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ORIGINAL ARTICLE

Does gradual detorsion protect the ovary against ischemia–reperfusion injury in rats?

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Abstract
Aim Adnexal torsion is an infrequent and serious gynecologic surgical emergency. Adnexal torsion may result from pre-existing tubal or ovarian pathology or hyperstimulation of the ovary during ovulation. Early diagnosis and emergency surgical treatment (detorsion) are important to preserve fertility and to prevent peritonitis or loss of the adnexa. However, during reperfusion, tissue damage is more severe than during ischemia because of oxygen-derived radicals. The present study aimed to investigate the protective effect of gradual detorsion on adnexal torsion.
Materials and methods Twenty-one adult female rats were divided into three groups as sham-operated (Sh group, $n = 7$); torsion + detorsion (TD group, $n = 7$); and torsion + gradual detorsion (TGD group, $n = 7$). A midline laparotomy was performed under anesthesia. In the TD and TGD groups, the left adnexa along with tubal and ovarian vessels were twisted three times in a clockwise direction and fixed to the abdominal wall. After 30 h, detorsion was performed on the mesenteries of both TD and TGD groups. In the TGD group, however, detorsion was performed gradually: the ovarian mesentery was detorsioned 360°, followed by a 5-min pause, then a repeat of the cycle until full detorsion was achieved. Rats were killed 1 week later. Left ovaries were removed and evaluated histopathologically.
Results The histopathological mean grade was significantly higher in the TD than in the TGD group ($p < 0.05$).
Conclusion Gradual detorsion can reduce reperfusion injury in a rat model of ovarian torsion. This method is easily applicable and may be a useful method for human patients with ovarian torsion.

Keywords Gradual detorsion · Ovary · Torsion · Reperfusion injury · Oxidative stress

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Introduction

Torsion of the ovary is a surgical emergency. It may occur in normal ovaries, but is more frequently associated with pre-existing ovarian masses, which are believed to potentiate the chance of torsion. It is most common in premenarchal girls and women of reproductive age [1, 2]. The degree and length of torsion are important factors for management and prognosis. Early diagnosis and immediate surgical correction are required to preserve fertility and to prevent loss of the adnexa [3, 4].

A decrease in blood flow to an organ or tissue causes hypoxia, resulting in elevated levels of lactic acid, hypoxanthine, and lipid peroxides. An increase in blood flow after lipid peroxidation leads to the formation of a large amount of oxygen-derived free radicals, which cannot be eliminated by enzymatic antioxidant defense systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). Paradoxically, therefore, reperfusion causes further damage in the ischemic tissue and is known as reperfusion injury [5–7].

Surgical management of ovarian torsion includes detorsion of the twisted segment, and if torsion is recognized before infarction, normal blood flow can be restored [8].

However, following detorsion, the reperfusion injury resulting from the large amounts of oxygen-derived free radicals leads to more tissue damage than the initial ischemia [6, 7].

Reperfusion injury thus plays a major role in ovarian damage-related to torsion of the ovary [4]. A variety of mechanisms have been described to prevent tissue damage during ischemia–reperfusion. It has been reported that gradual detorsion prevented reperfusion injury in the torsioned rat testis. We aimed to apply this method for torsioned adnex in an effort to provide a protective measure against ischemia–reperfusion injury.

The aim of the present study was to investigate the protective effect of gradual detorsion on adnexal torsion.

Materials and methods

The present experimental study was conducted at Adnan Menderes University. The study procedure was approved by the Local Ethics Committee. Twenty-one randomly selected adult female Wistar rats weighing between 200 and 300 g were used. Rats were maintained on a standard diet and water ad libitum, in a temperature- and light-controlled room. They were randomly divided into three groups as sham-operated (Sh group, $n = 7$); torsion + detorsion (TD group, $n = 7$); torsion + gradual detorsion (TGD group, $n = 7$).

Briefly, the rats were anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (3 mg/kg) and allowed to breathe room air spontaneously. A midline abdominal incision was performed. In the TD and TGD groups, the left adnexa along with tubal and ovarian vessels were twisted three times (1,080°) in a clockwise direction, and fixed to the abdominal wall. Since Doppler measurement showed partial flow at the 360° twisted adnexes, we twisted the adnexes three times to make sure that blood flow was totally occluded [9]. The abdominal wall was closed using interrupted 4/0 nonabsorbable sutures. Changes due to ischemic injury were shown to begin after 24 h of ischemia. For this reason we chose to wait for 30 h to detorse the twisted adnex. After a waiting period of 30 h, the rats were re-anesthetized, the detorsion was performed on the ovarian mesentery in the TD group. In the TGD group, detorsion was performed on the ovarian mesentery for a single 360° turn, followed by a 5-min waiting period, and this process was repeated until full detorsion was achieved. In the Sh group, the procedure included mobilization of the ovary under anesthesia without torsion, followed by a waiting period of 30 h. One week after detorsion, all rats were killed, and the left ovaries were removed and evaluated histopathologically.

Histopathologic evaluation

The specimens were fixed in 10 % formalin solution and then embedded in paraffin. The tissue samples were stained with hematoxylin and eosin (H&E). Specimens were evaluated by a single pathologist in a blinded fashion. Ovarian tissue damage was graded according to the scoring system described by Celik et al. [4]: Grade 0, normal; Grade 1, mild edema and vascular congestion, no hemorrhage and leukocytic infiltration; Grade 2, moderate edema and vascular congestion, no hemorrhage and leukocytic infiltration; Grade 3, severe edema and vascular congestion, minimal hemorrhage and leukocytic infiltration; Grade 4, severe edema, and vascular congestion, presence of hemorrhage and leukocytic infiltration.

Statistical analysis

Histopathologic score was evaluated by Chi-squared test. SPSS 14.0 for windows was used for statistical analyses.

Results

All rats were well fed and in normal activity throughout the study period. All adnexes were found in totally detorted position at the time of re-laparotomy after 1 week. Macroscopically, torsioned ovaries were in cherry-red color and had hemorrhagic appearance. Ovarian sections were assessed for tissue damage. The histological changes, graded as described by Celik et al. [4], are summarized in Table 1. The histologic grades for the ovaries of the TGD group were lower than those for the TD group ($p < 0.05$). The majority of the TGD group were Grade 1 and the majority of the TD group were Grades 2 and 3.

Figures 1, 2 and 3 illustrate the morphology of the ovaries from the three groups. The ovaries from the Sh group had a normal appearance (Fig. 1). Microscopic examination of the ovaries in the TD group revealed edema, vascular congestion and leukocytic infiltration (Fig. 2). Compared with TD group, edema, vascular

Table 1 The distribution of histological grades of the ovaries in all groups

Group	Grade 1	Grade 2	Grade 3	Grade 4
Sh	7	0	0	0
TD	0	4	2	1
TGD	5	1	0	1

Sh Sham-operated, *TD* torsion + detorsion, *TGD* torsion + gradual detorsion

* $p < 0.05$ compared with other groups

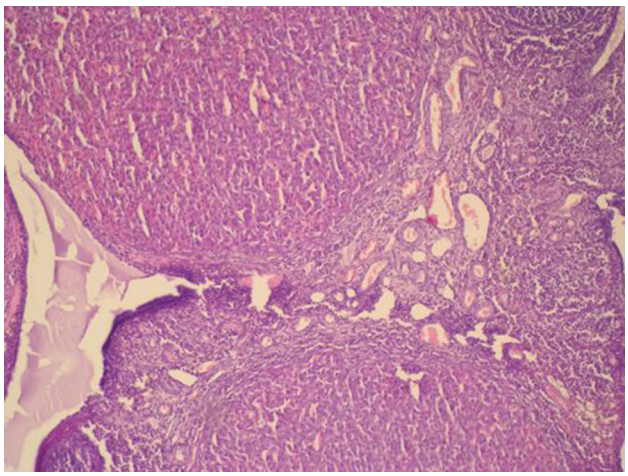

Fig. 1 Sham group showed normal ovarian morphology (H&E, ×400)

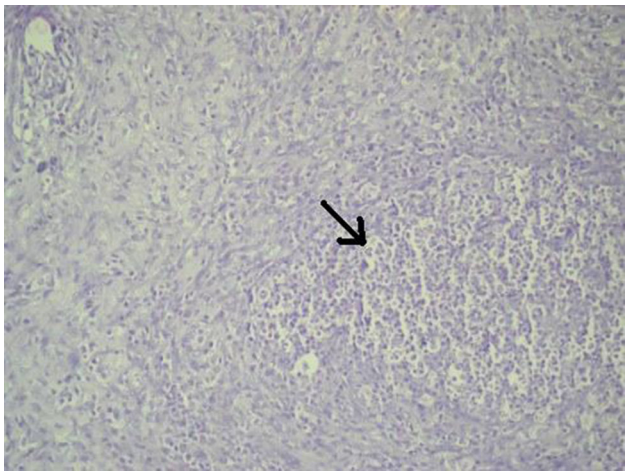

Fig. 2 Most of the specimens showed Grade 2 and 3 score in the TD group (H&E, ×400). There was significant neutrophilic infiltration (*arrow*)

congestion, hemorrhage and leukocytic infiltration were lower in the TGD group ($p < 0.05$) (Fig. 3).

Discussion

Torsion of the adnexa is the fifth most common surgical emergency in women of all ages. It is frequently observed in women of reproductive age and premenarchal girls and in association with a pre-existing ovarian mass. Such masses are believed to potentiate the chance of torsion [1, 10].

Oxygen-derived free radicals are produced in tissues and are scavenged by enzymatic antioxidant defense systems such as SOD, CAT, and GSH-Px [5]. When the blood flow is interrupted, free radicals lead to significant tissue injury [11].

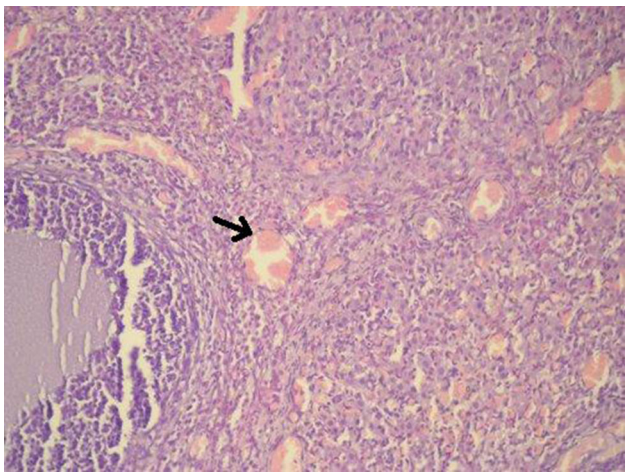

Fig. 3 Most of the specimens showed Grade 1 score in the TGD group (H&E, ×400). Most of the ovarian structures were protected. There was mild congestion (*arrow*)

Following reperfusion, large amounts of oxygen-derived free radicals lead to tissue damage and systemic complications may occur due to inflammatory responses [6, 7, 12]. Therefore, tissue damage that occurs during reperfusion is more severe than that which occurs during ischemia [7]. The degree of ischemia–reperfusion injury depends on the balance between oxygen free radicals and antioxidants [13].

Torsion of the ovarian vascular pedicle results in a compromised arterial supply and obstruction of venous and lymphatic drainage, which can cause ischemia and then infarction. Generally a twisted adnexa with a "black-bluish" or necrotic appearance is encountered during surgery, supporting the traditional approach of salpingo-oophorectomy [14]. However, recent reports have advocated the more conservative approach of releasing the pedicle and evaluating the tissue reperfusion [3, 14]. Taskin et al. [2] induced ischemia in rat ovaries for 4, 8, 12, 18, 24 and 36 h. At the end of the ischemic period they removed the ovaries and examined them histopathologically and biochemically. They found severe hemorrhage with infarct in the 24–36 h groups. The ovarian damage mostly occurred after 24 h in the study by Taskin et al. [2]; thus, in the present study we induced 30 h of ovarian ischemia.

As the majority of patients are in the reproductive age group, early diagnosis may prevent ovary loss. In contrast to traditional surgery, conservative interventions, including detorsion of the twisted segment, have become the treatment of choice in preserving the adnexa [8, 10, 15]. In this way, normal blood flow can be restored to the ovary. After performing detorsion to maintain ovarian circulation, a pathological process called reperfusion injury occurs and this is one of the most important factors in further injury [1, 2].

Reperfusion injury takes place in two phases. The first phase occurs immediately after reperfusion and extends for

a few hours. The second phase takes place over a longer period and is associated with irreversible tissue damage and inflammation, infiltration of neutrophils and accumulation of macrophages [5, 16].

If oxygen-derived free radicals are a major cause of tissue injury after reperfusion, it should be possible to prevent the sudden burst of free radical production and thereby to control damage in the ischemic area. Akçora et al. [17] performed partial detorsion, followed by torsion and full detorsion in their testicular torsion model and reported that gradual detorsion prevented the reperfusion injury in the torsioned rat testis. In the present study, gradual detorsion of the ovary was performed in a rat model of ovarian torsion. The detorsion was performed on the ovarian mesentery for a single 360° turn, followed by a waiting period of 5 min, and this process was repeated until achieving full detorsion. The sudden burst of reactive oxygen species in the first phase of reperfusion injury along with the resulting tissue damage were decreased in our model.

In the present study, ovarian tissue damage was graded according to the scoring system described by Celik et al. [4]. Using this scale, edema, vascular congestion, hemorrhage and leukocytic infiltration in the TGD group were found to be much lower compared with those in the TD group ($p < 0.05$). For this reason, we considered that gradual detorsion prevented the second phase of reperfusion injury by decreasing neutrophil infiltration and macrophage accumulation.

In conclusion, gradual detorsion can reduce reperfusion injury in the rat model of ovarian torsion and also ovarian damage in both early and late phases of reperfusion. Since this technique could be easily applied in rat model, it may also be easily applicable during ovarian surgery in humans. We therefore recommend further investigation into the possible use of gradual detorsion as a technique in human patients undergoing surgery for adnexal torsion.

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References

1. Meyer JS, Harmon CM, Harty MP et al (1995) Ovarian torsion: clinical and imaging presentation in children. J Pediatr Surg 30:1433–1436
2. Taskin O, Birincioglu M, Aydin A et al (1998) The effects of twisted ischaemic adnexa managed by detorsion on ovarian viability and histology: an ischaemia–reperfusion rodent model. Hum Reprod 13:2823–2827
3. Bayer AI, Wiskind AK (1994) Adnexal torsion: can the adnexa be saved? Am J Obstet Gynecol 171:1506–1511
4. Celik O, Turkoz Y, Hascalik S et al (2004) The protective effect of caffeic acid phenethyl ester on ischemia–reperfusion injury in rat ovary. Eur J Obstet Gynecol Reprod Biol 117:183–188
5. Filho DW, Torres MA, Bordin AL et al (2004) Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia–reperfusion injury. Mol Aspects Med 25:199–210
6. Akgür FM, Kilinç K, Aktuğ T (1993) Reperfusion injury after detorsion of unilateral testicular torsion. Urol Res 21:395–399
7. Liu X, Chen H, Zhan B et al (2007) Attenuation of reperfusion injury by renal ischemic postconditioning: the role of NO. Biochem Biophys Res Commun 359:628–634
8. Chen M, Chen CD, Yang YS (2001) Torsion of the previously normal uterine adnexa. Evaluation of the correlation between the pathological changes and the clinical characteristics. Acta Obstet Gynecol Scand 80:58–61
9. Shalev J, Mashiach R, Bar-Hava I et al (2001) Subtorsion of the ovary: sonographic features and clinical management. J Ultrasound Med 20:849–854
10. Kumtepe Y, Odabasoglu F, Karaca M et al (2010) Protective effects of telmisartan on ischemia/reperfusion injury of rat ovary: biochemical and histopathologic evaluation. Fertil Steril 93:1299–1307
11. Jefayri MK, Grace PA, Mathie RT (2000) Attenuation of reperfusion injury by renal ischaemic preconditioning: the role of nitric oxide. BJU Int 85:1007–1013
12. Ozmen S, Ayhan S, Demir Y et al (2008) Impact of gradual blood flow increase on ischaemia–reperfusion injury in the rat cremaster microcirculation model. J Plast Reconstr Aesthet Surg 61:939–948
13. Aguilar A, Alvarez-Vijande R, Capdevila S et al (2007) Antioxidant patterns (superoxide dismutase, glutathione reductase, and glutathione peroxidase) in kidneys from non-heart-beating-donors: experimental study. Transpl Proc 39:249–252
14. Pryor RA, Wiczyk HP, O'Shea DL (1995) Adnexal infarction after conservative surgical management of torsion of a hyperstimulated ovary. Fertil Steril 63:1344–1346
15. Sahin FK, Cosar E, Koken G et al (2008) Protective effect of aprotinin on ischemia–reperfusion injury in rat ovary. J Obstet Gynaecol Res 34:794–800
16. Turner TT, Brown KJ (1993) Spermatic cord torsion: loss of spermatogenesis despite return of blood flow. Biol Reprod 49:401–407
17. Akçora B, Altuğ ME, Balci A et al (2008) Gradual detorsion of torsioned rat testis attenuates ischemia reperfusion injury. J Pediatr Surg 43:1879–1884