Original Article

The effect of Suprogel® - macromolecule polysaccharide - on the formation of postoperative abdominal adhesions

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Abstract. Abdominal adhesion is one of the major causes of intestinal obstruction, primary and secondary female infertility, chronic pelvic pain, and inconveniences in the future operations. Postoperative adhesions develop after 90% of all laparotomies and they become mostly the reason for 54% to 74% of small bowel obstructions, which need additional surgical correction by the rate of 41% to 44%. The aim of this study is to evaluate the efficacy of Suprogel® - macromolecule polysaccharide - in the protection against formation of postsurgical adhesion in a standardized rat experimentally pathological model. Twenty rats with induced peritoneal adhesion were randomly divided into two groups. Rats in group 1 received no medication, while ingroup 2 rats Suprogel® was applied. Adhesions were evaluated blindly 21 days following the surgical operation. Adhesion tissue samples were microscopically evaluated. Tissue hydroxyproline and collagen concentrations were measured. The adhesion grading and histopathological adhesion score were significantly lower in Suprogel group, but peritoneal hydroxyproline levels did not differ significantly between the groups. Consequently, the present study suggested that intraperitoneal administration of Suprogel® might be effective to reduce the incidence of postoperative adhesions in this experimental model.

Key Words: Suprogel, abdominal adhesion, polysaccharide.

Peritoneal adhesions defined as fibrous bands of tissues that connect physiologically separated organs together are commonly seen as postoperative complications [1]. Abdominal adhesions are the major causes of intestinal obstruction, primary and secondary female infertility, chronic pelvic pain, and difficulties during some emerging pathologies in near future [2,3]. Postoperative adhesions are complicated following 90% of all laparotomies and they become mostly the reason for 54 to 74% of small bowel obstructions, which need additional surgical correction by the rate of 41 to 44% [1,3,4]. Even physiological wound healing process, ischemia, intestinal fistulas, foreign bodies, trauma, and infections are the major etiological factors for developing postoperative adhesions. Severity of complications can be decreased by the prevention of adhesions [3]. A wide variety of adjunctive treatments including fibrinolytic agents, anticoagulants, sodium citrate, corticosteroids, calcium channel blockers, antihistamines, pepsin, trypsin, non-steroidal anti-inflammatory drugs, tissue plasminogen activator, antibiotics, mechanical barriers created by instilling products such as Dextran and immunosupressor have been studied to prevent the formation of adhesions have been investigated [5-13].

Recently, 0.5% ferric hyaluronate gel was reported to reduce the number, severity, and extent of adhesions in experimental studies [2,14,15]. Suprogel® is a macromolecule polysaccharide in a kind of structure which is similar to body elements and possesses positive electricity. Polysaccharide plays a role as biological barrier. It promotes epithelial proliferation and restrains fibroblast growth, thus it reduces collagenous fibers and prevents adhesions. It activates process of clotting and stops the bleeding that intensifies the adhesions. Polysaccharides also enhance immunity and have antibacterial characteristics [16]. The aim of this study was to assess the efficacy of Suprogel in the protection against formation of postsurgical adhesion in a standardized rat experimentally pathological model. This study was performed in Gulhane Military Medical Academy between October 2006 and March 2007.

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Materials and methods

Experimental procedure
This study was performed in Experimental Animal Research and Development Center Laboratory of Gulhane Military Medical Faculty. A total of 20 male Wistar rats, weighing 180-200 g, were used. Sterile surgical procedures were applied during the entire experiment. After overnight fasting, all animals were anesthetized with intramuscular 75 mg/kg of ketamine (Ketalar® Parke-Davis, Detroit, Michigan) and xylazine (Rompun®, Bayer AG, Leverkusen, Germany), the abdomen was shaved and swabbed with povidone iodine. The peritoneal cavity was entered through a 3-cm midline incision. The terminal ileum and caecum of all animals were mobilized and placed onto wet gauze. Both sides of terminal ileum and caecum were brushed until serosal petechiae were seen on the intestinal surfaces.

Animals were separated into two groups. Rats in group 1 received no medication and group 2 rats were sprayed over the intraperitoneal organs with 5 ml of Suprogel® (Klas Medical, Istanbul, Turkey). The midline incision was then closed by continuous 3/0 propylene suture in two layers. All the rats were fed under the same conditions: at 24-26º of environmental temperature, 40% humidity, with an alternate 12 hour light/dark cycle, and free access to food and water. All animals gained weight and appeared healthy. There were no signs of impaired wound healing, bleeding and gastrointestinal complications.

All rats underwent a second laparotomy on the postoperative 21st day for evaluating a possible adhesion formation. Adhesions were then examined macroscopically and graded blindly according to Evans model [17] (Table 1).

Table 1. Adhesion grading system according to Evans model

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description of adhesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No adhesions</td>
</tr>
<tr>
<td>1</td>
<td>Spontaneously separating adhesions</td>
</tr>
<tr>
<td>2</td>
<td>Adhesions separating by traction</td>
</tr>
<tr>
<td>3</td>
<td>Adhesions separating by dissection</td>
</tr>
</tbody>
</table>

Morphologic evaluation
The injured terminal ileum and caecum walls with fibrous adhesions were taken out en bloc and fixed in 10% neutral buffered formaldehyde solution for 24 hours. Each sample was embedded in paraffin block by the standard method. Sections were cut at a thickness of 5 mm and stained with hematoxyline&eosine. Adhesion scores were evaluated as Zuhlke’s classification [14] (Table 2).

Table 2. Histopathological adhesion score according to the Zuhlke’s classification

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loose connective tissue, cell-rich, old and new fibrin, fine reticulin fibers</td>
</tr>
<tr>
<td>2</td>
<td>Connective tissue with cells and capillaries, few collagen fibers</td>
</tr>
<tr>
<td>3</td>
<td>Connective tissue firmer, fewer cells, more vessels, few elastic and smooth muscle fibers</td>
</tr>
<tr>
<td>4</td>
<td>Old firm granulation tissue, cell poor serosal layers hardly distinguishable</td>
</tr>
</tbody>
</table>

Hydroxyproline
The biopsy specimens were stored at -80ºC in air-tight tubes until homogenization. In order to assess the level of collagen in tissues, tissue hydroxyproline levels were spectrophotometrically determined as Jamall defined [15].

Statistical Analysis
Mann Whitney’s U-test used to compare the adhesion grading and histopathological adhesion scores in groups. Mann Whitney’s U-test was also used to determine the significance of differences in the hydroxyproline levels. The level for significance was accepted as p<0.05.

Results

Adhesion formation
As shown in Table 3, in the control group, there were no rats in grade 0 or 1, but there were two rats (20%) in grade 2 and eight rats (80%) in grade 3 (Figure 1). In Suprogel group, there were two rats (20%) in grade 0, seven rats (20%) in grade 1, one rat (10%) in grade 2, but there were no rats in grade 3. Mean adhesion grading was 2.8 ± 0.4 in control group and 0.9 ± 0.6 in Suprogel group. The adhesion grading were significantly lower when compared with Suprogel group (p<0.05).

Table 3. Adhesion grading

<table>
<thead>
<tr>
<th>Grade</th>
<th>Control group</th>
<th>Suprogel group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>2</td>
<td>2 (20%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>3</td>
<td>8 (80%)</td>
<td>-</td>
</tr>
</tbody>
</table>

Morphologic evaluation
Histopathological adhesion score was evaluated according to the Zuhlke’s classification (Table 4). In the control group, there were no rats in score 1,
there were two rats (20%) in score 2 and eight rats (80%) in score 3 but there were no rats in score 4. In Suprogel group, there were seven rats (70%) in score 1, three rats (30%) in score 2, and there were no rats in score 3 or score 4. Mean adhesion score was 2.8 ± 0.4 in control group and 1.3 ± 0.5 in Suprogel group (Figure 2). The histopathological adhesion scores were significantly lower in Suprogel group (p<0.05) (Figure 3). Histopathological sections obtained are illustrated in Figure 4 and 5.

Table 4. Histopathological adhesion score

<table>
<thead>
<tr>
<th>Score</th>
<th>Control group</th>
<th>Suprogel group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>2</td>
<td>2 (20%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>3</td>
<td>8 (80%)</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 1. The appearance of adhesions needing dissection for separation.

Figure 2. This graphic illustrates the adhesion grading of each group.

Figure 3. This graphic shows the comparative histopathological adhesion scores between groups.

Figure 4. This figure represents the loose connective tissue with cell-dense as a pathological marker for adhesion (HE, x100).

Figure 5. This pathological section is evidence showing adhesions rich in proliferated connective tissue with fewer cells and increased number of vessels (HE, x50).
Hydroxyproline
Peritoneal hydroxyproline levels did not differ significantly between the groups (p=0.631).

Discussion
The most common cause of peritoneal adhesion is originated from abdominal surgery performed previously, which generally seen in 93% of patients [1]. After abdominal and gynecological surgery, intraperitoneal adhesion formation may result with ileus, female infertility and pelvic pain. Adhesions also make the surgeons more difficult to manipulate on abdomen and causes difficulty in controlling bleeding, which results in deterioration of anatomical structures and an increase in operation time leading higher rates in morbidity and mortality [18-21].

Recent studies have been focused on either how adhesions are formed or how to prevent this adhesion formation. However, no ideal anti-adhesive agent was found against these two mentioned problems. The reason might be the unknown etiology in adhesion pathogenesis during the postoperative period. Since then, researches to find the best agent for minimizing or removal of abdominal adhesions are still continuing.

A variety of techniques and agents functioning against each of the steps in adhesion formation have been used in order to prevent postoperative adhesions. Antiadhesive agents have been applied for prevention of fibrin storage, removal of fibrin stores, and protection against fibroplasias and mechanical separation of visceral surfaces by adhesion barriers [22]. Dextran, heparin, sodium citrate and dicumarol have been used for prevention of fibrin storage; however, these agents increased hemorrhage [5-7].

Pepsin, trypsin and intraperitoneal lavage have also been applied to remove the fibrin storage, but these agents had limited beneficial effect to prevent the adhesion [5,9,14].

Steroids have been used against fibroplasia formation. In here, steroids increase cell membrane stability, regulate vascular permeability, prevent polymorphonuclear leukocyte cells immigration, but, steroids have also adverse effects on wound healing [22,23].

Halofuginone was used to prevent adhesions via administration either intraperitoneally or orally, and it was demonstrated that it reduced the abdominal adhesions by decreasing collagen α1 gene expression and inhibited only de novo synthesis of collagen without affecting the collagen deposited in the tissue previously [2].

Pentoxifylline, which inhibits glycosaminoglycans and collagen synthesis by dermal fibroblasts has been found to reduce the adhesion scores at the anastomotic sites, but has no effect on the strength of adhesions at sites other than the anastomosis in rats [24].

Cianidanol has also effects on the hydroxylation of the prolyl and lysyl residues of procollagen by proline and lysine hydroxylation due to its ability to bind Fe²⁺ and caused a decrease in the number of abdominal adhesions in rats [25].

Recently, absorbable anti-adhesive materials like sodium hyaluronate and carboxymethylcellulose, macromolecular polysaccharide have been improved. In a variety of animal models, Seprafilm® has reduced the intra-abdominal adhesion. Seprafilm® biodegradable, translucent adhesion barrier composed of two anionic polysaccharides, sodium hyaluronate and carboxymethylcellulose is placed between the tissues to prevent adhesion formation [26-29].

In the present study, Suprogel was studied for its efficacy in the prevention of postoperative adhesion formation in rat adhesion model. Suprogel® is an antiadhesive barrier produced from macromolecular polysaccharide. It is in a structure similar to body elements [30]. It activates cell functions and it ensures healing by increasing the regeneration of traumatized tissue. Suprogel® is a biological barrier; it reduces bleeding, prevents fibroblast development, and increases regeneration [31-33].

The adhesion grading and the histopathological adhesion score were significantly lower in Suprogel group (p<0.001). Hydroxyproline values were lower in Suprogel group, but no statistically significant difference was found between the groups.

It is solely supposed that Suprogel® prevents adhesive formation due to its physical barrier effect. In addition to that, it is both physical and biological barrier against adhesion development since a variety of studies reported that it contributed the activation of cellular functions and regeneration in traumatized tissue, and prevention of fibroblast proliferation [31-33]. From this point of view, we used Suprogel® instead of Suprofilm® and obtained similar results with that in Suprofilm study group.

Consequently, we have demonstrated that intraperitoneally administration of Suprogel® may reduce
the postoperative adhesions in this rat model. However, further investigations are required for its clinical use.

References
