bands in Group II, first 1 cc diluted solution of sterile talc powder was added into the abdominal cavity. Then, the abdominal cavity was washed with normal saline 5 cc and was evacuated. In Group III, similar to Group II, after laparotomy, 1 cc talc powder solution was used and then the area was washed with 5 cc normal saline solution and then suction was performed. Then, 100,000 IU/Kg equivalent to 20,000 units (IU) streptokinase was added into the abdominal cavity, and the abdominal cavity was closed. Once again, the skin was disinfected, and the rats were kept in similar conditions after anesthesia effect was disappeared, and a week later, the rats underwent another laparotomy. The findings of the presence or absence of adhesion bands were performed by a person who was unaware of the sample grouping. Adhesions were divided into three grades: grade 1 had only one adhesive band, Grade 2 had 2–3 adhesive bands, and Grade 3 had adhesion to the wall [Figure 1]. Then, in Group I and II, the abdomen was closed without specific intervention, and in Group III, 20,000 units (IU) streptokinase was added into the abdominal cavity, and the rats were taken back to the laboratory and were kept under similar circumstances. One month after the second operation, the rats underwent another laparotomy, and their abdominal cavity was fully investigated. Intestines were removed, and adhesive bands were observed and recorded.

Statistical analysis

Eventually, the data of adhesions in the abdominal cavity were recorded and entered SPSS software (version 16; SPSS Inc., Chicago, IL, USA) and were analyzed using the Fisher's exact test, Man-Whitney test, and Wilcoxon test while significance level was considered < 0.05.

Results

In this study, 30 male Wistar rats were operated on three occasions. One week after the first surgery, laparotomy results showed that based on the adhesion bands in the group without medication, only two rats (20%) had an adhesive band. In Group II, two rats (20%) had only one adhesive band, one rat (10%) had 2–3 adhesive bands and one rat (10%) had clear adhesive band to the wall, and 6 rats (60%) did not have adhesive band. It should be noted that one of the six rats suffered bleeding in the intestinal accessories during laparotomy that was controlled but expired 3 h after surgery. The results from examining 1 month after the second laparotomy indicated that in two rats with one adhesive band, we observed no increase in the number of bands. Furthermore, the rat with an adhesion in bands of 2–3 shows no change in this regard and the rat with an adhesive band to wall again shows the same adhesive band to wall as before. In other 5 rats, no adhesive band was observed. Furthermore, in Group III, we did not observe any adhesive band in the next month [Figure 2].

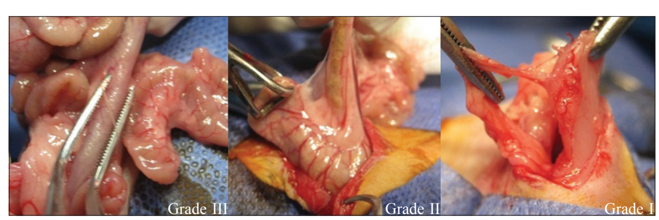

Figure 1: Grading adhesions in the abdominal cavity; Grade I had only one adhesive band, Grade II had II-III adhesive bands, and Grade III had adhesion to the wall

On the other hand, the mean of adhesion degree in Groups I, II, and III and in 1 week after laparotomy were equal to 1, 1.75, and 0, respectively, and the degree of adhesive bands by passing time and 1 month later remained without any change.

Statistically, we observed no change in the status of adhesive bands in all of these three groups in the range of 1 week to 1 month after laparotomy ($P = 1$). However, there was no difference between Group I and II in the mean of adhesion degree ($P > 0.05$); but the mean of adhesion degree in Group III was significantly lower than Groups I and II ($P < 0.05$) [Table 1].

A considerable point in the survey was that in the rats in Group III, the growth of the shaved hair on the abdomen after the 1st week was clearly more and the surgery scar in macroscopic terms was gentler and less thick and no hypertrophic scar was evident in Group III [Figure 3].

Discussion

Postoperative intra-abdominal adhesions represent a serious clinical problem. Adhesions cause difficulty in entering the abdomen, bleeding that is hard to control, anatomical structures deformity, delays in surgery period, and iatrogenic intestinal damages. Morbidity and mortality rates increase depending on all these.[18-20]

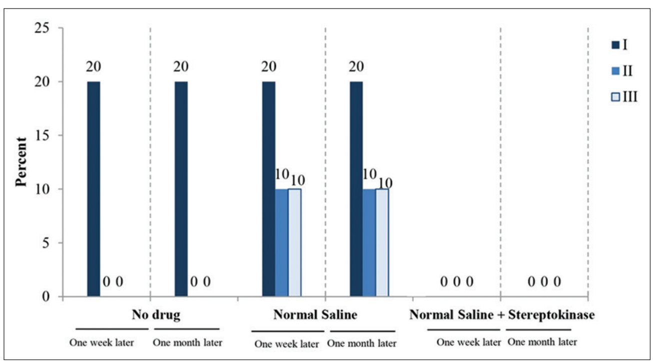

Figure 2: Grade of adhesions in the 7th day and 1 month after laparotomy in three groups

According to the results of this study, formation of postoperative abdominal adhesion reached the highest level in 7 days, and after a month, it did not change much.

Studies in literature report that trauma on peritoneal surfaces, ischemic areas, intestinal fistulas, infection, and foreign bodies (suture, powder, etc.,) play an important role in the formation of intra-abdominal adhesions.[20] Following trauma on peritoneal surfaces, vasoactive material and cytokine secretion take place and consequently, protein-rich liquid accumulates in the intraperitoneal area. This coagulum accumulated in the peritoneum causes adhesions. As a result, secretion of collagen by the fibroblasts accumulated in the medium beginning with day one and the maturation of adhesions happens. Adhesion reaches its top level in the first 7 days, and then, fibrinolytic activity becomes dominant after 7 days.[21,22]

The aim of this study was preventing the accumulation of collagen and involvement in the degradation of fibrin. For this purpose, streptokinase was used and compared with normal saline. According to results, rats receiving normal

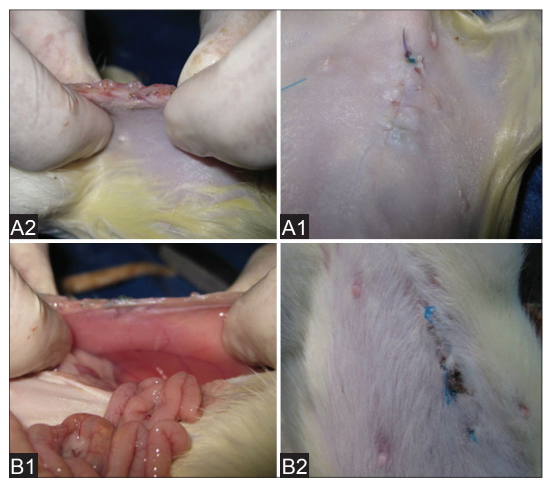

Figure 3: Images of scars and hair growth in Groups II and III. A1: the scar condition and A2: hair growth in rats receiving normal saline. B1: The scar condition and B2: Hair growth in rats receiving normal saline with streptokinase

Table 1: Comparison of adhesion grades of three groups

Follow-up	Group I (*n*=10)	Group II (*n*=10)	Group III (*n*=10)	*P** (Group I versus Group II)	*P** (Group I versus Group III)	*P** (Group II versus Group III)
7 days						
Mean	1	1.75	0	0.273	0.003	<0.001
Median	1	1.50	0			
SD	0	0.96	0			
1 month						
Mean	1	1.75	0	0.273	0.003	<0.001
Median	1	1.50	0			
SD	0	0.96	0			
*P*** (7 days versus 1 month)	1	1	1	-	-	-

*Used of Man–Whitney test, **Used of Wilcoxon test. Group I: The control group that received no drug, Group II: Received normal saline group, Group III: Received normal saline+streptokinase group. SD: Standard deviation saline with streptokinase did not have any adhesion 7th days and 1 month after surgery had a significant difference with other two groups ($P < 0.05$). The mean of adhesion degree was no difference between Group I and II ($P > 0.05$); but in Group III, the mean of adhesion degree was significantly lower than Groups I and II ($P < 0.05$). This in fact is an expression of streptokinase effect on preventing intra-abdominal adhesions.

In the line of the current study, Smaniotto *et al*. in 1997 found the rats that had received streptokinase by the intraperitoneal route or by the intraperitoneal and intravenous routes presented a significant reduction in the formation of adhesions in terms of number and thickness on the 3rd postoperative day and also in the organs involved when compared to controls injected with isotonic saline alone. As a result, significant difference was found in the number of diagnosed adhesions between the two control groups and streptokinase group.[9]

Furthermore, Jafari-Sabet *et al*. (2015) investigated the effects of pentoxifylline and streptokinase alone or both of them together on postoperative intra-abdominal adhesion formation in adult female NMRI mice. The results suggested that pentoxifylline with streptokinase may reduce postoperative intra-abdominal adhesion formation through improving local fibrinolytic activity.[23]

In fact, the use of streptokinase by the intraperitoneal route may determine the lysis of the fibrin clot by direct contact with the injured area; during the intravenous administration, the drug may act systemically and reaches the site of tissue damage simultaneously with fibrin deposition. When the two routes, intraperitoneal and intravenous, are combined, there may be a summation of the topic effect with the systemic effect.[9]

An ideal antiadhesive agent is still yet to be found. It is, however, possible to classify antiadhesive products being used into 4 main groups:

1. Preventing fibrin storage[24]
2. Removal of fibrin stores[25,26]
3. Mechanical separations of visceral surfaces by adhesion barriers[27,28]
4. Prevention of fibroplasias.[29]

Furthermore, previous studies have shown a beneficial effect of saline in preventing postoperative adhesion formation.[30,31] In a carbon dioxide pneumoperitoneum-enhanced adhesion model, normal saline and Ringer's lactate administration immediately after surgery was shown to reduce adhesion formation.[31] Furthermore, Ward and Panitch reviewed current or proposed therapies for abdominal adhesions. They found out with the current state of knowledge about many therapies for abdominal adhesion, a definitive therapy for prevention or reduction of abdominal adhesions still is required.[32]

Cakir *et al*. studied a total of 40 rates in four groups (Group 1: The control group; Group 2: Normal saline group; Group 3: Sterile Novuxol group; Group 4: Where the intraperitoneal and systematic effects of sterile Novuxol were examined). Finally, they suggested that sterile Novuxol can be a good antiadhesive agent due to its ease of use, nontoxicity, and effectiveness.[17]

Correia reviewed published surgical literature on epidemiology, pathogenesis, and various prevention strategies of adhesion formation and found out among several preventive measures, bioresorbable membranes as site-specific antiadhesion products may be more difficult to use in laparoscopy while liquids and gels with more widespread area of action are easier to use in laparoscopy. However, effective pharmacologic agents are under development. Most of the results in this study were contradictory. According to this study, none of modalities to reduce the risk of adhesion have become standard applications.[33]

Comparing the number of adhesions in each of the similar groups, 7 days and 1 month after surgery showed no significant difference ($P > 0.05$). In fact, according to the condition of the study that was conducted at two time levels, early and delayed postoperative band formation, it was observed that these bands significantly increased after 1 month. In other words, in the same rats with adhesive bands in the early stage, again the same amount of adhesion occurred a month later. This means that adhesions must have been formed at the beginning of the healing process. These findings agree with those reported by Wright *et al*., who observed a smaller number of adhesions in rabbits when they used streptokinase and streptodornase.[34] Similar data were reported by Luttwak *et al*., in a study of the prevention of adhesions in rats induced by the presence of talcum in the peritoneal cavity.[35]

Conclusion

Regarding the lack of adhesion and lack of delayed adhesion, it seems that for the formation of adhesion bands, in addition to the previously mentioned factors, perhaps genetic factors and inflammatory tissue reaction are effective in every living being.[36] Therefore, it suggested more and better investigations using more extensive sample size and more surgery operations within longer time to find out whether increased adhesive bands occurs over time or not. Moreover, whether fibrinolytic interventions can a play significant role in the process of hair growth or wound healing?

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Conflicts of interest

There are no conflicts of interest.

References

1. Trimbos-Kemper TC, Trimbos JB, van Hall EV. Adhesion formation after tubal surgery: Results of the eighth-day

laparoscopy in 188 patients. Fertility and sterility. 1985;43(3):395-400.
2. Kağızman SH, Belviranlı M, Şahin M, Vatansev C, Karahan Ö, Alptekin H. Mekanik intestinal obstrüksiyona bağlı opera edilmiş hastaların klinik analizi. T Klin. 1997;17:203-9.
3. Leach RE, Burns JW, Dawe EJ, SmithBarbour MD, Diamond MP. Reduction of postsurgical adhesion formation in the rabbit uterine horn model with use of hyaluronate/carboxymethylcellulose gel. Fertil Steril 1998;69:415-8.
4. Schnüriger B, Barmparas G, Branco BC, Lustenberger T, Inaba K, Demetriades D. Prevention of postoperative peritoneal adhesions: A review of the literature. Am J Surg 2011;201:111-21.
5. Monk BJ, Berman ML, Montz FJ. Adhesions after extensive gynecologic surgery: Clinical significance, etiology, and prevention. Am J Obstet Gynecol 1994;170(5 Pt 1):1396-403.
6. Ryan GB, Grobéty J, Majno G. Postoperative peritoneal adhesions. A study of the mechanisms. Am J Pathol 1971;65:117-48.
7. Schade DS, Williamson JR. The pathogenesis of peritoneal adhesions: An ultrastructural study. Ann Surg 1968;167:500-10.
8. Nisell H, Larsson B. Role of blood and fibrinogen in development of intraperitoneal adhesions in rats. Fertil Steril 1978;30:470-3.
9. Smaniotto B, Biondo-Simões MD, Artigas GV, Silva AD, Collaço LM, Ramasco GV. Effect of streptokinase in the prevention of intra-abdominal adhesions in the rat. Acta Cir Bras 1997;12:240-5.
10. Aldemir M, Oztürk H, Erten C, Büyükbayram H. The preventive effect of Rofecoxib in postoperative intraperitoneal adhesions. Acta Chir Belg 2004;104:97-100.
11. Cetin M, Ak D, Duran B, Cetin A, Guvenal T, Yanar O. Use of methylene blue and N,O-carboxymethylchitosan to prevent postoperative adhesions in a rat uterine horn model. Fertil Steril 2003;80 Suppl 2:698-701.
12. Alatas E, Günal O, Alatas O, Colak O. Octreotide prevents postoperative adhesion formation by suppressing peritoneal myeloperoxidase activity. Hepatogastroenterology 2000;47:1034-6.
13. Jackson JK, Skinner KC, Burgess L, Sun T, Hunter WL, Burt HM. Paclitaxel-loaded crosslinked hyaluronic acid films for the prevention of postsurgical adhesions. Pharm Res 2002;19:411-7.
14. Cashman J, Burt HM, Springate C, Gleave J, Jackson JK. Camptothecin-loaded films for the prevention of postsurgical adhesions. Inflamm Res 2004;53:355-62.
15. Nehéz L, Vödrös D, Axelsson J, Tingstedt B, Lindman B, Andersson R. Prevention of postoperative peritoneal adhesions: Effects of lysozyme, polylysine and polyglutamate versus hyaluronic acid. Scand J Gastroenterol 2005;40:1118-23.
16. 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.
17. Cakir M, Tekin A, Kucukkartallar T, Yilmaz H, Belviranli M, Kartal A. Effectiveness of collagenase in preventing postoperative intra-abdominal adhesions. Int J Surg 2013;11:487-91.
18. Khoury W, Abu-Abeid S, Person B, Klausner JM, Kariv Y. Missed inadvertent gastrointestinal injuries during abdominal operations: Characteristics, diagnosis, and treatment. Am Surg 2012;78:46-50.
19. Malik AM, Shah M, Pathan R, Sufi K. Pattern of acute intestinal obstruction: Is there a change in the underlying etiology? Saudi J Gastroenterol 2010;16:272-4.
20. Arung W, Meurisse M, Detry O. Pathophysiology and prevention of postoperative peritoneal adhesions. World J Gastroenterol 2011;17:4545-53.
21. Studer P, Mennicke M, Inderbitzin D. Adhesions and abdominal pain. Ther Umsch 2011;68:468-72.
22. VandeVord PJ, Matthew HW, DeSilva SP, Mayton L, Wu B, Wooley PH. Evaluation of the biocompatibility of a chitosan scaffold in mice. J Biomed Mater Res 2002;59:585-90.
23. Jafari-Sabet M, Shishegar A, Saeedi AR, Ghahari S. Pentoxifylline increases antiadhesion effect of streptokinase on postoperative adhesion formation: Involvement of fibrinolytic pathway. Indian J Surg 2015;77 Suppl 3:837-42.
24. Kirdak T, Uysal E, Korun N. Assessment of effectiveness of different doses of methylprednisolone on intraabdominal adhesion prevention. Ulus Travma Acil Cerrahi Derg 2008;14:188-91.
25. Sahin M, Cakir M, Avsar FM, Tekin A, Kucukkartallar T, Akoz M. The effects of anti-adhesion materials in preventing postoperative adhesion in abdominal cavity (anti-adhesion materials for postoperative adhesions). Inflammation 2007;30:244-9.
26. Stepanian S. The results of use of the antiadhesive seprafilm barrier in adhesive disease of abdomen. Georgian Med News 2011; 194:12-8.
27. Yeo Y, Bellas E, Highley CB, Langer R, Kohane DS. Peritoneal adhesion prevention with an *in situ* cross-linkable hyaluronan gel containing tissue-type plasminogen activator in a rabbit repeated-injury model. Biomaterials 2007;28:3704-13.
28. Jenkins ED, Melman L, Desai S, Brown SR, Frisella MM, Deeken CR, *et al.* Evaluation of intraperitoneal placement of absorbable and nonabsorbable barrier coated mesh secured with fibrin sealant in a New Zealand white rabbit model. Surg Endosc 2011;25:604-12.
29. Kucukozkan T, Ersoy B, Uygur D, Gundogdu C. Prevention of adhesions by sodium chromoglycate, dexamethasone, saline and aprotinin after pelvic surgery. ANZ J Surg 2004;74:1111-5.
30. Elkelani OA, Molinas CR, Mynbaev O, Koninckx PR. Prevention of adhesions with crystalloids during laparoscopic surgery in mice. J Am Assoc Gynecol Laparosc 2002;9:447-52.
31. Larsson B, Lalos O, Marsk L, Tronstad SE, Bygdeman M, Pehrson S, *et al.* Effect of intraperitoneal instillation of 32% dextran 70 on postoperative adhesion formation after tubal surgery. Acta Obstet Gynecol Scand 1985;64:437-41.
32. Ward BC, Panitch A. Abdominal adhesions: Current and novel therapies. J Surg Res 2011;165:91-111.
33. Correia AR. Adhesion prevention in laparoscopic surgery. J Gynecol Surg 2014;30:196-203.
34. Wright LT, Smith DH, Rothman M, Quash ET, Metzger WI. Prevention of postoperative adhesions in rabbits with streptococcal metabolites. Proc Soc Exp Biol Med 1950;75:602-4.
35. Luttwak EM, Feldman JD, Neuman Z. Effect of streptokinase-streptodornase on peritoneal talc adhesions and granulomas; experimental study. AMA Arch Surg 1954;68:69-75.
36. Hellebrekers BW, Trimbos-Kemper TC, Trimbos JB, Emeis JJ, Kooistra T. Use of fibrinolytic agents in the prevention of postoperative adhesion formation. Fertil Steril 2000;74:203-12.