Original Article

The Protective Effect of Dehydroepiandrosterone on Ovarian Tissues After Torsion-Detorsion Injury: A Stereological and Histopathological Study

Dehidroepiandrosteron'un Over Torsiyon-Detorsiyon Hasarı Üzerine Koruyucu Etkisi: Stereolojik ve Histopatolojik Bir Çalışma

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Abstract

Objective. Ovarian torsion is a rare problem in pre-pubescent girls that must be included in the differential diagnosis of patients with abdominal or pelvic pain. Current advice for treatment to support ovary preservation considers the macroscopic appearance of the ovary, but this is not a reliable indicator of the degree of injury in cases of ovarian torsion. The aim of this study was to determine the effects of dehydroepiandrosterone (DHEA) treatment on the rat ovary after torsion-detorsion injury using a histopathological approach and stereological methods.

Materials and Methods. Fifteen adult female Sprague Dawley rats were divided into three groups: sham operated (Control; n=5), torsion-detorsion with saline (Saline; n = 5), and torsion-detorsion with DHEA (DHEA; n = 5). Rats in the sham-operated group underwent a surgical procedure similar to the other groups, but the adnexa were not torsioned. The DHEA group was injected intraperitoneally 3 hours before detorsion and saline was administered to the saline group. After 24 h of adnexal detorsion, the rats in all groups were sacrificed and the adnexa were removed. The volume of ovarian stroma and edema were estimated using the Cavalieri Principle and were applied to serial paraffin sections. Ovary sections were also evaluated histopathologically.

Results. The volume of ovarian edema was 35.4% lower in the saline group compared to the control group ($p > 0.05$). The volume of ovarian edema increased by 73.4% in the DHEA group ($p<0.05$). In the experimental groups, stromal volume was 41.0% higher in the saline group and 52% higher in the DHEA group in comparison to the control group. Statistically significant differences were found when comparing the volume of both ovarian edema and stroma between experimental groups (Detorsion/Saline group – Detorsion/DHEA group; $p< 0.01$). Histopathologically, mononuclear cell infiltration and vascular dilatation, perivascular edema and common necrotic changes were obvious in the torsion-detorsion damaged ovary. These changes were partially ameliorated by DHEA treatment.

Conclusions. The present study shows that administration of DHEA has beneficial effects in the prevention of ischemia-reperfusion injuries of the ovaries.

Keywords: Ovarian torsion, DHEA, Stereology, Cavalieri Principle, Histopathology

Özet

Amaç. *Over torsiyonu puberte öncesinde, nadir görülen bir problem olup hastalığın tanısında abdominal ya da pelvic ağrı hikayesi önemlidir. Tedavi için overin makroskobik görünümü dikkate alınmalıdır. Ancak makroskobik görünüm hasarın derecesini tahmin için tek yeterli kriter değildir. Bu çalışmanın amacı dihidroepiandrosteron (DHEA) tedavisinin tosiyon-detorsiyon hasarı üzerine etkisini histolojik yaklaşım ve stereolojik yöntemle değerlendirmektir.*

Gereç ve Yöntem. *Bu çalışma için kullanılan 15 adet erişkin, Sprague Dawley cinsi sıçan Şam –operasyon grubu, torsiyon-detorsiyon-serum fizyolojik grubu ve torsiyon-detorsiyon-DHEA tedavi grubu olmak üzere 3 eşit gruba ayrıldı. Şam operasyon grubundaki sıçanlar diğer gruplarla torsiyon prosedürü hariç aynı cerrahi işlemlere tabi tutuldu. DHEA ve serum fizyolojik grupları detorsiyon işleminden önce intraperitoneal yolla serum fizyolojik ve DHEA aldı. Detorsiondan 24 saat sonra tüm gruplardaki denekler öldürülerek overleri çıkarıldı. Histolojik takip sonucu elde edilen doku örneklerinden alınan seri paraffin kesitler üzerinde over stroması ve ödem hacimleri hesaplandı. Ayrıca over kesitleri histopatolojik açıdan da değerlendirildi.*

Bulgular. *Bu çalışmadan elde edilen bulgulara göre torsiyon-detorsiyon işlemine tabi tutulan gruplardan serum fizyolojik uygulanan grupta over ödemi %35 azaldı. DHEA grubunda ise %73.4 arttı. Stroma hacmi ise serum fizyolojik grubunda %52 DHEA grubunda %41 arttı. Hem ödem hem de stroma hacmi açısından serum fizyolojik ve DHEA grupları arasında anlamlı fark bulundu. Tedavi edilmeyen torsiyon-detorsiyon grubunda, histopatolojik olarak; mononükleer hücre infiltrasyonu, vasküler dilatasyon, perivasküler ödem ve nekrotik değişiklikler gerçekleştiği gözlendi. Bu değişiklikler DHEA tedavisi ile kısmen düzeltilebil mekteydi.*

Sonuç. *Bu çalışmada DHEA'nın torsiyon-detorsiyon işleminden kaynaklanan iskemi-reperfüzyon hasarının tedavisinde faydalı olabileceği ilk kez gösterilmiştir.*

Anahtar Kelimeler: *Over torsiyonu, DHEA, Stereoloji, Cavalieri Prensibi, Histopatoloji*

Introduction

Ovarian torsion is a significant cause of acute and spasmodic lower abdominal pain in girls. Ovarian torsion may occur in previously normal ovaries, in ovaries with tumors (cystic teratoma) and in ovaries with cysts [1, 2]. Early diagnosis is important for the preservation of the affected ovary and for the prevention of more serious complications such as peritonitis and its sequelae. However, diagnosis poses a challenge because clinical symptoms are often nonspecific and misleading [3].

Torsion of the ovary requires immediate diagnosis, emergency surgery and treatment to avoid serious complications such as infertility. Torsion of normal ovaries occurs more commonly in young and adolescent girls than in adult women [4]. Traditional management involves oophorectomy, while conservative management includes detorsion of the twisted segment. However, detorsion of the torsioned adnexa can have local and systemic consequences due to reperfusion of ovaries [5-7]. During the detorsion process, an excess amount of molecular oxygen is supplied to the tissues and large amounts of reactive oxygen species (ROS) are produced. ROS have been implicated in the pathogenesis of tissue injury during ischemia-reperfusion (I/R) [8]. Use of free ROS after I/R has been shown to improve the status of various organs following ischemia [9, 10]. Reperfusion injury has been well documented in organs other than the ovary [11]. The main pathophysiology of the ovarian torsion is I/R injury of the ovary [12]. The severity of the injury depends on the duration of ischemia and subsequent reperfusion. Reperfusion is essential for the survival of ischemic ovary tissues, but causes additional damage. Large quantities of ROS are produced after reperfusion, leading to oxidative damage and dysfunction [13, 14]. Different experimental models have shown that antioxidant therapy can protect against oxidative damage by I/R [11].

Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) are the major steroid hormones secreted by the zona reticularis of the adrenal gland. Several studies have investigated the effect of DHEA on cellular antioxidant enzyme activities [15, 16]. The protective effect of DHEA has been shown in various forms of I/R injury, including kidney, liver and brain [9, 10]. However, no study has investigated the role of DHEA in ovarian I/R injuries.

Strereology is a serial toolbox based on mathematical and statistical principals that provides an unbiased method of evaluating the proporties of structures in 3D space from 2D microscope sections. On the other hand, the Cavalieri principle of stereological methodology is well suited to rapid and accurate volumetric evaluation based on standard 2D sections such as histological slides or radiological images. It can also be useful for examining changes in volume over time as an indicator of therapeutic effectiveness. In this study, we used the Cavalieri Principle to estimate the volume of stroma and edematous tissue in both torsioned ovaries and torsioned ovaries treated with DHEA.

The aim of this study was to determine the effects of DHEA treatment on rat ovaries after I/R injury that developed after torsion-detorsion using structural and stereological methods.

Materials and Methods

Animals and Experimental Procedure:

Fifteen adult female Sprague-Dawley rats weighing between 210 g and 240 g were used in this experiment. The rats were housed in a temperature-and light-controlled room and fed standard laboratory chow and water. Animal experiments were approved by the Ethical Committee of Ataturk University and followed the ethical guidelines provided. Rats were randomly divided into three groups of five animals. All surgical procedures were performed while the rats were under intraperitoneal ketamine HCL (50 mg/kg) and xylazine HCL (10 mg/kg) anesthesia induced via injection of 2.5 mg /100 g. All rats, except for the sham-operated group, were subjected to right unilateral adnexial torsion for 3h. Torsion-detorsion was performed as follows:

After midline laparotomy (Figure 1a), the right adnexa, including tubal and ovarian vessels, was rotated by 360o in a clockwise direction (Figure 1b). The rotated adnexa was fixed to the abdominal muscles with a 5/0 silk suture in the torsion and detorsion groups (Figure 1c). The skin was sutured with 5/0 silk. Three hours later, animals in all groups were re-anaesthetized and laparotomy was performed through the previous incision sites. In the Sham/Control group (n=5) the rats were killed and the ovaries were surgically removed after preparation of the adnexa (Figure 1d).

In the Detorsion/Saline group (n=5), the same surgical procedure was performed, and after 3 hours of torsion, the ovary was counter-rotated to its natural position. Saline was injected intraperitoneally 3 hours before detorsion, and detorsion was maintained for 24 hours before the rats were sacrificed. In the Detorsion/DHEA group (n=5), the same surgical procedure was performed, and in addition, 5 mg/100 g DHEA were injected intraperitoneally 3 hours before detorsion. At the end of the experiment, the rats were sacrificed with an overdose (200 mg/kg) of sodium pentobarbital. In each group, both ovaries were dissected for histopathological and stereological examination (Figure 1d). Ovaries were washed with cold saline, then dried and fixed in Bouin's solution (7.5 ml of saturated picric acid, 2.65 ml of glacial acetic acid, and 2.5 ml of 7 % formaldehyde).

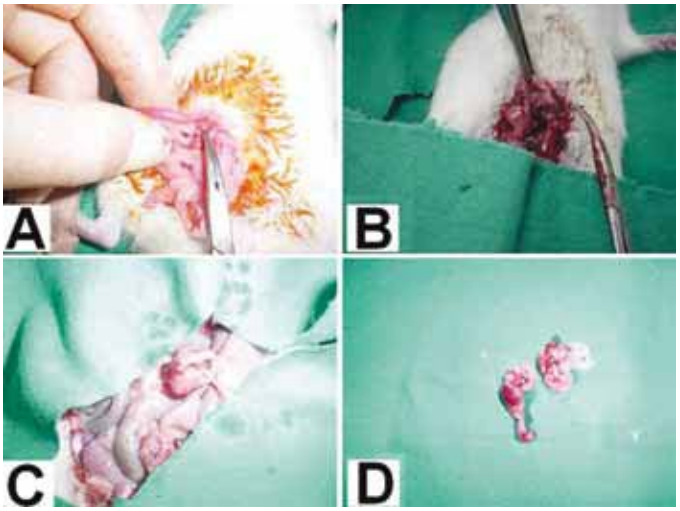

Fig. 1 — Surgical procedures applied in this study. A. Median laparotomy of female rats. B. Torsion process. C. Detorsioned ovary, attached to abdominal muscles. D. Ovaries removed for histopathological and stereological procedures.

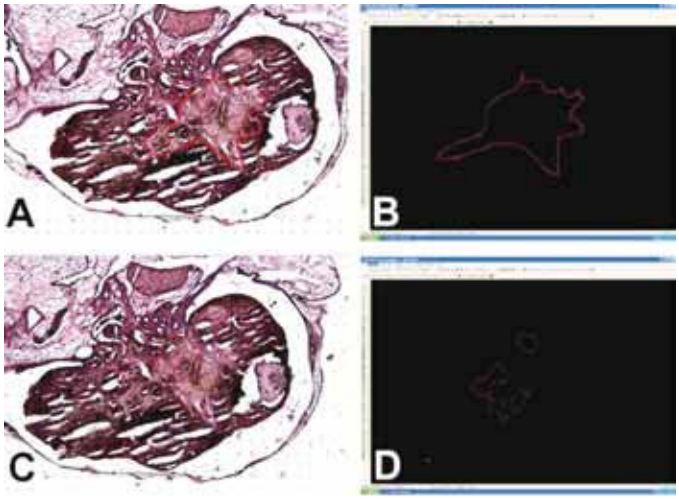

Fig. 2 — Stereological process involved in two different estimations by the Stereo Investigator. A. Line-drawn ovarian stroma. B. Estimation map of images in A. C. Line-drawn ovarian edema. D. Estimation map of images in C.

Briefly, groups in this study received treatments as follows: 1) Rats in the sham operated group were killed and the ovaries were dissected after preparation of the adnexa; 2) Rats in the Detorsion/Saline group were injected intraperitoneally with saline 3 hours before detorsion. Rats were killed after 24 hours of reperfusion and the ovaries were removed; and 3) Rats in the Detorsion/DHEA group received 5 mg/100 g DHEA injected intraperitoneally 3 hours before detorsion, and were killed after 24 hours of reperfusion.

Tissue Processing For Stereological and Microscopic Analyses

Fixed ovaries were dehydrated in graded alcohol series, embedded in paraffin wax, and serially sectioned using a Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany). Serial sections of 40-µm thickness were mounted onto glass slides. Sections were stained with Hematoxylin-Eosin (H-E).

Based on the results of a pilot study, every 4th ovary section was selected from the consecutive section using a systematic random sampling approach [17]. The Cavalieri method was applied to the light microscopic images for the stereological estimation of the volume of ovarian stroma and edema using Stereology software 6.0 (version 6.0, Microbrightfield, Colchester, VT). A point counting test grid was used to estimate the sectioned area in the ovary (Figure 2A, C). The point density of the point counting grid was designed to obtain an appropriate coefficient of error (CE) for images of the serial sections [18, 19]. The CE and the coefficient of variation (CV) were automatically estimated using Gundersen and Jensen' formula [18]. The test grid with a systematic array of points was randomly placed on a PC screen.

The volume of each ovary section was estimated using the following formula: Volume=T x a/p x P
(T, section thickness; "a/p", represents the area of each point on the point counting grid; and " P" is the total number of the points hitting the section surface area).

Then, the total volume of selected sections from each rat ovary was multiplied by four (the total fraction ratio) to estimate the total volume of selected sections.

Statistical Analyses

Microsoft® SPSS Version 13.0 for Windows was used for statistical analyses. An independent samples t-test was applied to compare the volumes of ovaries within the control and the treatment groups, while a one-sample t-test was used to compare between the experimental groups. Both tests were two tailed.

Results

Stereological Results:

Ovarian edema:

The estimated volumes of ovarian edema are summarized in Table 1 and in Figure 3. The volume of ovarian edema was 35.4% lower in the Detorsion/Saline group compared to the Sham/Control group ($p > 0.05$). Although the volume of ovarian edema was 73.4% higher in the Detorsion/DHEA group, this increase was not significant in comparison to the Sham/Control subjects ($p < 0.05$; Fig.3). Statistically significant differences were observed when comparing the volume of ovarian edema in the Detorsion/Saline group and the Detorsion/DHEA group ($p < 0.01$)

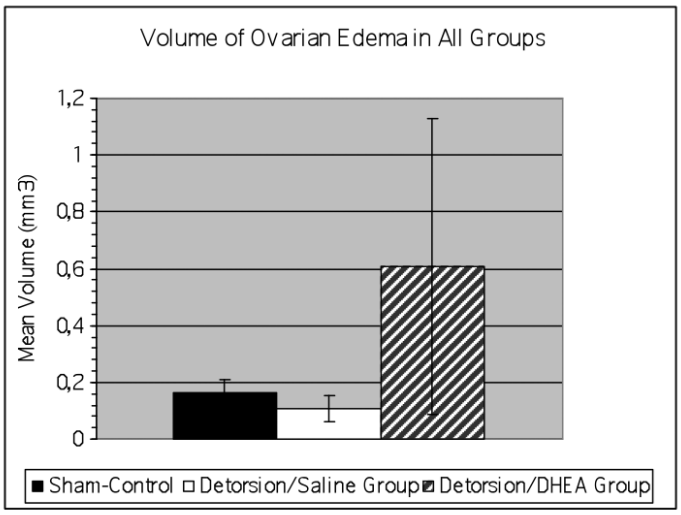

Fig. 3 — Volumetric values of ovarian edema ± SD in all groups.

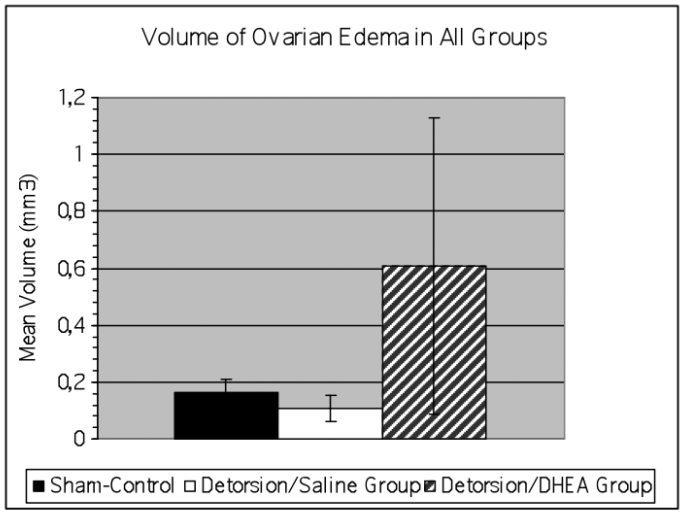

Fig. 4 — Volumetric values of ovarian stroma ± SD in all groups.

Stromal Volume:

Stromal volumes for the experimental and control groups are shown in Table 2 and in Figure 4. There were no significant differences between the experimental groups and the Sham/Control group (Fig. 4). The observed increases in the stromal volume of the experimental group in comparison to the Sham/Control with Detorsion/Saline group and the Detorsion/DHEA group were significant (P>0.05). In comparison to the Sham/Control group, the stromal volumes were 41.0% higher in the Detorsion/Saline group and 52% in the Detorsion/DHEA group. On the other hand, the volume of ovarian edema differed significantly between the Detorsion/Saline group and the Detorsion/DHEA group (p< 0.01).

Histopathological Results:

The structure of the ovaries from the sham operated group showed a normal histopathological appearance. Histopathological examination of the ovaries after ischemia showed diffuse hemorrhaging. Microscopic examination of the ovaries after I/R showed vascular dilatation and edema. DHEA administration reduced the morphological changes induced by I/R; in particular, edema and vascular dilatation were much lower, but were not totally prevented by i.p DHEA administration.

Discussion

Adnexa appear to be particularly prone to torsion in the early pubertal years. Functional ovarian cysts are very common during these pre-pubertal years and may cause a predisposal to adnexal twisting [20]. Torsion of the ovarian blood supply will result in venous congestion, hemorrhage and eventually necrosis of ovarian tissues [21, 22]. Although traditional treatment advocates the removal of the twisted adnexa, recent literature contains cases of conservative therapy [23]. In an experimental study performed on rats, a complete histological resuscitation was observed after reperfusion of ischemic ovaries, even after an ischemic period of 24 hours [24]. There was evidence of ovarian function in more than 90% of patients who underwent only detorsion [25].

Historically, detorsion of the ovary was avoided to prevent the theoretical risk of pulmonary embolism, leaving overtly necrotic tissue within the abdomen, which can lead to infectious complications. Another particular concern with

Table 1. Estimated volume of ovarian edema in all groups

Animals	Volume of Ovarian Edema (mm^3)		
	Sham/Control Group	Detorsion/Saline Group	Detorsion/DHEA Group
1	0.16	0	0.38
2	0.25	0.02	0.26
3	0.14	0.28	0.29
4	0.18	0.12	0.99
5	0.09	0.11	0.12
Mean	0.164	0.106	0.608

Table 2. Estimated volume of ovarian stroma in all groups

Animals	Volume of Ovarian Stroma (mm^3)		
	Sham/Control Group	Detorsion/Saline Group	Detorsion/DHEA Group
1	17.49	13.92	240.56
2	6.17	5.45	8.31
3	20.52	23.76	6.16
4	16.24	67.89	6.51
5	16.28	280.34	72.28
Mean	15.34	78.272	23.315

conservative management is the possibility of leaving malignancy in situ [22]. Although there is a theoretical concern in the literature about a potential risk of thromboembolism, the recovery was reported to be without thromboembolic complications or peritonitis [22, 23]. Also, long-term follow-ups in women treated conservatively indicated follicular activity on ultrasound scans [26].

In this study, we investigated that the mean volumetric values in both the Detorsion/Saline and Detorsion/DHEA groups and the Sham/Control group using the Cavalieri Principle [18, 19]. Sections used in stereological quantification were also examined histopathologically, with similar results obtained from both types of analysis. It may thus be suggested that DHEA is responsible for increased perfusion. Histopathological examination of DHEA treated ovaries revealed that the structure of ovarian tissues was preserved together with edematous changes. The results indicated that edema, developed as result of perfusion, may trigger a protection mechanism at the acute phase.

Protective mechanisms employed by the ovarian parenchyma, together with edematous changes, may occur via the following process.

If perfusion of any tissue or organ is deteriorated, free oxygen radicals will increase, leading to tissue injury, as observed in the Detorsion/Saline group. This is because while oxygen is essential for the survival of an organism, due to its critical role in the production of certain compounds, it may also have toxic effects on ovarian tissues. Many researchers have demonstrated that free radicals derived from oxygen play an instrumental role in the development of injury in post-ischemic cells and tissues [27]. This includes ovarian injuries resulting from torsion and detorsion.

In the DHEA-treated group, stereological observations revealed that the volume of edema increased and parenchyma was preserved histologically. In this situation, the effects of DHEA may be explained by the observation that free radicals decreased, as previously mentioned. DHEA exerts a dual effect on cell viability, first via its effects on oxidation [28], and second, via its provision of nutrients. Hence, DHEA may prevent irreversible tissue damage in acute stages of tissue injury.

Previous studies have claimed that ovarian torsion is followed by venous obstruction and hemorrhagic infarction [29]. Thus, the mean volume of the torsed ovary is, on average, 28 times that of the normal ovary, especially in terms of stroma [30]. Our results were in agreement with these previous findings.

Based on the data presented herein, it can be suggested that DHEA can prevent the deleterious effects of torsion/detorsion by increasing the perfusion rate. Thus, an improved treatment approach, in terms of the tissue preservation, would be to administer perfusion agents, such as DHEA, in cases of ovarian torsion before carrying out detorsion procedures.

Conflict interest statement The authors declare that they have no conflict of interest to the publication of this article.

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