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ORIGINAL ARTICLE

Losartan ameliorates ovarian ischaemia/reperfusion injury in rats: an experimental study

Ismet Hortu[a], Orkun Ilgen[b], Cagdas Sahin[a], Ali Akdemir[a], Gurkan Yigitturk[c] and Oytun Erbas[d]

[a]Department of Obstetrics and Gynaecology, School of Medicine, Ege University, Izmir, Turkey; [b]Department of Obstetrics and Gynaecology, School of Medicine, Dokuzeylul University, Izmir, Turkey; [c]Department of Histology and Embryology, School of Medicine, Mugla Sitki Kocman University, Mugla, Turkey; [d]Department of Physiology, School of Medicine, Istanbul Bilim University, Istanbul, Turkey

ABSTRACT

This study aimed to investigate the protective and antioxidant role of losartan in ovarian ischaemia and ischaemia/reperfusion injury in an experimental ovarian torsion model. Thirty adult female rats were used. Rats were separated randomly into five groups; Group 1: sham group (abdominal wall was only opened and closed), Group 2: torsion group with 3-hour ischaemia using atraumatic vascular clips. Group 3: torsion + losartan group with 3-hour ischaemia 30 minutes after the administration of 40 mg/kg of losartan via oral gavage. Group 4: torsion-detorsion group with 3-hour ischaemia and 3-hour reperfusion (vascular clips were removed). Group 5: torsion-detorsion + losartan group with 3-hour ischaemia followed by administration of 40 mg/kg of losartan 30 minutes prior to a 3-hour detorsion/reperfusion. Ovarian tissue damage was scored by histopathological analysis. Ovarian tissue malondialdehyde (MDA) and plasma pentraxin 3 (PTX 3) levels were measured biochemically. In comparison with the sham group, both the torsion and torsion-detorsion groups had significantly higher scores for follicular degeneration, vascular congestion, oedema, haemorrhage, and leukocyte infiltration ($p < .05$). The aforementioned parameters significantly decreased in the torsion-detorsion + losartan group ($p < .01$) compared to those in the torsion-detorsion group. MDA and plasma PTX 3 levels were notably higher both in the torsion and torsion-detorsion groups compared with those in the sham group ($p < .01$). The current experimental ovarian torsion study suggests a protective role for losartan upon ischaemia and ischaemia/reperfusion injury in rat ovaries. Losartan may be a novel agent for decreasing ovarian ischaemia/reperfusion injury in ovaries.

KEYWORDS

Ischemia-reperfusion injury; losartan; ovarian torsion

IMPACT STATEMENT

- **What is already known on this subject?** Among gynaecological emergencies, the diagnosis of ovarian torsion is highly difficult. A delayed diagnosis may lead to ovarian necrosis and subsequent loss of ovaries if timely surgical intervention is not performed, which is essential for the fertility and protection of ovarian functions in young patients. However, reperfusion of the ischaemic tissue might leads to more serious damage to the tissue than the damage caused by ischaemia.
- **What the results of this study add?** This study found that losartan, an Ang II type 1 receptor blocker which has been currently used for regulation of blood pressure, could be used experimentally to alleviate I/R injury in ovary through improving histological parameters, reducing tissue MDA and plasma PTX3 levels. To date, there is no study regarding the usage of losartan for alleviating I/R on ovary due to torsion.
- **What the implications are of these findings for clinical practice and/or further research?** Losartan may be suggested to have therapeutic value in patients with ovarian torsion. Further large clinical studies are necessary to prove the beneficial effect of losartan to prevent I/R injury on human ovaries.

Introduction

Ovarian torsion is one of the most prominent gynaecological emergencies with a frequency of 2.7–7.4% depending on the series and occurs most commonly during the reproductive years, with the average patient being in her mid-20s (Sasaki and Miller 2014). It is defined as the twisting of the ovary and/or tube around its own vascular axis. Patients with adnexal torsion present with acute, severe, unilateral lower abdominal and/or pelvic pain, and nausea in addition to vomiting. Hence, detorsion of the ovary is surgically implemented to maintain its proper blood supply (Soltani et al. 2017). Ovulation induction, ovarian and/or paratubal cysts, hyperlaxity of the infundibulopelvic or utero-ovarian ligaments have been considered as risk factors of this emergent condition. When diagnosed early, the adnexa can be unwound. However, the diagnosis is often delayed due to the inconsistent presenting symptoms and signs as well as intermittent pain. When the diagnosis is delayed, the adnexa

CONTACT Ismet Hortu ismethortu@yahoo.com Department of Obstetrics and Gynaecology, School of Medicine, Ege University, Izmir, Turkey

becomes congested, ischaemic, haemorrhagic, and necrotic. Misdiagnosis or delay in treatment can have permanent sequel including loss of an ovary which affects future fertility, peritonitis, pelvic thrombophlebitis, and even death (Shokri et al. 2018).

Pre-operative Doppler ultrasonography may be helpful in making a diagnosis. Surgical detorsion of the twisted ovary is recommended even if tissues are cyanotic (Becker et al. 2009). After detorsion, restoration of blood flow, results in ischaemia-reperfusion (I/R) injury which leads to the occurrence of morphological, histological, and biochemical alterations within the ovarian tissue. Reperfusion leads to the release of free oxygen radicals and reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals from neutrophils, macrophages, and dendritic cells that accelerate secondary tissue injury. Endothelial disruption, local inflammatory responses, DNA damage, and finally granulosa cell apoptosis can occur due to increased levels of ROS. Elevated levels of ROS cause oxidative stress that promotes cell death. Moreover, oxidative stress has a devastating effect on DNA, hence it is the base of injury in the epithelium of ovary (Shokoohi et al. 2019). Despite basic and clinical research efforts, a detailed mechanism of I/R injury has not been described (Wu et al. 2018). At this stage, the use of antioxidant agents helps to support the self-defence mechanism of cells affected by I/R injury. Pentraxin 3 (PTX3) is a member of a phylogenetically conserved group of acute-phase reactants that are involved in inflammation and innate immunity. PTX3 levels increase after I/R injury. Zhu H. et al. reported that both mRNA and protein expression levels of PTX3 were increased following myocardial I/R injury (Zhu et al. 2014). In another study, serum and tissue levels of PTX3 were similarly elevated after intestinal I/R injury (Souza et al. 2009). Malondialdehyde (MDA) is one of the end products of lipid peroxidation, which increases in oxidative stress. MDA, is also a prototype of the thiobarbituric acid reactive substances (TBARS). Previous studies reported that both tissue and serum MDA levels increased as a result of I/R injury (Abali et al. 2013).

Angiotensin II (Ang II), the main effector of the renin-angiotensin system (RAS) and its receptors appear to be strongly associated with I/R injury. Furthermore, Ang II induces oxidative stress injury by producing excessive amounts of reactive oxygen species (Kobori et al. 2007). Ang II type 1 receptor blockers (AT_1-R) are commonly used for the treatment of hypertension to prevent end-organ damage. Losartan, a blocker of the Ang II type 1 receptor, inhibits oxidative damage in cells resulting from I/R injury. Numerous Ang II type 1 receptor blockers can be used to protect tissues and alleviate the detrimental effect of oxidative injury (Takaya et al. 2006). To our knowledge, there are no known studies in the literature that have evaluated the protective role of losartan in ovarian ischaemia and I/R injury.

In this experimental study, we investigated the effect of losartan on the ischaemic stage as well as on reperfusion injury, using histopathological score, tissue MDA and plasma PTX3.

Methods

Animals

A total of 30 mature (12-week-old) female Sprague-Dawley albino rats weighing 200–220 g each were used in this study. The animals were fed *ad libitum* and housed in pairs in steel cages in a temperature-controlled environment (22 ± 2 °C) with 12-h light/dark cycles. The experimental procedures were approved by the Committee for Animal Research of Ege University, Izmir, Turkey (2019-053). All animal studies strictly conformed to the experimental guidelines of the Committee for Human Care (Ege University).

The oestrous stage of each rat was determined by taking a vaginal smear at an interval of 6 to 12 hours. Cell types in the smear were subsequently examined under a microscope according to the staining procedure of Papanicolaou. Thirty rats that had confirmed smears (oestrous stage) were included in the experiments.

Experimental design

Surgical process

Rats were anesthetised by the intraperitoneal injection of a combination of 50 mg/kg ketamine hydrochloride and 7 mg/kg xylazine hydrochloride (Alfazyne; Alfasan International BV, Woerden, Holland). The skin around the incision was shaved and disinfected by povidone iodine. A laparotomy was performed by making a 2-cm longitudinal incision in the midline area of the lower abdomen and the uterine horns and adnexa were detected. Animals were randomly divided into five groups.

In Group 1 ($n = 6$, sham-operated group), the abdominal wall was kept open for 1 min and then closed with 3/0 silk sutures. In Group 2 ($n = 6$, torsion group), ischaemia was induced for 3 h using a torsion model stimulated by applying atraumatic vascular clips (Vascu-Statt, Scanlan International, Inc., Minnesota) to the vascular pedicle 1 cm above and below the ovary. The incision was closed with 3/0 silk sutures. In Group 3 ($n = 6$, torsion + losartan group), torsion was induced as in Group 2 at 30 min after the administration (oral gavage) of 40 mg/kg of losartan (Cozaar, MSD) (Gokce et al. 2008). In Group 4 ($n = 6$, torsion/detorsion group), 3 h of ischaemia and 3 h of reperfusion were performed. In Group 5 ($n = 6$, torsion/detorsion + losartan group), torsion was induced for 3 h as in Group 2, followed by 40 mg/kg of losartan administration (oral gavage) 30 min prior to 3 h of detorsion/reperfusion. Tablets containing 50 mg losartan (Cozaar, MSD) were crushed and suspended in tap water to yield a concentration of 10 mg/ml. According to the weight of each rat, the suspended drug solution was brought up to 4 ml with tap water. The medications were given via orogastric tubes. Immediately after the reperfusion period, both ovaries were excised for histological scoring and biochemical evaluation of MDA and PTX3.

Histopathologic evaluation

Ovarian samples were prepared for routine light microscopy. The specimens were rapidly fixed in 10% buffered formalin

for 48 h, dehydrated in an ascending alcohol series, cleared in xylene, and embedded in paraffin. Tissue sections (4 μm in thickness) were stained with haematoxylin and eosin for general morphologic analysis. All sections were studied and photographed with an Olympus C-5050 digital camera mounted on an Olympus BX51 microscope (Olympus Corp., Tokyo, Japan). All primordial and developing follicles were examined histologically. Briefly, primordial follicles are localised just below the cortex and contain a single layer of squamous granulosa cells. Primary follicles consist of a single layer of cuboidal granulosa cells. Secondary follicles contain multiple layers of granulosa cells, and tertiary follicles are characterised by stratum granulosum and a fluid-filled antral space. The follicles were histologically classified as degenerated when they included an oocyte with a pyknotic nucleus and shrunken ooplasm and/or disorganised granulosa cells.

Follicular degeneration, vascular congestion, haemorrhaging, oedema, and infiltration by inflammatory cells were scored from 0 to 3 according to the severity of injury with 0 indicating no pathologic findings and 1, 2 and 3 representing pathologic findings of <33%, 33–66%, and >66% of the ovarian section, respectively (Guven et al. 2010).

Measurement of tissue lipid peroxidation

For biochemical analysis, ovaries were homogenised in ice-cold 150 mM KCl (potassium chloride) and centrifuged at 5000 g for 10 min. The supernatants were analysed for lipid peroxidation. Lipid peroxidation was assessed in each tissue sample by measuring the MDA level as thiobarbituric acid-reactive substances (TBARSs). Briefly, trichloroacetic acid and TBARS reagent were added to the tissue samples, which were then mixed and incubated at 100 °C for 60 min. After cooling on ice, the samples were centrifuged at 3,000 rpm for 20 min and the absorbance of the supernatant was read at 535 nm. The MDA level was calculated from a standard calibration curve using tetraethoxypropane and expressed as nmol/μg protein (Demougeot et al. 2000).

Measurement of the tissue protein levels

The total protein concentration in the tissue samples was determined according to Bradford's method using bovine serum albumin as a standard (Bradford 1976).

Evaluation of plasma PTX3 levels

Plasma pentraxin-3 (PTX3) levels were measured in each 100-μl sample by standard ELISA apparatus at 450 nm using a PTX3 kit (Uscn Life Science Inc., Wuhan, China). PTX3 levels were determined in duplicate according to the manufacturer's guide. The detection range for the PTX3 assay was 0.078–5 ng/ml.

Statistical analysis

GraphPad Prism 8.1.1 software (GraphPad Software, La Jolla, CA, USA) was used for all statistical analyses. Parametric variables (biochemistry data) were evaluated with one-way ANOVA tests where non-parametric variables (histopathology scores) were analysed using the Mann Whitney-U test. p values $<.05$ were considered as statistically significant. Results were expressed as the mean ±standard error of the mean (SEM).

Results

The histopathological results of ovarian tissue samples from the five groups are shown in Figure 1. Parameters, such as

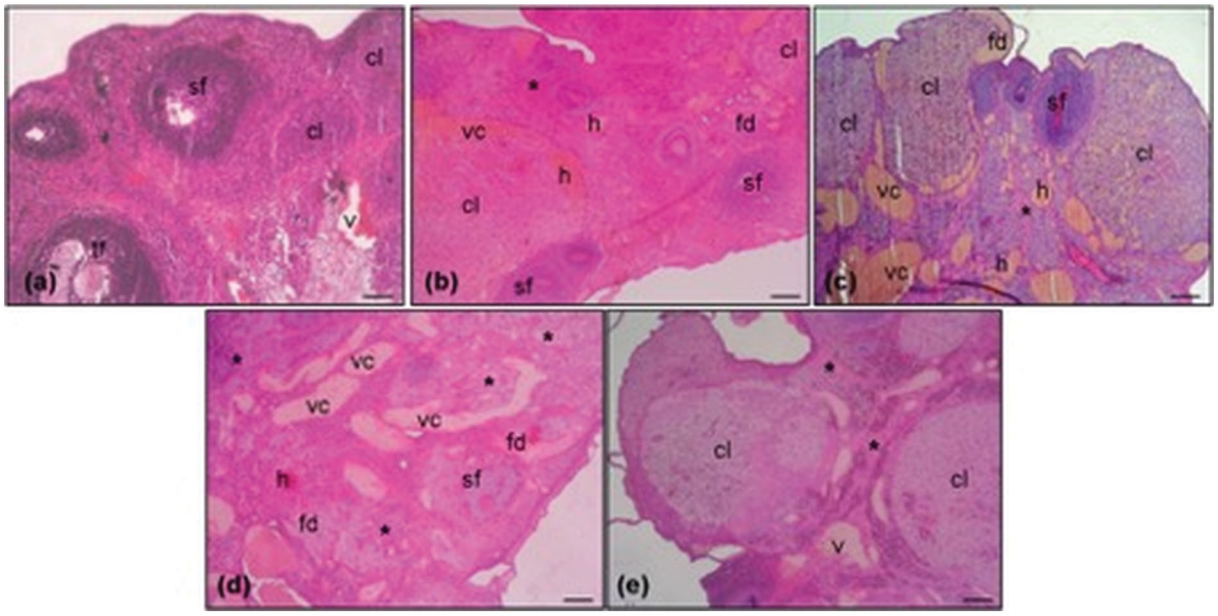

Figure 1. (a) No pathological changes were detected in the sham-operated animals. (v) vessel, (sf) secondary follicle, (tf) tertiary follicles, (cl) corpus luteum. (b) Follicular degeneration (fd), oedema (*), vascular congestion (vc) and haemorrhaging (h) were detected in the 3-h torsion group. (c) Oedema, follicular degeneration and haemorrhaging were observed in the torsion and losartan group. (d) Vascular congestion (vc), haemorrhaging (h), oedema (*), follicular degeneration (fd) were observed in the torsion/de-torsion group. (e) Decreased oedema (*) and no haemorrhaging and no follicular degeneration (fd) were detected in the torsion/de-torsion and losartan group. Haematoxylin and eosin staining was performed. Scale bars represent 250 μm.

follicular degeneration, haemorrhage, oedema, and inflammatory cells were evaluated for tissue injury. No histopathological changes were observed in the sham-operated group (group 1). Contrarily, increased follicular degeneration ($p < .000$), oedema ($p < .05$), vascular congestion ($p < .05$), haemorrhage ($p < .05$), and infiltration by inflammatory cells ($p < .05$) were observed in the torsion group (group 2) in comparison with group 1. In the torsion + losartan group (group 3), all these parameters except haemorrhage were significantly less ($p < .05$) compared with those associated with the torsion group (group 2). The reduction in the haemorrhage parameter in group 3 was not statistically significant ($p > .05$). In the torsion/detorsion group (group 4), follicular degeneration, oedema, vascular congestion, haemorrhage, and infiltration by inflammatory cells ($p < .01$) were significantly higher than in group 1. Follicular degeneration and vascular congestion ($p < .01$), oedema, haemorrhage, and leukocyte infiltration ($p < .05$) were remarkably reduced in the torsion/detorsion +losartan group (group 5) than in the torsion/detorsion group (group 4). The results of histopathological examination of the ovaries in each group are listed in Table 1.

Tissue MDA levels were significantly higher in group 2 and group 4 than in group 1 ($p < .01$). MDA levels were remarkably lower in the torsion + losartan group (group 3) than those in the torsion group (group 2; $p < .05$). Moreover, tissue MDA was higher in the torsion/detorsion group (group 4) than in the torsion/detorsion +losartan group (group 5; $p < .0001$). Differences of tissue MDA levels between groups are depicted in Figure 2.

Plasma PTX3 was significantly higher in the torsion group (group 2; $p < .01$) and torsion/detorsion group (group 4; $p < .01$) than in the sham group (group 1). When the losartan was administered before torsion, a significant decrease was observed in group 3 compared with group 2 ($p < .05$). Additionally, the levels of plasma PTX3 were significantly lower in the torsion/detorsion + losartan group (group 5) than those in the torsion/detorsion group (group 4; $p < .000$). Alterations of plasma PTX3 levels between groups are shown in Figure 3.

Discussion

The present study demonstrated that losartan, an Ang II type 1 receptor blocker currently used for blood pressure regulation, can alleviate experimental I/R injury in ovaries by improving histological parameters as well as by reducing tissue MDA and plasma PTX3 levels. The results demonstrated that losartan reduces I/R injury via antioxidant activity prior to reperfusion of the ovary. Conserving the ovary plays an important role in fertility. The current management of ovarian torsion includes a detorsion procedure to maintain the ovarian reserve even if the ovaries appear necrotic

Table 1. Histopathological scores of the ovaries between groups.

	Group 1 (control)	Group 2 (torsion)	Group 3 (torsion + losartan)	Group 4 (torsion/detorsion)	Group 5 (torsion/detorsion + losartan)
Follicular degeneration	0.16 ± 0.16	2.0 ± 0.26**	0.66 ± 0.33[a]	2.5 ± 0.34[b]	0.83 ± 0.31[††]
Vascular congestion	0.18 ± 0.16	1.8 ± 0.30*	0.84 ± 0.30[a]	2.33 ± 0.21[b]	1.0 ± 0.25[††]
Oedema	0.16 ± 0.16	1.8 ± 0.30*	0.83 ± 0.30[#]	2.16 ± 0.40[b]	1.3 ± 0.33[†]
Haemorrhage	0.33 ± 0.21	1.5 ± 0.22*	0.50 ± 0.21***	2.0 ± 0.36[b]	0.83 ± 0.31[†]
Infiltration by inflammatory cells	0.16 ± 0.16	0.83 ± 0.22*	0.33 ± 0.16[a]	1.50 ± 0.34[b]	0.66 ± 0.21[†]

*$p < .05$, Group 2 compared with Group 1.
**$p < .000$, Group 2 compared with Group 1.
***$p > .05$, Group 3 compared with Group 2.
[#]$p < .05$, Group 3 compared with Group 2.
[a]$p < .01$, Group 3 compared with Group 2.
[b]$p < .01$, Group 4 compared with Group 1.
[†]$p < .05$, Group 5 compared with Group 4.
[††]$p < .01$, Group 5 compared with Group 4.

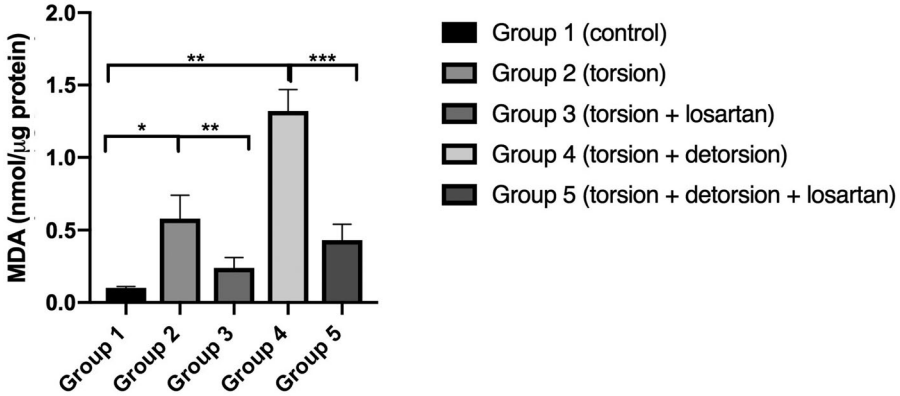

Figure 2. Comparison of tissue MDA. Data are expressed as the mean ± standard error of the mean, MDA indicates malondialdehyde. *$p < .01$, Group 2 compared with the Group 1; **$p < .05$, Group 3 compared with the Group 2; **$p < .01$, Group 4 compared with the Group 1; ***$p < .0001$, Group 5 compared with the Group 4.

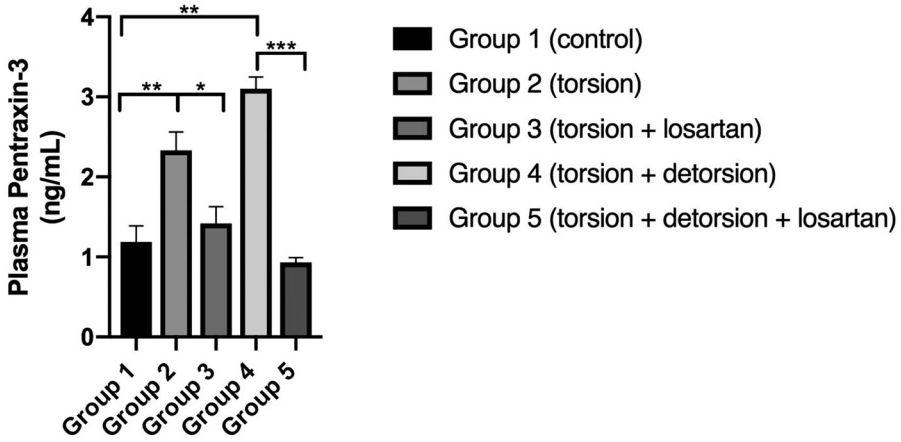

Figure 3. Comparison of plasma PTX 3 levels. Data are expressed as the mean ± standard error of the mean, PTX 3 indicates plasma pentraxin 3. *$p < .05$, Group 3 compared with the Group 2; **$p < .01$, Group 2 compared with the Group 1; **$p < .01$, Group 4 compared with the Group 1; ***$p < .000$, Group 5 compared with the Group 4.

(Huchon and Fauconnier 2010). After detorsion, re-establishment of oxygen into the ischaemic cellular environment can trigger tissue injury by damaging cellular membranes, including the mitochondria, by free radical formation, by the aggregation of leukocytes and inflammatory mediators, and by the activation of the pro-apoptotic signalling cascade and complement systems (Maneechote et al. 2017). The mechanisms of ovarian injury after I/R and cell death are not yet fully understood. Understanding the detailed mechanism of ischaemia-reperfusion injury may provide a strong foundation not only for novel therapeutic opportunities, but also for injury prevention. However, it has been demonstrated that oxygen free radical generation is a critical mechanism causing injury in post-ischaemic cells and tissues (Wu et al. 2018). When tissue expose I/R, proinflammatory cytokines produced by inflammatory cells can trigger adhesion and migration of circulating neutrophils to endothelial cells and generation of ROS, which increases neutrophil infiltration and results in ischaemic injury. ROS and their toxic products cause DNA damage and lipid peroxidation in the cellular and mitochondrial membranes, impairs ion channels, leading to cell damage and even death. Cell damage induced by prolonged ischaemia-reperfusion injury may lead to apoptosis, autophagy, necrosis, and necroptosis (Zhu et al. 2016). Moderate ischaemia-reperfusion injury may cause cell dysfunction by autophagy and activate recovery systems for survival. If damage is severe, cell death may be induced via apoptotic or necrotic pathways. In human physiology, ROS and antioxidants stay in equilibrium. The level of anti-oxidant agents in ischaemic cells crucial for overcoming the destructive effect of ROS. In parallel, the generation of ROS increases due to a lower concentration of antioxidative agents in ischaemic tissues (Kalogeris et al. 2014). Therefore, losartan which has a potential antioxidant role used in cardiovascular medicine can protect the destructive effect of I/R injury in the ovary. Compression of ovarian vessels because of stromal oedema and enlargement of the ovary first obstructs lymphatic and venous outflow and then arterial inflow. Then, ovarian arterial perfusion is blocked due to the stromal pressure, which results in infarction and necrosis in addition to microscopic haemorrhage in the ovarian tissue (Kurt et al. 2015). In the present study, with increased cellular damage, higher histopathological scores for haemorrhage and vascular congestion were observed in the torsion groups than in the sham group.

To date, there have been several pharmacological agents investigated for their protective role in ovarian I/R injury in experimental animal models (Kumtepe et al. 2010; Hortu et al., 2019). However, losartan is the first agent used in our study to evaluate its potential effect on ovarian I/R injury using a histopathological score, as well as ovarian tissue MDA, and plasma PTX3 levels as tissue and systemic I/R injury markers. Angiotensin II binds two major angiotensin receptor subtypes, AT_1-R and AT_2-R, to exert its physiological effects. As Ang II increases vascular permeability, stimulates inflammatory cell recruitment and activates pro-inflammatory chemokines and cytokines, several AT_1-R blockers, including losartan, have been shown to be protective in various experimental I/R models (Suzuki et al. 2003). Angiotensin II stimulation has been reported to produce ROS from miscellaneous vascular cell types (Babiker et al. 2016). The study of Dol et al. demonstrated that ARB alleviated atherosclerotic lesion formation in mice through decreasing chemokine expression and macrophage accumulation and by inhibiting low-density lipoprotein oxidation (Dol et al. 2001). Losartan has well-known inhibitory actions on neutrophils in various organs such as the heart, liver, intestine, and kidney (Riaz et al. 2004). It acts via suppression of neutrophil functions (rolling, adhesion, migration) and causes an eventual decrease in extravascular infiltration. According to these mechanisms, in our study, we found that after administration of losartan to rats, neutrophil infiltration was significantly less in the ovaries in group 5 (torsion/detorsion + losartan) than in group 4 (torsion/detorsion). In addition, leukocyte infiltration scores were significantly lower in group 3 (torsion + losartan) than those in group 2 (only torsion). Ovarian damage was reported to be more severe in the torsion/detorsion group than in the only torsion group, which is consistent with previously reported results (Ergun et al. 2010). Hence, losartan inhibits ovarian follicle loss through aforementioned antioxidant properties, such as decreasing apoptosis and autophagy.

Additionally, in the cases of ovarian torsion, losartan has beneficial effects on ovarian functions which have a great potential role for women in gynaecology practice in the reproductive period.

MDA, a marker of tissue injury, is a secondary product of oxidative damage formed during lipid peroxidation. It disrupts ionic transport as well as enzymatic activity, distorts cell membrane permeability and fluidity that separate cells and organelle contents (Rodrigo et al. 2013). In the present study, we observed significantly increased tissue MDA levels following ovarian I/R injury, which were decreased by losartan treatment. This result indicates that the ovarian protection conferred by losartan may be attributed to attenuating lipid peroxidation following I/R. Hence, our data suggest that losartan protects ovarian injury through the amelioration of oxidative stress.

PTX 3 is a member of the pentraxin family, and is also called the tumour necrosis factor-inducible gene 14 protein (TSGF-14). PTX 3 is produced by numerous cell types, including endothelial cells, epithelial cells, myocardial cells and mononuclear phagocytes and is present in very low amounts in serum and tissues of healthy subjects but rapidly increases in response to a variety of inflammatory stimuli (Souza et al. 2009). Our results showed that increased PTX3 expression was associated with ovarian I/R injury in a rat model. Plasma PTX 3 levels were significantly higher both in the torsion and torsion/detorsion groups compared with those in the sham group. Losartan also decreased ovarian MDA and plasma PTX 3 levels significantly both in the torsion + losartan and torsion/detorsion + losartan groups. Several studies report the ameliorative mechanisms of losartan on post-ischaemic and I/R injury in the literature (Ivanov et al. 2014). For example, Koh et al. suggest that losartan ameliorates I/R-induced liver damage through peroxisome proliferator-activated receptor gamma (PPAR-γ) (Koh et al. 2013). Guo et al. reported that Ang II inhibition prevents acute liver injury in a model of inflammation caused by ischaemia and reperfusion (Guo et al. 2004). In our study, losartan treatment showed significant protective effects by means of decreasing all oxidative stress parameters caused by ischaemia and reperfusion.

The current study had some limitations. The first is that we have not assessed total oxidant and antioxidant status, and other markers such as glutathione peroxidase, superoxide dismutase, and myeloperoxidase activity due to limited funding. The second is the fact that the present study was an animal model study and therefore, further comprehensive clinical human studies are necessary to draw definite conclusions before the results can be generalised to human beings. The third is the absence of observation of the long-term alterations (i.e. ovarian reserve via antral follicle number, anti-Müllerian hormone) in both histopathological and biochemical parameters. Further large-scale studies are required to determine drug dose, duration, and method of administration.

In conclusion, these observations suggest that pre-treatment with losartan has a significant protective role on ischaemia and ischaemia/reperfusion injury in rats with ovarian torsion. Our findings may provide an option for reducing the oxidative damage of the ovary against I/R injury in the field of gynaecology. In this regard, a fundamental and comprehensive understanding of action of losartan on reproductive organs is the key to future advances and applications. Larger clinical studies should stress the long-term effects of losartan on the human body including ovarian reserve and fertility.

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Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Ismet Hortu http://orcid.org/0000-0003-3833-0999

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