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The Effects of Anti-Adhesive Low Molecular Weight Na-Hyaluronate and Octreotide on Tissue Strength*

Wpływ środków przeciwzrostowych hialuronianu sodu o małej masie cząsteczkowej i oktreotydu na wytrzymałość tkanek

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Abstract

Background. The use of locally administered agents to prevent intraperitoneal adhesions is a popular research topic. The presumed effects of these agents on tissue matrix limit their use.

Objectives. To determine the effects of low-molecular-weight Na-hyaluronate and octreotide on tissue tensile strength and hydroxyproline levels.

Materials and Methods. Sprague-Dawley rats were anesthetized with ketamine and 10 longitudinal incisions, 2 to 3 cm in length each, were made in the right parietal peritoneum. A 2 × 1 cm peritoneal layer was excised from the left abdominal wall during the first laparotomy. Before closing the abdomen, intraperitoneal irrigation was performed, either with Na-hyaluronate solution at a concentration of 0.25 mg/kg (n = 10) or with octreotide solution at a dosage of 5 µg/ml (n = 10). On the 14th day after the operation, the abdomen was opened and abdominal adhesions were examined. Part of the abdominal wall (4 × 4 cm in size, including midline incision) was removed to measure the tensile strength and hydroxyproline level of the tissue on the suture line.

Results. Low-molecular-weight Na-hyaluronate significantly reduced the grade of adhesion (p = 0.005). Octreotide administration inhibited adhesion formation, although not to a statistically significant degree (p > 0.05). Tissue tensile strength and hydroxyproline levels were not affected by treatment with either LMW Na-hyaluronate or octreotide (p > 0.05).

Conclusions. Na-hyaluronate and octreotide, which are well known anti-adhesive agents, do not exert any negative effects either on tissue tensile strength or hydroxyproline level (Adv Clin Exp Med 2011, 20, 6, 711–715).

Key words: hyaluronate, octreotide, abdominal surgery, adhesion, tissue strength.

*This study was carried out without any financial support or grant.
Post-operative peritoneal adhesions are observed after abdominal operations in as many as 93% of the cases [1]. Adhesions account for 1% of the patients admitted to general surgery departments and for 3% of the laparotomies in one year [2].

Acquired adhesions secondary to foreign bodies left in the peritoneal cavity (such as talc, suture materials, operative dressings) and intestinal leakage can be minimized or completely eliminated by preventive measures. However, peritoneal injury and the formation of ischemic regions are inevitable during surgical interventions, and treatment modalities specifically aimed at preventing adhesions are therefore required [3].

Agents used for the prevention of post-operative peritoneal adhesions can be administered either systemically or intraperitoneally [4]. The main purpose of intraperitoneal application of these agents is to prevent adhesion by making the peritoneal surfaces slippery and by detaching them from each other, or to eliminate formed adhesions in the early stages [5]. In recent studies, both octreotide and Na-hyaluronate derivatives have been used to prevent adhesion [6, 7]. However, the effects of these agents on tissue tensile strength have not been studied yet.

The aim of this study was to investigate the effects of two well known intraperitoneally administered anti-adhesive agents – low-molecular-weight (LMW) Na-hyaluronate and octreotide – on the tensile strength of the rat abdominal wall.

Material and Methods

The experiments were conducted with adult male Sprague-Dawley rats weighing from 200 to 240 grams. The rats were kept at room temperature and provided with free access to standard chow and tap water. The entire study was carried out under the guidelines of the Selcuk University Institutional Animal Ethics Committee.

The Experimental Design

The rats were divided into three groups (n = 10 in each group) as follows: the control group, the LMW Na-hyaluronate group and the octreotide group. The animals were fasted for 12 hours before the experiments, but were allowed water ad libitum. They were anesthetized with ketamine HCl (5 mg/kg, intramuscular, Ketalar, Parke-Davis Inc., USA). Following anesthesia induction, a midline abdominal incision approximately 4 cm in length was performed under sterile conditions. Ten longitudinal incisions 2–3 cm in length were made on the right parietal peritoneal surface. A layer of the parietal peritoneum 2 × 1 cm in size was removed from the left side of the incision. The rats in the control group received intraoperative peritoneal irrigation with 6 ml of normal saline. The LMW Na-hyaluronate group received intraperitoneal irrigation with orthovisc (Anika Research, USA) at a concentration of 0.25 mg/kg in a total volume of 6 ml of normal saline solution. The octreotide group received intraperitoneal irrigation with 6 ml of octreotide solution (5 μg/ml in normal saline).

The rats were kept at an ambient temperature of 22°C after the surgical procedure described. They were fed with standard rat pellets for the next 13 days, and were fasted for 12 hours before the second operation. On the 14th day of the experiment, the animals were re-anesthetized with ketamine HCl (5 mg/kg, intramuscular, Ketalar, Parke-Davis Inc., USA) and the abdominal wall was opened by a suprapubic transverse incision perpendicular to the bottom end of the previous midline incision. The areas of peritoneal injury on the right and left sides of the abdomen and the suture line were examined for the presence of adhesions. The adhesions detected were graded according to the method described by Mazuji et al. [8]. After this, an abdominal wall segment 4 × 4 cm in size was removed by extending the incision in a “U” shape so that the previous suture line remained in the middle. After removing the sutures, the tensile strength of the suture line and of the right and left sides of the abdominal wall were determined according to the method described by Peacock [9, 10]. A 1-gram tissue sample was obtained from the suture line and from the right and left sides of the abdominal wall. The hydroxyproline level in the tissue was determined according to Bergman and Loxley’s method for each individual site [11, 12].

Tissue hydroxyproline levels and tensile strength values are expressed as mean and standard deviation. The values were analyzed with...
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If the differences were significant, Scheffe’s test was used to compare the groups. The values obtained from adhesion grading were expressed as medians and were analyzed with the Kruskal-Wallis test. If the differences were significant, the Mann-Whitney-U test with Bonferroni correction was used to compare the groups. P values less than 0.05 were considered significant.

**Results**

**Adhesion Grades**

In the control group, peritoneal adhesions were found extensively to the right of the midline incision. A similar distribution of adhesions in the abdominal cavity was seen in the LMW Na-hyaluronate and octreotide groups. The adhesion grade of the LMW Na-hyaluronate group was significantly lower than in the control group (p = 0.005). The reduction in adhesion grade in the octreotide group was insignificant compared to the control group (p > 0.05) (Table 1).

**Tissue Tensile Strength**

In the control group tensile strength was highest in the midline incision and lowest on the left side of the midline; values on the right side were in between. A similar distribution of tensile strength was observed in the other two groups. There was no statistically significant difference in tensile strength between the experimental groups (p > 0.05) (Table 2).

**Tissue Hydroxyproline Levels**

In each group, samples obtained from the midline incision contained higher hydroxyproline levels than either the right or left side of the midline incision. Insignificant differences between the groups were found when the hydroxyproline levels were compared (p > 0.05) (Table 3).

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**Table 1. Adhesion grades in the experimental groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>LMW Na-hyaluronate</th>
<th>Octreotide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>midline right left</td>
<td>midline right left</td>
</tr>
<tr>
<td>Grade 0</td>
<td>n = 1 n = 0 n = 2</td>
<td>n = 7 n = 5 n = 7</td>
</tr>
<tr>
<td>Grade I</td>
<td>n = 3 n = 2 n = 3</td>
<td>n = 2 n = 3 n = 3</td>
</tr>
<tr>
<td>Grade II</td>
<td>n = 3 n = 4 n = 4</td>
<td>n = 1 n = 2 n = 0</td>
</tr>
<tr>
<td>Grade III</td>
<td>n = 2 n = 3 n = 1</td>
<td>n = 0 n = 0 n = 0</td>
</tr>
<tr>
<td>Grade IV</td>
<td>n = 1 n = 1 n = 0</td>
<td>n = 0 n = 0 n = 0</td>
</tr>
</tbody>
</table>

p = 0.005 p > 0.05

**Table 2. Tissue tensile strengths (g)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Midline mean ± SD</th>
<th>Right side mean ± SD</th>
<th>Left side mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>724 ± 25.58</td>
<td>698 ± 18.6</td>
<td>678 ± 22.42</td>
</tr>
<tr>
<td>Na-Hyaluronate</td>
<td>717 ± 17.68</td>
<td>681 ± 11.52</td>
<td>672 ± 3</td>
</tr>
<tr>
<td>Octreotide</td>
<td>718 ± 24.4</td>
<td>701 ± 21.47</td>
<td>679 ± 13.5</td>
</tr>
</tbody>
</table>

p > 0.05

**Table 3. Tissue hydroxyproline levels (μg/mg tissue)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Midline mean ± SD</th>
<th>Right side mean ± SD</th>
<th>Left side mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.09 ± 0.57</td>
<td>3.03 ± 0.21</td>
<td>2.92 ± 0.47</td>
</tr>
<tr>
<td>Na-Hyaluronate</td>
<td>2.96 ± 0.36</td>
<td>2.91 ± 0.55</td>
<td>2.87 ± 0.39</td>
</tr>
<tr>
<td>Octreotide</td>
<td>2.91 ± 0.52</td>
<td>2.86 ± 0.85</td>
<td>2.84 ± 0.72</td>
</tr>
</tbody>
</table>

p > 0.05
Discussion

Adhesions are one of the major factors that are responsible for increased rates of mortality and morbidity following abdominal surgery, especially during relaparotomies [13]. Surgical trauma to the serosal surfaces is considered to be responsible for more than 90% of post-operative adhesions. Reduction in tissue oxygenation after surgical trauma plays a significant role in adhesion formation. The increased secretion of histamine after peritoneal injury causes accumulation of protein-rich fluid in the peritoneal cavity. All of these factors accelerate adhesion formation by blocking or inhibiting the activation of plasminogen activators [14, 15]. When combined with the above events, infection aggravates the development of adhesions by provoking inflammatory responses [16]. Despite preventive measures, adhesions cannot be completely eliminated. Ischemia is a major and inevitable factor in the formation of adhesions in abdominal surgery [17]. In all three experimental groups in the current study, adhesions were more observed in greater numbers on the right side of the midline incision, where the injury was more extensive. Despite the full-thickness injury at the midline incision site, adhesion development was lower on the midline. These results indicate that the extent of peritoneal injury is the most important factor in the formation of adhesions.

In a study by Miro et al., tissue tensile strength was found to be increased in injury sites where ischemia was more pronounced [10]. In addition, hydroxyproline levels in healthy tissues were reported to be lower than the injured site tissue samples. The differences between healthy and injured tissues were explained by the positive effect of an extensive inflammatory reaction on the re-modeling phase in the injured tissues. In the current study, tissue tensile strength was found to be higher around the midline incision than at the other sites. Similarly, tissue hydroxyproline levels were increased near the midline incision site, possibly secondary to excessive tissue ischemia.

No ideal agent to prevent peritoneal adhesion has been discovered to date. Several drugs and materials are applied locally or systemically for this purpose [7]. Hyaluron and its derivatives have been used in vitreoretinal surgery and in osteoarthritis. Since the development of cross-linked derivatives of hyaluron, their usage and indications have expanded, and their rate of success has increased [18, 19]. The use of hyaluron derivatives for the prevention of post-operative peritoneal adhesions seems to have encouraging results. In various studies, Seprafilm solution (Genzyme Co., Cambridge, MA, USA), a derivative of hyaluronic acid, has both prevented and reduced adhesion formation on serosal surfaces in animal models and in gynecologic operations without any side effects or signs of systemic toxicity [19]. Another form of hyaluronic acid derivative hardened with carboxymethylcellulose is called Seprafilm (Genzyme Co., Cambridge, MA, USA); it has been used for the prevention of adhesion formation [20, 21]. In the current study, a correlation was noted between tissue tensile strength and hydroxyproline levels and the intensity of the injury. The current results also indicate that the natural distribution of tissue tensile strength and hydroxyproline levels after injury remained unaffected by LMW-Na hyaluronate administration.

Various studies have shown that octreotide can inhibit local synthesis of insulin-like growth factor-1 after inflammatory responses and decrease the responsiveness of T lymphocytes, thereby inhibiting glycosaminoglycan synthesis in fibroblasts [22, 23]. The reduction of collagen production by fibroblasts in turn prevents fibrous band formation. Lai et al. and Alatas et al. showed that octreotide significantly reduced intraperitoneal adhesion formation in groups of subjects that which received octreotide intraperitoneally [24, 25]. In the present study, octreotide had no adverse effect on the distribution of tensile strength at different sites. Also, as in the LMW Na-hyaluronate group, tissue hydroxyproline levels were unaffected in the octreotide treated group. Despite controversies about the administration route and dosing, these results suggest that intraperitoneal administration of octreotide is successful in reducing the adhesion formation without any unwanted effects on the tissue matrix.

The authors conclude that intraperitoneal administration of LMW-Na hyaluronate and octreotide reduce adhesion formation after peritoneal insult. The effects of these agents on tissue tensile strength and hydroxyproline levels are similar. Further clinical studies are needed to strengthen the evidence for the well-known adhesion-preventing effects of hyaluron derivatives and octreotide.

References

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Conflict of interest: None declared

Received: 18.04.2011
Revised: 16.05.2011
Accepted: 7.12.2011