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The effects of ozone therapy on postoperative adhesions and ovarian functions: An experimental study

Ozon terapinin postoperatif adezyon ve over fonksiyonları üzerindeki etkisi: Deneysel bir çalışma

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Abstract

Aim: Numerous methods are used to prevent the development of postoperative adhesion formation. There are few studies on the effects of ozone therapy on postoperative intraabdominal adhesions and ovarian functions. This study aimed to investigate the effects of ozone treatment on postoperative intraabdominal adhesions and ovarian roles in rats.

Methods: Twenty female Wistar albino rats were randomly allocated into two groups as laparotomy (Group 1, n: 10) and laparotomy + intraperitoneal ozone (Group 2, n: 10). After laparotomy, parietal peritoneum, uterus and adnexal surfaces were scraped in both groups. Follicle-stimulating hormone (FSH), Estradiol (E2), Lactate Dehydrogenase (LDH), Urea, and Creatinine levels were measured, and histopathological evaluation was performed. Both groups were compared concerning histopathological and biochemical findings.

Results: In the ozone-treated group, antioxidant levels (P=0.012) were significantly higher, and E2 levels (P=0.005) were substantially lower than the control group. There was no statistically significant difference between the two groups regarding FSH, Urea, Creatinine, and LDH (P=0.12, P=0.72, P=0.45, and P=0.79, respectively). Histopathologically, postoperative intraabdominal adhesion rates between the two groups were statistically similar.

Conclusion: Although there was no statistically significant difference, ozone therapy had a decreasing effect on severe fibrosis and congestion rates. Although there was no difference in FSH, low levels of E2 in Group 2 suggest that ozone treatment may have a protective effect on the ovaries. However, further studies are needed concerning adhesion formation and the impact on ovarian functions.

Keywords: Rats, Ozone, Adhesion

Öz

Amaç: Postoperatif adezyon gelişiminin önlenmesi için çok sayıda yöntem kullanılmaktadır. Ozon tedavisinin postoperatif batın içi adezyonlar ve over fonksiyonları üzerine etkileriyle ilgili literatürde az sayıda çalışma mevcuttur. Bu çalışma, ozon tedavisinin ratlarda postoperatif batın içi adezyonlar ve over fonksiyonları açısından etkilerini araştırmayı amaçlamıştır.

Yöntemler: Yirmi adet dişi Wistar albino rat, laparotomi (Grup 1, n:10) ve laparotomi + intraperitoneal ozon (Grup 2, n:10) olacak şekilde rastgele iki gruba ayrıldı. Laparotomi sonrası her iki grupta parietal periton, uterus ve adneksiyal yüzeyler kazındı. Folikül uyarıcı hormon (FSH), Estradiol (E2) ve Laktat Dehidrojenaz (LDH), Üre ve Kreatinin seviyeleri ölçüldü, histopatolojik değerlendirme yapıldı. Her iki grup histopatolojik ve biyokimyasal bulgular açısından karşılaştırıldı.

Bulgular: Ozon tedavi grubunda antioksidan seviyeleri (P=0,012), kontrol grubuna göre anlamlı derecede yüksek, E2 seviyeleri (P=0,005) ise anlamlı derecede düşük bulundu. İki grup arasında FSH, Üre, Kreatinin, LDH açısından istatistiksel olarak anlamlı fark bulunmadı (sırasıyla P=0,12, P=0,72, P=0,45, P=0,79). Histopatolojik olarak her iki grup arasında postoperatif intraabdominal adezyon oranları istatistiksel olarak benzer saptandı.

Sonuç: Her ne kadar istatistiksel olarak anlamlı fark olmasa da ozon terapinin şiddetli fibrozis ve konjesyon oranlarında azaltıcı etkisinin olduğu tespit edildi. FSH açısından fark bulunmasa da Grup2'de E2 seviyelerinin düşük tespit edilmesi, ozon tedavisinin over fonksiyonları üzerinde koruyucu etki oluşturabileceğini akla getirmektedir. Ancak adezyon oluşumu ve over fonksiyonları üzerindeki etkiler açısından daha kapsamlı çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Rat, Ozon, Adezyon

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Introduction

Adhesions in the peritoneum are abnormal bonds emerging between the omentum, bowel loops, and the abdominal wall [1]. The etiology of peritoneal adhesions can be either congenital or acquired; among the acquired reasons are inflammatory events and surgical interventions [2]. Although almost 90-95% of the intra-abdominal adhesions develop after surgery, this debilitating process can also be expected after an intra-abdominal inflammatory process such as pelvic inflammatory disease (PID), diverticulitis, or spontaneous bacterial peritonitis [3-5].

Trauma to the peritoneal epithelium is blamed as the primary mechanism of adhesion formation, results in fibrin matrix accumulation in the damaged intra-abdominal planes. As a fibrinolytic agent, plasmin is not sufficient to prevent the process after surgery. Thus, the degradation collected deposits convert eventually to adhesions [3]. Diamond et al. [6], categorize peritoneal adhesions as two distinct forms. Type 1 entities involve adhesions at sites without any previous adhesions. They include type 1A without any past operative procedures at the area of the problem and type 1B with some preceding operative procedures at the zone of adhesions. Surgeons define adhesions depending on personal factors based on individual experiences and proficiency [7].

Peritoneal adhesions can be associated with many diseases ranging from enduring abdominal pain to infertility [8]. One of the most prevalent ailments observed is incomplete or complete bowel obstruction, which is usually located in the small bowel area. Almost 79% of the intestinal obstructions are due to post-surgical procedures [9].

Many studies have investigated the formation of peritoneal adhesions. However, there is no particular approach to prevent their development; there are controversies regarding the usefulness of the current preventive methods. Additionally, the majority of research concentrates on gynecological cases. Thus, there is a need for trustworthy recommendations and guidelines for patients receiving abdominal surgical interventions [10].

Researchers have investigated many agents for the prevention of post-surgical peritoneal adhesions. The main strategies in this effort were concerning fibrinolysis, coagulation, inflammation, collagen synthesis, or creating barriers between wound surfaces. The preventive approaches included mechanical barrier such as crystalloids, dextran, hyaluronic acid, cellulose, polytetrafluoroethylene, pure olive and and chemical agents such as non-steroidal anti-inflammatory medications, corticosteroids, calcium channel blockers, histamine antagonists, antibiotics, fibrinolytic substances, anticoagulants, antioxidants, hormones, vitamins, colchicine, and selective immunosuppressors [10,11].

Postoperative adhesions frequently develop after surgical procedures, and they most commonly affect the ovaries [5]. It was demonstrated that the reduction of adhesion reformation is more challenging than the prevention of adhesion formation, which can cause infertility [12].

Ozone (O_3) is a colorless gas, consisting of three oxygen atoms and a characteristic odor at room temperature [13]. Medical ozone is always used as a combination of ozone and oxygen with a concentration of 1 to 100 μg/ml (0.05–5 O_3). Besides its bactericidal, fungicidal, virostatic, and antioxidant properties, ozone improves blood circulation and activates the immune system [13]. Ozone therapy (OT) is widely used in medicine, such as for cardiovascular diseases, subcutaneous tissues, peripheral vascular diseases, neurological diseases, head and neck problems, as well as ailments of the orthopedic, gastrointestinal, or genitourinary systems [13]. However, few studies investigated the effects of ozone treatment on preventing postoperative intraabdominal adhesions [14].

Biochemical and histopathological findings suggest that ozone is effective against ovarian ischemia/reperfusion injury [15]. Ozone therapy could have beneficial effect on tubal occlusion, could protect from endometritis and vaginitis, might protect ovaries from ischemia and oocyte loss, and finally might lead to less formation of pelvic adhesions [16].

We hypothesized that ozone can prevent postoperative adhesion formation by preventing tissue damage caused by surgical interventions. Especially its antioxidant effects may help to obtain the desired results for a new clinical use. Thus, this study aimed to investigate the effects of ozone treatment on postoperative intraabdominal adhesions and ovarian roles in rats.

Materials and methods

Study design

The study was conducted in experimental design, at the operating room and research laboratories of the Faculty of Veterinary Medicine of Kafkas University between October and December 2016. Study reporting was done per the PREPARE guideline [17]. The study protocol was approved by the Kafkas University Animal Experiments Local Ethics Committee (IRB number:115, Date: 10/27/2016). The rats received appropriate care per the institution's guidelines, as described in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Twenty female Wistar rats weighing 200–240 g (4–6 months old, obtained from Atatürk University Experimental Animal Production and Research Center, Erzurum, Turkey), were used in the study.

Before commencing the experiment, the rats were fed with standard rat food and water as needed and housed in animal cages with controlled temperature and alternating 12h light/dark cycle for one week. They were randomly divided into two groups containing ten rats each: sham and OT groups. The sham group received no treatment in the postoperative period, while the OT group received ozone treatment as described below. The primary outcome variables of the study were the severity of inflammation and fibrosis in the endometrium and cervix.

The procedure

After general anesthesia, induced by 50 mg/kg ketamine (Ketalar; Parke Davis, Eczacıbaşı, Istanbul, Turkey), and 10 mg/kg xylazine (Rompun; Bayer AG, Leverkusen, Germany) (Figure 1), the abdominal area was shaved and disinfected with iodine. Under sterile conditions, the abdomen was opened with a 3cm incision at the midline (Figure 2). Then, laparotomy was performed on the parietal peritoneum, the surface of the uterus was scraped, and the abdomen was sutured according to the anatomical layers with 4/0 polyglycolic acid suture materials (Vicryl, Ethicon, Somerville, NJ). Right after closing the

abdomen, an ozone/oxygen mixture at a dose of 0.7 mg/kg was applied intraperitoneally once-a-day for consecutive 4 days, followed by a total of 8 doses of weekly intraperitoneal ozone. Later, all animals were put in a warm incubator at 33 C for two hours. After 2h, they were returned to their cages, where clear water was given as needed for 24h. Then, the animals were fed standard rat food and water ad libitum. Two months later, the rats were sacrificed by cervical dislocation.

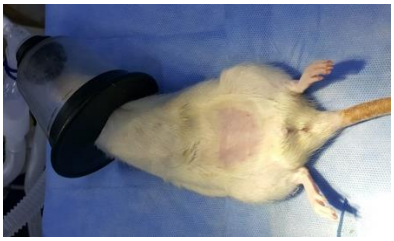

Figure 1: Administration of general anesthesia

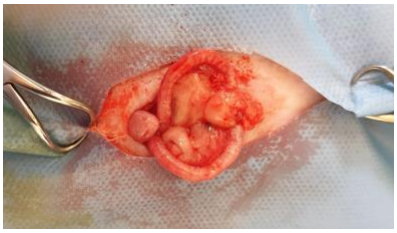

Figure 2: The surgical procedure

Ozone therapy

Ozone was produced by an ozone generator (Ozonosan Photonic 1014; Hansler GmbH, Iffezheim, Germany), permitting control of the gas flow rate and ozone concentration in real-time by a built-in UV spectrometer. The ozone flow rate was preserved at 3 L/min, representing a concentration of 60 mg/mL with a gas mixture of 97% oxygen+3% ozone. Tygon polymer tubes and single-use silicon-treated, ozone-resistant polypropylene syringes were used throughout the reaction to guarantee ozone containment and sustained concentrations.

Blood and tissue sampling

Blood samples were collected from sacrificed rats in the sham and OT groups. Antioxidant (Mm), follicle-stimulating hormone (FSH) (ng/mL), estradiol (E2) (ng/mL), urea (mg/dL), creatinine (mg/dL), and lactate dehydrogenase LDH (U/L) levels were measured.

The abdomen was re-opened to obtain tissue samples. The uterus, tubes, and ovaries were harvested and stowed at -80 C until histopathological examination. The tissues were homogenized in phosphate buffer (pH 7.4) employing a homogenizer (Heidolph Diax 900; Heidolph Elektro GmbH, Kelhaim, Germany) on an ice cube. The homogenized tissues were centrifuged at 7 530x*g* in 4 C for 10 minutes.

Variables

The primary outcome variables of the study were the severity (mild/moderate/severe) of inflammation and fibrosis in the endometrium and cervix. The secondary outcome variables were the macroscopic appearance of tuba, ovary, and intraabdominal adipose tissues. The independent study variable was study groups (sham and OT).

Statistical analysis

Data was entered into the computer and analyzed using the SPSS 25.0 software (SPSS Inc., Chicago, IL, USA). The findings were presented as frequencies, percentages, median, and Interquartile range (IQR). The normal distribution of the numerical data was analyzed by the Kolmogorov-Smirnov test. The homogeneity of the variances was examined by the Levene's test. For the comparison of sham and OT groups, the independent samples Mann-Whitney U test was used for antioxidant, FSH, E2, urea, creatinine, and LDH levels, while the Fisher's Exact Test was used for comparing the severity of inflammation, congestion, and fibrosis. All hypotheses were two-sided, and a *P*-value of <0.05 was considered statistically significant.

Results

Biochemical results

On the 19th day in the ozone-treated group and on the 11th day in the sham group, one rat died. Data of 9 rats in the ozone group and 9 in the sham group were analyzed. Antioxidant levels were significantly higher in the ozone group (median: 4.12, IQR: 0.93) than in the sham group (median: 2.86, IQR: 1.05) (Z=2.517, *P*=0.01). E2 levels were significantly lower in the ozone group (median: 108.96, IQR: 8.80) than in the sham group (median: 127.64, IQR: 17.77) (Z=2.782, *P*=0.005). There was no statistical difference between the other blood values between the groups (*P*>0.05). The comparison of biochemical blood values in the ozone and sham groups is given in Table 1.

Histological results

No statistically significant difference was found between the severity of congestion, inflammation, and fibrosis in the endometrium, cervix, and ovaries, in the ozone-treated and sham groups (*P*>0.05) (Table 2). Histological demonstrations of endometrium and cervix are shown in Figure 3A-D.

The appearance of all tubes was normal. Intraabdominal adipose tissues in the ozone and sham groups looked similar.

Table 1: Comparison of biochemical blood values in the ozone-treated and sham groups

	Groups					
	Ozone therapy		Sham			
	Median	IQR	Median	IQR	Z	*P*-value
Antioxidant (Mm)	4.12	0.93	2.86	1.05	2.52	0.012
FSH (ng/mL)	68.42	9.61	63.12	22.08	1.55	0.12
E2 (ng/mL)	108.96	8.80	127.64	17.77	2.78	0.005
Urea (mg/dL)	38.00	7.50	38.00	9.50	0.36	0.72
Creatinine (mg/dL)	0.50	0.08	0.47	0.04	0.75	0.45
LDH (U/L)	884.00	254.50	868.00	117.00	0.265	0.79

IQR: Interquartile range

Table 2: Comparison of inflammation, congestion, and fibrosis of tissues of ozone therapy, and sham groups

		Groups				
		Ozone therapy		Sham		
		n	%	n	%	*P*-value*
Endometrial inflammation	Mild	0	0.0	3	33.3	0.37
	Moderate	7	77.8	5	55.6	
	Severe	2	22.2	1	11.1	
Endometrial congestion	Mild	6	66.7	2	22.2	0.26
	Moderate	1	11.1	3	33.3	
	Severe	2	22.2	4	44.4	
Endometrial fibrosis	Mild	5	55.6	4	44.4	0.69
	Moderate	3	33.3	2	22.2	
	Severe	1	11.1	3	33.3	
Cervical inflammation	Mild	7	77.8	3	33.3	0.23
	Moderate	1	11.1	3	33.3	
	Severe	1	11.1	3	33.3	
Cervical fibrosis	Mild	5	55.6	4	44.4	0.69
	Moderate	3	33.3	2	22.2	
	Severe	1	11.1	3	33.3	
Ovarian congestion	Mild	3	33.3	5	55.6	0.35
	Moderate	0	0.0	1	11.1	
	Normal	6	66.7	3	33.3	

* Fisher's Exact Test

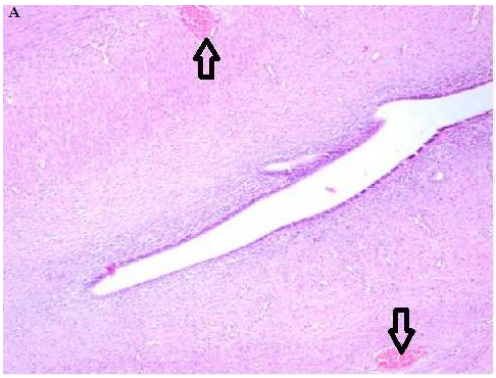

Figure 3A: Endometrial vascular congestion (black arrows) in SHAM group, H&E- 100x

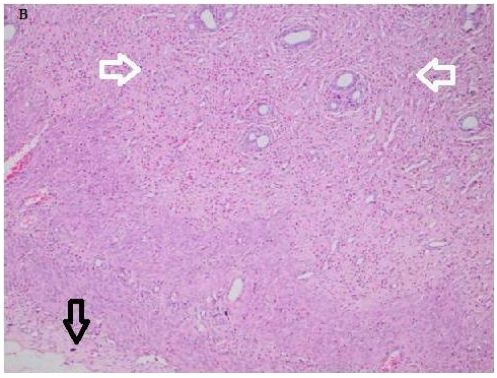

Figure 3B: Endometrial inflammation (white arrows) and fibrosis (black arrow) in the adhesion group, H&E- 100x

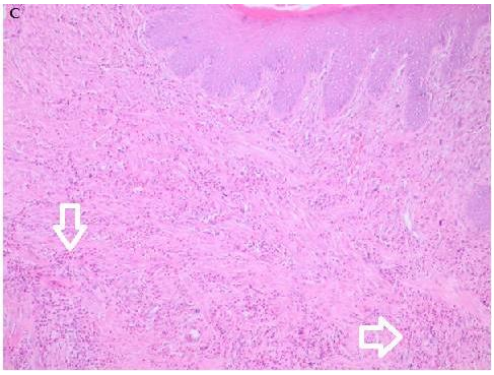

Figure 3C: Cervical inflammation (white arrows) in SHAM group, H&E- 100x,

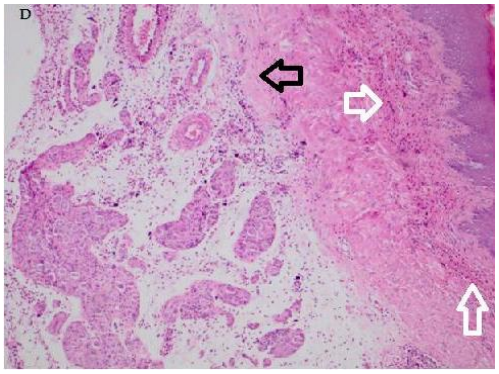

Figure 3D: Cervical inflammation (white arrows) and fibrosis (black arrow) in the adhesion group, H&E- 100x

Discussion

Our results showed significantly higher antioxidant levels in the OT group compared to the sham group. However, the E2 levels were meaningfully lower in the OT group. A recent study reported that SOD, CAT, and GPx activities increased, and MDA levels decreased in the ozone group [18]. These results suggest that ozone increases plasma antioxidant levels by triggering various biochemical pathways. Therefore, it is stated that ozone therapy can have positive impacts on preventing intraabdominal adhesions. In a study conducted in rats, it was reported that ozone exposure caused immunohistochemical staining for alpha and beta estrogen receptors, and dopamine beta-hydroxylase was reduced as were alpha and beta estrogen receptor protein levels [19]. Although ozone therapy is used in a broad spectrum of medical specialties [14], it is noteworthy that its effects on reproductive hormones have been under-examined.

Postoperative intraabdominal adhesion rates of the OT and sham groups were similar. A study conducted by Uysal et al. [14] reported that medical ozone treatment decreases postoperative uterine adhesions. Di Filippo et al. [20] reported the reduced formation of postoperative peritoneal adhesions after application of 300 Mm/kg ozone, associated with decreased levels of ubiquitin and 20S proteasome subunit within the adhered tissues.

Many agents were studied for their potential effects in preventing post-surgical peritoneal adhesions. The sought roles of these substances in achieving fibrinolysis, hindering coagulation, decreasing the inflammatory response, constraining collagen synthesis, or creating a boundary between neighboring wound surfaces. The preventive approaches are grouped into four categories: general principles, surgical techniques, mechanical barriers, and chemical agents [10]. Nonetheless, none of these methods were proven effective under all circumstances [21].

Our interpretations of this study confirmed that ozone improved oxidative stress and peritoneal adhesion formation in rats, which underwent a post-surgical experimental adhesion procedure. A possible explanation for the favorable effects of ozone is its modulation of oxidative and anti-oxidative status by stimulating endogenous superoxide dismutase (SOD) and glutathione peroxidase (GPX) [22-25]. In fact, the antioxidant effects of ozone administration are already proven in different organs such as the kidneys [24], esophagus [26], and intestines [27].

E2 levels in this study were significantly lower in the ozone group compared to the sham group. Although no studies could be retrieved concerning the effects of ozone, E2, and the ovaries, theoretically, hormonal alterations may be expected after procedures affecting the ovaries [28]. However, the connection of ozone treatment and decreased E2 levels in cases with peritoneal adhesions remains obscure.

Limitations

Our study has some limitations. The sample size is quite small. There are no human studies on the subject. In addition, our study is an experimental study and the follow-up period of the study was short.

Conclusion

Considering that the rate of endometrial and cervical fibrosis in our study was around 44%, it can be concluded that ozone application in postoperative intraabdominal adhesion is better than other methods, except for surgical treatment. Consequently, OT considerably prevents postoperative intra-abdominal adhesions in the experimental rat model. Thus, it seems logical that OT might be an add-on therapeutic modality in the prevention of postoperative intraabdominal adhesions. Nevertheless, further experimental, as well as clinical studies, are needed to determine the possible side effects and long-term

consequences before safely suggesting the use of ozone in the prevention of adhesions in humans.

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