Clinical Science

Preventing intraperitoneal adhesions with linezolid and hyaluronic acid/carboxymethylcellulose: a comparative study in cecal abrasion model

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KEYWORDS:
Intraperitoneal adhesion; Hyaluronic acid + carboxymethylcellulose; Linezolid

Abstract

BACKGROUND: We aimed to compare the effectiveness of linezolid in preventing intraperitoneal adhesions with hyaluronic acid + carboxymethylcellulose (Seprafilm).

METHODS: Thirty rats were divided randomly into 3 groups: Group I (control), untreated; Group II (Seprafilm); and Group III (linezolid). All rats were sacrificed on the 14th day after surgery. Macroscopic adhesion, inflammation, and fibrosis were evaluated.

RESULTS: The multiple comparisons between groups showed a statistically significant difference for adhesion. There were statistically significant differences between Group I and II and I and III, but no statistically significant difference between Group II and III. The multiple comparisons between the groups showed a statistically significant difference for inflammation and fibrosis. For inflammation and fibrosis, there was a statistically significant difference between Group I and II and I and III, but no statistically significant difference between Group II and III.

CONCLUSION: The efficiency of linezolid in reducing the formation of intraperitoneal adhesions was statistically significant compared with the control group.

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Approximately 90% of the patients who underwent laparotomy develops intraperitoneal adhesions and retreatment is needed in 5% to 20% of these cases.1 Because of these adhesions, patients are exposed to the risk of repetitive surgical interventions, which increased cost, morbidity, and mortality as well. Fibrinogen-rich fluid occupies the site of injury with the increase of bleeding and vascular permeability immediately after injury.1

At the same time, inflammatory process begins with the migration of inflammatory cells to the injury site, release of cytokines, and starting of coagulation cycle.1 Decreased fibrinolytic activity in the peritoneal cavity is currently accepted as a key mechanism for intra-abdominal adhesion formation by disrupting the balance between coagulation and fibrinolytic activity.1,2 After peritoneal injury, in the absence of fibrinolysis in the first 5 to 7 days, a transient fibrin matrix and collagen-secreting fibroblasts are organized and they lead to the
formation of intraperitoneal adhesions. Mechanical barriers such as hyaluronic acid + carboxymethylcellulose (Seprafilm), oxidized regenerated cellulose (interceed), and polylactic acid and also many anti-inflammatory agents such as 3-hydroxy 3-methylglutaryl coenzyme A reductase inhibitors (statins), melatonin, and collagen are used to prevent intraperitoneal adhesions after surgery. Linezolid, an antibiotic of the oxazolidine group, is used particularly for antibiotic-resistant Gram (−) positive coccus infections since 2000. Aytan et al. in their experimental study, stated that IV linezolid use after gynecologic surgery reduced intraperitoneal adhesions. The long-term use of linezolid may have a variety of clinical and laboratory adverse side effects in the literature. To avoid the side effects associated with the use of long-term linezolid, short-term use was thought to be more safe and this study was planned.

In our study, we aimed to evaluate the effects of intraperitoneal single-dose injection (100 mg/kg) of linezolid, comparing with hyaluronic acid + carboxymethylcellulose, which is previously proved to be effective in many clinical and experimental studies.

Patients and Methods

Animals and preoperative preparation

The study was approved by the Ondokuz Mayis University Local Ethics Committee for Animal Experiments on November 29, 2010 (number: 2010/73). Thirty Wistar Albino rats, female, weight ranging from 250 to 300 g were used in the study. A sample size of 10 animals for each group was calculated by using the mean difference of 1.5 units, standard deviation .8 units, confidence interval 95%, and at least 90% test power. All rats were fed ad libitum with standard rat chow and tap water. All subjects were shaved and painted with iodine skin preparations of 1.5 units, standard deviation .8 units, confidence interval 95%, and at least 90% test power. All rats were fed ad libitum with standard rat chow and tap water. All subjects were kept in 12-hour darkness and 12-hour light before and after study (at a standard temperature of −22°C). The subjects were under observation for at least 48 hours before they were included in the study. After 12 hours of starvation, the subjects were weighed and divided randomly into 3 groups.

Group I (control): operative procedure without further treatment
Group II (hyaluronic acid [HA] + carboxymethylcellulose [CMC] – Seprafilm; Genzyme Corporation, Cambridge, MA)
Group III (linezolid – Zyvoxid, 300 mL of infusion solution; Pfizer, Istanbul, Turkey)

Operative technique

A total of 50 mg/kg ketamine (Ketalar; Pfizer) with 10 mg/kg Xylazine HCl (Rompun; Bayer, Istanbul, Turkey) were given to all animals for anesthesia. The abdominal skins of all subjects were shaved and painted with iodine povidone under general anesthesia. All surgical procedures were performed under sterile conditions. Cecum abrasion model was used to create adhesions. Laparotomy was performed with midline incision of approximately 2 cm. After detecting the cecum, an abrasion of about 1 × 1 cm in diameter was performed with a sterile toothbrush on the antimesenteric surface (side) by the blinded surgeon until punctate bleeding and serosal petechiae on the intestinal surfaces occurred. In Group I, we only performed abrasion and in Group II we placed 2 × 1 cm of Seprafilm between cecum and abdominal wall after abrasion. In Group III, a solution of linezolid with a dose of 100 mg/kg was given to the abdominal cavity after abrasion. Midline incisions of all subjects were closed with 3/0 silk suture after surgical procedures. All rats were returned to their cages after the operation and kept at an ambient temperature of 22°C. They were fed with standard rat diet after surgery. None of the study animals died or had clinical evidence of adverse events (eg, signs of wound infection, anorexia, vomiting, diarrhea, or altered behavior). All rats were sacrificed with high dose of anesthetic on the 14th day after surgery. After being sacrificed, a repeat laparotomy through the same midline incision was performed immediately, and cecum and the abdominal sidewall were evaluated for adhesion.

Macroscopic evaluation

Macroscopic evaluation of intraperitoneal cavity was performed by a surgeon blinded to group allocation. Seprafilm is absorbed within 7 days. Therefore, the data analysis was performed in a blinded fashion. Majuzi classification is used for the evaluation of postoperative intraperitoneal adhesions. According to the classification, the following results were absorbed: Grade 0: no adhesion; Grade 1: very tiny and irregular adhesion; Grade 2: easily separable medium intensity adhesion; Grade 3: intense, not easily separable regular adhesion; and Grade 4: very hard, not easily separable, and homogeneous adhesion.

Histopathologic evaluation

After macroscopic evaluation, samples were taken from the fibrous bands between cecum and peritoneum and histopathologic examination was performed by pathologist blinded to groups. Tissue samples were fixed for 12 hours in a 10% buffered neutral formalin solution. After a routine follow-up, samples were embedded in paraffin blocks and sections of 4- to 5-μm thickness were stained with hematoxylin and eosin, and finally examined under light microscope. Semiquantitative scoring system was used to assess fibrosis and inflammation. According to the scoring system, fibrosis was scored as follows: 0: none, 1: minimal, loose, 2: moderate, and 3: florid dense; inflammation was scored as follows: 0: none, 1: giant cells, lymphocytes,
plasma cells, 2: giant cells, plasma cells, eosinophils, neutrophils, and 3: many inflammatory cells, microabcess.

**Statistical analyses**

Statistical analysis was performed using SPSS version 15.0 (SPSS, Inc, Chicago, IL) for Windows Vista. Data were expressed as mean ± standard deviation (SD) values. Kruskal–Wallis test and Mann–Whitney U test were used for multiple and binary comparisons of groups, respectively. P values of less than .05 were considered to be statistically significant.

**Results**

The macroscopic intraperitoneal adhesion scores (mean ± SD) for Groups I, II, and III were 3.0 ± .70, 1.3 ± .90, and 1.3 ± .82, respectively. In multiple comparisons of the groups, less adhesions were detected in Group II and III than in Group I and the difference between the groups was statistically significant (P = .001) (Table 1). In binary comparisons of the groups, there were statistically significant differences between Group I and II (P = .001) and Group I and III (P = .001), but no statistically significant difference between Group II and III was noted (P = .796) (Table 2).

After histopathologic evaluation, for Groups I, II, and III, the inflammation scores (mean ± SD) were 2.2 ± .67, .8 ± .60, and .9 ± .32 and the fibrosis scores were 2.0 ± .87, .7 ± .47, and .8 ± .42, respectively. Less inflammation and fibrosis were seen in Group II and III than in Group I and the differences were statistically significant (P < .001 and P = .002, respectively) (Table 1). In binary comparisons for inflammation, there were statistically significant differences between Group I and II (P = .002) and Group I and III (P = .001), but no significant difference between Group II and III was observed (P = .684).

In binary comparisons for fibrosis, statistically significant differences were detected between Group I and II (P = .005) and Group I and III (P = .009), but no significant difference between Group II and III was observed (P = .739) (Table 2, Figs. 1–3).

**Comments**

Strategies and researches destined to the prevention of postoperative intraperitoneal adhesions have a history for more than 100 years. Many clinical and animal experimental studies about therapy methods were realized to decrease the frequency and severity of intraperitoneal adhesion formation after peritoneal injuries. However, despite this great interest on this issue, the formation of intraperitoneal adhesions remains an important and common side effect of intra-abdominal surgery and there is still no clear strategy for the prevention of intraperitoneal adhesions. Proposed mechanisms to reduce the formation of adhesions are as follows: 1, to reduce the 1st inflammatory response and the release of exudate; 2, to block the coagulation of the exudate; 3, to enhance fibrinolysis; 4, to separate mechanically fibrin-coated surfaces; and 5, to prevent the proliferation of fibroblasts. The most effective way to reduce intraperitoneal adhesions is perhaps a meticulous hemostasis, to follow basic surgical principles to minimize the tissue trauma and ischemia, and to prevent infections.13–15 There are many experimental models to form peritoneal adhesions: the damaged uterine horn model, the ileal transection model, the large bowel anastomosis model, the peritoneal damage model, the bacterial peritonitis model, the scraping model, the suturing model, and the electrocautery model. The scraping model is very effective in causing peritoneal adhesions.16,17 A previous laboratory study on the development of post operative intra-abdominal adhesions showed that serosal scarification by abrasion is one of the best methods for inducing adhesion formation.18 Because the cecal model is one of the most widely used models in literature, we preferred the same model for inducing intra-abdominal adhesion in this study. Because a time period of 7th and 10th postoperative days is crucial in preventing adhesion formation,2 all rats were sacrificed at the 14th day after surgery as in the studies of Avsar et al,3 Kutlay et al,14 and Maghsoudi and Askary.16

Many local or systemic pharmacologic agents and many mechanical barriers were used to prevent intraperitoneal adhesions.14 Streptokinase, antioxidants, anti-inflammatory agents, antihistamines, nitric oxide, and recombinant tissue

| Table 1 | The results of multiple comparisons of the groups by Kruskal–Wallis test and Mann–Whitney U test
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<tr>
<td></td>
<td>Group I (mean ± SD)</td>
<td>Group II (mean ± SD)</td>
<td>Group III (mean ± SD)</td>
<td>P value</td>
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<tr>
<td>Adhesion</td>
<td>3.0 ± .70</td>
<td>1.3 ± .90</td>
<td>1.3 ± .82</td>
<td>.001 (S)</td>
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<tr>
<td>Inflammation</td>
<td>2.2 ± .67</td>
<td>.8 ± .60</td>
<td>.9 ± .32</td>
<td>.000 (S)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>2.0 ± .87</td>
<td>.7 ± .47</td>
<td>.8 ± .42</td>
<td>.002 (S)</td>
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S = significant; SD = standard deviation.

| Table 2 | The results of binary comparisons of the groups by Mann–Whitney test
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<td></td>
<td>Adhesion</td>
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<td>Group I/Group II</td>
<td>.001*</td>
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<td>Group I/Group III</td>
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<tr>
<td>Group II/Group III</td>
<td>.796†</td>
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*Significant.
†Nonsignificant.
Plasminogen activators are pharmacologic agents. Oxidized regenerated cellulose (interceed) and hyaluronic acid + carboxymethylcellulose (Seprafilm) are mechanical agents which are commonly used. In a meta-analysis of Cochrane database, it was expressed that Seprafilm had an effect to reduce the postoperative intra-abdominal adhesions. Seprafilm is a mechanical barrier that has been tested in both clinical and experimental studies and approved by US Food and Drug Administration. Although there are Class I and satisfactory evidences about Seprafilm reducing the incidence and severity of postoperative intra-peritoneal adhesions, there are very limited studies about small bowel obstructions. Fazio et al expressed that in 3.5-year follow-up of patients after intestinal resection, the rates of intestinal obstructions were similar in patients treated with and without Seprafilm (12% to 12%); however, patients with intestinal obstruction on whom Seprafilm was applied require surgical interventions statistically more rarely than others. In our study, the formation of macroscopic intraperitoneal adhesion in the Seprafilm group was statistically decreased. In addition, Seprafilm is used to reduce inflammation and fibrosis in microscopic examinations. There was no interobserver variable because the histopathologic evaluation was performed by a single pathologist and the histopathologic examination was repeated and confirmed at 3 different times (without regard to previous results) to overcome the interobserver variable. Because the semiquantitative scoring system has been widely used with confidence for scoring of inflammation and fibrosis in literature such as the researches of Hooker et al, Di Paola et al, and Gonzalez et al related with adhesions, wound healing, anastomoses, and so on, we preferred this method in our study also.

Linezolid, known as a quinolone included in the oxazolidine group, was shown to be a potentially important antibiotic against antibiotic-resistant mycobacteria and anaerobes. Linezolid is used to treat antibiotic-resistant Gram-positive infections and received US Food and Drug Administration approval in 2000. Bioavailability of intravenous or oral intake of linezolid is close to 100%. Clinical studies showed that linezolid is a safe and effective drug. However, post-treatment studies demonstrated that long-term linezolid use may be related with peripheral or optic neuropathy and hematologic abnormalities. One of the

**Figure 1** (A) Grade 3 fibrosis: in the control group, diffuse and dense fibrosis associated with intensive inflammation and microabscess formation (arrow) are present (H&E ×10). (B) Grade 3 inflammation: in the control group, many inflammatory cells including giant cells (arrow), neutrophils, eosinophils, plasma cells, and lymphocytes in fibrotic areas are present (H&E ×10). H&E = hematoxylin and eosin.

**Figure 2** (A) Grade 1 fibrosis: minimal and loose fibrotic areas in the HA + CMC group (H&E ×10). (B) Grade 1 inflammation: scattered lymphocytes and plasma cells of fibrotic areas in the HA + CMC group (H&E ×20). CMC = carboxymethylcellulose; HA = hyaluronic acid; H&E = hematoxylin and eosin.
possible mechanisms of linezolid in reducing postoperative adhesions is that linezolid has bacteriostatic effect on the contaminated peritoneal cavity. Another mechanism can be the suppression of synthesis of cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor-α by the immunomodulatory effect of linezolid. Probably because of this mechanism, inflammation was observed to be decreased in the linezolid group. The study by Aytan et al is known to be the only study about linezolid in the medical literature. In this study, they stated that the administration of intravenous (IV) linezolid during preoperative 3 days and postoperative 14 days reduces the formation of intraperitoneal adhesions and that only preoperative or postoperative application does not reduce adhesions compared with control subjects. However, long-term use of linezolid can give rise to resistant micro-organisms. In our study, we used the most effective dose of linezolid (100 mg/kg) as stated in the study of Aytan et al, but we applied one single dose intraperitoneally. Compared with the control group, the formation of macroscopic adhesions was statistically significantly reduced. Furthermore, linezolid was found to reduce inflammation and fibrosis in histopathologic examination.

Although the use of a single dose of intraperitoneal linezolid in our study may seem contradictory with the study of Aytan et al, there are many different studies related with the effective dose and usage length of linezolid. In the experimental study of Sacar et al, the use of linezolid at a dose of 50 mg/kg by IV route for 7 days was expressed to be effective in preventing bacterial reproduction. But, in other experimental study with linezolid, Sacar et al stated that a single intraperitoneal dose of 10 mg/kg was effective in preventing bacterial growth.

In summary, single-dose application of linezolid was shown to reduce macroscopic intraperitoneal adhesions significantly in experimental cecal abrasion model. When compared with HA + CMC combination, which was previously proven to be effective, there was no statistically significant difference. Considerably, the possible side effects of linezolid will be prevented with the use of a single dose of linezolid as in our study. In addition, linezolid seems to be more effective in reducing intraperitoneal adhesions in general histopathologic terms compared with the control group and has similar effects with HA + CMC. In the study, the intra-abdominal adhesion formation was determined to be less intense in the linezolid group than in the control group. As a consequence, linezolid might reduce the clinical complications of postoperative adhesions. However, as our study is the 1st experimental study ever in medical literature, further experimental and clinical researches are necessary.

References


**Figure 3**  (A) Grade 1 fibrosis: minimal and loose fibrotic areas in the linezolid group (H&E ×20). (B) Grade 1 inflammation: scattered lymphocytes and plasma cells of fibrotic areas in the linezolid group (H&E ×10). H&E = hematoxylin and eosin.