Zafirlukast alleviates ovarian histological and biochemical alterations induced by ischemia–reperfusion in rats

Sabreen M. Ahmed[a], Walaa Y. Abdelzaher[b]

[a]Departments of Anatomy, [b]Pharmacology, Faculty of Medicine, Minia University, Minia, Egypt

Correspondence to Sabreen M. Ahmed, MD, Department of Anatomy, Faculty of Medicine, Minia University, 61111, Minia, Egypt
Tel: + 20 109 597 2740;
e-mail: sabreen.hassan@mu.edu.eg

Received 8 August 2016
Accepted 5 October 2016

Kasr Al Ainy Medical Journal
2016, 22:129–135

Background
Torsion of the ovary is a gynecologic emergency syndrome, which has a vital role in the development of ovarian ischemia. The major aim of ischemia treatment is to recover tissue perfusion. However, ischemia–reperfusion (I/R) injury is associated with reactive oxygen species production. The aim of our work was to study the possible histological and biochemical changes due to I/R injury in rat ovaries and the probable protective effect of zafirlukast as an anti-inflammatory and antioxidant agent.
Materials and methods
Twenty-four adult female Wister albino rats were distributed into three groups: group I, the control group; group II, in which the rats were subjected to 3 h of ischemia followed by 1 h of reperfusion; and group III, in which the rats were subjected to 3 h of ischemia followed by zafirlukast administration (20 mg/kg, orally) and then 1 h of reperfusion. Groups II and III were divided into two smaller groups from which the ovaries were surgically removed either after 4 h or after 2 weeks of starting the experiment. Levels of malondialdehyde, glutathione, tumor necrosis factor α, and nitric oxide were determined, and histopathological changes were examined.
Results
Vascular congestion, hemorrhage, edema, increased caspase-3 immunoreaction, a rise in malondialdehyde, nitric oxide, and tumor necrosis factor α levels, and a decrease in reduced glutathione level were observed in the ovaries after I/R, which improved with zafirlukast administration, especially after 2 weeks.
Conclusion
Zafirlukast reduces the severity of ovarian I/R injury, probably through anti-inflammatory action and suppressing oxidative stress.

Keywords:
ischemia–reperfusion injury, ovary, oxidative stress, rat, zafirlukast

Kasr Al Ainy Med J 22:129–135

1687-4625

Introduction

Torsion of the ovary is the twisting of the ovary and/or tube around its blood vessels. It is a gynecologic syndrome that occurs mostly in adolescent girls and women [1]. Torsion of the ovary is a surgical emergency with a global incidence of 3%. Ovarian cysts, pregnancy, and extreme mobility of the adnexa due to long oviduct are the encouraging causes for ovarian torsion [2].

Torsion of normal ovaries more frequently happens in young girls than in women [3]. It is hard to detect ovarian torsion because its manifestations are sometimes comp arable to acute appendicitis [4]. Doppler sonographic findings are supportive in diagnosis [5].

Adnexectomy was the classical management of ovarian torsion without untwisting to avoid pulmonary embolism. However, it was conveyed that pulmonary embolism incidence after ovarian torsion is 0.2% [6]. Fertility impairment has been the important fear after unilateral adnexectomy. Thus, the current recommendation is ovarian detorsion [7].

The main pathological incident in ovarian torsion is ischemia followed by reperfusion; thus, ovarian torsion–detorsion is an ischemia–reperfusion (I/R) injury to the ovaries. The most important aim of ischemia treatment is to recover tissue perfusion [8]. I/R injuries result in free particles and activated neutrophil production [9] that release reactive oxygen species in tissues, which cause damage to the cell membranes [10].

Leukotrienes are purely active 5-lipoxygenase products of arachidonic acid metabolism. [11]. Cysteinyl leukotrienes (CysLTs) are responsible in many pathological situations – for example, asthma, allergic rhinitis, cancer, and urticaria [12]. CysLTs are inflammatory mediators that accompany I/R tissue injury [13].

 DOI: 10.4103/1687-4625.195894

Zafirlukast is a CysLT blocker that has anti-inflammatory and antiasthma effects [14]. Zafirlukast tolerance is good and it has limited side effects such as nausea and headache [15]. The ability of zafirlukast to alleviate inflammation in ovarian I/R injury has not been investigated.

Thus, this study aimed to inspect the possible histological and immunohistochemical changes due to I/R injury in adult rat ovaries and the probable protecting effect of zafirlukast.

Materials and methods

Animal model

Twenty-four adult female Wister albino rats (170–200 g) were obtained from the National Center of Research(El-Giza, Egypt). They were allowed access to water and chow (Nile Company, Egypt) for 1 week, for acclimatization, before the start of the experiment. All experimental measures were carried out as stated by the ethical principles approved by the Faculty of Medicine Committee, Minia University, Egypt.

Chemicals

Zafirlukast (20 mg tablet) was purchased from the Egyptian Group for Pharmaceutical Industries (El Obour City, Egypt). Formaldehyde, ethanol, xylene, paraffin wax, hematoxylin solution, and eosin solution were obtained from Sigma-Aldrich (Egypt). All other standard chemicals used for biochemical and histopathological reactions were of analytical grade.

Experimental design

The rats were distributed randomly to one of three groups (eight rats in each group). Rats were anesthetized with light ether anesthesia and then were placed in dorsal recumbent position. The abdominal area to be operated was prepared and cleaned using betadine. A longitudinal incision (2.5 cm) was made in the lower midline area of the abdomen. A small peritoneal incision was made and the adnexae located. All rats, except for the sham procedure group rats, were exposed to the bilateral adnexal rotation for 3 h. The torsion–detorsion process was achieved as follows: the bilateral adnexa was rotated by 360° in a clockwise track, including the tubal and ovarian vessels. The rotated adnexa was sutured to abdominal muscles by means of silk suture. The skin was stitched with silk. Three hours later, the animals in all groups were anesthetized and laparotomy was performed through the preceding incision sites.

Group I included control rats. Rats in this group were sham operated only or were sham operated with zafirlukast administration (20 mg/kg, orally by means of gavage) and killed either after 4 h or after 2 weeks.

Group II rats were subjected to a 3-h period of ischemia followed by 1 h of reperfusion. Group II was divided into subgroup IIA, in which rats were killed after 4 h, and subgroup IIB, considered the recovery group, in which rats were killed after 2 weeks of starting the experiment.

Groups III rats were subjected to a 3-h period of ischemia followed by zafirlukast administration (20 mg/kg, orally) by means of gavage and then 1 h of reperfusion. Group III was divided into subgroup IIIA, in which rats were killed after 4 h, and subgroup IIIB, considered the recovery group, in which rats were killed after 2 weeks of starting the experiment.

Before zafirlukast administration, it was dissolved in 0.2% carboxymethyl cellulose. The procedure and dose of zafirlukast administration were based on the prior studies that had been proven successful in other organ ischemia and perfusion [16].

Histological examination

One of the ovaries was purified from the congested soft tissues and stored in a freezer at −80°C for biochemical analysis and the other was fixed in 10% formalin solution, embedded in paraffin, and cut at 5 μm thickness. The sections were stained with hematoxylin and eosin to examine the structural changes. Ovary sections were deparaffinized with xylene and rehydrated, which was followed by antigen retrieval by heating them in citrate buffer (10 mmol/l, 10 min). This was followed by endogenous peroxidase blocking in 3% H_2O_2 for 10 min The sections were washed in PBS for 5 min; nonspecific binding of antibodies was blocked by incubating with normal goat serum (DAKO X 0907; Dako, Carpinteria, California, USA) with PBS (diluted 1 : 4). Thereafter, they were incubated in anti-caspase-3 (1 : 100; Ab4051) for 1 h. The sections were washed in PBS for 10 min. Thereafter, the sections were incubated with biotinylated anti mouse immunoglobulin-G (DAKO LSAB 2 Kit; Dako Denmark A/S, Glostrup, Denmark) and washed in PBS for 10 min, and then the sections were incubated with avidin–biotin peroxidase complex (DAKO LSAB 2 Kit; Dako Denmark A/S). After washing the slides with PBS, the sections were incubated with their respective secondary anti body at room temperature for 1 h. This was followed

by detection with 3-amino-9-ethylcarbazole, a chrom ogen. The slides were counterstained with hemat oxylin, and they were mounted under a coverslip in paramount [17].

Biochemical investigation of ovarian tissues

Lipid peroxides were determined using the thioba rbituric acid method described by Buegel and Aust [18]. It depends on measuring malondialdehyde (MDA), the breakdown products of lipid peroxides. The levels of tumor necrosis factor α (TNFα) and, glutathione (GSH) were measured using TNFα ELISA kit (IDlabsTM Inc. Biotechnology, Canada) and reduced glutathione kit (Biodiagnostic, Egypt), respectively, according to the manufacturers' instru ctions. Nitric oxide (NO) was determined using a spectrophotometric method previously described by Ridnour *et al.* [19]. It depends on direct detection and quantitation of NO.

Morphometric study

The criteria for ovarian injury, including follicular degeneration, vascular congestion, hemorrhage, edema, and positive immunoreaction for caspase-3, were examined in five microscopic fields at magnification, ×400. The changes were scored from 0 to 3 according to their severity, where 0 represents no pathologic finding, and 1, 2, and 3 represent pathologic findings of <33, 33–66, and >66% of the ovary, respectively.

Statistical analysis

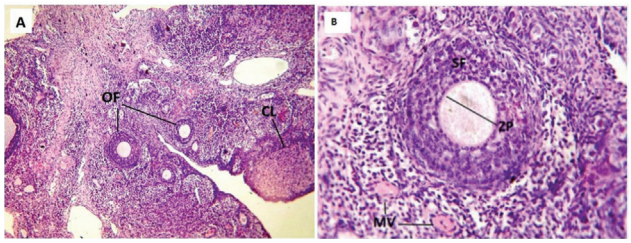
Data analysis was achieved with statistical package for social sciences, version 20. Means and SDs were used to describe the numerical variables. A Kolmogorov–Smirnov test was used to assess the distribution pattern of the data. For the comparisons between groups, the Mann–Whitney *U*-test was used. A *P* value less than 0.05 was accepted as significant.

Results

Histological results

The control group included either sham operated only rats or rats administered zafirlukast and killed either after 4 h or after 2 weeks; the rats showed no pathological changes, and the ovary was composed of two main parts, the cortex and the medulla. The cortex showed the corpus luteums and ovarian follicles (Fig. 1a). Ovarian follicles were composed of primary oocytes and granulosa cells surround them. Oocytes had normal morphology with intact zona pellucida. There are several blood vessels in the medullary connective tissue (Fig. 1b). There were no remarkable differences between different control subgroups.

Figure 1



Photomicrographs of the control group. (a) The ovarian tissue showing ovarian follicles (OF) and the corpus luteum (CL). (b) The tissue showing secondary follicle with the intact oocyte zona pellucida (ZP) and medullary vessels (MV) (hematoxylin and eosin; a, ×100; b, ×400).

In group II (I/R), macroscopically, I/R ovaries had a dark-red color and hemorrhagic appearance. Microscopically, in subgroup IIA, hemorrhage and edema in the stroma were seen. The oocytes displayed brown coloration and loss of the zona pellucida compared with the control group (Fig. 2a and b). These alterations were less evident after 2 weeks in subgroup IIB; the ovarian tissue had vascular dilatation, congestion, and disruption of follicular cells (Fig. 2c).

In group III (I/R+zafirlukast), the histological alterations were mild in subgroup IIIA. Fibroblasts and lymphocytic infiltration were perceived in the stroma (Fig. 3a).

After 2 weeks, in subgroup IIIB, general ovarian appearance was comparable to the control group. Follicular and stromal cells had a healthy appearance. No hemorrhage or vascular congestions were seen (Fig. 3b).

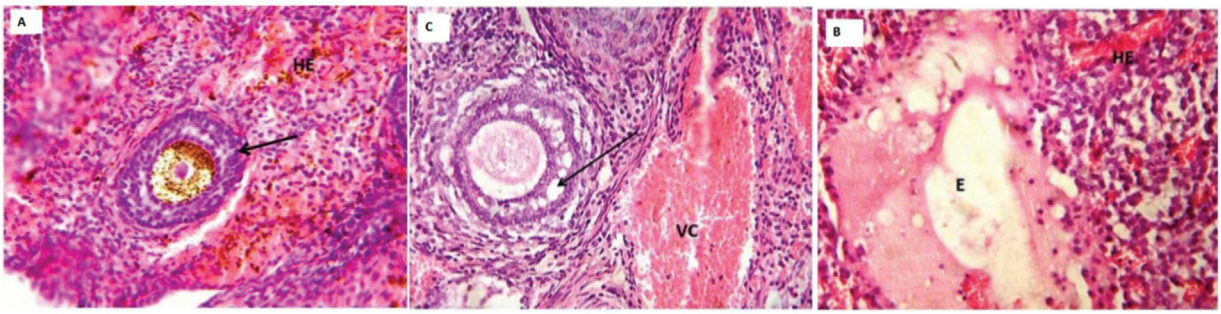
Immunohistochemical results

Immunohistochemical expression of caspase-3 in the ovarian tissue was scanty and hardly observed in the control group (Fig. 4). In contrast, extensive immunohistochemical caspase-3 expression was perceived in the I/R group in subgroup IIA (Fig. 5a), compared with decreased intensity to some extent after 2 weeks within the cells in subgroup IIB (Fig. 5b). Caspase-3 expression was found to be moderate in the I/R+zafirlukast group in subgroup IIIA (Fig. 6a), but was scanty and hardly observed after 2 weeks in subgroup IIIB (Fig. 6b).

Morphometric results

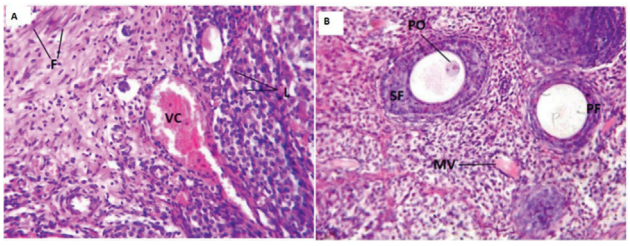
The histopathologic scores, the mean levels of the ovarian tissue MDA, GSH, NO, and TNFα activity for all groups and caspase-3 immunostaining as evaluated revealed a statistically significant variance between the groups ($P<0.05$). The results are

Figure 2



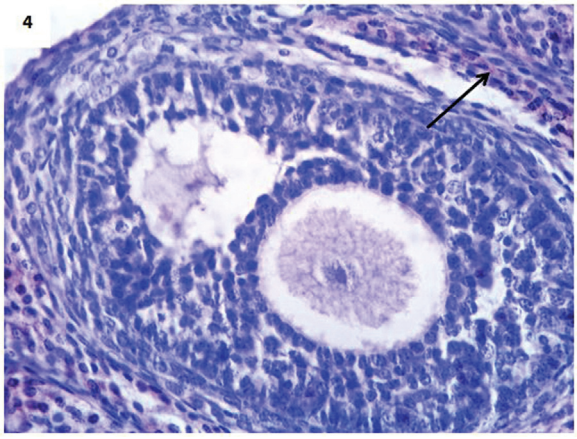
Photomicrographs of group II. (a, b) A tissue of subgroup IIA showing hemorrhagic areas (HE), oocyte with brown pigmentation (arrow), and edema (E). (c) A tissue of subgroup IIB showing congested vessels (VC) and disruption of follicle cells (arrow) (hematoxylin and eosin, ×400).

Figure 3



Photomicrographs of group III. (a) Tissue of subgroup IIIA showing congested vessels (VC), lymphocytes (L), and fibroblasts (F). (b) A tissue of subgroup IIIB showing intact vessels and healthy primary (PF) and secondary follicles (SF) with the primary oocyte (PO) (hematoxylin and eosin, ×400).

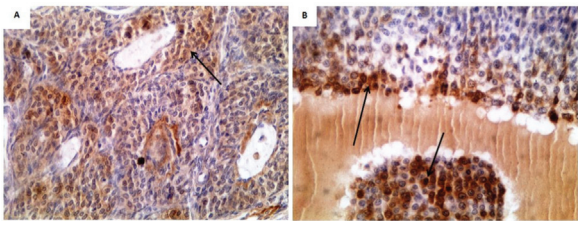
Figure 4



A photomicrograph of the control group showing scanty immunoreactivity for caspase-3 (arrow) (caspase-3 immunostaining, ×400).

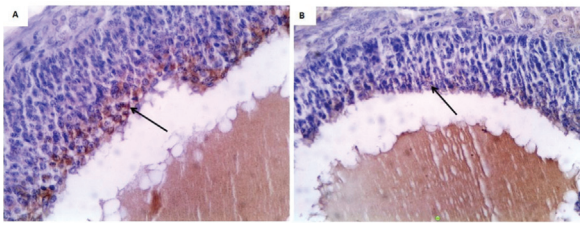
summarized in Tables 1–3. There were no statistically significant differences between the control group and the zafirlukast group IIIB ($P>0.05$). The scores were significantly higher for groups IIA, IIB, and IIIA compared with the control group and the zafirlukast group IIIB ($P<0.05$).

Figure 5



Photomicrographs of group II (I/R). (a) The ovarian tissue of subgroup IIA showing extensive immunoreactivity for caspase-3 (arrow). (b) The ovarian tissue of subgroup IIB showing strong immunoreactivity for caspase-3 (arrow) (caspase-3 immunostaining, ×400). I/R, ischemia–reperfusion.

Figure 6



Photomicrographs of group III (I/R+zafirlukast). (a) The ovarian tissue of subgroup IIIA showing moderate immunoreactivity for caspase-3 (arrow). (b) The ovarian tissue of subgroup IIIB showing scanty immunoreactivity for caspase-3 (arrow) (caspase-3 immunostaining, ×400). I/R, ischemia–reperfusion.

Discussion

The present work aimed to study the possible histological and immunohistochemical changes due to I/R injury in rat ovaries and the probable protective effect of zafirlukast as an antioxidant.

In the present study, the sections obtained from the control group, either sham operated only or with the administration of zafirlukast, showed the corpus luteums and ovarian follicles with no pathological changes.

Table 1 Histopathologic evaluation scores of the rat ovarian tissues in all groups (*n*=8)

Groups	Histopathologic score (mean±SD)			
	Congestion	Hemorrhage	Edema	Follicular degeneration
I. Control	0.3±0.5[a]	0.2±0.4[a]	0.3±0.5[a]	0.3±0.5[a]
IIA. I/R 4 h	2.8±0.4[b]	2.8±0.4[b]	2.7±0.5[b]	2.6±0.5[b]
IIB. I/R 2 weeks	2.3±0.8[b]	2.3±0.8[b]	2.2±0.7[b]	2.2±0.9[b]
IIIA. Zafirlukast 4 h	2±0.9[b]	2±0.6[b]	1.7±0.8[b]	2.1±0.7[b]
IIIB. Zafirlukast 2 weeks	1.5±0.5[a]	1.5±0.5[a]	1.5±0.5[a]	1.7±0.5[a]

I/R, ischemia–reperfusion. Different superscripts indicate statistically significant difference ($P<0.05$) from other groups.

Table 2 The mean levels of MDA, GSH, NO, and TNFα activity in the ovarian tissue in different groups (*n*=8)

Groups	MDA score (mean±SD) (nmol/g tissue)	GSH score (mean±SD) (mg/dl)	NO score (mean±SD) (nmol/dl)	TNFα score (mean±SD) (pg/dl)
I. Control	40.00±6.81[a]	56.48±4.30[a]	43.48±5.28[a]	27.58±5.18[a]
IIA. I/R 4 h	69.76±7.00[b]	23.98±4.57[b]	79.71±8.92[b]	122.92±9.14[b]
IIB. I/R 2 weeks	53.95±7.92[b]	31.78±5.53[b]	62.20±6.69[b]	82.75±10.51[b]
IIIA. Zafirlukast 4 h	63.32±5.27[b]	30.33±3.71[b]	72.28±8.42[b]	96.16±8.58[b]
IIIB. Zafirlukast 2 weeks	39.57±8.66[a]	49.83±5.18[a]	51.83±5.13[a]	38.78±9.65[a]

GSH, glutathione; I/R, ischemia–reperfusion;MDA, malondialdehyde; NO, nitric oxide; TNFα, tumor necrosis factor α. Different superscripts indicate statistically significant difference ($P<0.05$) from othergroups.

Table 3 The mean optical density of caspase-3 immunostaining in different groups (*n*=8)

	Groups				
	Group I (control)	Group IIA	Group IIB	Group IIIA	Group IIIB
Mean±SD	0.175±0.166[a]	2.800±0.163[b]	1.900±0.115[b]	0.850±0.191[b]	0.300±0.258[a]

Different superscripts indicate statistically significant difference ($P<0.05$) from othergroups.

Immunostaining for caspase-3 reaction, to determine apoptosis, revealed scanty positive immunoreactions in the ovarian tissue.

Group II, which was subjected to ischemia and then reperfusion demonstrated significant structural alterations in the ovary, such as vascular congestions, vascular dilatation, edema, disruption of the follicle cells, and oocytes with brown pigmentation. There was an increase in caspase-3 immunoreaction in the follicles and stroma. These changes improved further after 2 weeks compared with 1 h after reperfusion. Similar histologic alterations were previously reported following ovarian I/R [20,21]. Such damage could be attributed to the lipid peroxide accumulation and ATP breakdown, which cause lactic acid elevation [22]. Moreover, ischemia changes the tissue nature. Hence, with reperfusion, the tissue becomes more liable to inflammation [23] and disruption of endothelial cell junction, which end in increased vascular permeability [24]. After reperfusion, the accumulated neutrophils lead to reactive oxygen species release and oxidative stress [25].

Ovarian torsion with its clinical complication is an emergency case, which needs urgent surgical treatment. Physicians cannot protect and pretreat patients before the onset of the disease [26], but early treatment with detorsion to increase the ovarian blood flow should be carried out as early as possible to reduce ovarian injury and to keep ovarian reserve [27]. The protective role of anti-inflammatory and antioxidant agents is to neutralize the free radicals to prevent lipid peroxidation at the cell membrane and cell membrane damage [28].

There are many studies reporting the protective properties of numerous mediators against ovarian I/R injuries. Karaca *et al.* [10] reported that erythropoietin preserved the ovary from injury due to I/R. The protective properties of hesperetin were studied by Cakir Gungor *et al.* [20] in the ovarian I/R model of rats.

CysLTs increase the neutrophil activation and vascular permeability. Hence, they were considered as inflammatory agents [29]. CysLT receptor blockers protect different organs from I/R injury [30]. They have protective action in focal I/R [26], intestinal I/R [27], renal I/R [12], and ovarian I/R [31].

Zafirlukast is a CysLT blocker that has anti-inflammatory and antiasthma effects [14]. The protective properties of this drug may be due to its antioxidant activity on various free radicals. Zafirlukast inhibits neutrophil infiltration, lipid peroxidation, and

oxidative stress [16]. Further, it blocks the inflammatory cytokines such as p-selectin [32] and intracellular adhesion molecule-1 [33,34]. A previous study had indicated that zafirlukast has a protective effect against reperfusion injury in rat kidney [16]. There is limited information about the influence of zafirlukast on I/R injury. For this reason, in the present study, we choose zafirlukast as a protective antioxidant agent on the ovaries exposed to I/R injury.

After 3 h of ischemia, zafirlukast was given orally and reperfusion was performed for 1 h (group III), which resulted in a decrease in edema and congestion of the ovarian stroma. Caspase-3 immunostaining (which is a marker of apoptosis) showed a significant reduction in the mean area% of positive reaction compared with group II (the I/R only group), indicating a minimal amount of apoptosis. Thus, it could be assumed that zafirlukast can protect the ovary from I/R effect. There was also a considerable improvement in the zafirlukast group, which appeared to be as normal as that in the control group after 2 weeks in terms of general morphology and immunoreactivity to caspase-3. These results indicated that most of the ovarian stroma and follicles were preserved from I/R injury after zafirlukast administration.

These results were comparable to those of Akdemir *et al.* [35], who studied montelukast effects, a CysLT blocker on the ovary and reported that it reduced I/R injury of the ovary. Although the work by Akdemir *et al.* [35] support our results, there are some variances between the studies. In the present study, we examined the influence of zafirlukast, on ovarian I/R injury induced by torsion–detorsion of adnexa in rats. We determined the period of ischemia as 3 h and reperfusion for either 1 h or for 2 weeks as a recovery model. We, therefore, considered the effect of zafirlukast on ovarian I/R injury in rats by administering it after 3 h of ischemia and before induction of reperfusion by 30 min.Akdemir and colleagues examined the influence of montelukast on ovarian I/R injury induced by CO_2 pneumoperitoneum in a laparoscopic rat model with montelukast administrated 10 min before pneumoperitoneum. However, torsion of adnexa to generate ischemia would result in more ischemia than pneumoperitoneum.

It was reported that I/R causes acute inflammation with the rise in the inflammatory cytokines, especially TNFα [36,37]. In our study, there was a significant rise in TNFα, which was reversed with zafirlukast administration. TNFα can enhance neutrophil infiltration and inflammatory mediator production, such as platelet-activating factor and NO [37]. Thus, zafirlukast administration suppresses inflam matory mediators.

The present study revealed that MDA was significantly increased after I/R and attenuated with zafirlukast administration. Moreover, GSH was significantly decreased after I/R and increased with zafirlukast administration. These results are in harmony with Hagar *et al.* [16], who found that zafirlukast protects against reperfusion injury, through the reduction in neutrophil infiltration and suppression of oxidative stress.

The present study displayed that serum NO level has been significantly increased following I/R as related to the control group. This finding is in accordance with many earlier studies that addressed the elevated NO level as a measure of oxidative damage [38]. In the present study, zafirlukast improved the serum NO level; this result suggests that the ovarian protection of zafirlukast is linked to its antioxidant action.

The histologic and biochemical parameters in the current study confirmed that zafirlukast can help in protecting the ovaries from I/R injury due to detorsion, which is the basic conservative procedure for managing of ovarian torsion.

Conclusion

To the best of our knowledge, this is the first study that investigates the effects of zafirlukast in an ovarian I/R model. Zafirlukast reduced I/R injury by blocking neutrophil recruitment and suppressing oxidative stress. However, these observations are related to management with zafirlukast before reperfusion. Further studies that support our results should be performed with regard to the dose and timing of drug administration.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Oral A, Odabasoglu F, Halici Z, Keles ON, Unal B, Coskun AK, *et al.* Protective effects of montelukast on ischemia-reperfusion injury in rat ovaries subjected to torsion and detorsion: biochemical and histopathologic evaluation. Fertil Steril 2011; 95:1360–1366.

2 Huchon C, Fauconnier A. Adnexal torsion: a literature review. Eur J Obstet Gynecol Reprod Biol 2010; 150:8–12.

3 Berger RL, Robbins G. Torsion of the normal ovary. Am J Surg 1961; 102:716–719.

4 Germain M, Rarick T, Robins E. Management of intermittent ovarian torsion by laparoscopic oophoropexy. Obstet Gynecol 1996; 88:715–717.

5 Nizar K, Deutsch M, Filmer S, Weizman B, Beloosesky R, Weiner Z. Doppler studies of the ovarian venous blood flow in the diagnosis of adnexal torsion. J Clin Ultrasound 2009; 37:436–439.

6 McGovern PG, Noah R, Koenigsberg R, Little AB. Adnexal torsion and pulmonary embolism: case report and review of the literature. Obstet Gynecol Surv 1999; 54:601–608.

7 Tsafrir Z, Azem F, Hasson J, Solomon E, Almog B, Nagar H, *et al.* Risk factors, symptoms, and treatment of ovarian torsion in children: the twelve-year experience of one center. J Minim Invasive Gynecol 2012; 19:29–33.

8 Das DK, Maulik N. Antioxidant effectiveness in ischemia-reperfusion tissue injury. Methods Enzymol 1994; 233:601–610.

9 Aydogan H, Gurlek A, Parlakpinar H, Askar I, Bay-Karabulut A, Aydogan N, *et al.* Beneficial effects of caffeic acid phenethyl ester (CAPE) on the ischaemia-reperfusion injury in rat skin flaps. J Plast Reconstr Aesthet Surg 2007; 60:563–568.

10 Karaca M, Odabasoglu F, Kumtepe Y, Albayrak A, Cadirci E, Keles ON. Protective effects of erythropoietin on ischemia/reperfusion injury of rat ovary. Eur J Obstet Gynecol Reprod Biol 2009; 144:157–162.

11 Dengiz GO, Odabasoglu F, Halici Z, Cadirci E, Suleyman H. Gastroprotective and antioxidant effects of montelukast on indomethacin-induced gastric ulcer in rats. J Pharmacol Sci 2007; 105:94–102.

12 Sener G, Sehirli O, Velioglu-Ogunc A, Cetinel S, Gedik N, Caner M, *et al.* Montelukast protects against renal ischemia/reperfusion injury in rats. Pharmacol Res 2006; 54:65–71.

13 Ishikawa F, Miyazaki S. New biodefense strategies by neutrophils. Arch Immunol Ther Exp (Warsz) 2005; 53:226–233.

14 Kang SH, Shin KC, Kim WS, Bae TH, Kim HK, Kim MK. The preventive effect of topical zafirlukast instillation for peri-implant capsule formation in rabbits. Arch Plast Surg 2015; 42:179–185.

15 Twaites BR, Wilton LV, Shakir SA. Safety of zafirlukast: results of a postmarketing surveillance study on 7976 patients in England. Drug Saf 2007; 30:419–429.

16 Hagar HH, Abd El Tawab R. Cysteinyl leukotriene receptor antagonism alleviates renal injury induced by ischemia–reperfusion in rats. J Surg Res 2012; 178:25–34.

17 Elias JM, Margiotta M, Gaborc D. Sensitivity and detection efficiency of the peroxidase antiperoxidase (PAP), avidin-biotin peroxidase complex (ABC), and peroxidase-labeled avidin-biotin (LAB) methods. Am J Clin Pathol 1989; 92:62–67.

18 Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978; 52:302–310.

19 Ridnour LA, Sim JE, Hayward MA, Wink DA, Martin SM, Buettner GR, *et al.* A spectrophotometric method for the direct detection and quantitation of nitric oxide, nitrite, and nitrate in cell culture media. Anal Biochem 2000; 281:223–229.

20 Cakir Gungor AN, Gencer M, Karaca T, Hacivelioglu S, Uysal A, Korkmaz F, *et al.* The effect of hesperetin on ischemia–reperfusion injury in rat ovary. Arch Gynecol Obstet 2014; 290:763–769.

21 Li C, Jackson RM Reactive species mechanisms of cellular hypoxia-reoxygenation injury. Am J Physiol Cell Physiol 2002; 282:C227–C241.

22 Somuncu S, Cakmak M, Dikmen G, Akman H, Kaya M. Ischemia-reperfusion injury of rabbit ovary and protective effect of trapidil: an experimental study. Pediatr Surg Int 2008; 24:315–318.

23 Edgerton C, Crispin JC, Moratz CM, Bettelli E, Oukka M, Simovic M, *et al.* IL-17 producing CD4+ T cells mediate accelerated ischemia/reperfusion-induced injury in autoimmunity-prone mice. Clin Immunol 2009; 130: 313–321.

24 Gilmont RR, Dardano A, Young M, Engle JS, Adamson BS, Smith DJ Jr, *et al.* Effects of glutathione depletion on oxidant-induced endothelial cell injury. J Surg Res 1998; 80:62–68.

25 Teng JC, Kay H, Chen Q, Adams JS, Grilli C, Guglielmello G, *et al.* Mechanisms related to the cardioprotective effects of protein kinase C epsilon (PKC epsilon) peptide activator or inhibitor in rat ischemia/reperfusion injury. Naunyn Schmiedebergs Arch Pharmacol 2008; 378:1–15.

26 Meyer JS, Harmon CM, Harty MP, Markowitz RI, Hubbard AM, Bellah RD. Ovarian torsion: clinical and imaging presentation in children. J Pediatr Surg 1995; 30:1433–1436.

27 Chen M, Chen CD, Yang YS. Torsion of the previously normal uterine adnexa. Evaluation of the correlation between the pathological changes and the clinical characteristics. Acta Obstet Gynecol Scand 2001; 80:58–61.

28 Jassem W, Fuggle SV, Rela M, Koo DD, Heaton ND. The role of mitochondria in ischemia/reperfusion injury. Transplantation 2002; 73:493–499.

29 Guven S, Muci E, Unsal MA, Yulug E, Alver A, Kadioglu Duman M, *et al.* The effects of carbon dioxide pneumoperitoneum on ovarian blood flow, oxidative stress markers, and morphology during laparoscopy: a rabbit model. Fertil Steril 2010; 93:1327–1332.

30 Taskin O, Birincioglu M, Aydin A, Buhur A, Burak F, Yilmaz I, *et al.* The effects of twisted ischaemic adnexa managed by detorsion on ovarian viability and histology: an ischaemia-reperfusion rodent model. Hum Reprod 1998; 13:2823–2827.

31 Sagsoz N, Kisa U, Apan A. Ischaemia-reperfusion injury of rat ovary and the effects of vitamin C, mannitol and verapamil. Hum Reprod 2002; 17:2972–2976.

32 Singbartl K, Green SA, Ley K. Blocking P-selectin protects from ischemia/reperfusion-induced acute renal failure. Faseb j 2000; 14:48–54.

33 Ortiz AM, Troncoso P, Kahan BD. Prevention of renal ischemic-reperfusion injury using FTY 720 and ICAM-1 antisense oligonucleotides. Transplant Proc 2003; 35:1571–1574.

34 Troncoso P, Ortiz AM, Dominguez J, Kahan BD. Use of FTY 720 and ICAM-1 antisense oligonucleotides for attenuating chronic renal damage secondary to ischemia-reperfusion injury. Transplant Proc 2005; 37:4284–4288.

35 Akdemir A, Erbas O, Ergenoglu M, Ozgur Yeniel A, Oltulu F, Yavasoglu A, *et al.* Montelukast prevents ischaemia/reperfusion-induced ovarian damage in rats. Eur J Obstet Gynecol Reprod Biol 2014; 173:71–76.

36 Hayama T, Matsuyama M, Funao K, Tanaka T, Tsuchida K, Takemoto Y, *et al.* Benefical effect of neutrophil elastase inhibitor on renal warm ischemia-reperfusion injury in the rat. Transplant Proc 2006; 38:2201–2202.

37 Kher A, Meldrum KK, Hile KL, Wang M, Tsai BM, Turrentine MW, *et al.* Aprotinin improves kidney function and decreases tubular cell apoptosis and proapoptotic signaling after renal ischemia-reperfusion. J Thorac Cardiovasc Surg 2005; 130:662–669.

38 Bozkurt S, Arikan DC, Kurutas EB, Sayar H, Okumus M, Coskun A, *et al.* Selenium has a protective effect on ischemia/reperfusion injury in a rat ovary model: biochemical and histopathologic evaluation. J Pediatr Surg 2012; 47:1735–1741.