Effect of carboxymethylcellulose, icodextrin and hyaluronic acid solutions on postoperative intraabdominal adhesion formation in rabbits and the role of cytokines in intraabdominal adhesion formation

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Summary

In this study, the aim was to investigate the effects of 1% CMC, 4% ICO and 0.4% HA solutions in preventing postoperative IAs and their contribution to peritoneal wound healing, as well as the relationship of cytokines with the formation of postoperative IAs in rabbits. The material of the study consisted of 32 healthy rabbits. Rabbits underwent median laparotomy following general anesthesia. Serosal abraded was created at the antimesenteric border of the cecum. Next, a 3 × 2 cm peritoneum was excised on the right abdominal wall and the defect was closed using with a 2/0 silk suture. The rabbits were randomly assigned to either of the following four treatment groups: CNT (0.9% NaCl), ICO (4% icodextrin), HA (0.4% hyaluronic acid) and CMC (1% carboxymethyl cellulose). Both cecum and peritoneal surfaces were treated with 20 ml each of treatment solutions. On the 7th postoperative day, the rabbits were euthanized with a lethal dose of sodium pentobarbital and the degree of adhesion was evaluated. Samples taken from the peritoneal defect were examined histopathologically and tissue hydroxyproline levels were measured. Serum TNF-α, IL-1 and IL-6 levels were measured from blood samples taken before the surgery and at 1, 6, 24 and 48 hours after the surgery. It was observed that the adhesion grade in the HA group (p < 0.05) and CMC group (p < 0.01) was significantly lower than the control group. Although peritoneal tissue hydroxyproline levels were lower in the other groups compared to the control group, they were not statistically significant (p > 0.05). In rabbits with adhesion formation, it was determined that TNF-α levels increased at the 6th postoperative hour (p < 0.05), and IL-6 levels increased at the 6th, 24th and 48th postoperative hours (p < 0.001) compared to preoperative. In this study, treating tissues with 0.4% HA and 1% CMC solutions suppressed peritoneal inflammation, and this resulted in an increase in the levels of proinflammatory cytokines TNF-α and IL-6. It has been determined that the application of these solutions reduces postoperative adhesion formation. It was concluded that TNF-α and especially IL-6, which are proinflammatory cytokines, can be a non-invasive biomarker in determining postoperative IA formation and evaluating the adhesive process.

Keywords: intraabdominal adhesion, carboxymethylcellulose, icodextrin, hyaluronic acid, cytokine, rabbit

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with foreign bodies; tissue ischemia and infections are among the most important causes of postoperative IAs (12). The initial response to injury in the abdominal cavity is an inflammatory response in which cytokines are released and vascular permeability is increased, causing fibrinogen-rich exudate to leak from the vessel and accumulate in the injured area. The clot formed by the transformation of fibrinogen into fibrin causes fibrous adhesions between tissues within the first three hours following the reaction. During the normal healing process, fibrous adhesions occur with the shaping of the organization of collagen bundles and the formation of small vascular structures because of fibrinolytic activity in this fibrin matrix (11). Fibrous adhesions, which are completely organized approximately on the fifth postoperative day, cause intestinal and urethral obstructions and abdominal pain, intraabdominal abscesses, and infertility in females (12).

Currently, there is no ideal method for the prevention of IAs. Many treatment methods for prevention of IAs have been evaluated in humans, laboratory animals, sheep, and horses (13, 22). Numerous pharmacologic agents such as antibiotics, nonsteroidal anti-inflammatory drugs, corticosteroids, heparin, and tissue plasminogen activators have been used for this purpose and are reported to reduce IA formation (18, 25, 27). However, it has been emphasized that the use of these agents for the prevention of IAs may cause complications such as bleeding, ulcers, and impaired healing (25). In recent years, researchers have focused on barrier membranes and polymer solutions for the prevention of adhesions (36, 38). It has been suggested that barrier membranes used in the form of patches provide local protection but are not suitable for the closure of large mesothelial lesions caused by drying of the tissues during the operation or formed by the surgical instruments used (25). Studies have reported that intraoperative and postoperative administration of polymer solutions such as carboxymethylcellulose (CMC) (10, 17, 20, 21, 23, 28), hyaluronic acid (HA) (10, 17, 27, 28) and icodextrin ICO (4, 17, 25, 35) into the peritoneal cavity may reduce postoperative adhesion formation. CMC, a water-soluble high molecular weight polysaccharide polymer solution, is reported to significantly reduce adhesion formation in animals (13, 20, 21, 23, 25). It is stated that CMC solution, which acts as a physical barrier, reduces serosal trauma during manipulations and is effective in preventing adhesion by covering the surface of organs and preventing contact with each other in the early stages of postoperative healing (24). HA is an anionic, hydrophilic polysaccharide derivative that has an important role in cell biology and is found naturally in all tissues including connective tissue, skin, cartilage, synovial fluid and vitreous (12). HA solution has been reported to reduce postoperative adhesion formation in humans and animals (13). HA is thought to mechanically separate the wound surfaces in the abdominal cavity, contribute to wound healing and prevent adhesion formation by increasing fibrinolysis (26). ICO solution is an isoosmolar and α,1-4 linked glucose polymer used in peritoneal dialysis (3, 25). Various studies have reported that ICO solution can reduce postoperative adhesion formation (25, 35). It is thought that it prevents by providing flotation in the peritoneal cavity and separating the peritoneal surfaces (25).

Cytokines play an important role in IA formation and postoperative inflammatory response after intraabdominal operations. Inflammatory cells that infiltrate the region during the formation of IAs secrete cytokines and growth factors such as interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α). It has been suggested that the levels of TNF-α, IL-1 and IL-6 increase in both peritoneal fluid and circulation in the first stage of IA formation and are a biological indicator of IA formation (5, 12). In this study, the aim was to investigate the effects of 1% CMC, 4% ICO and 0.4% HA solutions in preventing postoperative IAs and their contribution to peritoneal wound healing, as well as the relationship of cytokines with the formation of postoperative IAs in rabbits.

### Material and methods

**Animals.** Thirty-two clinically healthy, local-breed, adult females rabbits weighing 2.1-3.0 kg were used. Rabbits were maintained in individual cages for at least 15 days before the experiments for acclimatization. They were fed commercial pellet food (Purina®) and freshwater ad libitum. Food, but not water, was withheld for at least 12 h before the experiments. Experiments were performed during the light phase of the cycle (between 08:30 and 11:00 hours). The study was conducted after the study protocol was approved by the ethical committee of the Faculty of Veterinary Medicine, University of Yuzuncu Yil.

**Surgery.** The animals were anesthetized with an intramuscular injection of a mixture of 50 mg/kg of ketamine (Ketamidor®, Richter pharma), and 5 mg/kg of xylazine (Rompun®, Bayer). The rabbits' abdomens were shaved and disinfected and the animals were prepared for sterile surgery. A midline laparotomy (6 cm) was performed, and the cecum were exteriorized. An antimesenteric border of cecum was abraded with scalpel until punctuate bleeding occurred approximately 5 × 2 cm in size, and two longitudinally parallel serosal incisions were made with the scalpel. After the bleeding was controlled, the cecum was rejected into the abdominal cavity. Next, a 3 × 2 cm peritoneum was excised on the right abdominal wall and the defect was closed using a 2/0 silk suture.

**Experimental design.** The rabbits were randomly assigned to either of the following four treatment groups (n = 8 per group): CNT control group (0.9% NaCl, Polifarma®), ICO (4% icodextrin, Adept®), HA (0.4% hyaluronic acid, Sigma®) and CMC (1% carboxymethyl cellulose, Sigma®). Both cecum and peritoneal surfaces were treated with 5 ml each of treatment solutions (10 ml in total) before creating the defect. Before the abdominal wall was closed, 10 ml of treatment solution was administered...
into the abdominal cavity. A total of 20 ml of treatment solution was used for each rabbit.

**Grading criteria for adhesions.** On postoperative day 7, the rabbits were euthanized with a lethal dose of sodium pentobarbital. The abdominal cavity was completely opened with a U-shaped incision, and adhesions were evaluated according to the classification of Zuhlke et al. (39) (Tab. 1).

**Estimation of hydroxyproline.** Samples removed from the peritoneal defect created on the right abdominal wall were stored at −18°C until the day of measurement. Tissue hydroxyproline levels were measured according to the Woessner method, which is based on the principle of oxidation of hydroxyproline with chloramine T and photometric measurement of the colored compound formed with p-dimethylaminobenzaldehyde (37).

**Histopathological studies.** Tissue samples removed from the parietal peritoneal defect created on the right abdominal wall were fixed in 10% formalin and 4-micron thick sections were taken from the prepared paraffin blocks. All sections were stained according to the hematoxylin-eosin (HE) method and examined under a light microscope. Peritoneal inflammatory process was evaluated. The scale was defined as follows: 0 = no inflammation, 1 = mild inflammation, 2 = moderate inflammation, 3 = severe inflammation.

**Cytokine levels.** Blood samples were taken from the lateral ear vein before the operation and at 1, 6, 24 and 48 hours after the operation to measure serum TNF-α, IL-1 and IL-6 levels. Sera obtained by centrifugation were stored at −18°C until the day of measurement. Serum TNF-α, IL-1 and IL-6 levels were measured on an ELISA device with an ELISA commercial kit (BioSource® Korean).

**Statistical analysis.** Among the different groups, each parameter was compared using independent-samples t test. Within the same group, parameters were compared using paired-samples t test. For all statistical comparisons, differences were considered significant when p value was < 0.05. The calculations were performed utilizing SPSS software. All values are presented as means ± standard deviations (SD).

**Results and discussion**

There were no complications or deaths in the rabbits after the operation. 1st and 2nd grade adhesions were found to occur between the traumatized cecum and mesenterium. It was observed that the majority of 3rd and 4th grade adhesions occurred between the traumatized cecum and the injury peritoneum and the median incision line. The total adhesion score, the number of animals with adhesion formation and the hydroxyproline levels of the peritoneal tissue were presented in Table 2. No statistical difference was detected between the ICO group and the control group in terms of adhesion grade. However, it was observed that the adhesion grade in the HA group (p < 0.05) and CMC group (p < 0.01) was significantly lower than the control group (Fig. 1).

**Tab. 1. Classification of adhesions according to Zuhlke et al. (39)**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No adhesions</td>
</tr>
<tr>
<td>I</td>
<td>Easy to separate by blunt dissection; no vascularization</td>
</tr>
<tr>
<td>II</td>
<td>Blunt dissection is possible but some sharp dissection necessary, beginning vascularization</td>
</tr>
<tr>
<td>III</td>
<td>Lysis of adhesions possible by sharp dissection only, clear vascularization</td>
</tr>
<tr>
<td>IV</td>
<td>Lysis of adhesions possible by sharp dissection only, organs strongly attached with severe adhesions, organ damage is inevitable</td>
</tr>
</tbody>
</table>

**Tab. 2. Total adhesion grade and number of animals with adhesion formation and hydroxyproline levels and histopathological inflammatory score of peritoneal tissue (X ± SD)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Adhesion grade</th>
<th>Number of animals with adhesions formed</th>
<th>Hydroxyproline levels (µg/mg)</th>
<th>Inflammatory score</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT (n = 8)</td>
<td>2.37 ± 0.56</td>
<td>5/8 (62.5%)</td>
<td>3.82 ± 0.29</td>
<td>2.50 ± 0.53</td>
</tr>
<tr>
<td>ICO (n = 8)</td>
<td>1.75 ± 0.52</td>
<td>4/8 (50%)</td>
<td>3.70 ± 0.43</td>
<td>2.37 ± 0.51</td>
</tr>
<tr>
<td>HA (n = 8)</td>
<td>0.75 ± 0.41</td>
<td>3/8 (37.5%)</td>
<td>3.64 ± 0.34</td>
<td>2.00 ± 0.75</td>
</tr>
<tr>
<td>CMC (n = 8)</td>
<td>0.25 ± 0.16</td>
<td>2/8 (25%)</td>
<td>3.24 ± 0.20</td>
<td>1.88 ± 0.83</td>
</tr>
</tbody>
</table>

Explanations: CNT – 0.9% NaCl; ICO – 4% icodextrin; HA – 0.4% hyaluronic acid; CMC – 1% carboxymethyl cellulose; a – p < 0.05, b – p < 0.01 compared to the control group

Fig. 1. Macroscopic evaluation of adhesion: a) no adhesion, b) 2nd grade adhesion, c) 3rd grade adhesion, d) 4th grade adhesion
Although peritoneal tissue hydroxyproline levels were lower in the other groups compared to the control group, they were not statistically significant (p > 0.05).

In rabbits with adhesion formation, it was determined that TNF-α levels increased at the 6th postoperative hour (p < 0.05), and IL-6 levels increased at the 6th, 24th and 48th postoperative hours (p < 0.001) compared to preoperative values. No statistical difference was detected in IL-1 levels. In rabbits without adhesion formation, no statistical difference was detected in postoperative serum TNF-α, IL-1 and IL-6 levels compared to preoperative values (Tab. 3).

No adverse effect of the solutions used in the study on peritoneal wound healing was detected. There was no statistically significant difference was observed between the groups in terms of the severity of inflammatory changes (p > 0.05). However, the severity of the inflammatory reaction was highest in the control group, then in the ICO, HA and CMC groups, respectively (Tab. 2, Fig. 2).

Despite numerous advances in surgery, postoperative IAs are still a persistent problem for surgeons. It significantly affects the quality of life of patients (31). Adhesion formation after abdominal surgery can be reduced by preventing fibroblastic proliferation and coagulation, reducing inflammation and exudate, increasing fibrinolysis, and mechanically separating the peritoneal surfaces from each other (38). For this purpose, to minimize peritoneal injury, protecting the tissues, moisturizing the tissues frequently, lavaging the peritoneal cavity with appropriate solutions, not suturing the serosal surfaces, and preventing contamination as much as possible minimizes the risk of adhesion (31, 38). In recent years, researchers have focused on polymer solutions that mechanically separate peritoneal surfaces from each other to prevent adhesions (3, 31, 38). Studies have reported that administration of polymer solutions such as CMC (14, 20, 21, 23, 24, 28), HA (27, 28) and ICO (25, 35) into the peritoneal cavity during and after the operation may reduce postoperative adhesion formation.

ICO is a biodegradable glucose polymer solution that plays a protective role against prolonged separation of peritoneal surfaces by hydroflotation and thus

Tab. 3. Serum TNF-α, IL-1 and IL-6 levels in rabbits with and without adhesion formation (X ± SD)

<table>
<thead>
<tr>
<th></th>
<th>no adhesion (n = 16)</th>
<th>adhesion formed (n = 14)</th>
<th>no adhesion (n = 16)</th>
<th>adhesion formed (n = 14)</th>
<th>no adhesion (n = 16)</th>
<th>adhesion formed (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before the surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>78.56 ± 3.05</td>
<td>76.43 ± 2.58</td>
<td>10.77 ± 0.62</td>
<td>11.54 ± 0.91</td>
<td>21.59 ± 1.02</td>
<td>22.37 ± 1.60</td>
</tr>
<tr>
<td>IL-1 (pg/ml)</td>
<td>82.43 ± 2.62</td>
<td>85.06 ± 2.56</td>
<td>11.82 ± 0.88</td>
<td>13.78 ± 1.45</td>
<td>24.23 ± 1.64</td>
<td>25.29 ± 1.68</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>84.81 ± 2.80</td>
<td>91.56 ± 3.43*</td>
<td>12.30 ± 0.64</td>
<td>12.48 ± 0.89</td>
<td>25.47 ± 1.40</td>
<td>48.63 ± 2.99*</td>
</tr>
<tr>
<td>Postoperative 6th hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>80.93 ± 2.56</td>
<td>87.50 ± 3.49</td>
<td>12.99 ± 0.51</td>
<td>12.64 ± 1.01</td>
<td>23.71 ± 0.93</td>
<td>36.52 ± 2.83*</td>
</tr>
<tr>
<td>IL-1 (pg/ml)</td>
<td>77.87 ± 3.29</td>
<td>78.93 ± 3.26</td>
<td>11.89 ± 0.87</td>
<td>12.87 ± 0.34</td>
<td>24.07 ± 1.05</td>
<td>28.11 ± 1.65*</td>
</tr>
</tbody>
</table>

Explanations: a – p < 0.05, b – p < 0.001 compared to the before surgery

Fig. 2. Histopathological evaluation of inflammation of the peritoneum: a) intact peritoneum, b) mild inflammation, c) moderate inflammation, d) severe inflammation
against IA (8, 19). It is also suggested that ICO minimizes tissue apposition during the critical period of postoperative mesothelial regeneration, thus providing a barrier against adhesion formation (19). In a study in which they created an adhesion model in rabbits, Müller et al. (25) and Verco et al. (35) investigated 4% ICO and reported that ICO was successful in preventing IAs. Catena et al. (7) also investigated the safety and effectiveness of 4% ICO in preventing IAs. The authors concluded that 4% ICO was more suitable to be applied to surfaces with damaged serosa compared to other anti-adhesion barriers (7). Contrary to the above results, some researchers (6, 10) showed that 4% ICO was not effective in significantly reducing adhesion formation. In this study, our results show that 4% ICO solution does not have a negative effect on parietal peritoneal healing but does not contribute significantly to the prevention of adhesion in rabbits compared to 0.9% NaCl, consistent with the findings of Ditzel et al. (10) and Bellon et al. (6) (p > 0.05).

HA, a natural polymer, is thought to prevent adhesions by covering the serosal surfaces when administered into the peritoneal cavity. HA, which is thought to have an anti-inflammatory effect, increases its anti-adhesion effect by supporting fibrinolysis, mesothelial cell proliferation and wound healing (9, 31). Eggleston et al. (13) in an experimental study in which they investigated the contributions of CMC and HA solutions to jejunum healing and adhesion development in horses, showed that CMC and HA solutions reduced adhesion formation, but HA solution was more effective than CMC solution in preventing adhesion.

Yoldemir et al. (38) reported in a study in which they investigated the role of various agents in preventing postoperative IAs following hysterotomy in rats, that HA solution was successful in preventing the drying of tissues and the erosion of damaged surfaces. In this study, similar to the findings of the researchers (12, 38), 0.4% HA contributed significantly to preventing adhesion compared to the control group (p < 0.05). As reported by some researchers (9, 25, 31), we think that HA plays a role in preventing adhesion formation by creating a physical barrier effect by separating the serosal surfaces and by having anti-inflammatory and fibrinolytic effects.

Koç et al. (21) reported that, in their trauma model with serosal damage on the cecum in rabbits, CMC solution could be used to prevent IAs due to its mechanical separating properties between traumatized surfaces. In a study conducted on calves, it was reported that 1% CMC solution reduced adhesion formation without any negative effects (33). Murphy et al. (23) experimentally applied CMC solution before and after creating typhlotomy and superficial trauma to the jejunum in ponies and investigated the effects of CMC solution on postoperative adhesion formation and wound healing, hematological and biochemical parameters. As a result, the researchers (23) determined that the CMC solution significantly prevented adhesion formation, had no negative effect on wound healing histopathologically, and had no change in hematological and biochemical parameters. In our study, when adhesion degrees were evaluated on the 7th postoperative day, 1% CMC solution prevented adhesion significantly compared to the control group (p < 0.01). Additionally, compared to all groups, this group had the lowest adhesion score. Similar to the researchers (31, 32), we believe that CMC solution is effective in preventing adhesion by acting as a physical barrier and preventing peritoneal surfaces from touching each other during the epithelial regeneration period. Studies have reported (18, 32) that inflammatory infiltration is milder in groups treated with HA and CMC than in groups treated with physiological saline. In this study, although there was no statistical difference between the groups, it was found that HA and CMC reduced inflammatory infiltration in the parietal peritoneum (Tab. 1). It shows that the applied HA and CMC solutions are also effective in preventing IA by preventing the maturation of adhesions through inhibition of inflammatory cell infiltration (32).

Hydroxyproline is an amino acid that forms the main component of collagen and plays an important role in the stabilization of collagen. Hydroxyproline level is directly proportional to the amount of collagen and is used as an indicator of the severity of adhesion. Increased hydroxyproline level leads to an increase in the severity of peritoneal adhesion (34). Contrary to the above information, some researchers (1) reported that HA-impregnated CMC membrane significantly reduced adhesion formation and increased peritoneal hydroxyproline levels. Although there was no statistical difference between the groups in this study, it was determined that peritoneal tissue hydroxyproline levels in the HA and CMC applied groups were relatively lower than the control group (Tab. 1). Therefore, in agreement with Uysal et al. (34), peritoneal tissue hydroxyproline level can be considered as an indicator of adhesion formation.

Peritoneal injury leads to an inflammatory response in which inflammatory cells release cytokines such as TNF-α and IL-1, IL-6. These cytokines induce the release of plasminogen activator inhibitor-1 and -2 from mesothelial cells, resulting in decreased activity of plasminogen activators. Plasminogen activator inhibitor suppresses fibrinolysis and the formation of adhesions develops as a result of collagen deposition (16). Temporary inflammation is normal after surgery during peritoneal healing, but persistent inflammation can lead to abnormal wound healing, making postoperative adhesions and fibrosis more likely. The increase of inflammatory cytokines such as IL-6 and TNF-α helps to increase some molecular mechanisms that trigger persistent inflammation and lead to fibrotic bands (2). In particular, it has been suggested that the levels of TNF-α, IL-1 and IL-6 increase both in the peritoneal fluid and in the circulation in the first
stage of IA formation, and that the increase in TNF-α, IL-1 and IL-6 cytokines is a biological indicator of IA formation (5, 11). It is emphasized that the levels of peripheral inflammatory markers and cytokines in both serum and peritoneum are associated with postoperative adhesion formation. Studies conducted in humans (29) and animals (11) reported that serum TNF (11, 29), IL-1 (29) and IL 6 (11) levels are correlated with the degrees of peritoneal adhesion after abdominal surgery. Experimental studies indicate that serum TNF-α and IL-6 are good quantitative and biological markers for postoperative peritoneal adhesion formation (5). Additionally, IL-1 has been shown to be at significantly elevated levels in the peritoneum and serum of postoperative patients (15), suggesting that IL-1 plays a role in adhesions. In our study, when serum TNF-α, IL-1 and IL-6 levels of rabbits with and without adhesion formation were evaluated, TNF-α in rabbits with adhesion formation was observed at the 6th postoperative hour (p < 0.05), while IL-6 was observed at the 6th and 24th postoperative hours (p < 0.05). It was determined that it increased statistically significantly compared to preoperative values at the 48th (p < 0.001) and 48th (p < 0.05) hours. However, no statistical difference was observed in IL-1 compared to preoperative values. In rabbits without adhesion, there was no statistical difference in serum TNF-α, IL-1 and IL-6 levels compared to preoperative values. We think that the increase in serum levels of TNF-α and IL-6 at the 6th postoperative hour and only IL-6 at the 24th hour may be a correlation with postoperative peritoneal adhesion formation (11, 29). We think that the lack of a significant difference in IL-1 may be related to the time of blood collection in the postoperative period.

In this study, treating tissues with 0.4% HA and 1% CMC solutions before manipulations in abdominal surgery and administering them to the abdominal cavity before closing the abdominal cavity suppressed peritoneal inflammation, and this resulted in an increase in the levels of proinflammatory cytokines TNF-α and IL-6. It has been determined that the application of these solutions reduces postoperative adhesion formation without causing any complications. As a result, it was concluded that TNF-α and especially IL-6, which are pro-inflammatory cytokines, can be a non-invasive biomarker in determining postoperative adhesion formation and evaluating the adverse process.

References


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