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Long-term outcomes of pure olive oil to prevent postoperative peritoneal adhesions in rats

Ratlarda postoperatif peritoneal adezyonların önlenmesinde saf zeytinyağının uzun dönem etkinliği

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Abstract

Aim: Postoperative peritoneal adhesion (PPA) that occur after abdominopelvic surgery are the current problems of surgeons. The aim of this study was to investigate the effect of pure olive oil in preventing PPA.

Methods: Thirty-two rats were randomly divided into four groups: (1) Sham group: 5 ml pure olive oil was injected percutaneously into the peritoneal cavity without laparotomy, (2) Adhesion group: A standard adhesion model was formed on jejunum, ileum and caecum. (3) Adhesion + olive oil: A standard adhesion model was formed on jejunum, ileum and caecum. Subsequently the area on jejunum, ileum and caecum was covered with 5 ml of pure olive oil. (4) Olive oil + adhesion: The area on jejunum, ileum and caecum was covered with 5 ml of pure olive oil. Subsequently the standard adhesion model was formed on this area. Four weeks later, abdominal cavities of rats were examined for PPA, using Evan's adhesion classification and Zühlke's histopathological grade scales.

Results: PPA was not found in Group 1 in which olive oil was injected intraperitoneally. PPA was present in all rats in Group 2. Microscopic adhesion scores in Group 3 and 4 were significantly lower than Group 2. The collagen fiber fractions were significantly lower and there was a significant decrease in fibrosis. There was no statistically significant difference in microscopic adhesion scores in Group 3 compared to Group 4.

Conclusion: We found that antiinflammation, tissue regeneration and hydroflotation effect of pure olive oil decreased PPA formation in rats by maintaining a long lasting effect on the wounded peritoneal surface. Thus olive oil, a cheap and easy to obtain product, can be used in cases of PPA in surgery clinics.

Keywords: Postoperative peritoneal adhesions, Olive oil, Surgery

Öz

Amaç: Abdominopelvik cerrahi sonrası ortaya çıkan postoperatif peritoneal adezyonlar (PPA) cerrahların güncel sorunlarındandır. Bu çalışmanın amacı, saf zeytinyağının PPA önlenmesindeki etkisini araştırmaktır.

Yöntemler: 32 rat randomize dört gruba ayrıldı: (1) Sham grubu: Laparotomisiz peritoneal kaviteye perkütan olarak 5 ml saf zeytinyağı enjekte edildi, (2) Adezyon grubu: Jejunum, ileum ve çekum üzerine standart bir adezyon modeli oluşturuldu. (3) Adezyon + zeytinyağı: Jejunum, ileum ve çekumda standart bir adezyon modeli oluşturuldu. Daha sonra jejunum, ileum ve çekum üzerindeki alan 5 ml saf zeytinyağı ile kaplandı. (4) Zeytinyağı + adezyon: Jejunum, ileum ve çekum üzerindeki alan 5 ml saf zeytinyağı ile kaplandı. Daha sonra bu alanda standart adezyon modeli oluşturuldu. Dört hafta sonra, sıçanların karın boşlukları, PPA için Evans'ın adezyon sınıflandırması ve Zühlke'nin histopatolojik derecelenme ölçekleri kullanılarak incelendi.

Bulgular: Zeytinyağının intraperitoneal olarak enjekte edildiği Grup 1'de herhangi bir adezyon bulunmadı. Grup 2'deki tüm ratlarda PPA mevcuttu. Grup 3 ve 4'ün mikroskopik adezyon skorları Grup 2'den istatistiksel olarak anlamlı düzeyde daha azdı. Kollajen lif fraksiyonları belirgin şekilde daha düşüktü ve fibrozisde belirgin bir azalma gözlendi. Grup 3, Grup 4 ile karşılaştırıldığında ise, mikroskopik adezyon skoru açısından istatistiksel olarak anlamlı fark yoktu.

Sonuç: Saf zeytinyağının antiinflamasyon, doku rejenerasyonu ve hidroflotasyon etkisinin, yaralanmış peritoneal yüzey üzerinde uzun süreli etki sağlayarak ratlarda PPA oluşumunu azalttığını bulduk. Bu nedenle, ucuz ve satın alınması kolay bir ürün olan zeytinyağı, cerrahi kliniklerinde PPA vakalarında kullanılabilir.

Anahtar kelimeler: Postoperatif peritoneal adezyon, Zeytinyağı, Cerrahi

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Introduction

Postoperative peritoneal adhesion (PPA) that occur after abdominopelvic surgery are the current problems of surgeons as they can lead to complications such as intestinal obstruction, infertility, pelvic pain and difficulties during re-operations. The best surgical techniques alone are insufficient to prevent PPA [1]. Approximately 90% of patients undergoing intraabdominal surgery develop PPA, and approximately 3% develop intestinal obstruction. PPA causes infertility approximately in 15-20% of women [2]. Intestinal obstruction is associated with PPA with a ratio of 75%, and this association might be higher up to 93% after multiple operations [3].

Damages to peritoneal and serosal surfaces, due to the chemical, thermal, foreign body reactions, infections or traumatic factors initiate the events resulting in adhesion formation [4]. Peritoneal or serosal injury results in release of histamine and vasoactive kinins from mast cells, leading to an increase in capillary permeability and accumulation of serous liquid [5]. The damage of mesothelial surface causes the contact of connective tissue with peritoneal fluid. This results in increased levels of leukotriene B4 (LTB4) and prostaglandin E2 (PGE2) in peritoneal fluid and inhibition of tissue plasminogen activator activity (tPAA). Increase in LTB4 and PGE2 stimulates the adhesion formation, whereas inhibition of tPAA reduces fibrin degradation; ultimately the balance changes further in adhesion formation. Peritoneal injury activates coagulation cascade, and a release in thromboplastin level and the fibrin formation occurs. If fibrin degradation is not enough, fibrin provides matrix for adhesion formation [6,7].

Olive oil has taken a focus of attention due to various effects of its phenolic components. Biological properties of olive oil have been evaluated extensively in vitro and in vivo animal models and human studies. Currently, anti-inflammatory, antioxidant, antineoplastic and antimicrobial effects of phenolic components have been documented in large number of studies [8-10]. There is also lubricant and mechanical barrier action of olive oil. Since pure olive oil is a highly viscous fluid, it is important that lubrication effect of olive oil on serosal and peritoneal surfaces may be used to minimize abrasion during surgical operations. Hence, olive oil may prevent abdominal adhesions due to mechanical separation (hydroflotation) effects between the traumatized surfaces.

In this study, we aimed to investigate the effect of pure olive oil in the prevention of PPA after long-term follow-up in rats undergoing an adhesion model.

Materials and methods

This study was carried out at pediatric surgery department. Animal trials were initiated after approval from the local ethics committee. A total number of thirty-two female Wistar-Albino rats (bodyweight 250-300 g) in eight groups were randomly selected in the study. Animals were kept at an ambient temperature of 22°C and 60±5% humidity with a 12-hr light/dark cycle and access to food and water ad libitum.

Pure olive oil preparation

Pure olive oil was produced in agricultural engineering with cold presecting method. Olive oil was sterilized by filtration through 0.45 nm porosity to sterile centrifuge tube. The pH value of olive oil was the same as that of peritoneal dialysis liquid (pH 6.8).

Experimental design and peritoneal adhesion model

Rats were given two weeks to acclimatize to the surroundings and handling prior to the experiments. Rats were starved for 12 hours prior to surgery, and experiment was started after appropriate anesthesia with ketamine 75 mg/kg (Ketalar, Pfizer, Istanbul, Turkey) and xylazine 10 mg/kg (Rompun, Bayer). All surgical procedures were performed under sterile conditions.

Experimental adhesion model was provided with a special designed device, with a movable arm for its floating action. We used a weight of 0.5 kg, an area of 2x2 cm², and applied the floating action ten times to the intestinal surface for standard adhesion model. Thus, a standardized peritoneal trauma was created on a selected intestinal surface with respect to weight, area and number.

Rats were divided randomly into four groups, each containing eight rats: Group 1 (sham group): 5 ml pure olive oil was injected percutaneously into the peritoneal cavity without laparotomy; Group 2 (adhesion group): Vertical midline incision of 3 cm was inserted, and a standard adhesion model was created with adhesion apparatus on jejunum, ileum and caecum; Group 3 (adhesion + olive oil): Vertical midline incision of 3 cm was inserted, and a standard adhesion model was created with adhesion apparatus on jejunum, ileum and caecum. Subsequently, the area on jejunum, ileum and caecum was covered with 5 ml of pure olive oil; Group 4 (olive oil + adhesion): Vertical midline incision of 3 cm was inserted, and the area on jejunum, ileum and caecum was covered with 5 ml of pure olive oil. Then the standard adhesion model was created with adhesion apparatus on this area.

Incisions were closed by continuous technique with 3/0 propylene suture. On the 30th postoperative day, the rats were sacrificed by cervical dislocation. Through the initial laparotomy scar, abdomen was re-opened using a midline incision from cranial to caudal to view the extent of intraabdominal adhesion formation. The adhesions were macroscopically graded according to Evans' adhesion classifications (Table 1), and microscopically graded according to Zühlke's histopathological classifications (Table 2) by an experienced pathologist who was blinded to the different treatment groups.

Macroscopic assessment of PPA

Macroscopic assessment of PPA was performed according to the method described by Evans [11]. A pathologist, who was uninformed about the groups, examined the intraabdominal adhesions of each rat. Through this examination, the interactions of the olive oil with the peritoneum and its efficacy in preventing adhesions were evaluated. PPA was classified according to the strength and area of adhesions (Table 1).

Histopathological assessment of PPA

The area of 2x2 cm2 on jejunum, ileum, anterior caecum from all rats, and adhesions on this surface were excised. The tissues were fixed in a 10% buffered formaldehyde solution and embedded in paraffin following dehydration. Tissue sections of 5 µm thickness were obtained and stained with hematoxylin

and eosin. These sections were evaluated using light microscopy at a magnification of 100x. The histopathological grade was evaluated according to histopathological grading of Zühlke's classification [12] (Table 2).

Table 1: Evans's scoring system

Adhesion area score (Grade)	Adhesion strength score (Grade)
0: no adhesion	0: no adhesion
1: 25% of the area has adhesion	1: spontaneously separating adhesions
2: 50% of the area has adhesion	2: adhesions separating by traction
3: complete area has adhesion	3: adhesion separated by dissection

Table 2: Zühlke's histopathological classification

Grade 1	Loose connective tissue, cell-rich, old and new fibrin, fine reticulin fibrils
Grade 2	Connective tissue with cells and capillaries, few collagen fibers
Grade 3	Connective tissue more firm, fewer cells, more vessels, few elastic and smooth muscle fibers
Grade 4	Old firm granulation tissue, cell-poor, serosal layers hardly distinguishable

Statistical analysis

SPSS 13.01 was used for statistical analysis. Kruskal Wallis test was used to evaluate the distribution of data. Categorical variables were expressed as n(%), continious date were as mean±standard deviation or median (interquartile range). Chi square test was for the analysis of categorical variables. Kruskal Wallis test was used for multiple group analysis and Bonferroni corrected Mann Whitney U test in posthoc subgroup comparisons. The results were evaluated at 95% confidence interval and at $p<0.05$ level.

Results

We did not find any peritoneal reaction, toxic effect or adhesion in group 1, in which olive oil was injected intraperitoneally without laparotomy. When group 3 and group 4 were separately compared to group 2, we found that the adhesion strength scores of group 3 and group 4 were statistically lower than group 2 ($p<0.001$). There was no statistically significant difference in the adhesion strength when compared group 3 to group 4 (Table 3). When group 3 and group 4 were separately compared to group 2, scores of adhesion areas in group 3 and group 4 were significantly lower than group 2 ($p <0.001$). When we compared group 3 to group 4, there was no significant difference in the score of adhesion area (Table 3).

The microscopic adhesion score evaluations of jejunum, ileum and caecum's were shown in Table 4. The microscopic adhesion scores of groups 3 and 4 were statistically lower than group 2 ($p <0.001$). There was no significant difference in microscopic adhesion score when compared group 3 to group 4.

Table 3: The macroscopic adhesion score in jejunum, ileum and caecum of the groups according to Evans's scoring system

Adhesion Strength Assessment, n (%)					
Group no	Score 0	Score 1	Score 2	Score 3	Total
2	0 (0%)	0 (0%)	1(12.5%)	7 (87.5%)	8 (100%)
3	5 (62.5%)	3 (37.5%)	0 (0%)	0 (0%)*	8 (100%)
4	7 (87.5%)*	1 (12.5%)	0 (0%)	0 (0%)*	8 (100%)
Adhesion Area Assessment, n (%)					
Group no	Score 0	Score 1	Score 2	Score 3	Total
2	0 (0%)	0 (0%)	6 (75.0%)	2 (25.0%)	8 (100%)
3	5 (62.5%)	3 (37.5%)	0 (0%)**	0 (0%)	8 (100%)
4	6 (87.5%)**	2 (12.5%)	0 (0%)**	0 (0%)	8 (100%)

* refers to the significant difference between group 3-4 and group 2 in adhesion strength scores ($p <0.001$)
** refers to the significant difference between group 3-4 and group 2 in scores of adhesion areas ($p <0.001$)

Table 4: The microscopic adhesion score in jejunum, ileum and caecum of the groups according to Zühlke's histopathologic classification

Group no	Score 0	Score 1	Score 2	Score 3
	Jejenum, n (%)			
2	0 (0%)	3 (37.5%)	1 (12.5%)	4 (50%)
3	6 (75%)*	2 (25%)	0 (0%)	0 (0%)
4	7 (87.5%)*	1 (12.5%)	0 (0%)	0 (0%)
	Ileum, n (%)			
2	0 (0%)	5 (62.5%)	2 (25%)	1 (12.5%)
3	7 (87.5%)**	1(12.5%)	0 (0%)	0 (0%)
4	8 (100%)**	0 (0%)	0 (0%)	0 (0%)
	Caecum, n (%)			
2	0 (0%)	1 (12.5%)	4 (50%)	3 (37.5%)
3	7 (87.5%)***	1 (12.5%)	0 (0%)	0 (0%)
4	7 (87.5%)***	1 (12.5%)	0 (0%)	0 (0%)

* refers to the significant difference between group 3-4 and group 2 in histopathologic adhesion in jejenum ($p <0.001$)
** refers to the significant difference between group 3-4 and group 2 in histopathologic adhesion in ileum ($p <0.001$)
*** refers to the significant difference between group 3-4 and group 2 in histopathologic adhesion in caecum ($p <0.001$)

Discussion

Adhesion is known as a type of physiologic peritoneal healing process. However, there are two main processes that can prevent the formation of PPA: decreasing the frequency of number of the peritoneal trauma, and performing less traumatic surgical techniques. It is much simpler and more effective to apply the first option. It's simple, because all should be done is to build a barrier that will prevent the formation of trauma. It's effective, since the process of wound healing after trauma is quite complicated. These options would have a low chance of success unless clarifying the underlying physiopathology. Despite the increasing number of laparoscopic surgeries over the years, an expected reduction in the percentage of adhesions has not been obtained. Clinical trials have shown that laparoscopic procedures reduce the area of adhesions but do not reduce the incidence of PPA [13].

Various experimental models such as abrasion, local peritoneal excision, ischemic damage, emplacement of foreign bodies into the peritoneal cavity, thermal damage and bacterial contamination have been developed to generate PPA [14,15]. Any manipulations performed by hand or surgical instruments during laparotomies are known as mechanical trauma and they are the most common cause of PPA [16,17]. We used abrasion model in this study because it simulated the mechanical trauma.

Fibrin is known to provide the matrix for adhesion formation [6,7]. The number of clinical and experimental studies demonstrating anti-adhesive and anti-phlogistic effects of materials that inhibit fibrin accumulation is not large. In an experimental study, no significant difference was found between aprotinin, dextran or lipid emulsion compared to the control group in preventing the frequency of PPA [18]. Isotonic NaCl and hypertonic dextrose solutions were used to remove fibrin, but their effects were limited because of their rapid absorbtion from peritoneum. In previous studies, pepsin, trypsin and papain, which remove fibrin by mechanical or enzymatic processes, have been recommended. However, subsequent studies have shown that these enzymes were rapidly neutralized by the peritoneum, and that there was no effect in preventing adhesions [19]. Sodium citrate, heparin, dicumarol, meclofenamate, tolmetin, ibuprofen, nimesulide, oxyphenbutazone, corticosteroid, aspirin, disodium cromoglycate, methylene blue, Mn-desferoxamine, allopurinol, mannitol, pentoxifylline, catalase and vitamin E were dosens of chemicals that had been used to prevent the

inflammatory response and prevent fibrinous exudate. Due to their barrier-forming properties which reduce fibroblastic activity and provide lubrication, soybean oil, aloe vera gel, honey, canola oil have been tried but not enough therapeutic results have been obtained [20,21].

Another method that was used to prevent PPA is the plication method in place of intestinal transmesenteric sutures. However, it presented operative difficulties and complications such as fistulas, prolonged postoperative ileus and abdominal pain. The simplest technique to decrease the frequency of contact between serosal surfaces was replacement of the omentum between intestines and abdominal wall. However, inability of omentum to prevent adhesions that may be occured between intestines has left this method inadequate [22,23]. In previous studies, oxygen, 32% dextran, 5% polyethylene glycol, paraffin, amniotic fluid, lanolin, dextrose solutions, silver foil, silk and silicone have been used as barriers to enhance the intraperitoneal fluid by increasing oncotic pressure and to reduce the frequency of contact between tissues [24].

In our study, we used olive oil because it is a low-cost product that can be easily obtained and used without too much processing. Previous studies regarding PPA prevention had focused on short-term treatment. Our study is the first survey that surveyed pure olive oil prevented PPA in the long term follow-up on jejenum, ileum and caecum in rats. We administered pure olive oil before or after forming an adhesion model and found that PPA was significantly reduced. This effect may depend on olive oil's anti-inflammatory, tissue regenerative and hydroflotting actions. We think that olive oil has long-term effect as a result of late absorption. We had two reasons for choosing pure olive oil to prevent PPA: the first fact was, phenolic components of pure olive oil had positive effects on wound healing due to its well-known anti-atherogenic, antioxidant, antineoplastic and anti-inflammatory effects. The second fact was because it is a liquid with high viscosity, so it can prevent PPA by the effect of hydroflotation. Anti-inflammatory effect is due to its major components including erythrodiol, beta sitosterol, squalene; and minor components including oleuropein, tyrosol, hydroxytyrosole and caffeic acid [9]. Cicerale et al have shown that the phenolic components exhibit antiinflammatory effects by reducing the release of thromboxane B2, LTB4, arachidonic acid, and inhibiting cyclooxygenase-1 and 2 [25]. Pure olive oil inhibited the stress ulcer formation by reducing tangential and shear forces, and prevented the wound dehiscence in immobilized patients [26].

In our study, we administered 5 ml of pure olive oil into the peritoneal cavity of rats in group-1 without laparotomy. Our aim was to explore the potential toxic effects of pure olive oil in the intact peritoneal cavity. However, we did not find any macroscopic or histopathological adhesions or any toxic reactions in the peritoneal cavity in this group. This result suggests that olive oil does not cause any inflammation in the peritoneal cavity in the long term.

The reason of thirty days-follow up is that there is a steady increase in collagen production till the 21st postoperative day. This is defined as maturation phase, known as the longest phase in wound healing. It indicates that chronic process begins with granulation tissue, re-epithelization and keratinocyte migration. After 21st day, a reduction in collagen synthesis and rearrangement of collagen is observed [27]. Hence we waited for the maturity phase to be completed before starting our assessments.

In our previous study of traumatic adhesion model in rats, we have found that 1 ml pure olive oil administered on a single adhesion area on caecum prevented PPA after a 10-days-follow up (a short-term study). Current study is a long-term study lasting up to four weeks. We found that olive oil is effective after four weeks indicating that it remains in the peritoneum without absorption and extends its hydroflotting action. In our long-term study, we showed that when abrasion was done before or after applying the olive oil, PPA was prevented in both.

On the other hand, the insignificant postoperative adhesions seen in groups 3 and 4 are due to the differences in individual fibronolithic processes. There is the possibility that individual genetic differences in fibrinolysis may result in PPA despite all the efforts to prevent their formation. These adhesions were grade 1 and simple adhesions that did not cause any intestinal obstructions.

Our study has some limitations. We did not evaluate the anti-inflammatory properties of olive oil on proinflammatory markers. In addition, we did not compare the positive effects of olive oil on adhesion-induced inflammation with a positive control group such as locally administrated non-steroidal antiinflammatory agents. However, according to the data in this experimental study, we conclude that administration of pure olive oil, an effective and readily available agent, prevented PPA.

It is necessary to evaluate the clinical usefulness of olive oil in surgery patients. As to light on the future studies, components of olive oil can be purified and individual effects may be investigated.

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