Kafkas Univ Vet Fak Derg
22 (3): 391-395, 2016
DOI: 10.9775/kvfd.2015.14795

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

More than an Uterotonic Agent: Oxytocin Prevents Peritoneal Adhesion

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Article Code: KVFD-2015-14795 Received: 04.12.2015 Accepted: 12.02.2016 Published Online: 12.02.2016

Abstract

Prevention of postoperative adhesions (PPA) has become an important issue. The aim is to investigate the effect of Oxytocin (OT) on PPAs. A total of thirty female Wistar-albino rats were randomly divided into three groups (10 rats/group). The cecal peritone of Group I rats (controls) were scraped, to trigger adhesion formation, and no treatment were given. After cecal scrubbing, 1 mL saline solution was applied to each rat in Group II (i.p. saline treated group) and 80 IU/kg of OT (Pituisan®, Ege Vet, Turkey) to Group III (i.p. OT treated group) intraperitoneally. All animals were sacrificed 10 days after surgery and adhesions graded in terms of severity and histopathologic characteristics. The median scores for the extent, severity, and degree of adhesions in Group I and Group II were statistically significant and considerably higher than those scores for Group III ($P<0.001$). The inflammation, neovascularization, and fibrosis scores for Group III were statistically significant and considerably lower than those scores for Groups I and II ($P<0.001$, $P<0.001$ and $P=0.002$ respectively). OT, significantly prevented adhesion formation improving wound healing possibly by suppressing adhesion formation with anti-inflammatory and antioxidant properties. OT may be useful in the prevention of PPA in humans.

Keywords: *Oxytocin, Postoperative, Peritoneal adhesion*

Uterotonik Bir Ajandan Fazlası: Oksitosin Postoperatif Adezyonları Önlüyor

Özet

Postoperatif adezyonların (PPA) önlenmesi önemli bir konu olmuştur. Bu çalışmada amaç Oksitosinin (OT) PPA üzerindeki etkisini araştırmaktır. Toplam 30 Wistar-albino dişi rat üç eşit gruba ayrıldı (10 rat/grup). Grup I ratlarda (kontrol) sekum peritonu adezyon oluşturmak amacıyla sıyrıldı ve hiçbir tedavi verilmedi. Grup II'ye (i.p. salin tedavi grubu) sekum sıyrılmasından sonra intraperitoneal 1 ml saline ve Grup III (i.p. OT tedavi grubu)'e 80I U/kg (Pituisan®, Ege Vet, Turkey) OT uygulandı. Bütün hayvanlar cerrahiden 10 gün sonra öldürüldü ve adezyonlar şiddeti ve histolojik özellikleri açısından derecelendirildi. Grup I ve II'deki adezyonların yaygınlık, şiddet ve derecelerinin medyan skorlarının Grup III'dekilere göre istatistiksel olarak arttığı görüldü ($P<0.001$). Grup III'deki inflamasyon, neovaskülarizasyon ve fibrozis skorları Grup I ve II'deki skorlara göre istatistiksel olarak anlamlı ve belirgin ölçüde düşük bulundu ($P<0.001$, $P<0.001$ and $P=0.002$ respectively). Oksitosin, anti-enflamatuarve anti- oksidan özellikleri ile adezyon oluşum sürecindeki basamakları baskılayarak, yara iyileşmesini düzenleyerek ratlarda adezyon oluşumunu ciddi ölçüde engellemiştir. OT insanlarda PPA önlenmesinde faydalı olabilir.

Anahtar sözcükler: *Oksitosin, Postoperatif, Peritoneal adezyone*

INTRODUCTION

Peritoneal adhesions occur due to the disruption of normal peritoneal healing generally after abdominal or pelvic surgery [1]. Postoperative peritoneal adhesions (PPA) can cause chronic pelvic pain, small bowel obstruction, dyspareunia, dysmenorrhea, or infertility. Reoperations due to adhesions can cause various complications such as iatrogenic organ damage and can sometimes make laparoscopic surgery impossible [1].

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Two mechanisms have been reported in peritoneal healing; normal physiological repair and adhesion formation. The inflammatory process begins due to the peritoneal insult, and then fibrin deposits occur. Activation of plasminogen degrades fibrinous exudate to plasmin. When fibrinolysis of the fibrinous exudate is inhibited, fibrin molecules organize, and then a collagen matrix and adhesions form [2].

Since adhesions cause many health problems and financial burdens, the prevention of PPA has become an important issue for health scientists. Numerous agents have been used to prevent PPA, but most of them are experimental.

Oxytocin (OT) is a neurohypophysial nonapeptide that is synthesized in the paraventricular and supraoptic nuclei in the hypothalamus. OT has central and peripheral effects. The main functions of OT are uterine contractions at parturition and myoepithelial contractions in the mammary gland. Also, OT receptors have been found in some peripheral tissues, such as the kidney, heart, thymus, pancreas, and adipocytes [3]. Experimental studies have shown that OT suppresses neutrophil infiltration and controls inflammatory cytokines [4,5]. Subcutaneous injection of OT has been shown to be helpful in wound healing by exerting anti-oxidant and anti-inflammatory properties [6]. In ischemia/reperfusion experimental models, OT has been found to be protective against many tissue injuries such as heart, liver, and skeletal muscle [7-9].

As far as we know, there has been no study of the effects of OT on PPAs. This study investigates whether OT prevents PPA formation.

MATERIAL and METHODS

The Animals and the Experimental Model

A total of 30 female Wistar-albino rats weighing 200±20 g were used for this study. The rats were housed in pairs in steel cages and were fed ad libitum with standard pelleted food approved by the Turkish Standards Institute and had free access to tap water. The room temperature (22±2°C) and humidity were controlled with 12-h light/dark cycles. All experimental procedures were approved by the Bulent Ecevit University School of Medicine Animal Care Committee with the ID number: 2013/09-1.

Rats were anesthetized with intraperitoneal injections of Ketamine hydrochloride (50 mg/kg, Ketalar; Pfizer, Istanbul, Turkey) and Xylazine hydrochloride (10 mg/kg, Rompun, Bayer, Istanbul, Turkey). After the disinfection and shaving of the anterior abdominal skin, a 2-cm midline incision was performed in the lower abdomen for each rat. Uterine horns and cecum were identified. To form adhesions the cecum abrasion model was used [10]. Cecums were scrubbed with a sterile sponge on the antimesenteric surface until punctate bleeding and serosal petechiae occurred. Rats were randomly divided into three groups each containing ten rats.

Group I (control): Only adhesion formation was performed without any treatment.

Group II (i.p. saline treated group): After cecal scrubbing, 1 mL saline solution wasapplied intraperitoneally.

Group III (i.p. OT treated group): After adhesion formation, 80 IU/kg of OT (Pituisan®, Ege Vet, Turkey) was applied intraperitoneally.

The midline incisions in the rats were closed with 3/0 monofilament sutures. All the rats were housed in cages under standard conditions with free access to food and water. Two weeks after the first operation, a second laparotomy wasperformed with a reverse U incision to evaluate PPA. Macroscopic evaluations of the adhesions were done according to Linsky's classification by the second author blinded to the groups [11]. Adhesions were evaluated for involvement, resistance, and severity. According to the involvement of adhesions, the scores were as follows: no adhesions=0, adhesions up to 25% of the scrubbed area=1, adhesions up to 75% of scrubbed area=2, adhesions on the whole scrubbed area=3. The scores for resistance were as follows: no adhesions=0, adhesions that can easily be separated=1, adhesions that can be separated with traction=2, adhesions that can be separated only by sharp dissection=3. For the severity of the adhesions, the scoring was as follows: no adhesions=0, filmy and avascular=1, moderately filmy and vascular=2, dense and significantly vascular=3. The total scores, calculated by summing the three scores, ranged between 0 and 18.

Histopathological Evaluation

Adhesion areas and cecum were excised, fixated in formal solution samples, and sent for histopathological evaluation. After dehydration with ethanol, specimens were embedded in paraffin blocks, and then 5 µm-thick sections were sliced from embedded tissues and stained with hematoxylin and eosin. A light microscope was used to evaluate fibrosis, inflammation, and vascularization. Histopathological evaluations of the samples were performed by a pathologist (B.D.G.) who was blind to groups. A light microscope was used for the evaluation of sections. To assess inflammation, neovascularization, and fibrosis, a semiquantitative scoring system was used [12]. The degree of inflammation was classified as grade 0 (absent or normal in number), grade 1 (slight increase), grade 2 (moderate infiltration), or grade 3 (massive infiltration); fibrosis as grade 0 (none), grade 1 (slight), grade 2 (moderate), or grade 3 (dense); and neo-vascularization as grade 0 (none), grade 1 (one to two vessels), grade 2 (three to nine vessels), or grade 3 (10 or more vessels).

Statistical Analysis

Statistical analyses were performed with SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Variables were expressed as median (minimum-maximum). Differences among the groups were analyzed by the Kruskal-Wallis test. Dual comparisons among groups with significant values were evaluated with Dunn's test after the Kruskal-Wallis test. A P-value of less than 0.05 was considered statistically significant for all of the tests.

RESULTS

During the study period, one rat from Group I (control group) and one rat from Group II (SF-treated group) were lost due to the operation. The remaining 28 rats all tolerated the study period. No complications were observed during the follow-up period.

The macroscopic and the histopathological adhesion scores of three groups are presented in *Table 1*. In Group I (control group), 22% of the rats had grade 2 adhesions, and 78% of the rats had grade 3 adhesions. In Group II (SF treated group), 33% of adhesions were grade 2, and 67% were grade 3, whereas in Group III (OT-treated group), 30% of the rats had no adhesions, and 70% had grade 1 adhesions with respect to macroscopic evaluation. The median scores for the extent, severity, and degree of adhesions in Group I and Group II were statistically significant and considerably higher than those scores for Group III (P<0.001).

In pathological examinations, the scores for Group III with respect to inflammation, neovascularization, and fibrosis were statistically significant and considerably lower than those scores for Groups I and II (P<0.001, P<0.001 and P=0.002 respectively). No statistically significant difference were observed between Group I and Group II with respect to the macroscopic and pathological adhesion scores. The microscopic evaluation of the inflammation, vascularization, and fibrosis are presented in *Fig. 1* as none, mild-moderate, and severe.

DISCUSSION

The present study reveals that intraperitoneal administration of 80 IU/kg OT reduces adhesion formation. Peritoneal adhesion formation after any kind of visceral surgery is still an important clinical problem although various preventive strategies against adhesion formation have been investigated. The mesothelial cell lining of the peritoneum has an important role in peritoneal healing [13,14]. In the case of peritoneal injury (bleeding, cauterization or local ischemia) peritoneal adhesions can

Table 1. *The macroscopic and the histopathological adhesion scores. Same letters denote similar groups with regards to adhesion scores*
Tablo 1. *Makroskopik ve histopatolojik adezyon skorları. Aynı harfler adezyon skorları açısından benzer grupları göstermektedir*

Adhesion Scores	Control (n = 9) median (min-max)	Saline Solution (n = 9) median (min-max)	Oxytocin (n = 10) median (min-max)	P
Extent	3 (2-3)[a]	3 (2-3)[a]	1 (0-1)[b]	< 0.001
Severity	3 (2-3)[a]	3 (2-3)[a]	1 (0-1)[b]	< 0.001
Degree	3 (1-3)[a]	3 (1-3)[a]	1 (0-1)[b]	0.001
Total Score	9 (7-9)[a]	8 (6-9)[a]	3 (0-3)[b]	< 0.001
Inflammation	3 (2-3)[a]	2 (2-3)[a]	1 (1-1)[b]	< 0.001
Neovascularization	3 (2-3)[a]	2 (2-3)[a]	1 (0-1)[b]	< 0.001
Fibrosis	2 (1-3)[a]	2 (2-3)[a]	1 (0-1)[b]	0.002

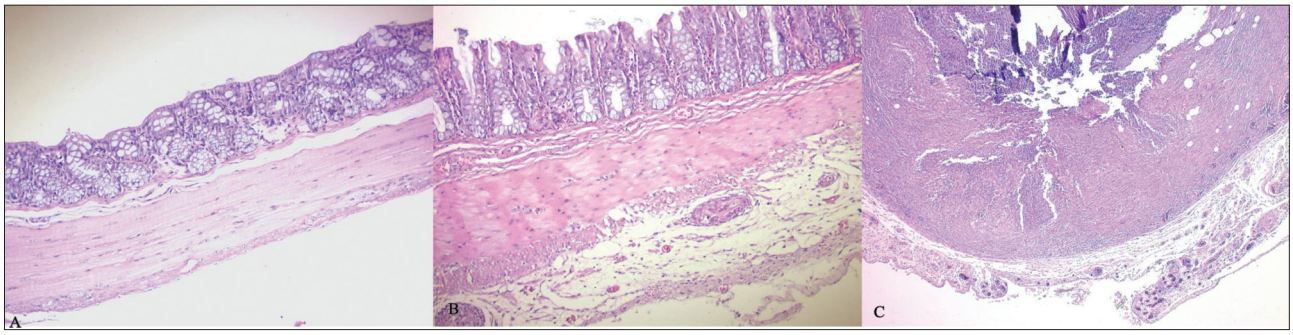

Fig 1. The histopatologic specimens with Hematoxylin-Eosin (HEx40) staining. **A-** no fibrosis, inflammation or vascular proliferation, **B-** mild-moderate inflammation, neo-vascularisation and fibrosis, **C-** severe inflammation, increased vascularisation and dense fibrosis

Şekil 1. Hematoxylin-Eosin (HEx40) ile boyanan histolojik kesitler **A-** fibrozis, inflamasyon ve vasküler proliferasyon yok, **B-** hafif-orta düzeyde inflamasyon, neovaskülazisyan ve fibrozis, **C-** şiddetli inflamasyon, artmış vaskülarizasyon ve yoğun fibrozis

occur since tissue plasminogen activator (tPA) expression is reduced and levels of plasminogen activator inhibitor types1 and 2 (from inflammatory, mesothelial, and endothelial cells) are increased. Normally, fibrinolytic activity provides remesothelization; however, in the case of ischemia, fibrinolytic activity decreases, causing fibrin deposition. In our study we performed peritoneal injury and ischemia via the cecal abrasion method.

Inflammation also plays a vital role in the formation of adhesions. Inflammatory mediators, such as cytokines, interleukins, and transforming growth factor-β, decrease fibrinolytic activity in the peritoneum, so that adhesion formation aggregates.

Reactive oxygen radicals (ROS) formed during hypoxia activate fibroblasts, causing the production of cytokines such as vascular endothelial growth factor (VEGF), tissue growth factor (TGF), and cyclooxygenase-2, which all increase adhesion formation [15,16].

Adhesions are complex structures composed of inflammatory cells, fibrosis, and new vessels. If the soft fibrin that results from adhesion formation is not removed by the fibrinolytic activity, dense fibrins will develop within 2 weeks [17]. In our study we performed the second operation 2 weeks after the first operation. The follow-up time was sufficient for adhesion maturation.

Many experimental methods have been used to introduce peritoneal adhesions such as uterine horn cauterization, large bowel anastomosis, ileal transection, peritoneal damage, scraping, and suturing [18,19]. Since scraping is one of the most effective methods for causing adhesions to form, in this study we used this model to evaluate the effects of OT on PPA.

Various treatment modalities have been used to prevent adhesion formation. The proposed mechanisms with these agents include reducing inflammation, increasing fibrinolysis capacity, preventing fibroplast proliferation, or separating the deperitonealized areas. Also, different antioxidant agents such as Vitamin C, Vitamin E, N-acetyl cysteine, and melatonin have been used to prevent adhesions [20,21]. Since oxytocin is known to have anti-inflammatory and anti-oxidant effects, we tried to evaluate its impact on the prevention of adhesions. Oxytocin had not been studied for the adhesion prevention effect before.

OT has been demonstrated to cause the release of nitric oxide (NO), which inhibits neutrophil infiltration and adhesion formation [22]. In the case of sepsis due to cecal ligation and puncture, massive inflammatory mediators such as NO are produced and it has been reported that via the central NO-cGMP pathway the arginine vasopressin (AVP) and OT gene expressions are increased [23]. Also Ahmed MA et al.[24] suggested that OT decelerated atherosclerosis by inhibiting proinflammatory responses. OT modulates immune and inflammatory response by decreasing interleukin-6 and TNF-α.levels, increasing prostacyclin, and insulin-like growth factor (IGF)-I levels [6]. OT treatment decreased macroscopic and microscopic scores for inflammation and fibrosis in our study.

Vascularization increases in adhesion formation [25]. The present study revealed that the mean neo-vascularization score of the OT-treated group was lower than those of the control and saline-treated groups. The negative effect of OT on neo-vascularization is proposed to be due to decreased levels of inflammatory cytokines including VEGF [26]. However we did not study VEGF levels, which was a limitation in our study.

OT has been known as an anti-oxidant agent that decreases ROS levels. Tas Hekimoglu A et al.[27] reported remote oxidative stress (OS) in liver tissue in renal ischemia reperfusion study. However, they suggested that OT application prevented the rising of OS. The previous studies also reported the protective effect of OT on ischemia reperfusion-related injuries in ovaries, heart and skeletal muscle [7,9,28]. In our study we also detected the protective effect of OT on inflammation, neo-vascularization, fibrosis, and then adhesion formation.

Oxytocin was used intraperitoneally at the dose of 80 IU/kg, which was the same dose used in the previous study [28]. It has been shown that high doses of OT have some side effects in humans such as cardiac arrhythmia, fatal afibrinogenemia, nausea, vomiting, premature ventricular contractions, subarachnoid hemorrhage, and hypertensive episodes. In the present study, we did not observe any OT-related systemic side effects with the dose given intraperitoneally.

Saline has been reported to decrease adhesion formation in some studies [29]. However, others argued that saline did not decrease adhesion formation [30]. Likewise in our study, adhesion formation was not prevented in the saline-treated group.

The present study showed that oxytocin prevented adhesion formation in rats. This effect of oxytocin would be suitable to protect human females against inflammation in the postpartum period especially those who gave birth by Cesarean section since oxytocin is released in large amounts and is also given to women intravenously in the postpartum period.

Conflict of Interest

The authors report no conflict of interest.

Acknowledgemenets

The authors thank to Füruzan Kokturk who performed the statistical analysis.

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