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SHORT COMMUNICATION

The effect of Resveratrol and Octreotide on peritoneal adhesions in a rat model

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Abstract

Introduction: The aim of this study was to investigate the efficacy of resveratrol and octreotide, agents that are used to prevent intra-abdominal adhesions in experimental models, in preventing intraperitoneal adhesions when used alone or in combination. *Materials and Methods:* The study employed 28 young female Wistar albino rats weighing 250-300 grams. An experimental adhesion model was created in each rat using serosal abrasion and peritoneal excision. They were divided into four groups, each comprising seven rats: Group 1, adhesion induction only; Group 2, resveratrol administration only; Group 3, octreotide administration only; and Group 4, administration of resveratrol and octreotide combination. The rats were monitored under appropriate conditions for 14 days and then underwent laparotomy. Macroscopic intensity and extensiveness of adhesions and microscopic changes in the granulation tissue (cellular intensity, reticular and collagen fibers, capillaries, elastic and smooth muscle fibers, fibrosis) were evaluated and graded. Kruskal-Wallis and Mann-Whitney U-test were used in statistical analysis and the level of statistical significance was established as $p < 0.05$. *Results:* There was no significant difference between the groups in terms of the intensity and extensiveness of macroscopic adhesions ($p=0.377$ and $p=0.319$). There was a statistically significant difference between the microscopic scores of the groups according to Zühlke's classification ($p=0.026$). The Bonferroni correction used to test for the differences revealed that the rats in Group 1 achieved significantly higher scores than the rats in Group 3 ($p=0.016$). *Conclusion:* Octreotide showed higher efficiency compared to the control group in microscopic classification; however, the two agents were not superior to each other or their combination was not superior in preventing intra-abdominal adhesions.

Keywords: Octreotide, resveratrol, intra-abdominal adhesions, experimental study

INTRODUCTION

Intra-abdominal adhesions continue to be one of the most widely encountered complications occurring in the postoperative period in various surgical branches and the possibilityof occurrence ranges from 67 to 93%.[1] The prevalence rate is 93-100% after upper abdominal surgeries, 67-93% after lower abdominal surgeries, and as low as 45% after laparoscopic surgeries.[2] Many mechanisms have been implicated in the formation of adhesions such as mechanical trauma to the peritoneal membrane, ischaemia associated with the sutures, and the use of electrocautery, foreign body reaction, tissue perfusion disorder, and infections.[3] Adhesions secondary to peritoneal inflammation produce many other medical problems such as intestinal obstruction, chronic abdominal pain, and infertility.[4,5] There are ongoing studies on the formation, prevention, and treatment of peritoneal adhesions. Although many experimental studies have been conducted to date, there is no method or product with proven efficiency.[6-8] Surgery is required in 15-18% of adhesions.[9] Apart from the use of appropriate surgical techniques, recent

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studies have also focused on pharmacological agents and physical barriers to prevent intra-abdominal adhesions.[10]

Resveratrol (3,5,4′-*o*-trihydroxy-stilbene) is a phenolic compound used in experimental models to prevent adhesions and it is a natural product with anti-inflammatory, anti-carcinogenic, and anti-oxidative properties found in grape seeds, cranberries, and blackberries.[11-13] Furthermore, it causes vasodilation, inhibits platelet aggregation, and inhibits the proliferation of cancer cells, in addition to its cardioprotective effects.[3] A few studies have suggested that it might be effective in reducing intra-abdominal adhesions.[3,12]

Octreotide is a somatostatin analogue with long-lasting effects that has similar properties to resveratrol. It also reduces the release of several growth factors and inhibits angiogenesis.[14] Octreotide decreases the concentration of inflammatory cells at the site of trauma and inhibits migration.[15] Some studies have suggested that intramuscular or peritoneal administration could reduce formation of intra-abdominal adhesions.[15,16] However, there is very limited data in the literature.

While ongoing experimental studies investigate the efficacy of octreotide and resveratrol, the aim of the present study was to investigate the effects of these agents in an intraperitoneal adhesion model when used alone or in combination. In addition, this is the first study in the literature to investigate the synergistic effects of these agents when used in combination to prevent intra-abdominal adhesions.

MATERIALS AND METHODS

Ethical approval

This study followed the Declaration of Helsinki on medical protocol and ethics and the regional Ethics Review Board issued approval for the study. The study was approved by Dokuz Eylül University Animal Studies Local Ethics Committee, protocol number: 07/12/2014, dated 11/08/2014.

Surgery

Twenty-eight young, female Wistar-albino rats, weighing 250-300 grams, fed with standard diet and allowed ad libitum access to water and food, were used in the present study. Surgical intervention was performed under sterile condition and the devices to be used during surgery were sterilised in a steam autoclave (Amsco, USA) at 134°C one night prior. General anaesthesia and analgesia were induced by intramuscular administration of 90 mg/kg ketamine (Ketalar®, Pfizer, USA) and xylazine (Rompun®, Bayer, İstanbul). After induction of general anaesthesia, the animals were placed in an *in vivo* analyzer in the supine position and their abdominal hair was removed. The incision site was cleaned with 10% povidone iodine and draped with sterile covering. A 3-4 cm midline incision was made using an 11 blade and the abdomen was exposed. The adhesion model was created using serosal abrasion by brushing the anterior wall of the caecum 20 times with a sterile tooth brush, and a 1x1 cm^2 peritoneal excision in the right lower quadrant was created. Four groups were created each comprising seven rats and the following models were created.

Group I - Rats that underwent standard adhesion modelling and received no medical therapy.
Group II - Rats that received intraperitoneal resveratrol, 10 mcg/kg only.
Group III - Rats that received intraperitoneal octreotide (Sandostatin®ampule, Novartis, Inc.), 10 mcg/kg only.
Group IV - Rats that received intraperitoneal resveratrol (mcg/kg) combined with octreotide (5 mcg/kg) with a final volume of 10 mcg/kg.

After this procedure, the fascia of all rats were closed with 3/0 PDS continuous sutures and the skin was closed with 3/0 silk sutures. The rats were fed with standard rat bait at the post-operative sixth hour and switched to full feed. The rats were monitored for 14 days in an environment complying with laboratory ethical conditions. The rats were sacrificed on the post-operative 14th day according to laboratory ethical standards under high dose ether anaesthesia, and laparotomy was performed. The intensity and extensiveness of adhesions were evaluated macroscopically and scored according to the Knightly classification (Table 1).[17]

Pathology

After macroscopic scoring, approximately 1x1x0.5 cm full-thickness excision was performed in the caecum and right lower quadrant. The specimen was numbered and sent to the pathology laboratory in 10% formalin solution. The specimens were left in formalin solution for at least eight hours after macroscopic examination. The specimens were embedded in paraffin blocks after routine tissue process. For each rat, tissues from caecum and the right lower quadrant were embedded in paraffin blocks separately. Five-micron thick

TABLE 1: Classification for the extent and severity of peritoneal adhesions

	Extent of peritoneal adhesions	Severity of peritoneal adhesions
0	No adhesions	complete absence of adhesions
I	Adhesions covering less than 1-25% of the traumatised area	single thin, easily separated adhesion
II	Adhesions covering 26-50% of the traumatised area	less extensive, but weak adhesions, which poorly withstood traction
III	Adhesions covering 51-75% of the traumatised area	numerous, extensive visceral adhesions, without visceroparietal extension
IV	Adhesions covering 76-100% of the traumatised area	numerous, extensive, dense adhesions that involved the adjacent mesentery, intestines, and omentum and extended to the abdominal wall

sections of paraffin blocks of the same rat were taken on the same slides. One of the sections prepared from the tissue blocks was stained with haematoxylin-eosin staining and the other section was stained with Mason's Trichrome (Masson's Trichrome Stain Kit- Methyl Blue, Atom Scientific Ltd, Code: RRSK20-100). The sections were examined by pathologists who were blind to the clinical data and the findings were scored according to Zühlke's microscopic adhesion classification (Table 2).[18]

Statistical analysis

The Kruskal-Wallis and Mann-Whitney U-test were used in the statistical analysis and the level of statistical significance was established as $p < 0.05$.

RESULTS

The study included seven rats in each group; however, one rat in group 4 died due to anaesthesia complications.

The mean macroscopic adhesion score in ascending order was as follows: Group 3<Group 4<Group 2<Group 1. The extensiveness of adhesions followed the same order. The mean macroscopic adhesion score was higher in the control group compared to the other groups. However, the p values were 0.377, 0.319 and 0.322, respectively, and there was no statistically significant difference ($p>0.05$) (Table 3).

In the macroscopic examination, the specimens of the eight rats had an oyster white colour, pale appearance, and were firm to palpation. The specimens of our rats had haemorrhagic and membranous appearance and were smooth to palpation. The macroscopic examination of the other tissues was not remarkable. Fibrosis was prominent in the histological examination of tissues that were firm to palpation and these tissues scored 4 according to Zühlke's classification. The score was 1 in tissues that were firm to palpation (Fig. 1 & 2) There was a statistically significant difference between the microscopic scores of the groups according to Zühlke's classification ($p=0.026$) (Table 3). The Mann-Whitney U-test with the Bonferroni correction used to test for the differences revealed that the rats in Group 1 achieved significantly higher scores than the rats in Group 3 ($p<0.016$).

When paired comparisons were performed for the intensity, extensiveness, and microscopic scores, there was a statistically significant difference between the extensiveness and microscopic scores in Group 1 ($p=0.039$). The other pairs did not show statistically significant differences ($p>0.05$) (Table 4).

TABLE 2: Histological features according to Zühlke classification

I	Loose connective tissue, cell-rich, old and new fibrin, fine reticulin fibers
II	Connective tissue with cells and capillaries, few collagen fibers
III	Connective tissue more firm, fewer cells, more vessels, few elastic and smooth muscle fibers
IV	Old firm granulation tissue, cell-poor serosal layers hardly distinguishable

TABLE 3: Analysis of macroscopic and microscopic adhesion classification scores between the groups

		Mean±SD	Min.-Max.	P value
Intensity (Macroscopic)	Group 1	2.43±1.27	1-4	0.377
	Group 2	2.29±0.95	1-4	
	Group 3	1.57±0.53	1-2	
	Group 4	2±0.63	1-3	
Extensiveness (Macroscopic)	Group 1	2.29±1.38	0-4	0.319
	Group 2	2.14±1.07	1-4	
	Group 3	1.29±0.95	0-2	
	Group 4	1.83±0.41	1-2	
Intensity and Extensiveness	Group 1	4.71±2.56	1-8	0.322
	Group 2	4.43±1.72	3-8	
	Group 3	2.86±1.46	1-4	
	Group 4	3.83±0.75	3-5	
Microscopic	Group 1	3.57±0.53	3-4	***0.026***
	Group 2	3.14±0.69	2-4	
	Group 3	2.29±0.95	1-4	
	Group 4	2±1.26	1-4	

Kruskal-Wallis H analysis

TABLE 4: Paired comparison of intensity, extensiveness and microscopic adhesions in each group

	Mean±SD	Mean±SD	P value
	Intensity (Macroscopic)	Extensiveness (Macroscopic)	
Group 1	2.43±1.27	2.29±1.38	0.564
Group 2	2.29±0.95	2.14±1.07	0.705
Group 3	1.57±0.53	1.29±0.95	0.157
Group 4	2±0.63	1.83±0.41	0.564
	Intensity (Macroscopic)	Microscopic	
Group 1	2.43±1.27	3.57±0.53	0.066
Group 2	2.29±0.95	3.14±0.69	0.161
Group 3	1.57±0.53	2.29±0.95	0.129
Group 4	2±0.63	2±1.26	1.000
	Extensiveness (Macroscopic)	Microscopic	
Group 1	2.29±1.38	3.57±0.53	***0.039***
Group 2	2.14±1.07	3.14±0.69	0.053
Group 3	1.29±0.95	2.29±0.95	0.066
Group 4	1.83±0.41	2±1.26	1.000
	Intensity and Extensiveness	Microscopic	
Group 1	4.71±2.56	3.57±0.53	0.230
Group 2	4.43±1.72	3.14±0.69	0.084
Group 3	2.86±1.46	2.29±0.95	0.339
Group 4	3.83±0.75	2±1.26	0.056

Wilcoxon Signed Ranks analysis

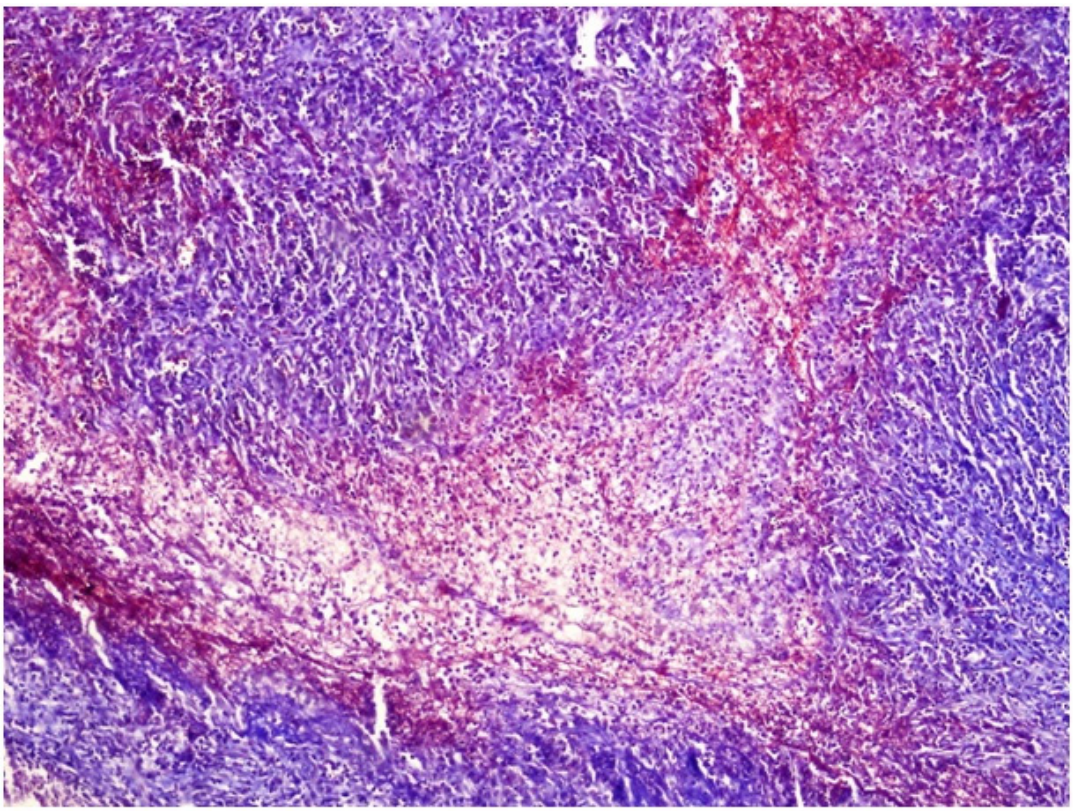

FIG. 1: Score 1 according to Zühlke's classification (Masson's Trichrome, x200).

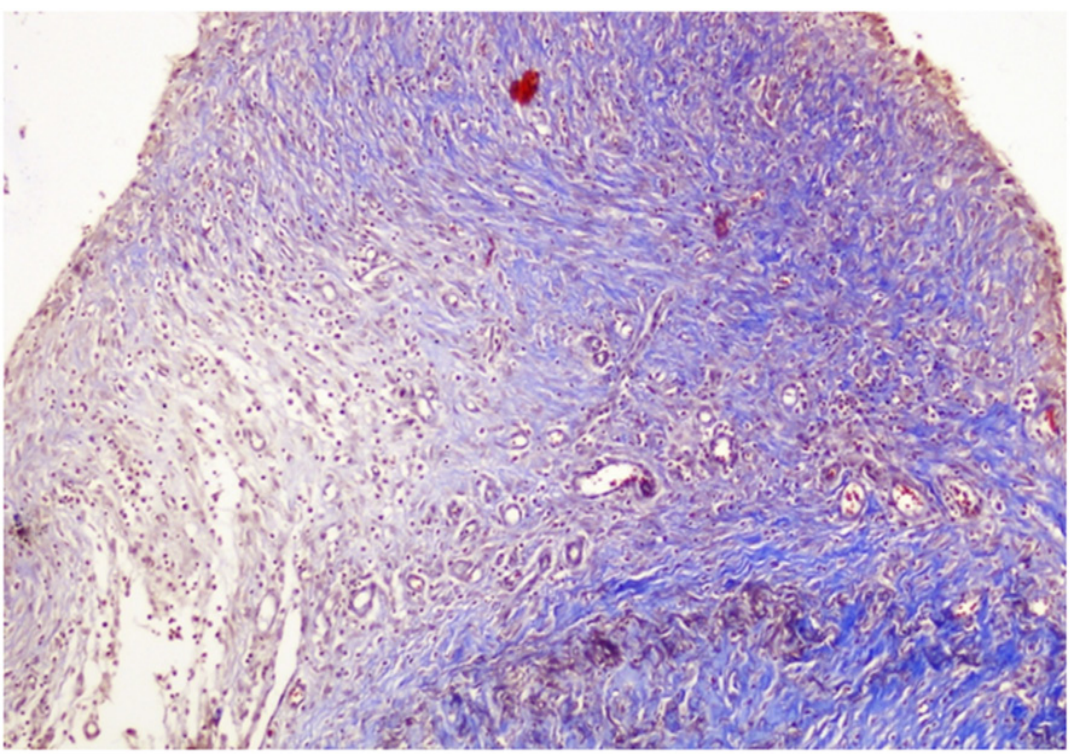

FIG. 2: Score 4 according to Zühlke's classification (Masson's Trichrome, x200).

DISCUSSION

Peritoneal adhesions occur as a result of mechanical trauma, foreign body reaction, ischaemia, and radiation within five to seven days after peritoneal injury.[19] The formation of adhesions is a complex process involving an inflammatory reaction, mesothelial cells, fibrin formation, and disordered fibrinolysis, remodelling, and phagocytic phase and for this reason, surgeons and veterinary surgeons plan many studies to prevent intra-abdominal adhesions using various products and methods.[20]

In the present study, group 3 was significantly superior to the control group considering the presence, intensity, extensiveness, and microscopic score of the adhesions. The intensity and extensiveness of adhesions in group 3 and 4 were not significantly different from group 1 and group 2 in terms of both microscopic and macroscopic scores. In general, all study groups achieved lower adhesion scores in all scoring systems compared to the control group. The lack of a statistically significant difference between these figures may be due to the small number of cases, as well as the intra peritoneal administration of the agents. A larger series using various administration routes may further elucidate the effects of the administration of resveratrol in combination with octreotide.

The agents inhibiting the effects of tissue factor in vascular cells can reduce the formation of adhesions. Resveratrol has been advocated to prevent adhesions through this mechanism.[4] Resveratrol exerts its effects by inhibiting platelet aggregation, increasing the production of nitric oxide, vascular cell permeability, and inhibiting the coagulation pathway over the tissue plasminogen activator.[3] Free oxygen radicals and inflammation mediators such as nitric oxide has been previously shown to be involved in the formation of adhesions. Thus, adhesions are considered to be caused by the damage of the cell membrane.[3] Possibly, as with other antioxidants, resveratrol reduces oxidative stress in the viable cells and maintain the integrity of the cell membrane.[13] Fibrin deposition is another well-known factor that plays a role in the formation of intra-abdominal adhesions and resveratrol by decreasing tissue factor expression, platelet aggregation, and thromboxane B2 synthesis.[21] Some of these mechanisms of actions have been demonstrated in experimental studies. Cakmak *et al*.[22] conducted an experimental study on rats and demonstrated its favourable effects on colonic wound healing by stimulating neovascularisation and they emphasized that the agent decreased inflammation and oxidative injury. Jha *et al*.[23] demonstrated that anti-inflammatory effects of this agent in a severe pancreatitis model.

Many studies have been conducted with the assumption that resveratrol prevented intra-abdominal adhesions; however, almost all of these studies have been conducted by gynaecologists in a uterine adhesion model (3, 12, 21). The study of Orçan *et al*.[4] suggested that resveratrol reduced postoperative adhesions when administered via the orogastric route. Similarly, Üstün *et al*.[21] reported favourable effects after subcutaneous administration. In the present study, resveratrol prevented intra-abdominal adhesions after intraperitoneal administration. The study of Sögütlü *et al*.[12] investigated both intraperitoneal and subcutaneous administration routes. They reported efficacy with subcutaneous administration but no efficacy with intraperitoneal administration, as in our study; however, they emphasized that research must be continued to investigate the effects of intraperitoneal administration. The debate continues on the administration route and effects of resveratrol. In addition, we consider that studies must be designed at a molecular level to determine the mechanism(s) through which the effects of the agent occur and whether one mechanism is superior to other.

Transforming growth factor β (TGFβ), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF-1) are important mediators in wound healing.[14] Octreotide is a neuro peptide, which is thought to reduce intra-abdominal adhesions by inhibiting cellular processes[16]. Baykal *et al*.[14] reported that octreotide was superior to normal saline in reducing peritoneal adhesions both after subcutaneous and peritoneal administration. Similarly, Lai *et al*.[16] reported that octreotide reduced formation of adhesions through epidermal growth factor receptor (EGF-R), tissue plasminogen activator (tPA), and plasminogen activator inhibitor (PAI-II). Octreotide was shown to reduce intraperitoneal adhesions in another experimental study by Lai and Chen.[24] Günal *et al*.[25] reported the efficacy of octreotide after intraperitoneal administration. These studies demonstrated the efficacy of different doses and administration routes of octreotide in reducing the intensity of adhesions. The finding that octreotide microscopically reduced adhesions compared to the control group in the

present study is consistent with the findings of the other studies. Despite these studies, there is still a debate over the efficacy of octreotide in preventing intra-abdominal adhesions.

These two agents that were advocated to have an efficacy in reducing adhesions in most studies were combined to be delivered via intraperitoneal route in the present study and produced no statistically significant difference, although adhesion scores were numerically lower compared to the control group. Our literature search revealed no study that used a combination of these products. This route of administration was employed since this was the first study on this subject and there were no previous data regarding the appropriate administration route of this combination. However, studies must be conducted using similar and different administration routes in order to make suggestions about the synergistic effects.

In conclusion, although there is a very limited body of knowledge in the literature to suggest that these two agents prevent or reduce formation of intra-abdominal adhesions, only octreotide showed a significant reduction in the microscopic adhesion score compared to the control group in the present study. Although the administration of these agents alone or in combination produced lower mean adhesion scores compared to the control group, intraperitoneal administration had no significant superior effect in reducing intra-abdominal adhesions. Experimental studies must be continued to investigate whether resveratrol and octreotide, when used alone or in combination, has efficiency in preventing or reducing intra-abdominal adhesions and to determine optimum doses and route of administration.

Conflict of interest: The authors report no conflicts of interest.

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