The Effect of Octreotide on Portal Vein and Peritoneal Fluid Cytokine Levels in Postoperative Adhesion Prevention

DENYESEL AMELİYAT SONRASI KARINİÇİ YAPİŞKLİK MODELİNDE OKTREOTİDİN PORTAL VEN VE PERİTON SIVISI Sİ TOKİN DÜZEYLERINE ETKİSİ

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Abstract

Objective: The aim of this study is to investigate the effect of octreotide on portal vein and peritoneal fluid TNF-α and IL-6 levels in the postoperative adhesion formation.

Material and Methods: Following an “adhesion model operation”, 10 rats were given octreotide subcutaneously (25 μg/kg/day) for 10 days (study group) while other 10 rats did not receive any treatment (control group). Ten rats had only a midline laparotomy as sham. Adhesion scores, portal vein and peritoneal fluid cytokine levels were determined on the postoperative 10th day.

Results: Octreotide significantly reduced the total adhesion score. It has also reduced the portal vein IL-6 and TNF-α (147 ± 2 pg/mL; 146.7 ± 2.6 pg/mL respectively) levels as compared to those of control group (193.5 ± 10 pg/mL; 195.8 ± 11.2 pg/mL respectively). There was no reduction (IL-6 132; 143 and TNF-α 132.8; 143.7) on contrary, elevations in peritoneal fluid cytokine levels, comparing to control.

Conclusion: Octreotide reduces postoperative intraabdominal adhesion formation through the mechanisms those mediated by intestinal rather than peritoneal cytokine production.

Key Words: Adhesion, cytokine, octreotide


Özet

Amaç: Bu çalışmanın amacı, oktrotidein postoperatif yapışıklık oluşumunda portal ven ve periton sıvısındaki TNF-α ve IL-6 seviyelerine etkisini araştırmaktır.

Gereç ve Yöntemler: Standart bir “adhezyon model operasyonu”nu takiben 10 adet erişkin szczana 10 gün süreyle subkutan yolla oktrotid (25 μg/kg/gün) uygulanırken (çalışma grubu) diğer 10 szczana herhangi bir tedavi verilmedi (kontrol grubu). On szczana sham operasyonu olarak sadece orta hat laparatomisi uygulandi. Postoperatif 10. gün adhezyon skorları, portal ven ve periton sıvısındaki sitokin seviyeleri değerlendirildi.

Bulgular: Oktrotid toptal adhezyon skorunu belirgin olarak azalttı. Ayrıca portal ven IL-6 (147 ± 2 pg/mL) ve TNF-α (146.7 ± 2.6 pg/mL) seviyeleri kontrol grubundakine (IL-6= 193.5 ± 10 pg/mL; TNF-α= 195.8 ± 11.2 pg/mL) göre azaldığı izlendi. Peritoneal sıvı seviyelerinde azalma olmayıp, tersine, kontrol grubuna kıyasla yükselme izlendi (IL-6 132; 143 ve TNF-α 132.8; 143.7).

Sonuç: Oktrotid amilyat sonrası karnıçılı yapışıklık oluşumunu peritonealden çok intestinal sitokin üretiminin yönendirildiği mekanizmaları etkilemektedir.

Anahtar Kelimeler: Adhezyon, sitokin, oktrotid

Postoperative intraabdominal adhesions are common sequelae of abdominal operations and lead to numerous complications including pain, small bowel obstruction, difficult reoperations and infertility.1,2 A great deal of efforts have been dedicated to reduce adhesion formation because of morbidity associated with adhesions. Despite these efforts, an ideal therapy or drug remains to be identified. Intraabdominal adhesion formation is a kind of aberrant wound healing process, which is working through some surrogate markers such as TNF-α and IL-6. TNF-α and IL-6 are mainly the products of activated macrophages.3 They may also be released from the mesothelial cells of the peritoneum as a response to trauma.4,7 Either of mesothelial cell or white blood cell origin, cytokine release to the peritoneal cavity and systemic circulation is inevitable after surgery. Intraabdominal adhesion formation follows the sequence of tissue inflammation, fibrin deposition,
fibrin organization, collagen formation and maturation with formation of adhesion.\textsuperscript{8,9} Intraperitoneal adhesion formation begins with an inflammatory reaction that can be interfered with drugs that have antiinflammatory activity such as octreotide.\textsuperscript{10-13}

Octreotide is an analogue of somatostatin, with the same biologic effects but a longer half-life. In clinical practice, it is generally used for its inhibitory effects on gastrointestinal peptides.\textsuperscript{14,15} This study was designed to investigate the effect of octreotide on postoperative adhesion formation and the cytokine levels of body fluids.

**Material and Methods**

Thirty 200-250 gr male, 12 months old Wistar-albino rats were divided into 3 groups. All rats lived at room temperature (27°C) and were fed by standard pellet diet and tap water. Rats were fasted before surgery and were allowed to feed orally 12 hours after surgery. This study was approved by the Animal Ethics Committee of Düzce Medical School.

**Adhesion model operation:** Rats were anesthetized with ether (Ether hydrochloride-Plantafarma-Düzce) inhalation. Induction of anesthesia was accomplished by keeping the rats in a glass chamber that contained a 10 mL ether absorbed pad on the floor. General anesthesia was maintained by ether inhalation through a mask containing an ether-absorbed pad. All groups underwent a standard midline laparotomy under sterile conditions. Both sides of the caecum were scraped with a fine sandpaper until the muscular layer appeared with a gentle handling. A 1 x 1 cm peritoneal patch excision from the right anterior abdominal wall opposing to the caecal abrasion site was performed. Abdomen was closed with 3.0 silk sutures en-bloc, continuously. On the tenth day, all rats had the second-look laparotomy for adhesion scoring and portal vein and peritoneal fluid samplings.

Sham group (n= 10) underwent a midline laparotomy only without any treatment. Control group (n= 10) had adhesion model operation and did not receive treatment. Octreotide group (n= 10) received a somatostatin analog octreotide 25 μg/kg (Sandostatin, Novartis Corp, İstanbul) once a day, subcutaneously for seven days, after adhesion model operation.

**Sampling and assays:** At second look laparotomy, immediately after the adhesion evaluation, 1 mL of blood was withdrawn from the portal vein. Two milliliters of saline was injected to the peritoneal cavity, was stirred and withdrawn with a syringe. All samples were then kept at -70°C until they were assayed. IL-6 and TNF-α levels of portal vein and peritoneal fluid were measured using commercially available ELISA kits (CYTELISA-Cytimmune Sciences inc. Maryland, USA). The limits of the sensitivity of the assays were 16.8 pg/mL and 18.2 pg/mL for IL-6 and TNF-α, respectively.

**Adhesion scoring:** The extent and strength of the fibrous bands at the peritoneal defect site were recorded. As previously defined, adhesion scores were determined according to the criteria shown in Table 1, with respect to the strength (severity) and extension of adhesions.\textsuperscript{16,17} Total adhesion score (TAS) was calculated by adding the mean of these two scores in each group.

**Statistical analysis**

All values were presented as mean ± standard error of mean. Adhesion scores were compared between control and octreotide groups with Mann-Whitney U test. Cytokine levels in portal vein

<table>
<thead>
<tr>
<th>Scores</th>
<th>Severity</th>
<th>Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No adhesions</td>
<td>No adhesions</td>
</tr>
<tr>
<td>1</td>
<td>Mild, easily dissectable adhesions</td>
<td>Adhesions on 25% of the traumatized area (by 2.5 mm)</td>
</tr>
<tr>
<td>2</td>
<td>Moderate adhesions, nondissectable, did not</td>
<td>Adhesions on 26% to 50% of the traumatized area (between 2.6-5 mm)</td>
</tr>
<tr>
<td></td>
<td>tear the organ</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Dense adhesions, nondissectable, tore organ</td>
<td>Adhesions on 51% to 100% of the traumatized area (between 6-10 mm)</td>
</tr>
<tr>
<td></td>
<td>when removed</td>
<td></td>
</tr>
</tbody>
</table>
blood and peritoneal fluid were compared by the Wilcoxon Signed Rank test. Difference between the three groups was analyzed with Kruskal Wallis test. A p value of < 0.05 was considered statistically significant.

**Results**

TAS was reduced in the octreotide group (n = 10) when compared to the control (n = 10) group (p = 0.053 not quite significant, U' = 9.00). Despite a significant reduction in adhesion extension (p = 0.0175, U' = 6.5), an equivocal alleviation in adhesion severity score could not be obtained in octreotide group (n = 10) (p = 0.1649, U' = 13.5) (Table 2).

Portal vein IL-6 (Table 3) and TNF-α (Table 4) levels were higher than the peritoneal fluid IL-6 and TNF-α levels in the control (n = 10) and sham (n = 10) groups. Portal vein IL-6 (p = 0.2188) and TNF-α and peritoneal fluid (p = 0.9999) levels were similar in the octreotide (n = 10) group (Table 3, 4).

The difference in IL-6 and TNF-α levels between the 3 groups was not statistically significant

**Table 2.** Adhesion scores obtained in the experimental groups. p; comparison of sham with control. p*; comparison of octreotide with control.

<table>
<thead>
<tr>
<th>Adhesion type</th>
<th>Control</th>
<th>Octreotide</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extension</td>
<td>2.4 (0.3)</td>
<td>1.2 (0.2)</td>
<td>0.0175</td>
</tr>
<tr>
<td>Severity</td>
<td>2.4 (0.4)</td>
<td>1.7 (0.3)</td>
<td>0.1649</td>
</tr>
<tr>
<td>Total Adhesion Scores</td>
<td>4.8 (0.6)</td>
<td>3.0 (0.4)</td>
<td>0.053</td>
</tr>
</tbody>
</table>

**Table 3.** Portal vein and peritoneal fluid IL-6 levels in different groups. p*; p value of Kruskal-Wallis analysis between the three groups. p; p value of Wilcoxon Signed Rank test between peritoneal fluid and portal vein blood levels. Values in parenthesis are s.e.m. values.

<table>
<thead>
<tr>
<th>IL-6 (pg/mL)</th>
<th>Portal vein blood</th>
<th>Peritoneal fluid</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>149.7 (10)</td>
<td>123 (8)</td>
<td>0.0156</td>
</tr>
<tr>
<td>Control</td>
<td>193.5 (10)</td>
<td>132 (12)</td>
<td>0.0156</td>
</tr>
<tr>
<td>Octreotide</td>
<td>147 (2)</td>
<td>143 (3)</td>
<td>0.2188</td>
</tr>
<tr>
<td>p*</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

(Kruskal-Wallis test; p = 1 for IL-6 and p = 1 for TNF-α). Increased peritoneal fluid IL-6 and TNF-α levels (Table 3 and 4) in the control (n = 10) group did not decrease with octreotide administration. However, significantly increased portal vein IL-6 and TNF-α levels in the control group displayed a considerable decrease in the octreotide group.

**Discussion**

The incidence of intraperitoneal adhesions ranges from 63 to 93 percent after general surgical abdominal operations and up to 97 percent after open gynecologic pelvic procedures. Abdominal adhesions account for 54-74% of small bowel obstructions. Approximately 80-90% of abdominal adhesions are due to surgical interventions, 5-20% arise from inflammatory causes and only 2-5% are congenital. An understanding of the adhesion formation pathophysiology helps us to define new methods of adhesion prevention.

Studies report that neutrophils take place in the granulation tissue that grows during the first three days of the peritoneal injury. Alatas showed that octreotide decreased the peritoneal adhesion score. In the same study, peritoneal tissue myeloperoxidase level-a marker of polymorphonuclear infiltration-also decreased. Lai demonstrated that octreotide reduced the incidence of postoperative intraperitoneal adhesions in rats. In addition, Baykal and colleagues suggested that octreotide had a beneficial effect in decreasing postoperative adhesion formation evaluated by scoring of adhe-
sions. Our results are in concordance with these two studies. In the former study, a single intraperitoneal dose of 120-150 μg/kg per rat or a concurrent intramuscular dose of 20 μg/kg for 14 days were administered. However, the latter study detected that much smaller doses such as a single intraperitoneal dose of 10 μg/kg was adequate for adhesion prevention. We used a subcutaneous dose of 25 μg/kg/day for 10 days. This dose was adequate to reduce the TAS from 4.8 to 3 in our study. There are several mechanisms to explain the effect of octreotide in decreasing postoperative adhesion formation. Angiopetin, a somatostatin analogue, decreases epidermal growth factor levels, insulin like growth factor-1 and platelet derived growth factor. Octreotide also inhibits angiogenesis.

Injury to the peritoneum leads to formation of fibrinous exudate. Adhesion formation starts with the formation of fibrin mesh followed by collagen bands at 3 to 5 days, increasing until day 14. Considering these data, we decided to evaluate adhesion formation on postoperative day 10. Prevention of permanent fibrous adhesions depends on the removal of this fibrin prior to its invasion by fibroblasts. However, we did not take any postoperative measure to clean the peritoneal cavity from the remaining fibrinous tissues such as blood. The fibrinous attachments naturally disappear by fibrinolysins released from peritoneal mesothelial cells. During the first 48 hours of healing of mesothelial defects, fibrinolytic activity is diminished by release of plasminogen activator inhibitors 1 and 2 from mesothelial, endothelial and inflammatory cells but thereafter it increases above normal levels. Drugs that interfere with fibrinolytic activity can affect adhesion formation at the fibrin organization phase; whereas antiinflammatory drugs such as steroids can modulate later phases of the process, interfering with collagen formation. Octreotide might interfere with plasminogen activator inhibitor. Nevertheless, blood alone will not be able to induce adhesions. These findings imply the presence of other factors in adhesion formation apart from those involved in fibrinogenesis theory. Postoperative adhesion formation may take place after all kinds of surgical traumas to the peritoneum irrespective of coagulation, suturing, abrasion or clamping of the tissues. Adhesions develop when the integrity of the mesothelium is damaged and the concurrent ischemia occurs. We created a peritoneal defect and visceral peritoneal injury by caecal abrasion for mesothelium damage and peritoneal trauma. This caused an almost complete adhesion formation that was prevented by octreotide therapy to some extent.

Although postoperative intraabdominal adhesions have largely been studied, limited information is available on the role of cytokines in adhesion formation. Kaidi, proposed TNF-α as a good biological marker for postoperative intraabdominal adhesion formation. A marker that can detect patients who have intraabdominal adhesions would be very beneficial. TNF-α is a short-lived cytokine, produced early in the disease process, and is thus often undetectable in the serum of patients following admission. Thus, we measured the portal vein and peritoneal fluid levels of cytokines. However, the level of IL-6 appears to be more stable and is raised in the serum of patients with acute pancreatitis, correlating to some degree with the severity of disease. Alternatively, IL-6 may simply be a marker of more important proinflammatory mediators. In our study, portal vein and peritoneal fluid TNF-α and IL-6 levels were higher in the control group compared to the sham group. Although the increased cytokine levels might be a harbinger of peritoneal adhesions, we did not look for a correlation between adhesion degree and cytokine levels. However, our result revealed that TNF-α and IL-6 levels increased due to the formation of intraperitoneal adhesion. In our study, despite a slight increase in peritoneal fluid TNF-α and IL-6 levels in the control groups compared to the sham group, the difference was not statistically significant. Octreotide also did not cause a reduction in peritoneal fluid cytokine levels. Reports suggest that somatostatin analogues have significant anti-inflammatory effects in vivo, associated with the suppression of proinflammatory cytokines and neuropeptides. In inflammatory conditions
such as acute pancreatitis, excessive production of IL-6 from peripheral blood mononuclear cells may be altered by therapeutic intervention and could conceivably influence outcome.\(^\text{35}\) Our results, although they were not statistically significant, showed that portal vein IL-6 and TNF-\(\alpha\) levels were both considerably reduced by subcutaneous octreotide treatment. However, this effect of octreotide was not observed on peritoneal fluid IL-6 and TNF-\(\alpha\) levels. This implied that TNF-\(\alpha\) and IL-6 response to peritoneal injury was not mainly mesothelial cell origin.

Reports suggest that somatostatin analogues have significant antiinflammatory effects in vivo, associated with suppression of proinflammatory cytokines and neuropeptides.\(^\text{36}\) Besides, it decreases the release of some growth factors, inhibits angiogenesis and prevents cell proliferation.\(^\text{37,39}\) This also implies that somatostatin analogues such as octreotide may inhibit the inflammatory reaction which leads to intraperitoneal adhesion formation. Chao, showed that octreotide caused a significant reduction in the release of hydrogen peroxide, nitric oxide, and TNF-\(\alpha\) from the macrophages.\(^\text{40}\) Marton, detected a decrease in serum TNF-\(\alpha\) and IL-6 levels in octreotide-treated rats with taurocholic acid-induced pancreatitis.\(^\text{41}\) Chao, demonstrated in an in vivo study, that Kupffer cells treated with somatostatin, released significantly less amount of TNF-\(\alpha\) than the untreated controls.\(^\text{42}\) All studies of which TNF-\(\alpha\) and IL-6 levels were measured in systemic blood demonstrate the suppressive effect of octreotide on cytokines. We also showed the suppressive effect of octreotide on portal vein blood TNF-\(\alpha\) and IL-6 levels. This difference between the portal vein blood and peritoneal fluid cytokine response to peritoneal trauma revealed a selective effect of octreotide in the prevention of adhesion formation.

In conclusion, postoperative adhesion formation causes a significant increase in portal vein blood TNF-\(\alpha\) and IL-6 levels. Octreotide has a selective effect on portal vein TNF-\(\alpha\) and IL-6 levels and may prevent postoperative adhesion formation through the suppression of portal vein cytokine levels.

**REFERENCES**