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Antioxidant effect of erdosteine and lipoic acid in ovarian ischemia–reperfusion injury

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ABSTRACT

Objective: To investigate the effects of erdosteine and alpha lipoic acid (ALA) in a rat model of ovarian ischaemia–reperfusion injury.
Study design: Forty-eight female Wistar albino rats were separated, at random, into six groups of eight rats. The groups were classified as: sham, torsion, detorsion, detorsion + erdosteine 100 mg/kg, detorsion + alpha lipoic acid (ALA) 100 mg/kg, and detorsion + erdosteine + ALA. The investigators executing the biochemical and histological analyses were blinded to the randomization until the end of the study.
Results: The TOS (Total Oxidant Status) and OSI (Oxidative Stress Index) levels are higher in the Torsion and Detorsion groups when compared with the ones in the Sham group ($p < 0.05$). Strong correlation was found between OSI and total histological score in the sham, torsion and detorsion groups ($r = 0.765$, $p < 0.001$). The mean levels of TOS and OSI in the rats that received erdosteine and/or ALA were significantly lower compared with the sham, torsion and detorsion groups ($p < 0.05$). Mean TOS and mean OSI were lower in the detorsion + erdosteine + ALA group compared with the detorsion + erdosteine and detorsion + ALA groups ($p < 0.05$). In comparison with the detorsion group, the numbers of primordial follicles ($p = 0.006$) and primary follicles ($p = 0.036$) were increased in the groups that received erdosteine and/or ALA.
Conclusions: Erdosteine and ALA decreased ischaemia–reperfusion injury in an experimental rat ovarian torsion model; combination treatment had a greater effect than either agent alone. Treatment with erdosteine and/or ALA was found to preserve the loss of reproductive capacity normally observed after ovarian torsion.

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Introduction

Ovarian torsion is a gynaecological emergency that results in decreased or lack of blood flow due to partial or complete rotation of the ovary around its own ligamentous supports [1]. Although rare in clinical practice, it represents 2.7% of gynaecological surgical emergencies, affecting both children and adolescents [2].

Early diagnosis and treatment for detorsion of the involved segments are very important for the preservation of ovarian function [3,4]. The primary pathophysiological event in ovarian torsion–detorsion causes ovarian ischaemia–reperfusion (I/R) injury [5,6]. Ovarian tissues contain release of free radicals and cytokines, neutrophil and thrombocyte activation, nitric oxide and apoptosis due to I/R injury [7–10]. During the I/R period, reactive oxygen species are generated, such as hydroxyl radicals, hydrogen peroxide and superoxide. However, separate assessment of these markers is impractical and also inadequate for comprehensive evaluation. The total antioxidant status (TAS) has been used to appraise the overall antioxidant situation [11]. At the same time,

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total oxidant status (TOS) is used to determine the full oxidation state [12]. The oxidative stress index (OSI) was evaluated as the proportion of TOS to TAS; this is considered to be a more sensitive indicator of oxidative stress on the tissue.

Erdosteine [N-(carboxymethylthioacetyl)-homosyteine thiolactone], a mucolytic drug, is used widely in clinical practice. Functioning erdosteine metabolites show free radical depuration activity, with sulphydryl groups released following the catabolism of erdosteine in the liver; these groups account for the antioxidant activity of erdosteine [13,14]. However, to the authors' knowledge, no studies have investigated the effects of erdosteine in an ovarian I/R injury rat model.

Alpha lipoic acid (ALA) plays an important role in mitochondrial dehydrogenase reactions, which occur in all types of eukaryotic and prokaryotic cells, and has received attention as an antioxidant [15]. ALA and its reduced form dihydrolipoic acid work as antioxidants by fighting free radicals and reprocessing other cellular antioxidants [16]. The curative impact arises from free, unbound ALA from other sources. Free ALA has not been determined in humans due to protein binding. After alternative utilization of different forms, free ALA may be found in the circulation [17].

The aim of this study was to investigate the effects of erdosteine and ALA on ovarian I/R injury in a rat model. It was hypothesized that combination treatment, with enhanced cellular strength during the oxidative period due to ALA and free radical depletion due to erdosteine, would provide greater protection of ovarian tissue and reproductive capacity in the ovarian I/R injury model than single-agent treatment.

Materials and methods

Animals

The experimental protocol was approved by the Animal Ethics Committee of Mustafa Kemal University (Approval No. 40595970/29). Forty-eight female adult Wistar albino rats, weighing 250–300 g, were obtained from the Experimental Animal Laboratory of Hatay Mustafa Kemal University. Rats were caged individually in a controlled environment at 20–22 °C temperature and humidity and 12-h light/dark cycles, and fed ad libitum. The rats were given 5 days to acclimatize to the environment before the study commenced. The rats were separated, at random, into six groups of eight rats. The groups were: sham (Group I), torsion (Group II), detorsion (Group III), detorsion + erdosteine (Group IV), detorsion + ALA (Group V), and detorsion + erdosteine + ALA (Group VI).

Chemicals

Erdosteine (Erdostin suspension) was obtained from Ilsan-iltas Pharmaceuticals (Istanbul, Turkey), and ALA was obtained from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Ketamine hydrochloride (Ketalar 50 mg/ml) was obtained from Pfizer (Turkey), and xylasine hydrochloride (Rompun) was obtained from Bayer (Turkey).

Surgical technique

All rats were anaesthetized with intramuscular 50 mg/kg Ketalar and 10 mg/kg Rompun. Pre-operative sterilization was performed, and a midline incision (2.0 cm) was made to the lower abdomen. In Group I, the laparotomy was performed and the incision was closed with 3,0 nylon suture. After a 3-h period, a re-laparotomy was performed and both ovaries were extracted. In this study, the adnexal torsion (AT) technique was performed as follows: the adnexa were rotated 360° in a clockwise direction, including ovarian vessels and tubes, and then fixed to the abdominal wall [18]. In Group II, AT was performed and both ovaries were extracted following re-laparotomy after a 3-h period of ischaemia. In Group III, both AT (3 h of ischaemia) and adnexal detorsion (AD) (3 h of reperfusion) were performed. After 6 h, both ovaries were extracted following re-laparotomy. In Group IV, rats received erdosteine 150 mg/kg by oral gavage. After 24 h, both AT (3 h of ischaemia) and bilateral AD (3 h of reperfusion) were performed. Both ovaries were extracted after these procedures. In Group V, both AT (3 h of ischaemia) and AD (3 h of reperfusion) were performed, and ALA 100 mg/kg was applied to the peritoneum 30 min before AD. Both ovaries were extracted after these procedures. In Group VI, rats received erdosteine 150 mg/kg by oral gavage [19]. After 24 h, both AT (3 h of ischaemia) and AD (3 h of reperfusion) were performed, and ALA was applied to the peritoneum 30 min before AD. After surgery, all rats were killed. One of the ovaries from each rat was cleaned of adherent soft tissues and stored in a freezer at −80° C for biochemical analysis; the other ovary was fixed in 10% formaldehyde solution for histological examination.

Histological analysis

Ovarian tissues were fixed in 10% formalin for 48 h, cleaned, dehydrated and embedded in paraffin. The paraffin blocks were sectioned using a microtome (Leica RM2125RTS, Nussloch-Germany) at a thickness of 4 mm, and stained with haematoxylin and eosin. A light microscope (Olympus Clinical Microscope BX45, Tokyo-Japan) was used to analyze all sections. Five microscopic areas were evaluated to determine the scores of the specimens semiquantitatively. The criteria for ovarian injury were vascular congestion, haemorrhage, follicular cell degeneration (granulosa cells) and inflammation (neutrophil infiltration). The findings were classified separately for each parameter as follows: Grade 0, no findings; Grade 1, findings $<$33%; Grade 2: findings of 33–66%; and Grade 3, findings $>$66% [20]. One pathologist examined all of the ovarian sections in a blinded fashion. The numbers of primordial, primary, pre-antral (secondary) and antral (tertiary) follicles in each ovarian section were counted [21].

Biochemical steps and analyses

For determination of serum TOS and TAS, rats were killed and blood samples were collected on ice at 4 °C. Serum samples were separated from cells by centrifugation at 3000 rpm for 15 min, stored at −80 °C, and used for the analysis of TAS and TOS. Serum TAS and TOS were determined using Rel Assay Diagnostics kits (Mega Tip, Turkey), and OSI values were calculated.

Measurement of TAS

Erel developed a new automated measurement technique to determine serum TAS. This technique measures the antioxidant effect of the sample against the potent free radical reactions initiated by hydroxyl radicals. The assay had sensitivity of $<$3%. The findings are expressed as μmol Trolox Eq/l [11].

Measurement of TOS

Erel also developed an automated measurement technique to determine serum TOS. The colour density, which can be measured spectrophotometrically, indicates the total quantity of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and findings are described for micromolar hydrogen peroxide equivalent per litre (μmol H_2O_2 Eq/l) [12].

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